ΕΛΛΗΝΙΚΗ ΝΕΥΡΟΛΟΓΙΚΗ ΕΤΑΙΡΕΙΑ
ΑΛΚΜΑΝΟΣ 10, 115 28, ΑΘΗΝΑ
ΕΝΤΥΠΟ ΚΛΕΙΣΤΟ ΑΡ. ΑΔΕΙΑΣ 815/94 ΚΕΜΠ ΚΡ.
ΚΩΔΙΚΟΣ ΕΝΤΥΠΟΥ: 017159

ISSN: 2732-9119

ΕΛΛΗΝΙΚΗ ΝΕΥΡΟΛΟΓΙΚΗ ΕΤΑΙΡΕΙΑ
HELLENIC NEUROLOGICAL SOCIETY
www.enee.gr

Αρχεία Κλινικής Νευρολογίας

www.jneurology.gr

ΕΠΙΣΗΜΟ ΠΕΡΙΟΔΙΚΟ ΤΗΣ ΕΛΛΗΝΙΚΗΣ ΝΕΥΡΟΛΟΓΙΚΗΣ ΕΤΑΙΡΕΙΑΣ

Τόμος 31 - Τεύχος 1

Vol. 31 - Issue 1

Ιανουάριος - Φεβρουάριος 2022 / January - February 2022

ΕΙΔΙΚΟ ΤΕΥΧΟΣ / SPECIAL ISSUE
ΕΝΕ: ΘΕΡΑΠΕΥΣΙΜΑ ΓΕΝΕΤΙΚΑ ΝΕΥΡΟΛΟΓΙΚΑ ΝΟΣΗΜΑΤΑ / TREATABLE GENETIC NEUROLOGICAL DISEASE

ΑΡΘΡΑ / ARTICLES

Διμηνιαία έκδοση της
Ελληνικής Νευρολογικής Εταιρείας
Αλκμάνος 10, Αθήνα 115 28
Τηλ.: 210 72.47.056 - Fax: 210 72.47.556
www.enee.gr info@jneurology.gr

ΙΔΙΟΚΤΗΣΙΑ

ΕΛΛΗΝΙΚΗ ΝΕΥΡΟΛΟΓΙΚΗ ΕΤΑΙΡΕΙΑ
Διεύθυνση: Αλκμάνος 10,
Αθήνα ΤΚ 115 28

ΠΑΡΑΓΩΓΗ ΕΝΤΥΠΗΣ ΕΚΔΟΣΗΣ ΚΑΙ ΗΛΕΚΤΡΟΝΙΚΩΝ ΑΡΧΕΙΩΝ

Λυχνία Α.Ε.
Ανδραβίδας 7
136 71, Χαμόμυλο Αχαρνών
Τηλ.: 210 34.10.436 - 1, Fax: 210.34.25.967
www.lyhnia.com

ΣΥΝΔΡΟΜΕΣ

Μέλη της ΕΝΕ Δωρεάν

Κωδικός Διεύθυνσης Εποπτείας ΜΜΕ: 7159ISSN 1106 - 3106

Αρχεία Κλινικής Νευρολογίας

www.jneurology.gr

Τόμος 31, Τεύχος 1, Ιανουάριος - Φεβρουάριος 2022

Περιεχόμενα

Official Journal of the
Hellenic Neurological Society
10, Alkmanos str., Athens
Tel.: 210 72.47.056 - Fax: 210 72.47.556
www.enee.gr info@jneurology.gr
e-submission: submission@jneurology.gr

PRINTED EDITION AND PDFs

Lychnia S.A.
7 Andravidas str., Athens
136 71, Hamomilo Aharnon
Tel.: 210 34.10.436 - 1, Fax: 210.34.25.967
www.lyhnia.com

SUBSCRIPTION FEES

HNS Members Free

Archives of Clinical Neurology

www.jneurology.gr

Volume 31:1 January - February 2022

Contents

Editorial for the Special Issue "Treatable Genetic Neurological Diseases"

We are pleased to announce a Special Issue of the "Archives of clinical Neurology" *Journal of the Hellenic Neurological Society* on the topic of "Treatable Genetic Neurological Diseases".

Advances in genetics have provided efficient techniques for the diagnosis of genetic neurological diseases. Moreover, in recent years, apart from the increasing application of neurogenetics in the clinical evaluation of several disorders, the available therapeutic options have also dramatically expanded. Identifying the causative genetic loci responsible for the various phenotypic expressions of nervous system pathology is of great importance for both highlighting their pathophysiology processes and improving their treatment efficacy.

In the present Special Issue, we have collected review articles regarding on-going information focusing on the pathophysiology, clinical manifestations, diagnosis, and treatment approaches of various genetic neurological diseases, encompassing all the newest advancements. We believe that this collection offers a useful and up-to-date guide for clinical neurologists concerning the diagnosis and the treatment of neurological diseases with a genetic basis. Moreover, we hope that future studies on the therapeutic approaches of genetic neurological diseases will attempt to provide novel ways to manage these patients and lead to a personalized, effective treatment.

We would like to thank all authors who have contributed to this special issue with these valuable review articles.

Efthimios Dardiotis
Department of Neurology, Laboratory of Neurogenetics,
Faculty of Medicine, University of Thessaly, Larissa, Greece

Συντακτική Ομάδα (Editorial Board)

Συντακτική Ομάδα (Editorial Board)

Συντακτική Ομάδα (Editorial Board)

25. Χ. Κρόγιας (Ruhr University of Bochum, Bochum, Germany)
26. Β. Λιούτας (Harvard University, Boston, USA)
27. Π. Μήτσιας (Πανεπιστήμιο Κρήτης, Ηράκλειο & Wayne State University, Detroit, USA)
28. Γ. Ρούντολφ (Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη)
29. Α. Σαφούρης (Νοσοκομείο Metropolitan, Πειραιά)
30. Κ. Σπέγγος (Νοσοκομείο Υγεία, Αθήνα)
31. Γ. Τσιβγούλης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών & University of Tennessee Health Sciences Center, Memphis, USA)

Παιδονευρολογία

1. Β. Δάρας (Harvard University, Boston, USA)
2. Α. Ευαγγελίου (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
3. Δ. Ζαφειρίου (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
4. Α. Παπαβασιλείου (Ιασώ Παίδων, Αθήνα)

Κλινική Νευροφυσιολογία

1. Ε. Αναγνώστου (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
2. Π. Ζης (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)
3. Ι. Καράκης (Emory University, Atlanta, USA)
4. Β. Κιμισκίδης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
5. Π. Κοκότης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
6. Α. Κωδούνης (251 Γενικό Νοσοκομείο Αεροπορίας, Αθήνα)
7. Α. Μπονάκης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
8. Μ. Παπαδοπούλου (Πανεπιστήμιο Δυτικής Αττικής)
9. Χ. Πιπερίδου (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)
10. Ε. Σταμπουλής (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
11. Δ. Τσίπτσιος (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)
12. Ε. Χρόνη (Πανεπιστήμιο Πάτρας, Πάτρα)

Άνοια

1. Π.Ιωαννίδης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
2. Ε.Καπάκη (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
3. Χ.Μπούρας (University of Geneva, Geneva, Switzerland)
4. Γ.Παρασκευάς (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
5. Ν.Σκαρμέας (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
6. Μ.Τσολάκη (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)

Επιληψία

1. M. Reuber (University of Sheffield, UK)
2. Α. Αγαθονίκου (Γενικό Νοσοκομείο Αθηνών ΚΑΤ, Αθήνα)
3. Α. Αρζιμάνογλου (University Hospital of Lyon, Lyon, France)
4. Ι. Καράκης (Emory University, Atlanta, USA)
5. Β. Κιμισκίδης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
6. Μ. Κουτρουμανίδης (Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom)
7. Χ. Πιπερίδου (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)
8. Π. Πολυχρονόπουλος (Πανεπιστήμιο Πάτρας, Πάτρα)
9. Αικ.Τερζούδη (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)

Συντακτική Ομάδα (Editorial Board)

Κεφαλαλγία
1. Χ.Αρβανίτη (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
2. Θ. Αβραμίδης (Γενικό Νοσοκομείο Αθηνών Ερυθρός Σταυρός, Αθήνα)
3. Μ. Βικελής (Αθήνα)
4. Κ. Γυμνόπουλος (Γενικό Νοσοκομείο Θεσσαλονίκης Άγιος Λουκάς, Θεσσαλονίκη)
5. Π. Μήτσιας (Πανεπιστήμιο Κρήτης, Ηράκλειο & Henry Ford Hospital - Wayne State University, Detroit, USA)
6. Δ. Μητσικώστας (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
7. Γ. Ρούντολφ (Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη)

Ιστορία της Νευρολογίας
1. Α. Καράβατος (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
2. Λ. Τριάρχου (Πανεπιστήμιο Μακεδονίας, Θεσσαλονίκη)

Επεμβατική Νευρολογία
1. N. Goyal (University of Tennessee Health Sciences Center, Memphis, USA)
2. A. Sarraj (The University of Texas McGovern Medical School, Houston, USA)
3. Α. Σαφούρης (Νοσοκομείο Metropolitan, Πειραιά)

Κινητικές Διαταραχές
1. Μ. Αρναούτογλου (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
2. Ζ.Μ. Κεφαλοπούλου (Πανεπιστήμιο Πάτρας, Πάτρα)
3. Σ. Κονιτσιώτης (Πανεπιστήμιο Ιωαννίνων, Ιωάννινα)
4. Σ. Μποσταντζοπούλου (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
5. Μ. Πολίτης (University of Exeter, UK)
6. Μ. Σταμέλου (University of Marburg, Germany)
7. Λ. Στεφανής (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)

Νευρογενετική
1. K. Kleopa (Cyprus Institute of Neurology and Genetics, Cyprus)
2. Ε. Δαρδιώτης (Πανεπιστήμιο Θεσσαλίας, Λάρισα)
3. Γ. Κούτσης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
4. Δ. Μόνος (University of Pennsylvania, Philadelphia, USA)
5. Γ. Ξηρομερίσιου (Πανεπιστήμιο Θεσσαλίας, Λάρισα)
6. Γ. Χατζηγεωργίου (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)

Νευροανοσολογία
1. R. Gold (Ruhr University of Bochum, Bochum, Germany)
2. M. Hadjivassiliou (University of Sheffield, UK)
3. Κ. Βουμβουράκης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
4. Ν. Γρηγοριάδης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
5. Ε. Δαρδιώτης (Πανεπιστήμιο Θεσσαλίας, Λάρισα)
6. Γ. Δερετζή (Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη)
7. Ε.Μ. Ευαγγελοπούλου (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
8. Ι. Ηλιόπουλος (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)
9. Λ. Κάππος (University of Basel, Basel, Switzerland)
10. Κ. Κυλιντηρέας (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
11. Δ. Μόνος (University of Pennsylvania, Philadelphia, USA)
12. Μ. Μποζίκη (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
13. Τ. Ντόσκας (Ναυτικό Νοσοκομείο Αθηνών, Αθήνα)
14. Π. Παπαθανασόπουλος (Πανεπιστήμιο Πάτρας, Πάτρα)

15. Γ. Τζάρτος (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
16. Γ. Χατζηγεωργίου (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)

Νευροεντατική
1. J. Chang (MedStar Washington Hospital Center)
2. T. Steiner (University of Heidelberg, Heidelberg, Germany)
3. Π. Βαρελάς (Albany Medical College, Albany, USA)
4. Κ. Δημητριάδης (Ludwig-Maximillians University Munich, Germany)
5. Δ. Κάζης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
6. Χ. Κρόγιας (Ruhr University of Bochum, Bochum, Germany)
7. Γ. Ρούντολφ (Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη)

Εκπαίδευση στη Νευρολογία
1. Θ. Αβραμίδης (Γενικό Νοσοκομείο Αθηνών Ερυθρός Σταυρός, Αθήνα)
2. Κ. Βαδικόλιας (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)
3. Π. Βαρελάς (Albany Medical College, Albany, USA)
4. Κ. Βουμβουράκης ((Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
5. Ν. Γρηγοριάδης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
6. Ε. Δαρδιώτης (Πανεπιστήμιο Θεσσαλίας, Λάρισα)
7. Γ. Δερετζή (Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη)
8. Π. Ζης (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)
9. Κ. Κυλιντηρέας (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
10. Π. Μήτσιας (Πανεπιστήμιο Κρήτης, Ηράκλειο & Henry Ford Hospital - Wayne State University, Detroit, USA)
11. Ι. Μυλωνάς (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
12. Γ. Ρούντολφ (Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη)
13. Λ. Στεφανής (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
14. Γ. Τσιβγούλης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών & University of Tennessee Health Sciences Center, Memphis, USA)
15. Γ. Χατζηγεωργίου (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)

Νευρομυϊκές διαταραχές
1. C. McDermott (University of Sheffield, UK)
2. Θ. Αβραμίδης (Γενικό Νοσοκομείο Αθηνών Ερυθρός Σταυρός, Αθήνα)
3. Π. Δαβάκη (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
4. Θ. Ζαμπέλης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
5. Β. Ζούβελου (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
6. Π. Ζης (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)
7. Ι. Μαυρομάτης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
8. Γ. Παπαδήμας (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
9. Α. Παπαδημητρίου (Πανεπιστήμιο Θεσσαλίας, Λάρισα)
10. Δ. Παρίσης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
11. Ε. Σταμπουλής (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
12. Ν. Τάσκος (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)

Νευρο-ογκολογία
1. Α. Κυρίτσης (Πανεπιστήμιο Ιωαννίνων, Ιωάννινα)

Νευρο-οφθαλμολογία
1. Ε. Αναγνώστου (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
2. Ι. Ευδοκιμίδης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)

Συντακτική Ομάδα (Editorial Board)

3. Ι. Ηλιόπουλος (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)

Νευροψυχολογία – Νευροψυχιατρική
1. Γ. Δελατόλας (Universite Paris Descartes, Paris, France)
2. Ε. Καπάκη (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
3. Α. Καράβατος (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
4. Χ. Μπακιρτζής (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
5. Χ. Μπούρας (University of Geneva, Geneva, Switzerland)
6. Ν. Ρομπάκης (Mounti Sinai, New York, USA)
7. Μ. Συγγελάκης (Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη)

Νευροακτινολογία και Νευρουπερηχογραφία
1. M. Rubiera (Hospital Universitari Vall d'Hebron, Barcelona, Spain)
2. M. Rubin (University of Tennessee Health Sciences Center, Memphis, USA)
3. Ν. Αρτέμης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
4. Κ. Βαδικόλιας (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)
5. Α. Βαλαβάνης (University of Zurich, Zurich, Switzerland)
6. Ν. Βλαϊκίδης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
7. Σ. Γιαννόπουλος (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
8. Ι. Ηλιόπουλος (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)
9. Θ. Καραπαναγιωτίδης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
10. Α. Κατσάνος (McMaster University, Hamilton, Canada)
11. Σ. Κόλλιας (University of Zurich, Zurich, Switzerland)
12. Χ. Κρόγιας (Ruhr University of Bochum, Bochum Germany)
13. Β. Λιούτας (Harvard University, Boston, USA)
14. Π. Μήτσιας (Πανεπιστήμιο Κρήτης, Ηράκλειο & Henry Ford Hospital - Wayne State University, Detroit, USA)
15. Μ. Πολίτης (University of Exeter, UK)
16. Θ. Τέγος (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
17. Γ. Τσιβγούλης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών & University of Tennessee Health Sciences Center, Memphis, USA)
18. Α.Χαριτάντη-Κουρίδου (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)

Πόνος
1. A. Paladini (L'Aquila University, Italy)
2. G. Varrassi (Paolo Procacci Foundation, Italy)
3. Π. Ζης (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)

Ιατρική του Ύπνου
1. Α. Βγόντζας (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)
2. Π. Μπαργιώτας (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)
3. Α. Μπονάκης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
4. Αικ. Τερζούδη (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)

Διεθνής Εκπροσώπηση
1. Π. Ζης (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)
2. Α. Κατσάνος (McMaster University, Hamilton, Canada)

Editorial Board

Editorial Board

Editorial Board

15. Tzartos J (National & Kapodistrian University of Athens, Athens, Greece)
16. Voumvourakis K (National & Kapodistrian University of Athens, Athens, Greece)

Neurointensive care

1. Dimitriadis K (Ludwig-Maximillians University Munich, Germany)
2. Chang J (MedStar Washington Hospital Center)
3. Kazis D (Aristotle University of Thessaloniki, Thessaloniki, Greece)
4. Krogias C (Ruhr University of Bochum, Bochum, Germany)
5. Rudolf J (Papageorgiou Hospital, Thessaloniki, Greece)
6. Steiner T (University of Heidelberg, Heidelberg, Germany)
7. Varelas P (Albany Medical College, Albany, USA)

Neurology Education

1. Avramidis T (Red Cross Hospital, Athens, Greece)
2. Dardiotis E (University of Thessaly, Larissa, Greece)
3. Deretzi G (Papageorgiou Hospital, Thessaloniki, Greece)
4. Grigoriadis N (Aristotle University of Thessaloniki, Thessaloniki, Greece)
5. Hadjigeorgiou GM (University of Cyprus, Nicosia, Cyprus)
6. Kilidireas K (National & Kapodistrian University of Athens, Athens, Greece)
7. Milonas I (Aristotle University of Thessaloniki, Thessaloniki, Greece)
8. Mitsias P (University of Crete, Heraklion, Greece & Wayne State University, Detroit, USA)
9. Rudolf J (Papageorgiou Hospital, Thessaloniki, Greece)
10. Stefanis L (National & Kapodistrian University of Athens, Greece)
11. Tsivgoulis G (National & Kapodistrian University of Athens, Athens, Greece & University of Tennessee Health Sciences Center, Memphis, USA)
12. Vadikolias K (Democritus University of Thrace, Alexandroupolis, Greece)
13. Varelas P (Albany Medical College, Albany, USA)
14. Voumvourakis K (National & Kapodistrian University of Athens, Athens, Greece)
15. Zis P (University of Cyprus, Nicosia, Cyprus)

Neuromuscular disorders

1. Avramidis T (Red Cross Hospital, Athens, Greece)
2. Chroni E (University of Patras, Patras, Greece)
3. Davaki P (National & Kapodistrian University of Athens, Greece)
4. McDermott C. (University of Sheffield, UK)
5. Mavromatis I (Aristotle University of Thessaloniki, Greece)
6. Papadimas G (National & Kapodistrian University of Athens, Athens, Greece)
7. Papadimitriou A (University of Thessaly, Larissa, Greece)
8. Parissis D (Aristotle University of Thessaloniki, Greece)
9. Stamboulis E (National & Kapodistrian University of Athens, Athens, Greece)
10. Taskos N (Aristotle University of Thessaloniki, Greece)
11. Zouvelou V (National & Kapodistrian University of Athens, Athens, Greece)
12. Zis P (University of Cyprus, Nicosia, Cyprus)

Neurooncology

1. Kyritsis A (University of Ioannina, Ioannina, Greece)

Neuro-opthalmology

1. Anagnostou E (National & Kapodistrian University of Athens, Athens Greece)
2. Evdokimidis I (National & Kapodistrian University of Athens, Athens, Greece)
3. Iliopoulos I (Democritus University of Thrace, Alexandroupolis, Greece)

Άρθρα...

DOPA-RESPONSIVE DYSTONIA COMPLEX: CLINICAL CHARACTERISTICS, DIAGNOSIS, MANAGEMENT

Iro Boura[1, 2], Cleanthe Spanaki[1, 3]

[1] *Medical School, University of Crete*
[2] *Neurology Department, General Hospital of Chania*
[3] *Neurology Department, University Hospital of Heraklion, Crete, Greece*

Abstract

Dopa – responsive dystonia (DRD) is a clinically and genetically heterogeneous condition that is caused by the deficiency of enzymes involved in dopamine biosynthesis. Autosomal dominant mutations in GTP cyclohydrolase 1 account for most cases. DRD typically manifests in childhood or adolescence with dystonia of the lower limb, which might spread gradually during the following decades to other body parts. Symptoms exhibit a characteristic diurnal fluctuation and show a remarkable response to low doses of levodopa, rendering DRD a treatable disorder. Atypical cases have also been described with more severe phenotypes linked to various genotypes. Diagnosis is eventually based on appropriate targeted or non-targeted genetic analysis. Long delays in diagnosis are not a rare phenomenon, thus, a levodopa trial is always advisable in suspicious cases. Here, we present the DRD complex according to the new dystonia classification system of 2013.

Introduction

Dopa – responsive dystonia (DRD) is a genetically heterogeneous, treatable movement disorder, which is caused by the deficiency of enzymes involved in dopamine biosynthesis. As such, it is considered a biochemical, rather than a neurodegenerative movement disorder [1]. While dystonia is its most typical clinical characteristic, DRD can present with additional motor and non-motor symptoms.

The DRD prevalence is estimated at 0.5-1/1,000,000 [2]. The generic term of DRD was introduced by Nygaard et al. in 1988, in order to distinguish the condition from other forms of childhood- or adolescence-onset dystonia or juvenile Parkinson's disease (JPD) [3]. However, case reports of the most prevalent subtype [4], mediated by the inheritable by the autosomal dominant pattern, deficiency of the enzyme GTP cyclohydrolase 1 (GCH1), also known as Segawa disease, were described more than a decade earlier [5]. In 1998, Lee et al. suggested the term "DRD-plus" to describe atypical DRD cases with additional symptoms that did not respond well to dopamine substitution[6].This term, although frequently encountered in the literature of movement disorders [7], was abandoned after the introduction of the recent 2013 dystonia classification system, which integrates two axes: the clinical (axis I) and the subjacent etiology (axis II) [8].

Here, we present DRD based on the new dystonia classification system [8], with relevant terms highlighted in bold throughout the text.

Clinical characteristics (Axis I)

1. Age at onset

DRD typically appears in **childhood or adolescence** [1], although many atypical cases have been reported, with symptoms starting from early infancy [9] to late adulthood [10] (Table 1). Women usually present symptoms at a younger age [11]. DRD is three times more common in women compared to men [9], partly due to the fact that GCH-1 gene mutations' prevalence and penetrance are higher among females [1]. Fever has been recently described as a triggering factor preceding symptoms onset [2].

2. Body distribution

The classic initial presentation of DRD is limb dystonia, most commonly of the lower extremity (**focal dystonia**) [1]. It usually develops as an **action-specific** dystonia of the lower limb, leading to equinovarus foot posturing that often results in walking impairment [1]. In case of upper limb dystonia, focal hand dystonia is the most common manifestation [12]. Within the next two decades, dystonia may spread to adjacent body parts and evolve to **segmen-**

Table 1. Age of onset in DRD

Author, date	Sample characteristics	Sample size	Age		Notes
			($\bar{x}$ ± SD) (y)	(range)	
Trender-Gerhard, 2009 [12]	DRD & GCH-I deficiency	34	8.5	0-48y	Adult onset in 4 patients ($\bar{x}$ =37y).
Tadic, 2012 [11]	DRD & GCH-I deficiency	352	11.6±13.4	–	Homozygous cases excluded.
Tadic, 2012 [11]	DRD & GCH-I deficiency	28	9.4±7.7	–	–
Segawa, 2013 [13]	DRD & GCH-I deficiency	28	6.9±2.9	16mo-13y	A 58y old excluded.
Dobricic, 2017 [14]	DRD	47	18.7±13.6	1-50y	GCH-1 mutations in 11/47 (12.0±9.77).
Ahn, 2019 [15]	DRD & GCH-I deficiency	39	9.4	–	–

DRD: Dopa-Responsive Dystonia; GCH-I: GTP Cyclohydrolase I; mo: months; SD: standard deviation; $\bar{x}$: mean; y: years

tal or generalized dystonia, with or without leg involvement [1]. Absence of dystonia, especially in the adult-onset cases, is also possible [13].

3. Temporal pattern

DRD is a **progressive** disorder that reaches a plateau in the fourth decade [13].Dystonic symptoms show remarkable **diurnal** fluctuation (in >80% of cases) [16], which typically involves evening worsening, exacerbation with physical exercise [17], and improvement with sleep or rest [6]. These fluctuations become less frequent with time and disappear by the third decade [1].

4. Associated features

DRD can present as an **isolated dystonia**, although it is usually considered a **combined dystonia** [18]. Mild parkinsonism often accompanies dystonic symptoms in adult-onset cases but only rarely in children [6]. Less often, bradykinesia, rigidity or postural and rest tremor, might dominate the clinical picture [2] or even be the presenting features [19]. Age at disease onset has also been reported to affect the presenting clinical picture. In contrast to childhood-onset patients, who typically develop lower limb dystonia at disease onset, patients with symptoms onset after 15 years of age may present with parkinsonism without dystonia [12, 19]. Moreover, a wide range of pyramidal signs might be noticed, ranging from brisk reflexes in some patients [17, 20] to spastic quadriparesis [17, 19] and abnormal plantar reflexes in others [21].

Many atypical manifestations have been occasionally described including psychomotor retardation, developmental arrest [9, 22, 23], hypotonia [20, 24], mental retardation [17, 19], scoliosis [17, 25], cerebellar dysfunction [17], tics [26, 27], myoclonus [28], or oculogyric crisis [13]. There was an interesting report of a child presenting with waddling gait and proximal weakness, mimicking a myopathy [29].

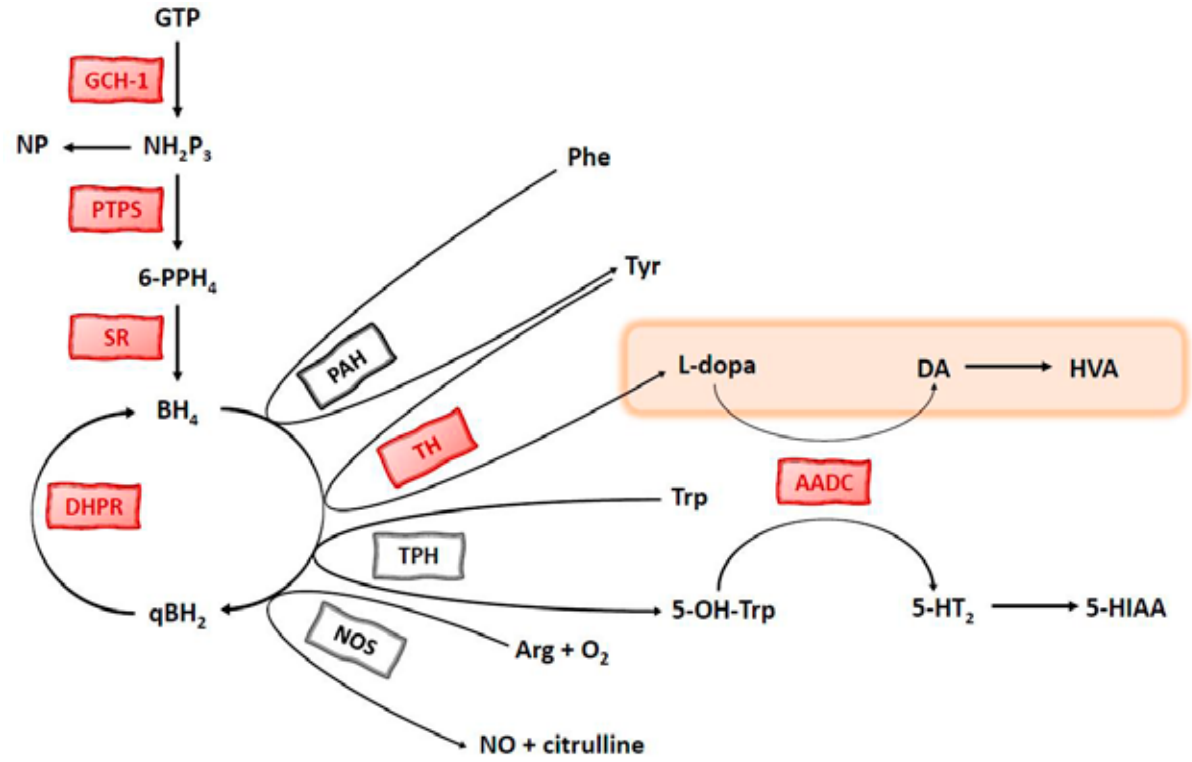
The disorder may also present with a variety of non-motor symptoms that include psychiatric problems, such as mood swings, depression, suicidality [12, 30], anxiety, agoraphobia, obsessive-compulsive disorder [12, 31, 32], as well as fatigue [30], pain [19], constipation, urinary retention, drooling [33], and sleep problems, including somnolence, intense and frightening dreams, difficulty in sleep initiation, or fragmented sleep pattern [31, 32]. Some of them, such as depression, obsessive compulsive disorders and anxiety, are thought to be due to downstream monoaminergic deficiencies [7, 34].

Etiology (Axis II)

1. Nervous system pathology

Symptoms in DRD derive from genetic defects that lead to various degrees of deficiency in enzymes involved in dopamine biosynthesis, in the absence of nigral cell loss [1] (Figure 1). In typical cases, patients present **no evidence of degeneration or structural lesions** in the striatum or substantia nigra. Dopamine levels are lower in the nigrostriatal terminals, but remain normal in the pars compacta of the substantia nigra [1]. However, there have been recent reports showing structural changes in the gray and white matter in the brain of DRD patients, implying alterations of the cortico-subcortical network, al-

Figure 1. Dopamine biosynthesis pathway



AADC: Aromatic L-amino Acid Decarboxylase; **Arg**: arginine; **qBH$_2$**: dihyrdobiopterin; **BH$_4$**: tetrabiopterin; **DA**: dopamine; **DHPR**: dihydropterin reductase; **GCH-1**: GTP cyclohydrolase 1; **GTP**: guanosine 5'-triphosphate; **5-HIAA**: 5-hydroxy-indoleacetic acid; **5-HT$_2$**: serotonin; **HVA**: homovanillic acid; **L-dopa**: levodopa; **NO**: nitric oxide; **NOS**: nitric oxide synthetase; **NP**: neopterin; **PAH**: phenylalanine hydroxylase; **O$_2$**: oxygen; **Phe**: Phenylalanine; **6-PPH$_4$**: 6-pyruvoyl tetrahydropterin; **PTPS**: pyruvoyl-tetrahydropterin synthase; **SR**: sepiapterin reductase; **TH**: tyrosine hydroxylase; **TPH**: tryptophan hydroxylase; **Trp**: tryptofan; **Tyr**: tyrosine.

though it remains unclear if this finding is a primary or secondary effect to dopamine deficiency [35].

Up to now, mutations in six genes have been associated with typical or atypical DRD phenotypes. These genes encode enzymes involved in either tetrabiopterin (BH$_4$) synthesis and recycling, or in neurotransmitter production (Table 2).

2. Inheritance

The enzyme GCH1 is the initial and rate-limiting step in the biosynthesis of BH$_4$, an essential cofactor that mediates the degradation of several amino acids, such as phenylalanine, tyrosine and tryptophane, and the production of monoamine neurotransmitters, like dopamine and serotonin [39].

Mutations of the GCH1 gene are the most common cause of DRD. Both **autosomal dominant and recessive mutations** have been identified. Patients with **autosomal dominant GCH1 mutations** usually maintain some residual enzyme activity and present the benign typical DRD phenotype. In contrary, autosomal **recessive GCH1 mutations** may result in complete absence of functional GCH1 protein and are associated with greater reductions in BH$_4$, hyperphenylalaninemia, and depletion of serotonin and dopamine [6, 7]. Hence, patients with recessive GCH1 mutations may present with a more severe phenotype that may include atypical features depending on the amount of residual enzyme activity [12].

DRD cases due to **autosomally recessive inherited mutations** in tyrosine hydroxylase (TH), sepiapterin reductase (SR) or pyruvoyl-tetrahydropterin synthase (PTPS) genes have also been described. Such cases are much less common and are characterized by an earlier age at symptoms onset and more complex clinical features [2, 4, 40]. PTPS and SR are also involved in the biosynthesis of BH$_4$, while TH constitutes the initial rate-limiting step in the catecholamine biosynthesis pathway [41] (Figure 1). Mutations in dihydropterin reductase (DHPR), an enzyme involved in the regeneration of BH$_4$, have also been linked to DRD [42].

No safe assumptions can be made for a patient's underlying causative mutation based solely on the clinical picture. One specific mutation can be asso-

Table 2. DRD – associated mutations

Gene name	Chromosome location	Enzyme coded	Number of reported mutations [36, 37]
Enzymatic defects of BH_4 synthesis or recycling			
GCH1	14q22.2	GTP cyclohydrolase 1 (GCH1)	192
PTS	11q23.1	Pyruvoyl-tetrahydropterin synthase (PTPS)	34
SPR	2p13.2	Sepiapterin reductase (SR)	19
QDPR	4p15.32	Quinoid dihydropterin reductase (DHPR)	15
Primary neurotransmitter synthesis defects			
TH	11p15.5	Tyrosine hydroxylase (TH)	77
AADC	7p12.2-p12.1	Aromatic L-amino Decarboxylase Decarboxylase*	79

* AACD deficiency results in a more complex phenotype than DRD. It is included here, as patients often present dystonia that respond to dopaminergic agents [38].
BH_4: tetrabiopterin; DRD: Dopamine-Responsive Dystonia; GTP: Guanosine 5'-Triphosphate.

ciated with various degrees of penetrance and residual enzyme function, even in twins [21]. As such, a wide spectrum of phenotypes may be linked to the same genotype, including asymptomatic carriers [6] or even completely different conditions (i.e. GCH1 pathogenic variants in PD patients) [43-45]. In conclusion, it seems that the severity and pattern of DRD phenotype (typical or atypical) is determined mainly by the type and severity of the enzymatic defect and the amount of residual functional protein, rather than the underlying genotype.

Diagnosis

Delays in DRD diagnosis, exceeding 15 years, have been reported in the literature [11]. A common diagnostic pitfall is parkin related-PD especially in cases of adult-onset DRD [46].

DRD chameleons that may warrant a levodopa trial include cases of cerebral palsy (especially among early-onset cases) [9, 22, 23], hereditary spastic paraplegia [21, 47], muscular dystrophy [2], and cervical myelopathy [23]. On the other hand, cases of hereditary spastic paraplegia [48], spinocerebellar ataxia type 3 [49, 50] and ataxia telangiectasia [51] have been reported as DRD mimics as well.

A suggested diagnostic algorithm is depicted in Figure 2 [1, 6, 34] and is analyzed below.

Step 1: Defining the phenotype

In the process of diagnosing DRD, it is helpful to characterize patients' symptoms as suggestive of the typical or atypical DRD phenotype. This distinction can guide further workup to a targeted genetic analysis, as patients with the classic DRD phenotype usually carry an autosomal dominant GCH1 mutation. Those with an atypical phenotype (previously noted as DRD-plus) may harbor genetic defects (usually recessive) on any of the enzymes involved in the dopamine synthesis pathway, which can be missed with the commercially available gene panels.

Step 2: Verifying an excellent levodopa response

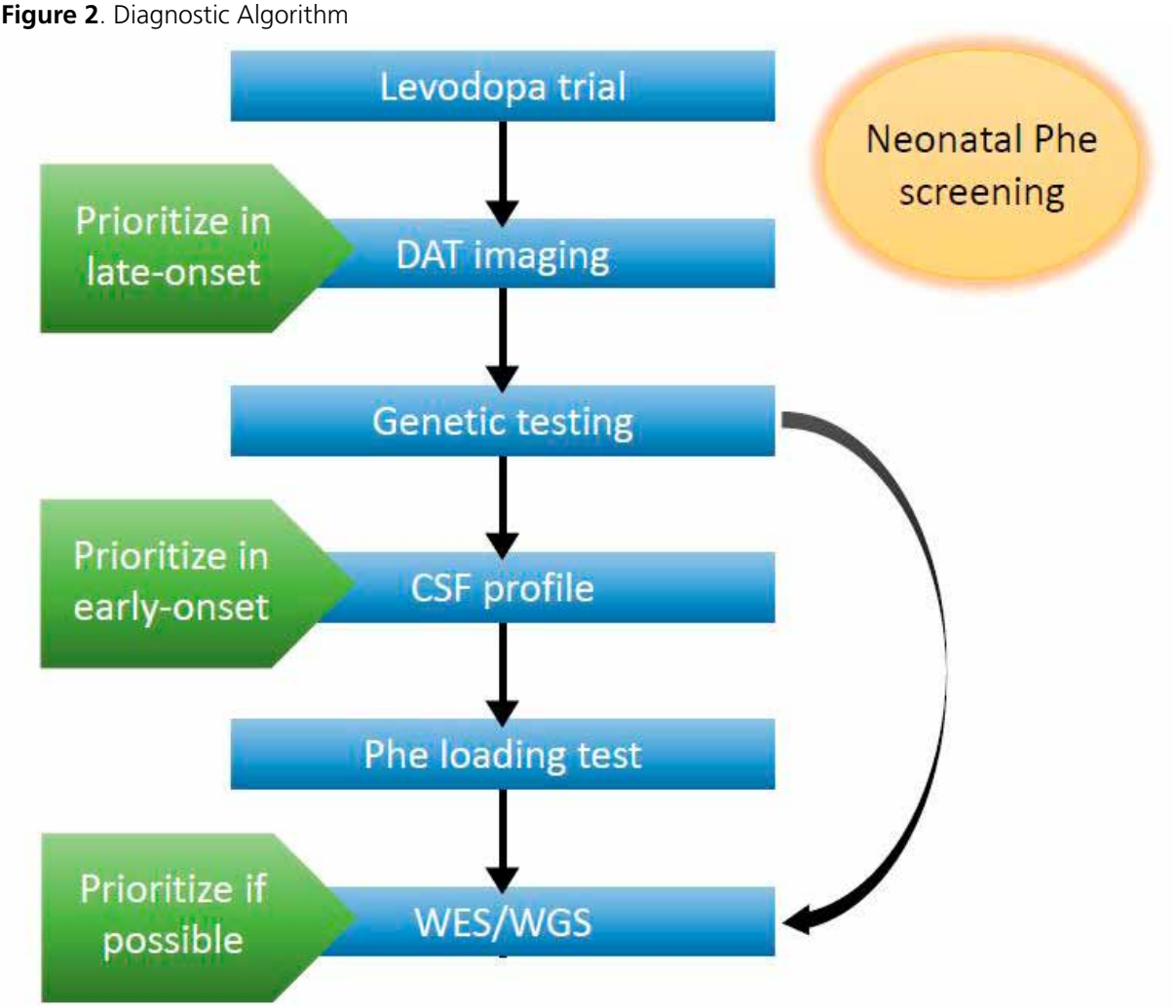
Typical DRD shows a striking and sustained response to small doses of levodopa [6]. Therefore, a levodopa trial should be attempted in all cases of childhood- or adolescence-onset dystonia early in the diagnostic process, even in atypical cases, and despite the absence of lower limb involvement, diurnal fluctuations or a positive family history (see following field of *Treatment*). However, opposing views of DRD over-diagnosis have been expressed, underlying the need for genetic confirmation [52].

Step 3: Ruling out DRD mimics

In patients with the typical DRD phenotype and a good response to levodopa, a targeted genetic analysis should be performed early in the diagnostic process, for the identification of GCH1 mutations.

In patients with atypical DRD symptoms, an inconclusive targeted genetic analysis or suboptimal response to levodopa, further workup is needed. This may include:

Figure 2. Diagnostic Algorithm



CSF: cerebrospinal fluid; **DAT**: Dopamine Transporter; **Phe**: phenylalanine; **WES/WGS**: whole exome/genome sequencing

- ***Imaging with DaTSCAN to rule out nigrostriatal neurodegeneration***

Molecular imaging of the nigrostriatal pathway with DaTSCAN can rule out neurodegenerative disorders of the substantia nigra (SN). A normal result would exclude PD and support a DRD diagnosis [53]. In the rare cases of patients with clinically presumed PD and normal DaTSCAN, often referred to as SWEDD (scans without evidence for dopaminergic deficit) [54], GCH1 mutations are not often encountered [55]. Atypical DRD cases displaying tracer reduction in DaTSCAN have been reported in the literature but are rare [56].

An interesting clinical feature that may be helpful in differentiating DRD from PD is the rarity of levodopa-induced motor complications in DRD patients. In contrast to PD cases, typical DRD patients do not present dyskinesias or fluctuations and do not require levodopa dose titration with disease progression [57]. Delayed levodopa-induced dyskinesias have been occasionally described in up to 20% of DRD patients, however, they are usually mild and quickly subside with levodopa dose reduction, without subsequent motor deterioration [58-60].

- ***Cerebrospinal Fluid (CSF)Studies: Measurement of metabolites***

In DRD patients, determination of neopterin and biopterin levels, 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA), and HIV in the CSF, may significantly contribute to the diagnostic process. Not only can they be of merit in ruling out PD, but they can also help in identifying the underlying enzyme deficiency, as levels vary depending on the relevant enzyme position in the biopterin biosynthesis pathway (Table 3).

Low CSF levels of both neopterin (<20 %) and biopterin (Figure 3) is a typical finding of GCH1 deficiency. PD patients also present low levels of these proteins, however, neopterin is expected to

Table 3. CSF and blood neurotransmitters profile [1, 6]

Condition	CSF				Blood	
	Neopterin	Biopterin	HVA	5-HIAA	Phenylalanine	Phenylalanine loading test
GCH1 deficiency	↓	↓	↓	↓	~ *	↑
PTPS deficiency	↑	↓	↓	↓	↑	N/A
SR deficiency	~	↑	↓	↓	~	↑
TH deficiency	~	~	↓	~	~	~
DHPR deficiency	N/A	N/A			↑	N/A
AADC deficiency	~	~	↓	↓	~	~
PD	↓	↓			~	~

*might be high in recessive forms.
CSF: cerebrospinal fluid; **DHPR**: dihydropterin reductase; **GCH1**: GTP cyclohydrolase 1; **N/A**: non applicable; **PD**: Parkinson's Disease; **PTPS**: pyruvoyl-tetrahydropterin synthase; **SR**: sepiapterin reductase; **TH**: tyrosine hydroxylase

be higher than 20% of normal levels, in contrast to GCH1 deficiency [6]. In patients with defects in enzymes that function more distally than GCH1 in the BH_4 biosynthetic pathway, such as SR, salvage pathways are activated that by-pass the enzymatic deficiency and result in normal neopterin and high biopterin levels [7].

Measurement of 5-HIAA and HVA in the CSF can be useful in differentiating DRD from other conditions with similar phenotypes, especially in atypical cases. For example, TH deficiency is characterized by normal neopterin and biopterin levels (distinguishing it from GCH1 and PD), low HVA and normal 5-HIAA levels (Table 3) while AADC deficiency, which is also characterized by normal neopterin and biopterin levels, results in low levels of both HVA and 5-HIAA (Table 3).

• *Blood studies: Phenylalanine Loading Test*

Since BH_4 is a cofactor for phenylalanine hydroxylase (Figure 1), disorders of BH_4 synthesis may present with hyperphenylalaninemia, as a result of impaired phenylalanine metabolism in the liver. Increased blood levels of phenylalanine are a typical finding in the more severe autosomal recessive or compound heterozygous forms of GCH1, PTPS and DHPR deficiencies, thus these conditions are usually diagnosed during neonatal screening and treated timely and accordingly [7, 34]. In autosomal dominant GCH1, TH and SR deficiencies, blood phenylalanine levels at baseline are usually normal [61]. However, hyperphenilalaninemia might arise, if patients are enforced to process a high amount of phenylalanine, as done during the phenylalanine loading test.

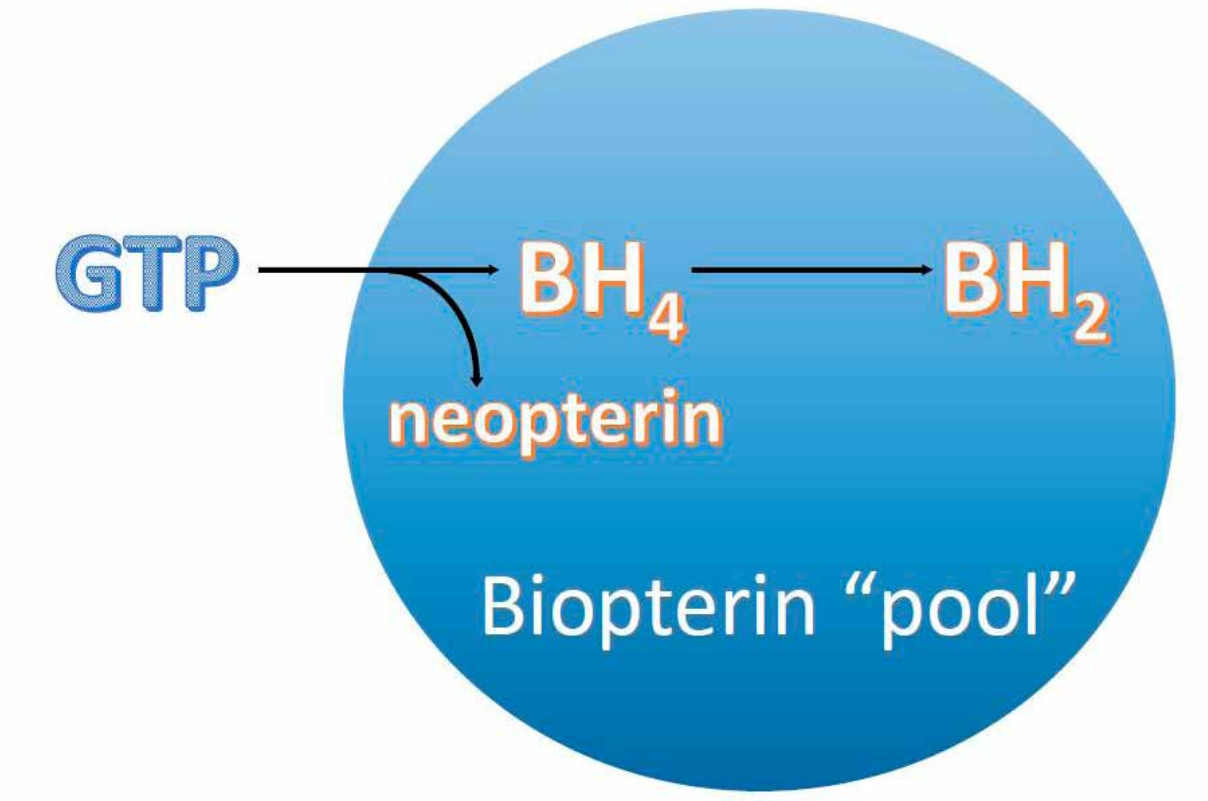
Challenge with Phenylalanine: Adult patients are advised to have a low-protein breakfast approximately two hours before the test. Blood samples are collected for baseline plasma phenylalanine and tyrosine concentration measurements [62]. Then, a loading dose of 100mg/kg of phenylalanine diluted in 100mL of water is administered to the patients [63]. Serial blood tests are performed, and the blood phenylalanine/tyrosine ratio is calculated several times for a period of 4-8 hours [34]. In patients with GCH1 or SR deficiency, an increase in phenylalanine levels will be noted after 1-2 hours, lasting up to 6 hours [34, 63], while the test won't have any effect on those with DRD not related to BH4 synthesis defects such as PD and TH or AADC deficiency [34, 63] (Table 3). In patients with TH or AADC deficiency, the enzymatic defect is located after BH4 production, thus phenylalanine can be normally converted to tyrosine.

The challenge with phenylalanine is particularly useful when lumbar puncture and CSF analysis are not possible [62, 63]. However, false negative and false positive results have been reported [64].

• *Targeted and non-Targeted Genetic Analysis*

Genetic testing plays a fundamental role in DRD diagnosis. GCH1 deficiency constitutes by far the most common form of the disorder. Different types

Figure 3. Biopterin components



BH_2: dihyrdobiopterin; **BH_4**: tetrabiopterin; **GTP**: Guanosine 5'-Triphosphate

of mutations have been reported, including nonsense and missense point mutations, deletions, and duplications, while a significant number of them are sporadic [65]. GCH1 mutations can be detected through commercially available kits [66]. Kits for TH deficiencies are also available in specific clinical settings [1]. However, due to the continuously increasing number of pathogenic mutations identified, this approach leaves room for omissions. Whole exome (WES) or genome sequencing (WGS) is probably the most cost-effective and rewarding type of genetic analysis in the diagnostic process of DRD. However, results should be read with caution, as large deletions, duplications and repeat expansions can be missed. Additionally, special consideration should be given to confirm the relevance of any identified likely pathogenic or novel variants with the condition under investigation [66]. It is worth mentioning that in a cohort of 64 DRD patients, about 17% of them carried no known mutation, suggesting that many causative genetic defects linked to DRD remain to be discovered [4].

Furthermore, patients and their families with a genetic diagnosis of DRD should receive pre- and post-diagnostic genetic counselling. This might not be a straightforward procedure. As penetrance of GCH1 mutations can vary significantly (a 30% penetrance has been reported), some mutations may not necessarily result in a DRD phenotype [67].

Treatment

Regardless of the underlying enzyme deficiency, administration of levodopa plus a peripheral decarboxylase inhibitor, carbidopa or benserazide, is the cornerstone of DRD treatment [1]. DRD patients, especially those with autosomal dominant GCH1 deficiency, show an excellent response to levodopa, with doses significantly lower than those used for PD [68]. Therefore, a levodopa trial is recommended in all childhood- or adolescence-onset dystonia cases, but also in patients with undiagnosed dystonic movement disorders of the adulthood.

Clinicians are advised to "start low and go slow" with levodopa treatment. In children, levodopa is initiated at 1mg/kg/day in divided doses, reaching optimal symptoms' response typically at around 4-5mg/kg/day in the majority of cases [69]. In adults, one should start with 25mg per day and titrate slowly until a satisfactory effect is achieved, or tolerability issues arise [1, 70]. Administration of up to 10mg/kg/day of levodopa in divided doses is recommended for children. Higher doses of levodopa may be required in adults, reaching 600mg/day [71, 72], although

Table 4. Dopa-Responsive Dystonia Treatment [70]

	First-line treatment			Second-line treatment		
	Levodopa	**5-HTP**	**BH_4**	**Anticholinergics**	**DAs**	**MAOIs**
				Trihexyphenidil	**Pramipexole**	**Selegiline**
AD GCH1	+			+		
AR GCH1	+	+	+		+	
PTPS	+	+	+		+	
SR	+	+				
TH	+					+
DHPR	+	+	+		+	
AADC				+	+	+

AD: autosomal dominant; **AR**: autosomal recessive; **BH_4**: tetrabiopterin; **DAs**: Dopamine Agonists; **DHPR**: dihydropterin reductase; **GCH1**: GTP cyclohydrolase 1; **5-HTTP**: 5-hydroxyryptophan; **MAOIs**: monoamine oxidase inhibitor; **PTPS**: pyruvoyl-tetrahydropterin synthase; **SR**: sepiapterin reductase; **TH**: tyrosine hydroxylase

typical cases respond to significantly lower doses (50-300mg). The final dose as well as the time and magnitude of symptoms response are highly individualized and depend on the underlying genetic defect [7]. DRD cases due to enzyme deficiency other than GCH1, with the exception of SR, might need higher levodopa doses, although treatment initiation and titration should always follow the "start low and go slow" principle [70].

As delayed responses have been reported, usually in atypical cases, a levodopa trial should be maintained for three months before considering it unsuccessful [7]. The majority of DRD patients have a long-lasting improvement under a stable levodopa scheme, which is not expected to change over time. However, patients with TH deficiency have been reported to require increasing levodopa doses as the disease progresses [73].

Dyskinesias can rarely appear when initiating treatment with levodopa, especially in atypical cases, and usually signify the need for a lower dose (0.5-1mg/kg daily) [20, 70]. Dyskinesias might also appear later in the disease course, especially in SR and TH deficiency, but usually respond well to levodopa dose reduction or spreading of the doses throughout the day. For persisting dyskinesias, amantadine could be administered at a dose of 4-6mg/kg daily [74].

If motor symptoms are not sufficiently controlled with levodopa, anticholinergic agents, such as trihexyphenidil, can be used either as an add-on treatment or as an alternative monotherapy, in doses ranging from 2-10mg daily [70]. Similarly to levodopa, the initiation dose should be low and the titration slow with regular follow-ups to determine optimal dose.

Dopamine agonists have also been used in selected cases of atypical DRD as second-line treatment. More specifically, in autosomal recessive GCH-1, PTPS and DHPR deficiency, pramipexole in a daily maintenance dose of 0.02-0.04mg/kg was found to be effective [70].

Selegiline, a selective monoamine oxidase (MAO)-B inhibitor has been used as a second-line treatment in TH deficiency cases in daily doses of 0.2-0.4mg/kg, and had a complementary role to levodopa [70].

Residual motor symptoms might persist despite optimal medical therapy. Botulinum toxin can be used to treat focal dystonic phenomena, which are not well controlled with dopaminergic medications [12]. Finally, deep brain stimulation of the globus pallidus internus has been tried in DRD patients with a good response of motor and some non-motor symptoms, such as anxiety and depression, but not cognition [75, 76].

While motor symptoms usually respond perfectly to levodopa, non-motor neuropsychiatric and cognitive symptoms do not. In the more severe autosomal recessive forms of enzyme deficiencies, neuropsychiatric non-motor symptoms usually develop either due to the toxic effect that high phenylalanine levels exert on brain function, or in the context of serotonin deficiency [7]. In such patients, a diet poor in phenylalanine, combined possibly with BH_4 and 5-hydroxytryptofan (5-HTP), a precursor of serotonin, can improve symptoms [7].

Isolated BH_4 therapy fails in restoring neurotransmitter deficiencies, due to poor blood brain barrier permeability and is therefore used in combination with levodopa and 5-HTP. In light of these considerations, a combination treatment of levodopa with 5-HTP has been used as a first-line therapy in cases of SR deficiency and a triple scheme of levodopa, 5-HTP and BH_4, has been successfully tried in patients with

PTS, DHPR or autosomal recessive GCH1 deficiencies [70]. The suggested initial dosage of BH_4 is 1-2mg/kg/day, slowly escalated up to 5-10mg/kg /day. The exact maintenance dose of BH_4 should be adjusted according to serum phenylalanine levels, which have to be maintained at levels lower than 120μmol/L [77].

Treatment with folic acid and pyridoxine should also be considered in certain DRD syndromes. Patients with DHPR deficiency should receive folic acid at doses of 10-20mg per day, as DHPR is required for normal folic acid blood levels maintenance. Pyridoxine should be administered in cases with AADC deficiency,as excess amounts of the enzyme's cofactor can boost residual AADC activity [34].

Conclusion

DRD is a genetic dystonia with very characteristic dystonic symptoms and a good response to treatment. A growing number of underlying causative genetic defects are currently being detected in DRD patients and linked to typical and atypical disease phenotypes. Various degrees of penetrance have been associated with the classic mutations, rendering genetic counseling in carrier families very challenging [78].

Given that DRD is a treatable condition, the diagnosis should always be examined and a low threshold for a levodopa trial up to 600mg sustained for 3 months is recommended as early as possible in all childhood- or adolescence-onset dystonia cases. A timely therapeutic intervention in DRD patients is of paramount importance since treatment can markedly improve patients' quality of life. Nevertheless, even in undiagnosed dystonia cases, reconsideration of the initial diagnosis and a levodopa trial is always of merit, as delayed diagnosis does not exclude a significant improvement following levodopa treatment.

References

[1] Wijemanne S, Jankovic J. Dopa-responsive dystonia-clinical and genetic heterogeneity. *Nat Rev Neurol.* 2015;11:414-424.

[2] Chen Y, Bao X, Wen Y, et al. Clinical and Genetic Heterogeneity in a Cohort of Chinese Children With Dopa-Responsive Dystonia. *Front Pediatr.* 2020;8:83.

[3] Nygaard TG, Marsden CD, Duvoisin RC. Dopa-responsive dystonia. *Adv Neurol.* 1988;50:377-384.

[4] Clot F, Grabli D, Cazeneuve C, et al. Exhaustive analysis of BH4 and dopamine biosynthesis genes in patients with Dopa-responsive dystonia. *Brain.* 2009;132:1753-1763.

[5] Segawa M, Hosaka A, Miyagawa F, et al. Hereditary progressive dystonia with marked diurnal fluctuation. *Adv Neurol.* 1976;14:215-233.

[6] Lee WW, Jeon BS. Clinical spectrum of dopa-responsive dystonia and related disorders. *Curr Neurol Neurosci Rep.* 2014;14:461.

[7] Cherian A, Paramasivan NK, Divya KP. Dopa-responsive dystonia, DRD-plus and DRD look-alike: a pragmatic review. *Acta Neurol Belg.* 2021;121:613-623.

[8] Albanese A, Bhatia K, Bressman SB, et al. Phenomenology and classification of dystonia: a consensus update. *Mov Disord.* 2013;28:863-873.

[9] Nygaard TG, Waran SP, Levine RA, et al. Dopa-responsive dystonia simulating cerebral palsy. *Pediatr Neurol.* 1994;11:236-240.

[10] Nygaard TG, Takahashi H, Heiman GA, et al. Long-term treatment response and fluorodopa positron emission tomographic scanning of parkinsonism in a family with dopa-responsive dystonia. *Ann Neurol.* 1992;32:603-608.

[11] Tadic V, Kasten M, Brüggemann N, et al. Dopa-responsive dystonia revisited: diagnostic delay, residual signs, and nonmotor signs. *Arch Neurol.* 2012;69:1558-1562.

[12] Trender-Gerhard I, Sweeney MG, Schwingenschuh P, et al. Autosomal-dominant GTPCH1-deficient DRD: clinical characteristics and long-term outcome of 34 patients. *J Neurol Neurosurg Psychiatry.* 2009;80:839-845.

[13] Segawa M, Nomura Y, Nishiyama N. Autosomal dominant guanosine triphosphate cyclohydrolase I deficiency (Segawa disease). *Ann Neurol.* 2003;54 Suppl 6:S32-45.

[14] Dobričić V, Tomić A, Branković V, et al. GCH1 mutations are common in Serbian patients with dystonia-parkinsonism: Challenging previously reported prevalence rates of DOPA-responsive dystonia. *Parkinsonism Relat Disord.* 2017;45:81-84.

[15] Ahn TB, Chung SJ, Koh SB, et al. Residual signs of dopa-responsive dystonia with GCH1 mutation following levodopa treatment are uncommon in Korean patients. *Parkinsonism Relat Disord.* 2019;65:248-251.

[16] Kim W, Cho JS, Shim YK, et al. Early-onset autosomal dominant GTP-cyclohydrolase I deficiency: Diagnostic delay and residual motor signs. *Brain Dev.* 2021;43:759-767.

[17] Chaila EC, McCabe DJ, Delanty N, et al. Broadening the phenotype of childhood-onset dopa-responsive dystonia. *Arch Neurol.* 2006;63:1185-1188.

[18] Balint B, Bhatia KP. Dystonia: an update on phenomenology, classification, pathogenesis and treatment. *Curr Opin Neurol.* 2014;27:468-476.

[19] Tassin J, Dürr A, Bonnet AM, et al. Levodopa-responsive dystonia. GTP cyclohydrolase I or parkin mutations? *Brain.* 2000;123 (Pt 6):1112-1121.

[20] Furukawa Y, Kish SJ, Bebin EM, et al. Dystonia with motor delay in compound heterozygotes for GTP-cyclohydrolase I gene mutations. *Ann Neurol.* 1998;44:10-16.
[21] Varghaei P, Yoon G, Estiar MA, et al. GCH1 mutations in hereditary spastic paraplegia. *Clin Genet.* 2021;100:51-58.
[22] Cheyette BN, Cheyette SN, Cusmano-Ozog K, et al. Dopa-responsive dystonia presenting as delayed and awkward gait. *Pediatr Neurol.* 2008;38:273-275.
[23] Mishra S, Mallick AK, Panigrahy D, et al. Series of Dopa Responsive Dystonia Masquerading as Other Diseases with Short Review. *Journal of pediatric neurosciences.* 2020;15:421-425.
[24] Eye PG, Horvat D, Wade KT, et al. Pure hypotonia in a four-year-old patient: An atypical presentation of Dopa-responsive dystonia. *J Neurol Sci.* 2019;403:125-126.
[25] Furukawa Y, Kish SJ, Lang AE. Scoliosis in a dopa-responsive dystonia family with a mutation of the GTP cyclohydrolase I gene. *Neurology.* 2000;54:2187.
[26] Romstad A, Dupont E, Krag-Olsen B, et al. Dopa-responsive dystonia and Tourette syndrome in a large Danish family. *Arch Neurol.* 2003;60:618-622.
[27] Yaltho TC, Jankovic J, Lotze T. The association of Tourette syndrome and dopa-responsive dystonia. *Mov Disord.* 2011;26:359-360.
[28] Leuzzi V, Carducci C, Carducci C, et al. Autosomal dominant GTP-CH deficiency presenting as a dopa-responsive myoclonus-dystonia syndrome. *Neurology.* 2002;59:1241-1243.
[29] Kong CK, Ko CH, Tong SF, et al. Atypical presentation of dopa-responsive dystonia: generalized hypotonia and proximal weakness. *Neurology.* 2001;57:1121-1124.
[30] Hahn H, Trant MR, Brownstein MJ, et al. Neurologic and psychiatric manifestations in a family with a mutation in exon 2 of the guanosine triphosphate-cyclohydrolase gene. *Arch Neurol.* 2001;58:749-755.
[31] Van Hove JLK, Steyaert J, Matthijs G, et al. Expanded motor and psychiatric phenotype in autosomal dominant Segawa syndrome due to GTP cyclohydrolase deficiency. *Journal of neurology, neurosurgery, and psychiatry.* 2006;77:18-23.
[32] Timmers ER, Kuiper A, Smit M, et al. Non-motor symptoms and quality of life in dopa-responsive dystonia patients. *Parkinsonism Relat Disord.* 2017;45:57-62.
[33] Wang W, Xin B, Wang H. Dopa-Responsive Dystonia: A Male Patient Inherited a Novel GCH1 Deletion from an Asymptomatic Mother. *J Mov Disord.* 2020;13:150-153.
[34] Opladen T, López-Laso E, Cortès-Saladelafont E, et al. Consensus guideline for the diagnosis and treatment of tetrahydrobiopterin (BH4) deficiencies. *Orphanet Journal of Rare Diseases.* 2020;15:126.
[35] Kostić VS, Agosta F, Tomic A, et al. Brain structural alterations in patients with GCH1 mutations associated DOPA-responsive dystonia. *J Neurol Neurosurg Psychiatry.* 2021;92:332-333.
[36] Klein Christine MC, Lohmann Katja. MDSGene. *Movement Disorders Society.* 2019.
[37] Himmelreich N, Montioli R, Bertoldi M, et al. Aromatic amino acid decarboxylase deficiency: Molecular and metabolic basis and therapeutic outlook. *Mol Genet Metab.* 2019;127:12-22.
[38] Pearson TS, Gilbert L, Opladen T, et al. AADC deficiency from infancy to adulthood: Symptoms and developmental outcome in an international cohort of 63 patients. *Journal of inherited metabolic disease.* 2020;43:1121-1130.
[39] Nasser A, Møller LB. GCH1 variants, tetrahydrobiopterin and their effects on pain sensitivity. *Scand J Pain.* 2014;5:121-128.
[40] Opladen T, Hoffmann G, Hörster F, et al. Clinical and biochemical characterization of patients with early infantile onset of autosomal recessive GTP cyclohydrolase I deficiency without hyperphenylalaninemia. *Mov Disord.* 2011;26:157-161.
[41] Daubner SC, Le T, Wang S. Tyrosine hydroxylase and regulation of dopamine synthesis. *Archives of biochemistry and biophysics.* 2011;508:1-12.
[42] Zouvelou V, Yubero D, Apostolakopoulou L, et al. The genetic etiology in cerebral palsy mimics: The results from a Greek tertiary care center. *Eur J Paediatr Neurol.* 2019;23:427-437.
[43] Mencacci NE, Isaias IU, Reich MM, et al. Parkinson's disease in GTP cyclohydrolase 1 mutation carriers. *Brain.* 2014;137:2480-2492.
[44] Rudakou U, Ouled Amar Bencheikh B, Ruskey JA, et al. Common and rare GCH1 variants are associated with Parkinson's disease. *Neurobiol Aging.* 2019;73:231.e231-231.e236.
[45] Pan HX, Zhao YW, Mei JP, et al. GCH1 variants contribute to the risk and earlier age-at-onset of Parkinson's disease: a two-cohort case-control study. *Transl Neurodegener.* 2020;9:31.
[46] Niemann N, Jankovic J. Juvenile parkinsonism: Differential diagnosis, genetics, and treatment. *Parkinsonism Relat Disord.* 2019;67:74-89.
[47] Wassenberg T, Schouten MI, Helmich RC, et al. Autosomal dominant GCH1 mutations causing spastic paraplegia at disease onset. *Parkinsonism Relat Disord.* 2020;74:12-15.
[48] Wijemanne S, Shulman JM, Jimenez-Shahed J, et al. SPG11 Mutations Associated With a Complex Phenotype Resembling Dopa-Responsive

Dystonia. *Movement disorders clinical practice.* 2015;2:149-154.
[49] Wilder-Smith E, Tan EK, Law HY, et al. Spinocerebellar ataxia type 3 presenting as an L-DOPA responsive dystonia phenotype in a Chinese family. *J Neurol Sci.* 2003;213:25-28.
[50] Nandagopal R, Moorthy SG. Dramatic levodopa responsiveness of dystonia in a sporadic case of spinocerebellar ataxia type 3. *Postgrad Med J.* 2004;80:363-365.
[51] Charlesworth G, Mohire MD, Schneider SA, et al. Ataxia telangiectasia presenting as dopa-responsive cervical dystonia. *Neurology.* 2013;81:1148-1151.
[52] Maas R, Wassenberg T, Lin JP, et al. l-Dopa in dystonia: A modern perspective. *Neurology.* 2017;88:1865-1871.
[53] Brajkovic LD, Svetel MV, Kostic VS, et al. Dopamine transporter imaging (123)I-FP-CIT (DaTSCAN) SPET in differential diagnosis of dopa-responsive dystonia and young-onset Parkinson's disease. *Hell J Nucl Med.* 2012;15:134-138.
[54] Erro R, Schneider SA, Stamelou M, et al. What do patients with scans without evidence of dopaminergic deficit (SWEDD) have? New evidence and continuing controversies. *J Neurol Neurosurg Psychiatry.* 2016;87:319-323.
[55] De Rosa A, Carducci C, Carducci C, et al. Screening for dopa-responsive dystonia in patients with Scans Without Evidence of Dopaminergic Deficiency (SWEDD). *J Neurol.* 2014;261:2204-2208.
[56] Eggers C, Volk AE, Kahraman D, et al. Are Dopa-responsive dystonia and Parkinson's disease related disorders? A case report. *Parkinsonism Relat Disord.* 2012;18:666-668.
[57] Nygaard TG, Marsden CD, Fahn S. Dopa-responsive dystonia: long-term treatment response and prognosis. *Neurology.* 1991;41:174-181.
[58] López-Laso E, Beyer K, Opladen T, et al. Dyskinesias as a Limiting Factor in the Treatment of Segawa Disease. *Pediatric Neurology.* 2012;46:404-406.
[59] Hwang WJ, Calne DB, Tsui JK, et al. The long-term response to levodopa in dopa-responsive dystonia. *Parkinsonism Relat Disord.* 2001;8:1-5.
[60] Bendi VS, Shou J, Joy S, et al. Motor fluctuations and levodopa-induced dyskinesias in dopa-responsive dystonia. *Parkinsonism Relat Disord.* 2018;50:126-127.
[61] Brennenstuhl H, Jung-Klawitter S, Assmann B, et al. Inherited Disorders of Neurotransmitters: Classification and Practical Approaches for Diagnosis and Treatment. *Neuropediatrics.* 2019;50:2-14.
[62] Hyland K, Fryburg JS, Wilson WG, et al. Oral phenylalanine loading in dopa-responsive dystonia: a possible diagnostic test. *Neurology.* 1997;48:1290-1297.
[63] Opladen T, Hoffmann GF, Kühn AA, et al. Pitfalls in phenylalanine loading test in the diagnosis of dopa-responsive dystonia. *Mol Genet Metab.* 2013;108:195-197.
[64] Saunders-Pullman R, Blau N, Hyland K, et al. Phenylalanine loading as a diagnostic test for DRD: interpreting the utility of the test. *Mol Genet Metab.* 2004;83:207-212.
[65] Ichinose H, Suzuki T, Inagaki H, et al. Molecular genetics of dopa-responsive dystonia. *Biol Chem.* 1999;380:1355-1364.
[66] Pozojevic J, Beetz C, Westenberger A. The importance of genetic testing for dystonia patients and translational research. *Journal of neural transmission (Vienna, Austria: 1996).* 2021;128:473-481.
[67] Nygaard TG, Trugman JM, de Yebenes JG, et al. Dopa-responsive dystonia: the spectrum of clinical manifestations in a large North American family. *Neurology.* 1990;40:66-69.
[68] Bledsoe IO, Viser AC, San Luciano M. Treatment of Dystonia: Medications, Neurotoxins, Neuromodulation, and Rehabilitation. *Neurotherapeutics.* 2020;17:1622-1644.
[69] Malek N, Fletcher N, Newman E. Diagnosing dopamine-responsive dystonias. *Pract Neurol.* 2015;15:340-345.
[70] Kim R, Jeon B, Lee W-W. A Systematic Review of Treatment Outcome in Patients with Dopa-responsive Dystonia (DRD) and DRD-Plus. *Movement disorders clinical practice.* 2016;3:435-442.
[71] Weissbach A, Saranza G, Domingo A. Combined dystonias: clinical and genetic updates. *J Neural Transm (Vienna).* 2021;128:417-429.
[72] Bradley M, Lyons S, Coleman S, et al. A Case of GCH-1 Mutation Dopa-Responsive Dystonia Requiring High Doses of Levodopa for Treatment. *Tremor and other hyperkinetic movements (New York, NY).* 2021;11:23-23.
[73] Li XY, Yang YM, Li LB, et al. Identification of TH Variants in Chinese Dopa-Responsive Dystonia Patients and Long-Term Outcomes. *Frontiers in neurology.* 2021;12:644910.
[74] Pons R, Syrengelas D, Youroukos S, et al. Levodopa-induced dyskinesias in tyrosine hydroxylase deficiency. *Mov Disord.* 2013;28:1058-1063.
[75] Dong W, Luo B, Qiu C, et al. Deep Brain Stimulation for the Treatment of Dopa-Responsive Dystonia: A Case Report and Literature Review. *World Neurosurg.* 2020;136:394-398.e395.
[76] Daida K, Nishioka K, Shimo Y, et al. Deep brain stimulation shows high efficacy in two patients with GCH1 variants. *Parkinsonism Relat Disord.* 2019;65:277-278.

[77] Ye J, Yang Y, Yu W, et al. Demographics, diagnosis and treatment of 256 patients with tetrahydrobiopterin deficiency in mainland China: results of a retrospective, multicentre study. *J Inherit Metab Dis.* 2013;36:893-901.

[78] Nedelea F, Veduta A, Duta S, et al. Prenatal Genetic Testing for Dopa-Responsive Dystonia - Clinical Judgment in the Context of Next Generation Sequencing. *J Med Life.* 2018;11:343-345.

DUCHENNE MUSCULAR DYSTROPHY: CLINICAL CHARACTERISTICS, DIAGNOSIS AND MANAGEMENT

George-Konstantinos Papadimas, Sophia Xirou, Evangelia Kararizou, Constantinos Papadopoulos

Department of Neurology, Brigham and Women's Hospital and Jamaica Plain Veterans Administration Hospital, Harvard Medical School

Abstract

Duchenne muscular dystrophy (DMD) is the most common form of dystrophinopathy, followed by the milder Becker muscular dystrophy and the DMD-associated dilated cardiomyopathy. DMD is inherited in an X-linked recessive manner, caused by mutations in DMD gene encoding for dystrophin, and presents in early childhood with muscle weakness and gait impairment. Respiratory involvement is a major cause of mortality, and the use of steroids and non-invasive ventilation have significantly increased survival. Dilated cardiomyopathy is another big challenge, especially for the older patients with DMD, carrying a poor prognosis. Despite the important efforts and progress that have been made over the last years, curing DMD is still a far-reaching goal. However, strict application of the current guidelines and emerging genetic treatments have decisively improved the clinical course of the disease and provide reasonable hope for a much better outcome in the future.

Key words: duchenne muscular dystrophy, myopathy, dystrophinopathty

INTRODUCTION, EPIDEMIOLOGY AND GENETICS

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disorder caused by mutations in dystrophin (*DMD*) gene, located in the short arm of the X chromosome. It is the most frequent inherited myopathy and one of the most common debilitating muscular diseases, with a birth prevalence of 15.9-19.5 per 100000 live male births [1-4]. In addition to DMD, dystrophinopathies also include Becker Muscular Dystrophy (BMD), which is a milder but rarer disease than DMD, with one case per 6000-8000 live male births [2, 5], DMD-associated dilated cardiomyopathy (DCM), and the female carriers of DMD mutations, who may occasionally be mildly to moderately symptomatic. The predominant primary presenting symptom in most forms of dystrophinopathies is skeletal muscle weakness. However, cardiac muscle is also very often involved and remains one of the most common causes of morbidity and mortality [6-8]. The predominant primary presenting symptom in most forms of dystrophinopathies is skeletal muscle weakness. However, cardiac muscle is also very often involved and remains one of the most common causes of morbidity and mortality [6-8]. It is noteworthy that there are two hotspots in the *DMD* gene, located mostly in exons 45-55 and secondarily in exons 2-19 [9]. On the contrary, in BMD, deletions are found in 60-70% of patients, duplications in approximately 20% and only 5-10% are point mutations, small deletions or insertions [4, 10, 11]. The predominant primary presenting symptom in most forms of dystrophinopathies is skeletal muscle weakness. However, cardiac muscle is also very often involved and remains one of the most common causes of morbidity and mortality [6-8].

The diagnosis of *DMD* is suspected based upon the clinical symptoms, biochemical findings, especially the CK (creatine kinase) increase and the possible presence of a positive family history, and is finally confirmed by genetic testing. Prenatal diagnosis and counselling in female carriers of a known pathogenic *DMD* mutation is of outmost importance, in order to avoid the birth of an affected boy. However, even with the application of very clear prenatal recommendations and genetic counselling for at risk women of reproductive age, the birth of affected boys cannot be completely avoided, since in one-third of DMD and BMD have a *de novo* mutation with negative family history [12-14]. Finally, it is important to note that the mothers of DMD/BMB children, who are not somatic carriers of a *DMD* mutation, exhibit a higher possibility of birthing another affected boy, due to germline mosaicism [15].

PATHOPHYSIOLOGY

The predominant primary presenting symptom in most forms of dystrophinopathies is skeletal muscle weakness. However, cardiac muscle is also very often involved and remains one of the most common

causes of morbidity and mortality [6-8]. Dystrophin has four important domains: a) the acting-binding domain, which attaches to F actin, providing a linkage between dystrophin and the subsarcolemmal actin network, b) the central rod domain, which contains 24 spectrin repeats and mediates the dystrophin interaction with microtubules, c) a cysteine rich domain, and d) a carboxyl-terminal domain. The latter is the dystroglycan-binding end, providing the connection to the dystroglycan complex within the membrane that is anchored to extracellular matrix [16-18].

The disease starts very early and muscle inflammation can be observed soon after birth, while muscle fibrosis usually starts to develop even within the first year of life. Muscle degeneration and necrosis are the primary features of DMD. Several hypotheses on the pathophysiology of the disease have been elaborated, but according to the most prevailing theory, DMD is caused by a structural of functional defect of dystrophin [19, 20]. The absence of dystrophin results in lack of integrity within the muscle cells causing progressive damage particularly during muscle contraction, while the loss of linkage with the dystroglycan complex (α-dystroglycan and β-dystroglycan) leads to disruption of transmembrane signaling [16, 21]. The integrity of the sarcolemma is dependent on the normal function of the dystrophin-associated protein complex (DAPC). The DAPC disassembly results in weakening of the muscle membrane, which can no longer withstand the strong mechanical stress produced by repeated contraction and relaxation of the sarcomeres, leading to sarcolemma ruptures. Muscle enzymes, such as creatine kinase (CK), aldolase, and transaminases leak through these membrane tears into the bloodstream [22, 23].

A dysregulation of calcium homeostasis is highly implicated in the pathogenesis of muscular dystrophies and particularly DMD. An abnormal increase in calcium influx and intracellular calcium concentration is well known from very early studies in dystrophic animal models and is associated with muscle fiber hypercontraction and myonecrosis [24-27]. The increased intracellular calcium concentration may originate either from an enhanced calcium influx through calcium channels, such as TRPC mechanosensitive voltage-independent calcium channels, which are highly expressed in DMD and plasma membrane calcium ATPases, or from microscopic sarcolemma microtears and sodium-calcium exchangers [4, 28, 29]. Another source of elevated cytocolic calcium is the sarcoplasmic reticulum (SR), which permits calcium release through the defective ryanodine receptors (RYR1) of the dystrophic muscle [30]. RYR1 is destabilized due to an aberrant binding with calstabin, with a subsequent opening of the channel and intracellular calcium leakage. In addition, the activity of sarco/endoplasmic reticulum calcium ATPase (SERCA), which normally functions to mediate calcium re-entry to SR, is reduced due to sarcolipin-induced down regulation, further contributing to increased intracellular calcium [30-32].

An additional crucial role of dystrophin is to anchor nNOS (neuronal nitric oxide synthase) to the sarcolemma, and thus muscle damage may be further aggravated by a functional ischemia caused by the mislocalization of nNOS in DMD, which is necessary for vasodilation during muscle contraction in order to normally supply exercising muscle with oxygen. [33, 34]. Muscle ischemia may in turn lead to activation of different parallel pathomechanisms, such as the release of inflammatory cytokines, calcium overload and an overproduction of ROS (reactive oxygen species) [35, 36], which may be in turn exacerbated by the microtubule-associated protein Rac1 activation of NADPH oxidase 2 (NOX2), with a subsequent severe free radical injury [37].

Recent data also support a possible mitochondrial dysfunction, implied by an aberrant mitochondrial morphology in dystrophic mice, which in fact precedes the onset of muscle fiber damage. Thus, a link between dystrophin and mitochondrial function is highly suspected but larger studies are needed to identify the underlying mechanisms [38-41].

In the early stages of the disease, muscle fibers have a greater regenerative capacity, which gradually decreases due to a progressive depletion of satellite cells [42]. Regenerative fibers often display a branched morphology that may further increase their susceptibility to damage. Moreover, muscle fiber branching may contribute to channel dysfunction and excessive calcium influx, creating a vicious cycle and maximizing muscle failure [26, 43].The progressive muscle fiber replacement with fat and fibrotic tissue further limits the ability of muscle regeneration.

CLINICAL CHARACTERISTICS

Although DMD and BMD are allelic disorders, they have also many differences as shown in Table 1. DMD is a continuum and although the diagnosis could be occasionally made in the first 2 years of life, the vast majority of patients are diagnosed at the age of 4-5 years. The disease is relentlessly progressive and initially leads to loss of the ability to run, to walk and then to ambulate, and DMD affected children finally end up wheelchair bound, approximately by the age of 10 years. The patients' autonomy is further limited by the concomitant loss of arm function. The early recognition of symptoms and signs of DMD becomes more necessary nowadays in the era of new evolving therapeutic approaches.

In a very early presymptomatic stage, there may be some indications of delayed developmental mile-

Table 1. Main differences between Duchenne and Becker muscular dystrophy

	Incidence	CPK levels	Onset (age)	Wheelchair dependency	Cardiomyopathy	Median survival	Muscle biopsy Immuno-histochemistry (dystrophin staining)	Muscle biopsy Western blot (dystrophin quantity)
DMD	15.9-19.5 per 100000 live male births	>10 normal	2-5 years	Before age 13	100% after age 18	27 yrs	Complete/ almost complete absence	0-5% dystrophin
BMD	1 per 6000 - 8000 live male births	>5 normal	Usually >7 years	After age 16 (if present)	30-70% of patients overall	Mid 40s	Normal appearing, or reduced/patchy intensity	

Abbreviations: DMD, Duchenne muscular dystrophy; BMD, Becker muscular dystrophy; CPK, Creatine phosphokinase

stones. Therefore, any difficulty in the acquisition of motor skills, such as a poor head control, the inability of a child to walk independently by the age of 18 months or to run by the age of 3 years, or a difficulty to jump, to climb stairs, or to get up easily from the floor should be considered as potential early indications of DMD [44, 45]. In addition, the presence of speech and language delay (no words spoken at the age of 18 months, unable to speak sentences by age 3), the detection of learning difficulties, the occurrence of behavioral issues or the recognition of an autistic spectrum disorder within the appropriate clinical context, may also raise the suspicion of an underlying dystrophinopathy [46-49]. An early ambulatory phase follows with affected children manifesting some signs of the disease, such as calf enlargement or pseudohypertrophy, which is usually asymmetric, due to adipose and connective tissue replacement, toe walking and difficulty standing up from a squatting position (Gower's sign). The patients usually also adopt a curved posture, to account for weaker chest and pelvic muscles. In a late ambulatory stage, the patients may exhibit a clumsy gait with frequent falls, and an increasing loss of walking ability. The patients can no longer climb stairs and an intermittent wheelchair use may be necessary. The early non-ambulatory stage is characterized by an absolute dependence on wheelchair and the development of scoliosis, while in the late non-ambulatory phase, the upper extremity function is severely impaired and there is also limited postural maintenance [12, 44, 50, 51].

It is also very important to emphasize that high levels of muscle enzymes, such as CK, LDH, ALT, AST and aldolase, may be incidentally detected at a presymptomatic stage and may be the first sign of the disease. There are also rare reports of a pseudometabolic phenotype associated with an underlying dystrophinopathy. These patients may present exertional myalgia and/or rhabdomyolysis, and usually run a more benign clinical course [52, 53].

The disease progression towards an increased need for ambulation support coincides with a rapid peak of fibrotic tissue approximately at the age of 7 years, with a concomitant loss of muscle tissue ability to regenerate, which should impact the decision of starting treatment [54]. Restrictive lung disease is also very common in DMD patients and pulmonary function progressively deteriorates due to respiratory muscles involvement, including the diaphragm [55-57]. Three distinct stages in the progression of respiratory function have been identified in DMD patients based on forced vital capacity (FVC) measurements: an initial annual rise in the ambulatory phase of the disease, a subsequent plateau during the early non-ambulatory stage, and finally a progressive decline during the late non-ambulatory period. An FVC reduction of less than 1 L is associated with a significantly higher mortality risk [56, 58, 59]. It has been shown that corticosteroids and particularly the use of respiratory support through mechanical ventilation resulted in a robust increase in the life expectancy of DMD patients, improving median survival from late teen years to 27.0 years of age [60-62].

Cardiac involvement in dystrophinopathies, although common, is not necessarily related to the severity of myopathy and in some cases of BMD, it may be predominant even with minimal muscular disease [63]. In DMD, at a preclinical stage of the disease, the heart manifestations are very subtle, with mild ECG abnormalities, some degree of diastolic dysfunction, or wall motion abnormalities. However, at a more advanced clinical stage, the progressively worsening dilatation of heart chambers and subendocardial fibrosis eventually lead to over 60% of DMD patients from adolescence onwards developing symptoms suggestive of heart failure and dilative cardiomyopathy with left ventricular ejection shortening (LVES) less than 28%, and left ventricular ejection fraction (LVEF) less than 45% [64-66]. Despite the severity of cardiac involvement, DMD patients are not considered good candidates for cardiac transplantation due to the shortage of donor availability and their poor prognosis [67-69].

Table 2. Clinical manifestations of female carriers

Signs/Symptoms	DMD mutations	BMD mutations
None	76%	81%
Muscle weakness	19%	14%
Myalgia/cramps	5%	5%
Left-ventricle dilation	19%	16%
Dilated cardiomyopathy	8%	0

Abbreviations: DMD, Duchenne muscular dystrophy; BMD, Becker muscular dystrophy
Adapted from Darras BT et al. [138].

Finally, there is increasing data supporting the genetic predisposition for the outcome of both cardiac and respiratory function. More specifically, a better cardiac prognosis was observed in association with mutations in the dystrophin Dp116 coding region [70] and in patients carrying the polymorphisms rs28357094 in the SPP1 promoter, rs10880 and the VTTT/IAAM haplotype in LTBP4, which are also associated with age at loss of acquired motor skills [71]. Moreover, DMD patients amenable to skipping exon 44 seem to have a better respiratory function with higher FVC% and a slower rate of decline [72, 73].

Female carriers of *DMD* and *BMD* mutations may rarely have symptoms of myopathy or even cardiac involvement. Table 2 summarizes their main clinical characteristics

DIAGNOSTIC ALGORITHM

The typical myopathic presentation in a young boy combined with a significantly high CK are key features for coonsidering DMD. Though while DMD may be easily recognized in patients at an older age with the typical signs and symptoms of the disease, the diagnosis at an early stage is usually more difficult and requires a high suspicion index. A positive family history may be helpful, but as previously mentioned, there is a high proportion of patients carrying a *de novo* mutation. The high CK levels may be a very useful diagnostic clue, especially if randomly found at a preclinical stage. Although a CK increase is non specific and may be observed in various neuromuscular diseases and other conditions, the stable and very high levels can significantly narrow down the differential diagnosis [74].

In case that DMD is suspected, the initial diagnostic step is to perform genetic testing. Since deletions and duplications are the cause for the great majority of patients, it is considered cost-efficient to initially check for these mutations by using MLPA (multiplex ligation-dependent probe amplification) analysis or array comparative genome hybridization (array CGH) [75, 76]. In case of a positive result, the diagnosis is considered established, whereas if the mutation is not found, genetic testing must be completed with Sanger sequencing of the 79 exons of the *DMD* gene, in order to possibly detect a small causative mutation. However, this technique is laborious, time consuming and expensive, and not performed by all genetic laboratories [76]. If the results are still negative, but DMD remains highly suspected, there is also the rare possibility of deep intronic mutations that cannot be identified by the aforementioned techniques and may be picked up with more elaborate approaches, such as next generation sequencing (NGS) [76-78].

The need for muscle biopsy, which was historically the initial step for diagnosing DMD, has a limited role now. Although protein analysis through immunohistochemistry and western blot can provide further insights on the location, abundance and molecular size of dystrophin, the need for genetic testing is absolute, especially in the era of evolving specific genetic treatments, which require an accurate molecular diagnosis. Moreover, muscle biopsy is an invasive procedure and affected children at a young age have to undergo general anesthesia, which may pose an increased risk, given their cardiorespiratory status [76, 79, 80]. However, in case that a thorough genetic testing does not yield positive results, muscle biopsy should be considered to confirm or rule out the diagnosis [5, 74].

MANAGEMENT

Although there is currently no radical cure for DMD, there are many modern therapeutic approaches. In recent years, there has been a very large number of clinical trials investigating the safety and efficacy of multiple compounds with different mechanisms in DMD patients. They can be broadly divided into primary therapies, aiming to restore the missing or dysfunctional dystrophin, and secondary therapies,

targeting parallel pathophysiological processes due to the absence of dystrophin. An update on drug development for the treatment of DMD is provided on Table 3 and current information can be found at ClinicalTrials.gov.

However, the tremendous progress of genetic treatments and gene therapy in particular, should not downplay the importance of compliance to the standards of care, which have been updated in 2018 after their initial publication in 2010 by Bushby et al. [5, 44, 81-83]. The recent guidelines include more detailed recommendations for management of other aspects of the disease, such as endocrine abnormalities and bone health, and also emphasize the transition from childhood to adulthood care. Especially for the latter, an early transition planning is vital in order to assist DMD patients in better adjusting to the demands of the new setting. The participation of the individual in transition planning and decision making is also very important and ensures the maximum degree of independency a patient can achieve [84, 85].

The strict adherence to multidisciplinary management guidelines has decisively modified the natural course of the disease and can better control the symptoms of DMD patients, as they improve their quality of life and prolong their lifespan [4, 86]. It should be mentioned, however, that most guidelines are not evidence-based, due to the lack of large-scale randomized controlled studies for DMD, and are the result of expert opinions based on the available evidence rather than statistical approaches [44, 82, 83].

Respiratory complications

The strict application of respiratory guidelines with ventilatory support through non-invasive ventilation brought about improvements in the survival of DMD patients by approximately 10 years [60]. Respiratory assessment must be annually performed after the confirmation of the diagnosis. Many different methods are routinely used to assess lung function. Forced vital capacity % predicted (FVC%) is one of the most useful outcome measures of respiratory progression and when it is below 50%, there is an increased risk of sleep disordered breathing, while maximum expiratory and inspiratory pressure (MEP, MIP) are more specific for the evaluation of expiratory and inspiratory muscle function [55-57]. Especially in the early ambulatory stages of the disease, where the very young affected children cannot cooperate well in performing lung function tests, peak expiratory flow percentage predicted (PEF%) has proved a reliable and useful surrogate marker of respiratory progression [57, 87]. Sleep studies are also strongly recommended on suspicion of nocturnal hypoventilation and the use of mechanically assisted coughing and ventilation is highly advised when needed [57, 82].

Cardiac complications

The improvement of lifetime expectancy in DMD patients, mainly due to the best respiratory care, resulted in the emergence of cardiac complications and in an increase in cardiac-associated deaths owing to heart failure and conduction abnormalities. Current guidelines suggest starting cardiac monitoring with echocardiogram at the age of 6 years, which is later supplemented by cardiovascular MRI. It is also recommended to initiate angiotensin-converting enzyme (ACE) inhibitors or ACE blockers by the age of 10 years regardless of the presence of symptoms, which emphasizes the importance of a proactive approach [82, 88, 89].

Orthopaedic complications

Scoliosis, joint contractures, and a low bone mineral density due to impaired bone metabolism are commonly encountered in DMD patients. In ambulant patients, physiotherapy, occupational therapy and orthotics or other appropriate assistive devices are strongly encouraged to help them move and perform daily tasks. Especially the prevention of contractures developmt is of outmost importance for maintaining a patient's gait. In non-ambulant patients, the emphasis should be placed on the correct sitting position, to avoid worsening of scoliosis and to maintain as much as possible the upper limb function [82, 90]. Although the use of steroids has prevented the early development of severe scoliosis, it continues to be a common complication of the disease contributing to respiratory deterioration. In presence of scoliosis, radiological assessment should be performed at least annually, and any surgical intervention should be cautiously decided on a multidisciplinary basis [82, 91].

Other system complications

Gastrointestinal motor function disturbances due to visceral smooth muscle involvement, seem to be quite common in DMD patients, especially at an advanced age. Gastroparesis, constipation and gastroesophageal reflux disease (GERD) are the most prevalent manifestations [92]. Dietary guidelines and symptomatic treatment with the administration of osmotic and stimulant laxatives for bowel dysmotility or histamine 2 receptor antagonists and proton-pump inhibitors for GERD are highly recommended [44].

Urological management is also frequently required to address problems such as bladder hyperactivity, detrusor sphincter dyssynergia, and urinary tract infections. Pharmacological interventions may alleviate symptoms and improve quality of life. Special caution should also be paid to renal dysfunction, which may be observed in the late stages of the disease [93, 94].

Endocrinological monitoring for growth problems,

Table 3. Drug Development Pipeline for Duchenne Muscular Dystrophy

		Preclinical	Phase I	Phase I/II	Phase II	Phase III	Approved
PRIMARY THERAPIES (dystrophin restoration or replacement)							
Genetic treatments							
Non sense mutation readthrough	Ataluren (Translarna) *PTC Therapeutics*						EMA*
Exon Skipping	Golodirsen (exon 53) *Sarepta Therapeutics*						FDA*
	Eteplirsen/Exondys51 (exon 51) *Sarepta Therapeutics*						FDA*
	Viltepso (Viltolarsen/NS-065/NCNP-01 (exon 53)) *NS, Pharma, Inc.*						FDA/MHLW Japan*
	Casimersen (exon 45) *Sarepta Therapeutics*					√	
	SRP-5051 (exon 51) *Sarepta Therapeutics*				√		
	DS-5141b (exon 45) *Daiichi Sankyo*				√		
	NS-089/NCNP-02 (exon 44) *NS Pharma, Inc.*				√		
	scAAV9.U7.ACCA (exon 2) *Audentes Therapeutics*				√		
Gene Therapy	AAV9.microdystrophin (PF-06939926) *Pfizer*					√	
	rAAVrh74.MHCK7.micro-dystrophin (SRP-9001) *SareptaTherapeutics*				√		
	AAV9.microdystrophin (SGT-001) *Solid Biosciences*				√		
	GALGT2 genetherapy (rAAVrh74.MCK.GALGT2) *Nationwide Children's Hospital, Columbus, United States*				√		
Celltherapies	CAP-1002				√		
	Bone Marrow-derived autologous Stem Cells *Stem Cells Arabia*				√		
	Myoblasts *CHU de Quebec-Universite Laval, Canada*				√		
SECONDARY THERAPIES targeting...							
fibrosis	Pamrevlumab *FibroGen*					√	
inflammation	EMFLAZA (Deflazacort)-steroid *PTC Therapeutics*						FDA
	Vamorolone (VBP15) - steroid alternative *Santhera Pharmaceuticals*					√	
	Tamoxifen -SERM *University Hospital of Basel*					√	
	ATL1102 - antisense oligonucleotide *Antisense Therapeutics*				√		
	Canakinumab (ILARIS) - monoclonal antibody *Children's Research Institute*			√			
calciumhomeostasis	Rimeporide *EspeRare Foundation*			√			
musclegrowthand-protection	Givinostat Follastatin enhancement *ItalfarmacoSpA*					√	

Table 3. Continuity

		Preclinical	Phase I	Phase I/II	Phase II	Phase III	Approved
	Carmeseal-MD Membrane Sealant *PhrixusPharaceuticals*			√			
	EDG-5506 Muscle stabilizer *Edgewise herapeutics*		√				
	Spironolactone vs Prednisolone Aldosterone antagonist *Nationwide Children's Hospital*		√				
mitochondrial-function	EPM-01 mitochondrial biogenesis *Epirium Bio*		√				
	ASP0367 (MA-0211) Cellular function improvement Astellas Pharma Inc.		√				
cardiacfunction	Ifetroban Cardiomyocyte protection *Cumberland Pharmaceuticals*				√		
	Bisoprolol fumarate *Hoffmann-La Roche, Peking Union Medical College Hospital,* China					√	
	Nebivolol *Assistance Publique - Hopitaux de Paris, France*					√	

hypogonadism, delayed puberty and nutritional assessment should be regularly performed as well. Moreover, bone health and glucose metabolism should be given special attention, especially due to the long-term corticosteroid administration [44].

Moving on, neuropsychological status and neurodevelopmental progression should be carefully monitored in DMD patients, due to the high incidence of cognitive issues and psychiatric manifestations, such as anxiety, depression, autism, and attention deficit/hyperactivity disorder [95]. Regular neuropsychological and psychiatric evaluations and pharmacological treatment, when needed, should be provided. Moreover, specific educational programs could improve cognitive skills of patients, especially if applied early.

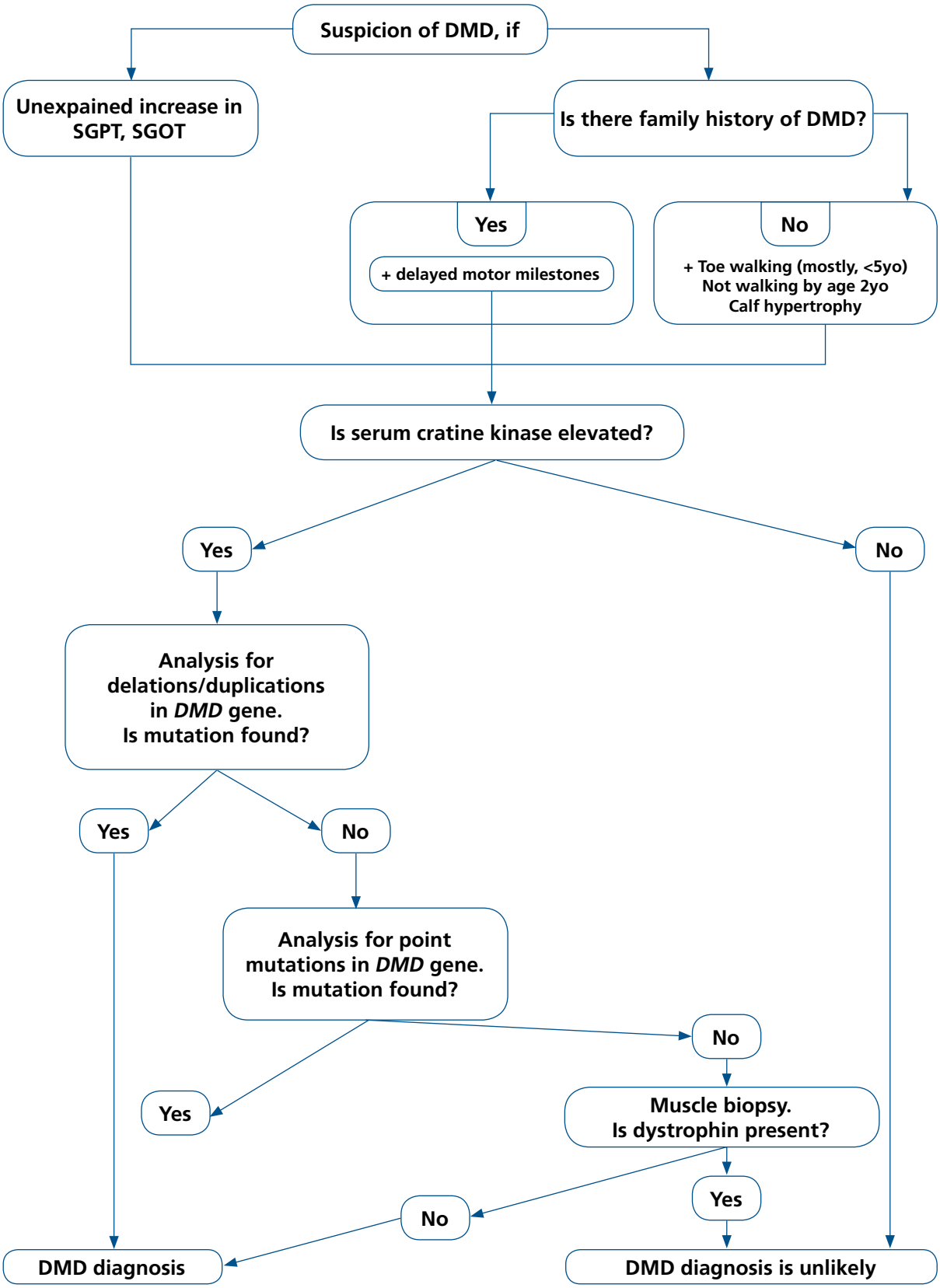
Steroids

Steroids have been shown to have a beneficial effect primarily on the respiratory function and in muscle strength maintenance. The early administration of steroids in children with DMD is included in the SOCs and aims at prolonging ambulation at least for 3 years, which is also very important for respiratory function, as it seems that there is a good correlation between loss of ambulation and respiratory function decline. Retaining ambulation may further delay spinal deformities, which is a major concern in DMD patients [44]. Despite the strict recommendations for steroid administration in paediatric DMD patients, there are no clear guidelines for the adult patients, and the treating physician must weigh the pros and cons of continuing treatment.

A significant body of evidence from recent clinical studies suggest that the early administration of steroids, before the age of 10 years, may increase the pulmonary function testing measures with a subsequent delay in the onset of decline, compared to naïve DMD patients. On the other hand, if given at a later stage, after the onset of respiratory deterioration, they do not seem to have any beneficial impact on the progression of the disease [72, 73, 96]. Previous studies have also shown that steroids slow the progression of scoliosis and delay the need for spinal surgery. Given the association of scoliosis and pulmonary function, it would be expected that the positive effect of steroids on spinal pathology may also indirectly impact the respiratory function [97, 98]. The role of steroids in the cardiac function of DMD patients is quite controversial. In a large retrospective study investigating the role of genetic modifiers in DMD, steroid treatment did not significantly affect the onset of dilated cardiomyopathy, which occurred at a mean age of 20 years [71]. Nevertheless, older studies suggest that steroids may delay progression of heart failure and can improve survival [99, 100].

Lastly, a further matter of interest is the potential different effect of frequently used corticosteroids and

Figure 1. Diagnostic algorithm for Duchenne muscular dystrophy



Abbreviations: DMD, Duchenne muscular dystrophy ; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, Serum glutamic pyruvic transaminase
Adapted from Birnkrant DJ et al. [44].

their various regimen schedules on the progression of cardiorespiratory function. According to a recent retrospective longitudinal study, steroids, either deflazacort or prednisone administered either daily or intermittently, had a significantly positive impact on both respiratory function and cardiomyopathy [73]. Notably, deflazacort is associated with less weight gain than prednisone and is the first glucocorticoid with a full FDA approval for DMD patients older than 5 years of age [44, 101]. A current ongoing trial is now comparing benefits and adverse effects between deflazacort and prednisone [102].

Genetic Treatments

Genetic therapies have attracted increasing attention and where indicated, are incorporated into the treatment plan. The approval of the first genetic drugs, ataluren by EMA in August 2014, and eteplirsen by FDA in September 2016, are considered important milestones in the treatment of the disease [44, 103].

➢ **Stop codon read through therapies**

In DMD, 11-30% of patients have a nonsense mutation in the *DMD* gene, resulting in a premature mRNA stop codon, which leads to termination of the translation before a full-length functional dystrophin is generated. Therefore, DMD patients carrying this type of mutation are eligible for ataluren, an orally administered small molecule, which promotes ribosomal read-through of mRNA with a premature stop codon, restoring the production of a full-length protein. Despite the failure to achieve the primary endpoints of improved walking distance in the 6-minute walk test (6MWT) after 48 weeks of treatment in two randomized, double-blind, placebo-controlled trials, there was a clear improvement in timed function tests and a significant 29-meter increase in 6MWT, which formed the basis of a conditional approval by EMA since 2014 [104-106]. On the other hand, ataluren has not gained approval from FDA yet, mainly because the 9% increase in dystrophin production induced by the drug was not considered statistically significant.

Finally, the efficacy and safety of ataluren has also been confirmed by the European Drug Registry (STRIDE), while another placebo-controlled study evaluating the effect of ataluren is underway [107].

➢ **Exon skipping therapies**

Exon skipping technology is being extensively used over the past few years in DMD. The aim is to restore the reading frame by converting an out-of-frame to an in-frame mutation, leading to a partially functional dystrophin and a milder BMD-like phenotype. Exon skipping is induced by the intravenous administration of antisense oligonucleotides (ASOs), which are short single-stranded nucleic acids that can bind to the pre-messenger RNA mutation preventing it from being included in the mature mRNA [108, 109].Obviously, the knowledge of the accurate genetic diagnosis is critical, as any frameshift mutation can be amenable to certain exon skipping therapy. Since deletions cluster in hotspots of the *DMD* gene, skipping of certain exons may be applied to a great majority of DMD patients [110]. More specifically, skipping of exon 51 is applicable to approximately 14% of patients, of exon 45 to 8%, of exon 53 to 8% and of exon 44 to 6%, respectively [111, 112]. Conditional approval has been already given by FDA to four exon skipping therapies: firstly eteplirsen (ExonDys51) in September 2016, to skip exon 51, golodirsen in December 2019 and vitolarsen in August 2020 to target exon 53 and more recently in March 2021, casimerson for skipping exon 45 [113-116]. Similarly, conditional approval has been granted to vitolarsen by the Japanese Ministry of Health, Welfare and Labour. Current studies are now assessing the long-term clinical effect of those compounds in order to obtain final approval.

➢ **Gene therapy**

Gene transfer therapy is an evolving therapeutic strategy for monogenic disorders, including DMD. The first double-blind placebo-controlled gene transfer therapy clinical trial for DMD patients (NCT03769116) started in 2018.

The aim of gene therapy is to prevent or slow the progression of the disease and relies on the use of viral vectors for efficient gene delivery. The vectors that are usually used for transferring functional genes are adenoviruses, adeno-associated viruses (AAVs), and lentiviruses, and are the most important determinants of safety and transduction efficiency. In DMD, the AAV9 and AAVrh74 vectors are suitable candidates for targeting both muscle and heart [117-119]. The AAV-induced immune response varies over time following administration. The first response is observed very early, hours to days after the injection and is mediated by innate immunity, while the adaptive immunity is activated later, weeks to months after drug delivery and may persist in the form of antigen-specific T and B cells [120, 121]. The most common adverse reactions of gene therapy may include an increase in transaminases, platelet reduction, nausea, vomiting, loss of appetite, diarrhea, increase of troponin and creatine kinase, fever and myalgia, while extremely rarely more serious side effects such as, liver, respiratory or heart failure, hemolytic uremic syndrome, intestinal bleeding, tumorgenicity, dorsal root ganglia toxicity, septicemia and death, have been reported [122-124]. An important concern with AAVs is that the delivery of gene therapy may be

prevented by neutralizing antibodies that block AAV entry into the cells. The pre-existing antibodies are mainly acquired through environmental exposure to wild-type AAVs and more rarely through AAV-based vaccination or AAV-based treatments [125-127].

A major issue of gene therapy for DMD is the huge size of *DMD* gene. As such, the dystrophin cDNA of 14kb far exceeds the 5kb packaging capacity of AAVs [128-130]. This problem was addressed with the discovery of microdystrophin, a shorter version of the *DMD* gene, which contains the important information for the production of a functional dystrophin protein, especially the coding region for binding to actin and to sarcoglycan complex. Therefore, it is expected that the expression of microdystrophin can keep patients at a stable state for a long period of time [131, 132]. Despite some initial promising results, an important question to be answered is the durability of gene transfer therapy. However, the treatment benefit will be potentially long term, since skeletal muscle cells are non-dividing and long-lived, while cardiomyocytes have a low turnover, with less than 50% of them being exchanged during a normal lifespan and the rate even decreases exponentially with age. A realistic goal would be an improvement of the disease trajectory of DMD patients compared to what could be expected from natural history studies [133-136]. However, further investigations are needed, particularly because in parallel with the effect of gene transfer therapy, there may be some extent of ongoing degeneration, which may lead to a clinical deterioration. Moreover, since AAV vectors are not integrating in the genome, the AAV-mediated dystrophin expression may decrease over time. There is currently no possibility of repeating gene transfer therapy, mainly due to the existence of neutralizing antibodies following the initial dose, which may affect subsequent administrations [137].

CONCLUSION

DMD should be regarded as a continuum, with signs and symptoms that may manifest very early and go unnoticed if there is no high suspicion. Although DMD still remains an incurable condition, significant progress has been made, especially in the field of genetic therapies and as such, an early diagnosis becomes more important, since it allows patients to receive timely any available modifying treatment or to participate in clinical trials. The main therapeutic goal is firstly to delay the progression to each milestone, especially to prolong ambulation as much as possible, and to partially restore respiratory, cardiac and skeletal muscle function. Finally, adhering to the guidelines and the international standards of care for DMD in a multidisciplinary approach should be strongly encouraged.

REFERENCES

[1] Mercuri E, Bonnemann CG, Muntoni F. Muscular dystrophies. *Lancet.* 2019;394:2025-2038.

[2] Ryder S, Leadley RM, Armstrong N, et al. The burden, epidemiology, costs and treatment for Duchenne muscular dystrophy: an evidence review. *Orphanet J Rare Dis.* 2017;12:79.

[3] Aartsma-Rus A, Van Deutekom JC, Fokkema IF, et al. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve.* 2006;34:135-144.

[4] Duan D, Goemans N, Takeda S, et al. Duchenne muscular dystrophy. *Nat Rev Dis Primers.* 2021;7:13.

[5] Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. *Lancet Neurol.* 2010;9:77-93.

[6] Zhong J, Xie Y, Bhandari V, et al. Clinical and genetic characteristics of female dystrophinopathy carriers. *Mol Med Rep.* 2019;19:3035-3044.

[7] Kamdar F, Garry DJ. Dystrophin-Deficient Cardiomyopathy. *J Am Coll Cardiol.* 2016;67:2533-2546.

[8] Soltanzadeh P, Friez MJ, Dunn D, et al. Clinical and genetic characterization of manifesting carriers of DMD mutations. *Neuromuscul Disord.* 2010;20:499-504.

[9] López-Hernández LB, Gómez-Díaz B, Luna-Angulo AB, et al. Comparison of Mutation Profiles in the Duchenne Muscular Dystrophy Gene among Populations: Implications for Potential Molecular Therapies. *International Journal of Molecular Sciences.* 2015;16.

[10] Garcia S, de Haro T, Zafra-Ceres M, et al. Identification of de novo Mutations of Duchénnè/Becker Muscular Dystrophies in Southern Spain. *International Journal of Medical Sciences.* 2014;11:988-993.

[11] Kesari A, Pirra LN, Bremadesam L, et al. Integrated DNA, cDNA, and protein studies in Becker muscular dystrophy show high exception to the reading frame rule. *Hum Mutat.* 2008;29:728-737.

[12] Chen WJ, Lin QF, Zhang QJ, et al. Molecular analysis of the dystrophin gene in 407 Chinese patients with Duchenne/Becker muscular dystrophy by the combination of multiplex ligation-dependent probe amplification and Sanger sequencing. *Clin Chim Acta.* 2013;423:35-38.

[13] Yu H, Chen YC, Liu GL, et al. A De novo Mutation in Dystrophin Causing Muscular Dystrophy in a Female Patient. *Chin Med J (Engl).* 2017;130:2273-2278.

[14] Caskey CT, Nussbaum RL, Cohan LC, et al. Sporadic occurrence of Duchenne muscular dystrophy: evidence for new mutation. *Clin Genet.* 1980;18:329-341.
[15] Helderman-van den Enden AT, de Jong R, den Dunnen JT, et al. Recurrence risk due to germ line mosaicism: Duchenne and Becker muscular dystrophy. *Clin Genet.* 2009;75:465-472.
[16] Gao QQ, McNally EM. The Dystrophin Complex: Structure, Function, and Implications for Therapy. *Compr Physiol.* 2015;5:1223-1239.
[17] Prins KW, Humston JL, Mehta A, et al. Dystrophin is a microtubule-associated protein. *J Cell Biol.* 2009;186:363-369.
[18] Stone MR, O'Neill A, Catino D, et al. Specific interaction of the actin-binding domain of dystrophin with intermediate filaments containing keratin 19. *Mol Biol Cell.* 2005;16:4280-4293.
[19] Moser H. Duchenne muscular dystrophy: pathogenetic aspects and genetic prevention. *Hum Genet.* 1984;66:17-40.
[20] Hoffman EP, Brown RH, Jr., Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell.* 1987;51:919-928.
[21] Allen DG, Whitehead NP, Froehner SC. Absence of Dystrophin Disrupts Skeletal Muscle Signaling: Roles of Ca2+, Reactive Oxygen Species, and Nitric Oxide in the Development of Muscular Dystrophy. *Physiol Rev.* 2016;96:253-305.
[22] Aartsma-Rus A, van Putten M. Assessing functional performance in the mdx mouse model. *J Vis Exp.* 2014.
[23] Mokri B, Engel AG. Duchenne dystrophy: electron microscopic findings pointing to a basic or early abnormality in the plasma membrane of the muscle fiber. *Neurology.* 1975;25:1111-1120.
[24] Turner PR, Westwood T, Regen CM, et al. Increased protein degradation results from elevated free calcium levels found in muscle from mdx mice. *Nature.* 1988;335:735-738.
[25] Reeve JL, McArdle A, Jackson MJ. Age-related changes in muscle calcium content in dystrophin-deficient mdx mice. *Muscle Nerve.* 1997;20:357-360.
[26] Mareedu S, Million ED, Duan D, et al. Abnormal Calcium Handling in Duchenne Muscular Dystrophy: Mechanisms and Potential Therapies. *Front Physiol.* 2021;12:647010.
[27] Vallejo-Illarramendi A, Toral-Ojeda I, Aldanondo G, et al. Dysregulation of calcium homeostasis in muscular dystrophies. *Expert Rev Mol Med.* 2014;16:e16.
[28] Vandebrouck C, Martin D, Colson-Van Schoor M, et al. Involvement of TRPC in the abnormal calcium influx observed in dystrophic (mdx) mouse skeletal muscle fibers. *J Cell Biol.* 2002;158:1089-1096.
[29] Constantin B. Dystrophin complex functions as a scaffold for signalling proteins. *Biochim Biophys Acta.* 2014;1838:635-642.
[30] Bellinger AM, Reiken S, Carlson C, et al. Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle. *Nat Med.* 2009;15:325-330.
[31] Kushnir A, Wajsberg B, Marks AR. Ryanodine receptor dysfunction in human disorders. *Biochim Biophys Acta Mol Cell Res.* 2018;1865:1687-1697.
[32] Voit A, Patel V, Pachon R, et al. Reducing sarcolipin expression mitigates Duchenne muscular dystrophy and associated cardiomyopathy in mice. *Nat Commun.* 2017;8:1068.
[33] Lai Y, Thomas GD, Yue Y, et al. Dystrophins carrying spectrin-like repeats 16 and 17 anchor nNOS to the sarcolemma and enhance exercise performance in a mouse model of muscular dystrophy. *J Clin Invest.* 2009;119:624-635.
[34] Sander M, Chavoshan B, Harris SA, et al. Functional muscle ischemia in neuronal nitric oxide synthase-deficient skeletal muscle of children with Duchenne muscular dystrophy. *Proc Natl Acad Sci U S A.* 2000;97:13818-13823.
[35] Kalogeris T, Baines CP, Krenz M, et al. Ischemia/Reperfusion. *Compr Physiol.* 2016;7:113-170.
[36] Kuroda Y, Togashi H, Uchida T, et al. Oxidative stress evaluation of skeletal muscle in ischemia-reperfusion injury using enhanced magnetic resonance imaging. *Scientific Reports.* 2020;10:10863.
[37] Khairallah RJ, Shi G, Sbrana F, et al. Microtubules underlie dysfunction in duchenne muscular dystrophy. *Sci Signal.* 2012;5:ra56.
[38] Moore TM, Lin AJ, Strumwasser AR, et al. Mitochondrial Dysfunction Is an Early Consequence of Partial or Complete Dystrophin Loss in mdx Mice. *Front Physiol.* 2020;11:690.
[39] Nghiem PP, Bello L, Stoughton WB, et al. Changes in Muscle Metabolism are Associated with Phenotypic Variability in Golden Retriever Muscular Dystrophy. *Yale J Biol Med.* 2017;90:351-360.
[40] Vila MC, Rayavarapu S, Hogarth MW, et al. Mitochondria mediate cell membrane repair and contribute to Duchenne muscular dystrophy. *Cell Death Differ.* 2017;24:330-342.
[41] Barker RG, Wyckelsma VL, Xu H, et al. Mitochondrial content is preserved throughout disease progression in the mdx mouse model of Duchenne muscular dystrophy, regardless of taurine supplementation. *Am J Physiol Cell Physiol.* 2018;314:C483-C491.
[42] Dumont NA, Rudnicki MA. Targeting muscle

stem cell intrinsic defects to treat Duchenne muscular dystrophy. *npj Regenerative Medicine.* 2016;1:16006.
[43] Burr AR, Molkentin JD. Genetic evidence in the mouse solidifies the calcium hypothesis of myofiber death in muscular dystrophy. *Cell Death Differ.* 2015;22:1402-1412.
[44] Birnkrant DJ, Bushby K, Bann CM, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. *Lancet Neurol.* 2018;17:251-267.
[45] van Dommelen P, van Dijk O, de Wilde JA, et al. Early developmental milestones in Duchenne muscular dystrophy. *Dev Med Child Neurol.* 2020;62:1198-1204.
[46] Cyrulnik SE, Fee RJ, De Vivo DC, et al. Delayed developmental language milestones in children with Duchenne's muscular dystrophy. *J Pediatr.* 2007;150:474-478.
[47] Parsons EP, Clarke AJ, Bradley DM. Developmental progress in Duchenne muscular dystrophy: lessons for earlier detection. *Eur J Paediatr Neurol.* 2004;8:145-153.
[48] Fujino H, Saito T, Matsumura T, et al. Autism spectrum disorders are prevalent among patients with dystrophinopathies. *Neurol Sci.* 2018;39:1279-1282.
[49] Hendriksen JG, Vles JS. Neuropsychiatric disorders in males with duchenne muscular dystrophy: frequency rate of attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder, and obsessive--compulsive disorder. *J Child Neurol.* 2008;23:477-481.
[50] Emery AE. The muscular dystrophies. *Lancet.* 2002;359:687-695.
[51] Chen YW, Nagaraju K, Bakay M, et al. Early onset of inflammation and later involvement of TGFbeta in Duchenne muscular dystrophy. *Neurology.* 2005;65:826-834.
[52] Allen NM, Ewer A, Nakou V, et al. Unusual Presentations of Dystrophinopathies in Childhood. *Pediatrics.* 2018;141:S510-S514.
[53] Serratrice J, Chabrol B, Attarrian S, et al. Dystrophinopathie pseudométabolique sans anomalie immunohistochimique. *Revue neurologique (Paris).* 2000;156:175-178.
[54] Peverelli L, Testolin S, Villa L, et al. Histologic muscular history in steroid-treated and untreated patients with Duchenne dystrophy. *Neurology.* 2015;85:1886-1893.
[55] Khirani S, Ramirez A, Aubertin G, et al. Respiratory muscle decline in Duchenne muscular dystrophy. *Pediatr Pulmonol.* 2014;49:473-481.
[56] Mayer OH, Finkel RS, Rummey C, et al. Characterization of pulmonary function in Duchenne Muscular Dystrophy. *Pediatr Pulmonol.* 2015;50:487-494.
[57] Finder J, Mayer OH, Sheehan D, et al. Pulmonary Endpoints in Duchenne Muscular Dystrophy. A Workshop Summary. *Am J Respir Crit Care Med.* 2017;196:512-519.
[58] LoMauro A, Romei M, Gandossini S, et al. Evolution of respiratory function in Duchenne muscular dystrophy from childhood to adulthood. *Eur Respir J.* 2018;51.
[59] Sheehan DW, Birnkrant DJ, Benditt JO, et al. Respiratory Management of the Patient With Duchenne Muscular Dystrophy. *Pediatrics.* 2018;142:S62-S71.
[60] Passamano L, Taglia A, Palladino A, et al. Improvement of survival in Duchenne Muscular Dystrophy: retrospective analysis of 835 patients. *Acta Myol.* 2012;31:121-125.
[61] Rall S, Grimm T. Survival in Duchenne muscular dystrophy. *Acta Myol.* 2012;31:117-120.
[62] Abresch R, McDonald C, Henricson E, et al. P.11.11 Pulmonary function characteristics of boys with Duchenne Muscular Dystrophy by age groups, ambulatory status and steroid use. *Neuromuscular Disorders.* 2013;23:801-802.
[63] Mavrogeni SI, Markousis-Mavrogenis G, Papavasiliou A, et al. Cardiac Involvement in Duchenne Muscular Dystrophy and Related Dystrophinopathies. *Methods Mol Biol.* 2018;1687:31-42.
[64] D'Amario D, Amodeo A, Adorisio R, et al. A current approach to heart failure in Duchenne muscular dystrophy. *Heart.* 2017;103:1770-1779.
[65] D'Amario D, Gowran A, Canonico F, et al. Dystrophin Cardiomyopathies: Clinical Management, Molecular Pathogenesis and Evolution towards Precision Medicine. *J Clin Med.* 2018;7.
[66] Adorisio R, Mencarelli E, Cantarutti N, et al. Duchenne Dilated Cardiomyopathy: Cardiac Management from Prevention to Advanced Cardiovascular Therapies. *J Clin Med.* 2020;9.
[67] Papa AA, D'Ambrosio P, Petillo R, et al. Heart transplantation in patients with dystrophinopathic cardiomyopathy: Review of the literature and personal series. *Intractable Rare Dis Res.* 2017;6:95-101.
[68] Perri G, Filippelli S, Adorisio R, et al. Left ventricular assist device as destination therapy in cardiac end-stage dystrophinopathies: Midterm results. *J Thorac Cardiovasc Surg.* 2017;153:669-674.
[69] Ryan TD, Jefferies JL, Sawnani H, et al. Implantation of the HeartMate II and HeartWare left ventricular assist devices in patients with duchenne muscular dystrophy: lessons learned from the first applications. *ASAIO J.* 2014;60:246-248.

[70] Yamamoto T, Awano H, Zhang Z, et al. Cardiac Dysfunction in Duchenne Muscular Dystrophy Is Less Frequent in Patients With Mutations in the Dystrophin Dp116 Coding Region Than in Other Regions. *Circ Genom Precis Med.* 2018;11:e001782.
[71] Barp A, Bello L, Politano L, et al. Genetic Modifiers of Duchenne Muscular Dystrophy and Dilated Cardiomyopathy. *PLoS One.* 2015;10:e0141240.
[72] Bello L, D'Angelo G, Villa M, et al. Genetic modifiers of respiratory function in Duchenne muscular dystrophy. *Ann Clin Transl Neurol.* 2020;7:786-798.
[73] Trucco F, Domingos JP, Tay CG, et al. Cardiorespiratory Progression Over 5 Years and Role of Corticosteroids in Duchenne Muscular Dystrophy: A Single-Site Retrospective Longitudinal Study. *Chest.* 2020;158:1606-1616.
[74] van Ruiten HJ, Straub V, Bushby K, et al. Improving recognition of Duchenne muscular dystrophy: a retrospective case note review. *Arch Dis Child.* 2014;99:1074-1077.
[75] Lalic T, Vossen RH, Coffa J, et al. Deletion and duplication screening in the DMD gene using MLPA. *Eur J Hum Genet.* 2005;13:1231-1234.
[76] Aartsma-Rus A, Ginjaar IB, Bushby K. The importance of genetic diagnosis for Duchenne muscular dystrophy. *J Med Genet.* 2016;53:145-151.
[77] Okubo M, Minami N, Goto K, et al. Genetic diagnosis of Duchenne/Becker muscular dystrophy using next-generation sequencing: validation analysis of DMD mutations. *J Hum Genet.* 2016;61:483-489.
[78] Schussler SC, Gerhalter T, Abicht A, et al. Rare intronic mutation between Exon 62 and 63 (c.9225-285A>G) of the dystrophin gene associated with atypical BMD phenotype. *Neuromuscul Disord.* 2020;30:680-684.
[79] Anderson LV, Davison K. Multiplex Western blotting system for the analysis of muscular dystrophy proteins. *Am J Pathol.* 1999;154:1017-1022.
[80] Anthony K, Arechavala-Gomeza V, Taylor LE, et al. Dystrophin quantification: Biological and translational research implications. *Neurology.* 2014;83:2062-2069.
[81] Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. *Lancet Neurol.* 2010;9:177-189.
[82] Birnkrant DJ, Bushby K, Bann CM, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: respiratory, cardiac, bone health, and orthopaedic management. *Lancet Neurol.* 2018;17:347-361.
[83] Birnkrant DJ, Bushby K, Bann CM, et al. Diagnosis and management of Duchenne muscular dystrophy, part 3: primary care, emergency management, psychosocial care, and transitions of care across the lifespan. *Lancet Neurol.* 2018;17:445-455.
[84] Hamdani Y, Mistry B, Gibson BE. Transitioning to adulthood with a progressive condition: best practice assumptions and individual experiences of young men with Duchenne muscular dystrophy. *Disabil Rehabil.* 2015;37:1144-1151.
[85] Andrews JG, Conway K, Westfield C, et al. Implementation of Duchenne Muscular Dystrophy Care Considerations. *Pediatrics.* 2018;142.
[86] Saito T, Kawai M, Kimura E, et al. Study of Duchenne muscular dystrophy long-term survivors aged 40 years and older living in specialized institutions in Japan. *Neuromuscul Disord.* 2017;27:107-114.
[87] Ricotti V, Selby V, Ridout D, et al. Respiratory and upper limb function as outcome measures in ambulant and non-ambulant subjects with Duchenne muscular dystrophy: A prospective multicentre study. *Neuromuscul Disord.* 2019;29:261-268.
[88] McNally EM, Kaltman JR, Benson DW, et al. Contemporary cardiac issues in Duchenne muscular dystrophy. Working Group of the National Heart, Lung, and Blood Institute in collaboration with Parent Project Muscular Dystrophy. *Circulation.* 2015;131:1590-1598.
[89] Duboc D, Meune C, Pierre B, et al. Perindopril preventive treatment on mortality in Duchenne muscular dystrophy: 10 years' follow-up. *Am Heart J.* 2007;154:596-602.
[90] Suthar R, Reddy BVC, Malviya M, et al. Bone density and bone health alteration in boys with Duchenne Muscular Dystrophy: a prospective observational study. *J Pediatr Endocrinol Metab.* 2021;34:573-581.
[91] Yilmaz O, Karaduman A, Topaloglu H. Prednisolone therapy in Duchenne muscular dystrophy prolongs ambulation and prevents scoliosis. *Eur J Neurol.* 2004;11:541-544.
[92] Lo Cascio CM, Goetze O, Latshang TD, et al. Gastrointestinal Dysfunction in Patients with Duchenne Muscular Dystrophy. *PLoS One.* 2016;11:e0163779.
[93] Bertrand LA, Askeland EJ, Mathews KD, et al. Prevalence and bother of patient-reported lower urinary tract symptoms in the muscular dystrophies. *J Pediatr Urol.* 2016;12:398 e391-398 e394.
[94] Matsumura T, Saito T, Fujimura H, et al. [Renal dysfunction is a frequent complication in patients with advanced stage of Duchenne muscular dystrophy]. *Rinsho Shinkeigaku.* 2012;52:211-217.

[95] Banihani R, Smile S, Yoon G, et al. Cognitive and Neurobehavioral Profile in Boys With Duchenne Muscular Dystrophy. *J Child Neurol.* 2015;30:1472-1482.
[96] McDonald CM, Gordish-Dressman H, Henricson EK, et al. Longitudinal pulmonary function testing outcome measures in Duchenne muscular dystrophy: Long-term natural history with and without glucocorticoids. *Neuromuscul Disord.* 2018;28:897-909.
[97] Alman BA, Raza SN, Biggar WD. Steroid treatment and the development of scoliosis in males with duchenne muscular dystrophy. *J Bone Joint Surg Am.* 2004;86:519-524.
[98] Kurz LT, Mubarak SJ, Schultz P, et al. Correlation of scoliosis and pulmonary function in Duchenne muscular dystrophy. *J Pediatr Orthop.* 1983;3:347-353.
[99] Barber BJ, Andrews JG, Lu Z, et al. Oral corticosteroids and onset of cardiomyopathy in Duchenne muscular dystrophy. *J Pediatr.* 2013;163:1080-1084 e1081.
[100] Markham LW, Kinnett K, Wong BL, et al. Corticosteroid treatment retards development of ventricular dysfunction in Duchenne muscular dystrophy. *Neuromuscul Disord.* 2008;18:365-370.
[101] Griggs RC, Miller JP, Greenberg CR, et al. Efficacy and safety of deflazacort vs prednisone and placebo for Duchenne muscular dystrophy. *Neurology.* 2016;87:2123-2131.
[102] Guglieri M, Bushby K, McDermott MP, et al. Developing standardized corticosteroid treatment for Duchenne muscular dystrophy. *Contemp Clin Trials.* 2017;58:34-39.
[103] Stephenson AA, Flanigan KM. Gene editing and modulation for Duchenne muscular dystrophy. *Prog Mol Biol Transl Sci.* 2021;182:225-255.
[104] Bushby K, Finkel R, Wong B, et al. Ataluren treatment of patients with nonsense mutation dystrophinopathy. *Muscle Nerve.* 2014;50:477-487.
[105] Haas M, Vlcek V, Balabanov P, et al. European Medicines Agency review of ataluren for the treatment of ambulant patients aged 5 years and older with Duchenne muscular dystrophy resulting from a nonsense mutation in the dystrophin gene. *Neuromuscul Disord.* 2015;25:5-13.
[106] McDonald CM, Campbell C, Torricelli RE, et al. Ataluren in patients with nonsense mutation Duchenne muscular dystrophy (ACT DMD): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet.* 2017;390:1489-1498.
[107] Mercuri E, Muntoni F, Osorio AN, et al. Safety and effectiveness of ataluren: comparison of results from the STRIDE Registry and CINRG DMD Natural History Study. *J Comp Eff Res.* 2020;9:341-360.
[108] Harding PL, Fall AM, Honeyman K, et al. The influence of antisense oligonucleotide length on dystrophin exon skipping. *Mol Ther.* 2007;15:157-166.
[109] Niks EH, Aartsma-Rus A. Exon skipping: a first in class strategy for Duchenne muscular dystrophy. *Expert Opin Biol Ther.* 2017;17:225-236.
[110] Aartsma-Rus A, Fokkema I, Verschuuren J, et al. Theoretic applicability of antisense-mediated exon skipping for Duchenne muscular dystrophy mutations. *Hum Mutat.* 2009;30:293-299.
[111] Bladen CL, Rafferty K, Straub V, et al. The TREAT-NMD Duchenne muscular dystrophy registries: conception, design, and utilization by industry and academia. *Hum Mutat.* 2013;34:1449-1457.
[112] Wang RT, Barthelemy F, Martin AS, et al. DMD genotype correlations from the Duchenne Registry: Endogenous exon skipping is a factor in prolonged ambulation for individuals with a defined mutation subtype. *Hum Mutat.* 2018;39:1193-1202.
[113] Alfano LN, Charleston JS, Connolly AM, et al. Long-term treatment with eteplirsen in nonambulatory patients with Duchenne muscular dystrophy. *Medicine (Baltimore).* 2019;98:e15858.
[114] Mendell JR, Goemans N, Lowes LP, et al. Longitudinal effect of eteplirsen versus historical control on ambulation in Duchenne muscular dystrophy. *Ann Neurol.* 2016;79:257-271.
[115] Drug U. FDA grants accelerated approval to first targeted treatment for rare Duchenne muscular dystrophy mutation. 2019. 2019.
[116] Casimersen (Amondys 45) for Duchenne muscular dystrophy. *Med Lett Drugs Ther.* 2021;63:e104-e105.
[117] Colella P, Ronzitti G, Mingozzi F. Emerging Issues in AAV-Mediated In Vivo Gene Therapy. *Mol Ther Methods Clin Dev.* 2018;8:87-104.
[118] Bulcha JT, Wang Y, Ma H, et al. Viral vector platforms within the gene therapy landscape. *Signal Transduct Target Ther.* 2021;6:53.
[119] Lau CH, Suh Y. In vivo genome editing in animals using AAV-CRISPR system: applications to translational research of human disease. *F1000Res.* 2017;6:2153.
[120] Mingozzi F, High KA. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. *Blood.* 2013;122:23-36.
[121] Capasso C, Garofalo M, Hirvinen M, et al.

The evolution of adenoviral vectors through genetic and chemical surface modifications. *Viruses.* 2014;6:832-855.
[122] Bolt MW, Brady JT, Whiteley LO, et al. Development challenges associated with rAAV-based gene therapies. *J Toxicol Sci.* 2021;46:57-68.
[123] Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov.* 2019;18:358-378.
[124] Buning H, Schmidt M. Adeno-associated Vector Toxicity-To Be or Not to Be? *Mol Ther.* 2015;23:1673-1675.
[125] Long BR, Sandza K, Holcomb J, et al. The Impact of Pre-existing Immunity on the Nonclinical Pharmacodynamics of AAV5-Based Gene Therapy. *Mol Ther Methods Clin Dev.* 2019;13:440-452.
[126] Calcedo R, Wilson JM. Humoral Immune Response to AAV. *Front Immunol.* 2013;4:341.
[127] Boisgerault F, Mingozzi F. The Skeletal Muscle Environment and Its Role in Immunity and Tolerance to AAV Vector-Mediated Gene Transfer. *Curr Gene Ther.* 2015;15:381-394.
[128] Naso MF, Tomkowicz B, Perry WL, 3rd, et al. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. *BioDrugs.* 2017;31:317-334.
[129] Ferrer A, Foster H, Wells KE, et al. Long-term expression of full-length human dystrophin in transgenic mdx mice expressing internally deleted human dystrophins. *Gene Ther.* 2004;11:884-893.
[130] Lostal W, Kodippili K, Yue Y, et al. Full-length dystrophin reconstitution with adeno-associated viral vectors. *Hum Gene Ther.* 2014;25:552-562.
[131] Zhao J, Kodippili K, Yue Y, et al. Dystrophin contains multiple independent membrane-binding domains. *Hum Mol Genet.* 2016;25:3647-3653.
[132] Mendell JR, Sahenk Z, Lehman K, et al. Assessment of Systemic Delivery of rAAVrh74.MHCK7.micro-dystrophin in Children With Duchenne Muscular Dystrophy: A Nonrandomized Controlled Trial. *JAMA Neurol.* 2020;77:1122-1131.
[133] Asher DR, Thapa K, Dharia SD, et al. Clinical development on the frontier: gene therapy for duchenne muscular dystrophy. *Expert Opin Biol Ther.* 2020;20:263-274.
[134] Partridge TA. Cells that participate in regeneration of skeletal muscle. *Gene Ther.* 2002;9:752-753.
[135] Spalding KL, Bhardwaj RD, Buchholz BA, et al. Retrospective birth dating of cells in humans. *Cell.* 2005;122:133-143.
[136] Yin H, Price F, Rudnicki MA. Satellite cells and the muscle stem cell niche. *Physiol Rev.* 2013;93:23-67.
[137] Ilyinskii PO, Michaud AM, Roy CJ, et al. Enhancement of liver-directed transgene expression at initial and repeat doses of AAV vectors admixed with ImmTOR nanoparticles. *Sci Adv.* 2021;7.
[138] Darras BT, Urion DK, Ghosh PS. Dystrophinopathies. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews((R)).* Seattle (WA), 1993.

LEBER HEREDITARY OPTIC NEUROPATHY. CLINICAL CHARACTERISTICS, DIAGNOSIS AND MANAGEMENT

Konstantina Riri, Evangelia Tsironi-Malizou, Maria Kotoula, Eleni Papageorgiou

Department of Ophthalmology, General University Hospital of Larissa, Mezourlo Area, Larissa, Greece

Abstract

Leber hereditary optic neuropathy (LHON) is a rare, maternally inherited mitochondrial disorder, which affects the retinal ganglion cells. LHON usually presents in young males with progressive visual decline due to optic neuropathy. Visual acuity decrease progresses to legal blindness in a large number of patients. The diagnosis of LHON is based on history, visual acuity, perimetry, fluorescein angiography, optical coherence tomography, electrophysiology, and on the molecular confirmation of a pathogenic mtDNA mutation. Currently, the treatment of LHON includes genetic counseling, avoidance of certain environmental risk factors, and medical treatment with idebenone for subacute and dynamic cases. Recently, gene therapy using adeno-associated virus (AAV) vectors and mitochondrial replacement therapy are also showing promising results. This review will address the pathophysiology, clinical presentation, diagnostic procedures and current management of LHON.

Key words: leber hereditary optic neuropathy, LHON, mitochondrial disease, optic atrophy, gene therapy, idebenone, adeno-associated virus vectors

1. Introduction

Leber's hereditary optic neuropathy (LHON) is the most common primary mitochondrial DNA (mtDNA) disease [1-4]. LHON is a maternally inherited condition, which is associated with defective cellular energy production by the retinal ganglion cells. More than 90% of LHON patients harbor one of the three mtDNA point mutations. The prevalence of LHON is 1 in 30.000-50.000 in North Europe, with an estimated incidence of 1 in 1.000.000 in Japan [3, 5, 6]. LHON affects mainly males (80%-90%), usually between 15 to 35 years [7]. In most cases painless acute or subacute central visual loss occurs in both eyes within a few weeks to months. Most patients remain legally blind with visual acuity less than 20/200 for the rest of their lives. LHON is characterized by a preferential loss of retinal ganglion cells within the papillomacular bundle, which results in dense central scotomas [8]. In severe cases, the entire visual field can be affected [3, 5, 6]. In the last few years, advances in molecular medicine have led to substantial understanding of the genetic basis of LHON. Gene therapy trials have shown promising results in reducing the impact and progression of LHON. Idebenone, which is a synthetic analog of coenzyme Q_{10} has been recently implement in clinical trials for treatment of LHON. However, optimal management of LHON still remains a major challenge in the field of inherited mitochondrial diseases.

This review will address the pathophysiology, clinical presentation, diagnostic procedures and current management of LHON. A PubMed search of all articles published from January 1991 to September 2021 on etiology, clinical characteristics and treatment of LHON was performed. Searches included a combination of the following terms: "Leber's hereditary optic neuropathy", "visual field", "LHON mutations", "natural history", "electrophysiology", "retinal ganglion cell function", "prognosis", "LHON treatment", "gene therapy", "idebenone". The resulting references were then reviewed for pertinent articles. Selected key papers of historical importance published before 1991 were also included.

2. Etiology

2.1. *Genetics*

Three primary point mitochondrial mutations, m.3460G>A (MTND1), m.11778G>A (MTND4), and m.14484T>C (MTND6) are detected in approximately 90% of all LHON patients in multiple and ethnically divergent pedigrees. The remaining 10% of LHON cases are due to less common pathogenic mtDNA mutations, which have been documented in single case reports. The 11778G>A mutation is the most common cause of LHON worldwide, and is associated with a severe phenotype of LHON and the poorest visual recovery rates between 11-14% [3, 8]. It is detected in 70% of LHON cases in Northern Europe

Table 1. The 3 main LHON mutations; Phenotypic correlation and lifetime risk for visual loss

Mutations	Phenotypic correlation	Risk for visual loss		Median age at onset	M: F Ratio	Visual recovery	References
		M	F				
m.3460G>A	Intermediate course	32%-49%	15%-28%	20-22 y	1.7-4.3:1	15% - 25%	[11-14, 17]
m.11778G>A	Severe clinical form of LHON-poorest visual recovery rates The most common mutation	43%-51%	9-%11%	22-24 y	3.7-5.1:1	14% of persons of all ages; 11% of those aged ≥15 y	[12, 15, 17]
m.14484T>C	The most optimal visual outcome	47%	8%	20 y	7.7:1	37% - 64%	[13, 16, 26]

F = female, **M** = male, **y** = years

and 90% in Asia. The 14484T>C mutation is associated with the most optimal prognosis and best long-term visual outcome. In 37-64% of patients, some degree of visual recovery is achieved after reaching a visual "nadir". This mutation has been commonly described in French Canadians [9, 10]. The mutation 3460G>A presents with an intermediate course and the visual recovery rates between 15-25% [9, 10]. Phenotypic correlation and lifetime risk for vision loss for each of the three main mitochondrial mutations are summarized in Table 1.

2.2. *Sex*

LHON is characterized by incomplete penetrance. Sex and age are major risk factors for visual loss. A mtDNA mutation exists in all maternal related relatives of LHON patients, however most of the patient's relatives will never experience any symptom. Although wide variability exists across different families, the average lifetime risk of optic neuropathy and visual loss in male carriers is 50%, and in females it is only 10%.

The predominance of vision loss in male LHON patients is explained by a vision loss susceptibility allele on the X-chromosome. Patients with the 11778 and 14484 mutations at Xp21 chromosome were 35-fold more likely to lose their vision than patients without such mutations [7].

2.3. *Age*

Regarding age, more than 95% of male patients are affected before the age of 50 years, for all three main mutations. Consequently, a 50-year-old male without symptoms has <5% chances of vision loss [3].

2.4. *Heteroplasmy*

An additional factor that may influence the phenotypic expression of LHON is heteroplasmy, i.e. each cell contains many mitochondria and some of them with pathogenic mtDNA that cause LHON are mixed with other mutated and wild-type forms of mtDNA [17]. According to some studies, individuals with a "mutation load" less than 60-75%, may never experience vision loss. Tissue heteroplasmy leads to a variety in phenotypes in patients with similar mitochondrial genotypes, as subjects at risk may have different amounts of mutant mtDNA in their optic nerves [18]. Furthermore, due to heteroplasmy, the right and left eye may have different amounts of affected mtDNA [19]. Presymptomatic testing for quantifying the level of heteroplasmy is not widely used, because mtDNA in peripheral blood cells might not predict the mutation load of the retinal ganglion cells (RGCs). Additionally, most LHON individuals are homoplasmic, i.e. they have 100% of mutant mtDNA. Only 10%-15% of subjects carrying a LHON mutation are heterosplasmic [3, 9, 20].

2.5. *Other genetic factors*

Additionally, polymorphisms in nuclear genes related to mitochondrial regulation likely explain the variable penetrance and phenotypic expression of LHON. The correlation between LHON and multiple sclerosis (LHON-MS, known as "Harding disease") has been explained on the basis of immunologic factors explain [21]. The HLA-DR locus is not a main factor for the development of vision loss, however the resulting pathological condition has a characteristic phenotype, suggesting a mechanistic interaction. The course of LHON-MS is more aggressive and prognosis and management should be guarded [22].

2.6. *Environmental factors*

Finally, it has been suggested that environmental factors could be associated with the primary mtDNA

Table 2. Extraocular manifestations of LHON

Extraocular manifestations of LHON	
Cardiac abnormalities	Cardiac arrhythmias Wolff-Parkinson-White (WPW)
Neurologic abnormalities	Dystonia Postural tremor Peripheral neuropathy Movement disorders Multiple sclerosis-like illness Nonspecific myopathy

pathogenic mutation and affect the course of the disease which varies from optic nerve dysfunction to total visual failure. Factors that may affect phenotypic expression of the disease are systemic illnesses, nutritional deficiencies, trauma, medications, smoking, alcohol, and drug-induced mitochondrial toxicity [2].

3. Molecular Pathophysiology

Retinal ganglion cells of the papillomacular bundle are the main target tissue of damage, resulting in degenerated cell bodies and axons. The ensuing demyelination extends to the lateral geniculate bodies. Retinal pigment epithelium and photoreceptors are not affected [23]. In LHON, complex I subunit genes in the respiratory chain are affected from mitochondrial mutations hence RGCs are degenerated selectively. Specifically, the majority of mutations in LHON involve a single subunit of mitochondrial NADH dehydrogenase (MTND), an enzyme partially responsible for the oxidative phosphorylation pathway, causing impairment of complex I of the electron transport chain (ETC) [7, 8]. Therefore, the mitochondrial respiratory chain produces less ATP, the reactive oxygen species increase, the glutamate transport is affected, and these factors synergistically contribute to retinal ganglion cell apoptosis and optic atrophy within a year of disease onset [24]. Though the genetic phenotype is well-described, the pathophysiology of selective damage of the retinal ganglion cell layer in LHON is not fully clarified yet [2].

4. History/Clinical Presentation

Patients with LHON usually present with unilateral, painless, subacute, central visual loss, with the fellow eye being affected within the following 6 months, and in more than 97% of patients within one year [25]. In approximately 25% of cases, both eyes are affected on initial presentation. Symptoms begin between 15-35 years of life, with an average onset age at 22-24 years for the 11778A mutation, and at 20 years for the 14484C mutation [3, 21, 26]. However, LHON has been reported in patients from 2-87 years of age [27-30].

LHON is four to five times more common in males than females. However, the timing and severity of the initial vision impairment is not significantly influenced by either sex or mutational status [23]. The disease is transmitted strictly by maternal inheritance.

A history of trauma, alcohol-tobacco abuse, drug intake, systemic illnesses and increased intraocular pressure are potential precipitating factors for vision decline in subjects at risk for LHON. LHON Plus disease refers to coexisting neurologic or cardiac deficits, hence correlated symptoms or signs, such as arrhythmias, cardiac conduction abnormalities, tremor, dystonia, movement disorders, nonspecific myopathy, weakness, and multiple sclerosis-like illness, should be investigated (Table 2). Leigh syndrome may also correlate with LHON [31].

5. Physical Examination

The course of LHON has been divided into three clinical stages, depending on the duration of vision loss: the subacute stage (less than 6 months), the dynamic stage (6-12 months) and the chronic stage (>12 months) based on both structural and functional changes [32].

Visual acuity loss may be mild in early stages, but typically deteriorates to acuities worse than <20/200, or counting fingers [33]. Usually, the unaffected eye becomes involved within weeks to months, however the interval between initial and fellow eye involvement may be longer, with the longest interval between eyes being eighteen years after the initial attack. When the disease is asymmetric, an afferent pupillary defect is present. Color vision is severely affected and color testing shows deficits in red-green discrimination. Contrast sensitivity is also reduced, the pattern or multifocal visual evoked potentials (VEPs) is clearly impaired, and the electroretinogram may be subnormal [34]. Perimetry may reveal central or cecocentral scotomas, and subclinical visual disorders in the fellow seemingly unaffected eye. Initially

Figure 1. Fundus images of a 16-year-old male patient during the acute phase of LHON. There is bilateral disk hyperemia, peripapillary retinal nerve fiber layer edema, increased vascular tortuosity and retinal telangiectasia (From Tsironi E, Editor. Basic Principles of Ophthalmology, Konstadaras Publications, Athens, 2018).

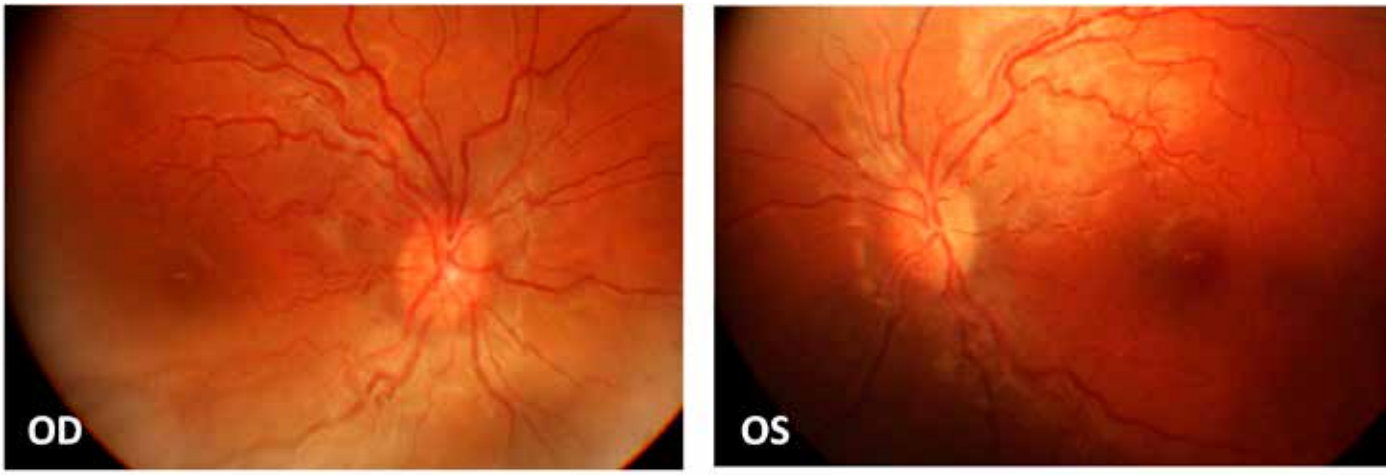
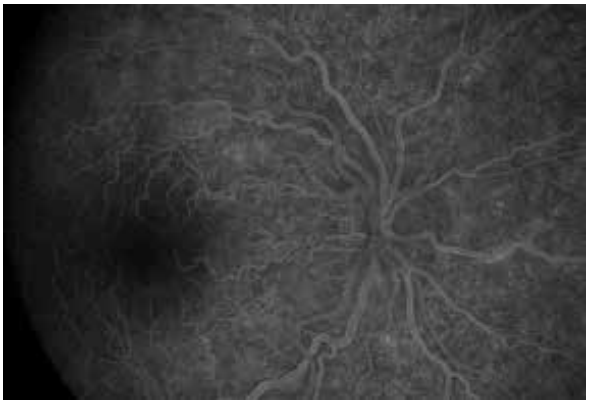


Figure 2. Fluorescein angiography of the right eye of the 16-year-old male patient with LHON. There is no dye leakage from the disk in the peripapillary region, distinguishing LHON from true optic disk edema (From Tsironi E, Editor. Basic Principles of Ophthalmology, Konstadaras Publications, Athens, 2018)



the scotomas may be relative, but with time they become absolute and extend at least 25-30 degrees across the central visual field [35].

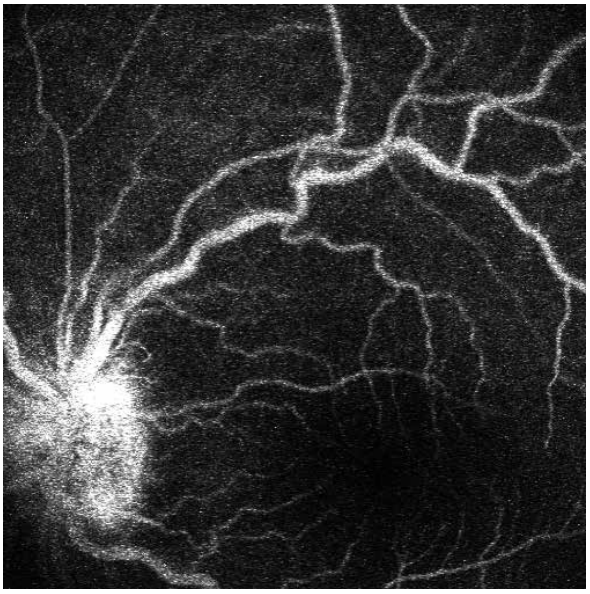
In the acute phase of LHON, funduscopy may reveal a pathognomonic triad of funduscopic abnormalities: circumpapillary telangiectatic microangiopathy with hyperemia and vessel tortuosity, elevation of the optic nerve head, and a thickened peripapillary nerve fiber layer (pseudoedema) (Figure 1). In LHON, there is no dye leakage on fluorescein angiogram, as opposed to true optic disk swelling. In about 20% of affected individuals, the optic disks appear normal in the acute phase, which can delay the diagnosis. With disease progression, the telangiectatic microangiopathy and pseudoedema of the disk resolve. Subsequently, there is rapid RGC axonal loss and optic atrophy with termporal pallor of the optic disk (36). The retinal nerve fiber layer dropout is first observed in the papillomacular bundle and months later, the whole nerve fiber layer becomes atrophic[37], The severe loss of the cells originating the papillomacular bundle is reflected on the thinning of the macular RGC layer on optical coherence tomography (OCT), which is completed after 4-6 months [32] Regarding retinal nerve fiber layer (RNFL) thickness, there is swelling in the first 6 months on OCT, followed by gradual quadrant specific thinning [32]. During the subacute stage visual acuity decreases and then usually stabilizes at approximately 6 months after onset of symptoms, while visual field defects and OCT abnormalities stabilize between 6 months to one year after symptom onset [38].

Vision loss is usually permanent, but partial spontaneous recovery of visual acuity can be observed. Slowly progressive variants of the disease course have been also described [39, 40]. Ocular symptoms and signs of LHON are outlined in Table 3.

6. Diagnostic Procedures

In suspected LHON, patients should undergo visual acuity testing, color vision assessment, dilated funduscopy, visual field examination, and OCT. As described above, fluorescein angiography is performed to distinguish true optic disk edema from pseudoedema. In LHON individuals, there is no dye leakage, although the optic disk may appear swollen (Figure 2, Figure 3). OCT is used for assessment of optic disk elevation in the early stages and for optic atrophy in the late stages of LHON. Specifically, the loss of macular RGCs is observed before the clinical disease onset and in about 4 months, maximal loss

Figure 3. Fluorescein angiography of the left eye of a 12-year-old male patient with panuveitis shows leakage of the disk in the peripapillary region ("hot disk") and enlargement and tortuosity of the retinal vessels (image courtesy of Dr. Kotoula)



has occurred [41]. In OCT, the RNFL appears thicker in the early stages of LHON and thinner in atrophic LHON, while in patients with visual recovery the RNFL seems to be preserved. The temporal fibers (papillomacular bundle) are the first and most severely affected, while the nasal fibers are partially preserved until the late stages of LHON [42]. A recent study of unaffected mutation carriers who converted to affected status found an early RNFL increase before conversion, suggesting that structural changes occur before clinically detectable vision loss [25]. Multifocal VEPs in LHON show impaired neural conduction along the visual pathway, with primary impairment of axons representing the central retina when compared to axons from the mid-peripheral retina [43]. OCT angiography in LHON individuals may demonstrate vascular dilation and tortuosity, in correlation with funduscopy findings [44].

Neuroimaging is essential to exclude intracranial disorders, compressive optic neuropathies, and demyelinating diseases. It should be performed in the presence of additional neurological symptoms, extracranial disease, or strictly unilateral findings with negative family history. MRI is often normal, but may demonstrate optic nerve enhancement, white matter lesions, or chiasmal enlargement and enhancement [45-48]. In the chronic phase, decrease of grey matter volume in the primary visual cortex and reduction of white matter volume in the optic chiasm, optic tract and optic radiations have been also documented [45, 46].

Gene testing may identify one of the three most Lfrequent LHON mutations, which are present in 90% of affected individuals. Targeted mtDNA testing for these three primary mutations is commercially available. A diagnostic algorithm of LHON is presented in Figure 4.

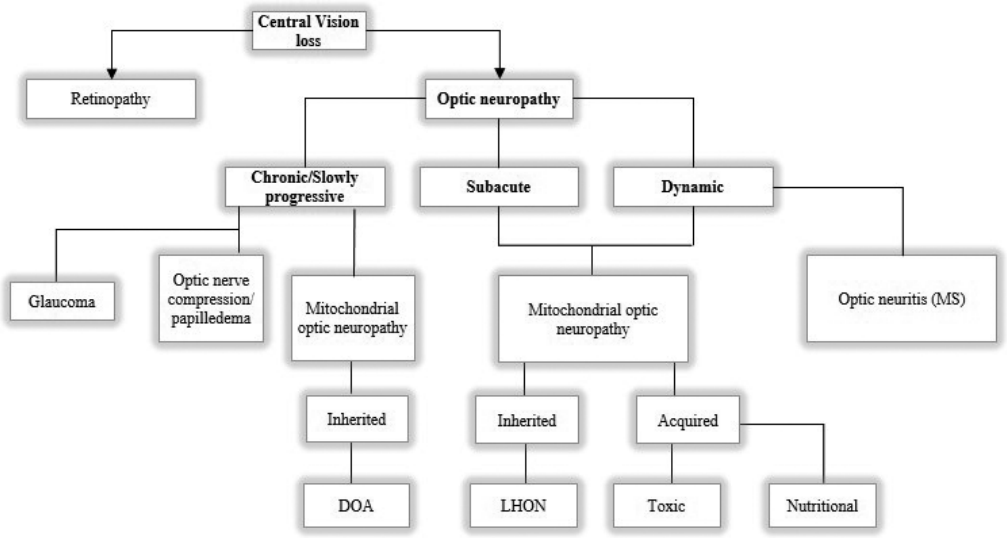
7. Differential Diagnosis

LHON should be included in the differential diagnosis of bilateral optic neuropathy. Unilateral involvement is exceptionally rare in LHON. Demyelinating optic neuritis, compressive optic neuropathy, toxic optic neuropathy, autosomal dominant optic atrophy, other inherited optic atrophies, normal tension glaucoma and bilateral anterior ischemic optic neuropathy should be excluded. In clinical practice, presentation and evolution of the disease should be taken under consideration and additional examinations such as autoantibody testing, screening for vasculitis, evaluation of oligoclonal bands in the cerebrospinal fluid and neuroimaging might be necessary. In the acute phase of LHON, the differential diagnosis includes a wide variety of non-genetic causes. However, in the

Table 3. Ocular symptoms and signs of LHON

Ocular symptoms and signs of LHON
Bilateral, painless subacute visual loss • Visual acuity < 20/200 • Visual fields: enlarging dense central or centrocecal skotomas
Triad of fundoscopic abnormalities: • circumpapillary telangiectatic microangiopathy with hyperemia and vessel tortuosity • elevation of the optic nerve head • thickened peripapillary nerve fiber layer (pseudoedema)
Note: Approximately 20% of affected individuals show no fundal abnormalities in the acute stage. • Thinning of RNFL - macular RGC layer • Optic disc atrophy

Figure 4. Diagnostic algorithm of LHON - Adapted from [32, 49, 50]



Diagnostic algorithm of LHON - Adapted from [32, 49, 50]

DOA = dominant optic atrophy; LHON = Leber's hereditary optic neuropathy; MS = multiple sclerosis.

chronic phase, the diagnosis of LHON is more challenging as optic atrophy is a multivariate condition. In these cases, neuroimaging and molecular genetic testing are necessary to establish the diagnosis [17].

8. Management

In 2017 a consensus on the clinical characteristics and treatment of LHON has demonstrated, that during the dynamic phase, 6-12 months after onset, there are progressive RNFL changes, while there is stability regarding the RGC loss in the macula [32, 51]. Hence the consensus group concluded that the chances for visual recovery are probably better during the subacute phase in comparison with the dynamic phase [32].

The recommended investigations for patients with LHON in both diagnostic and follow up visits, include measurement of the best-corrected visual acuity, static or kinetic perimetry, assessment of color vision, contrast sensitivity, measurement of macular RGC layer and RNFL thickness with OCT, electrophysiology, ECG findings with or without cardiac symptoms, neurological screening for symptoms and signs, genetic consultation and in some cases neuroimaging and/or lumbar puncture. The recommended follow-up visits should be performed in 3-month intervals during the subacute and dynamic phases, then in 6-month intervals during the second year after disease onset, and then in annual intervals.

Regarding preventive measures, individuals with established LHON should avoid smoking and excessive alcohol consumption [52]. Although there is not sufficient evidence about the environmental risk factors, it is necessary to avoid industrial toxins, drug-induced mitochondrial toxicity and other aggravating visual loss factors [53]. Several agents are recommended: Vitamins and cofactors including vitamin B12, CoenzymeQ$_{10}$ (CoQ$_{10}$), riboflavin, creatine, folic acid and L-carnitine, electron acceptors such as vitamin C, free radical scavengers such as vitamin E, alpha lipoic acid, EPI-743 and curcumin, and inhibitors of toxic metabolites, such as dicholoroacetate. However, the efficacy of these interventions is unclear [54].

8.1. *Mitochondrial neuroprotection: idebenone*

The ubiquinone family, including Coenzyme Q$_{10}$, has shown protective effects in other inherited mitochondrial diseases, in which its deficiency causes encephalomyelopathy [55]. However, due to the inability of coenzyme Q$_{10}$ to cross the blood-brain barrier after oral ingestion, a beneficial effect of Coenzyme Q$_{10}$ in LHON has been reported in only a few cases [56].

In order to overcome this limitation, idebenone, a synthetic hydrosoluble analog of coenzyme Q$_{10}$, was introduced [57]. The first LHON patient treated with idebenone was a 10-year-old boy who received 90 mg of idebenone daily. Pre-treatment visual acuity was 6/90 on either eye and reached 6/6 in the right eye after 4 months and in the left eye after 7 months [58]. Further case reports and case series have shown promising results, and idebenone (Raxone®)

was approved for LHON in 2015 by the European Medicines Agency in adults and adolescents at 900 mg/day given as three equally divided doses [32, 59].

Raxone was instituted as treatment of choice in LHON through the RHODOS study (Rescue of Hereditary Optic Disease Outpatient Study) [60]. This study was a prospective, double-blind, randomized placebo-controlled trial which was completed recently. RHODOS randomized 85 patients with genetically confirmed LHON and visual loss <5 years to receive either idebenone 900 mg/day (300 mg three times daily) or placebo. Treatment duration was 24 weeks, the primary endpoint was the best recovery in visual acuity and the secondary endpoint was the change in best visual acuity. During the trial, no safety concerns were raised. The authors found no significant differences in best recovery in visual acuity between idebenone and placebo. However, there was a trend in favor of idebenone regarding change in best visual acuity and after exclusion of patients with the 14488 mutation, who have better chances for spontaneous recovery [60]. After two years, the treatment effect persisted in 60 of 85 patients from the first study [61]. Visual benefit in patients treated with idebenone was likely to be higher when treatment was initiated within the first year of disease onset [10, 62, 63]. The aim of the ongoing "Post-Authorisation Safety Study with Raxone® in LHON Patients" (PAROS) study (NCT02771379) is to assess the long-term safety of idebenone.

Current treatment algorithms recommend initiation of 900 mg/day idebenone as soon as possible in patients with subacute and dynamic disease (less than 1 year from symptom onset). Treatment with idebenone is introduced for at least 1 year aiming to a positive response, or until an improvement plateau is documented. A clinically relevant visual response to idebenone is the improvement of 2 lines of visual acuity and automated perimetry (mean deviation). If a clinically relevant improvement is observed, and a plateau has been reached, the treatment is continued for another year. Idebenone should be discontinued if no visual recovery is documented and is currently not offered in patients during the chronic stage of LHON [60].

8.2. *Medical neuroprotective treatments*

EPI-743 and MTP-131 (elamipretide) are neuroprotective and antioxidant drugs, which represent potential treatment candidates for LHON [62]. Four out of five LHON patients who started EPI-743 within the first 4 months after the onset of visual decline, demonstrated visual improvement [65]. MTP-131 is under investigation in 12 patients with LHON with disease duration between 1-10 years (https://clinicaltrials.gov/ct2/show/NCT02693119).

Cyclosporine A inhibits mitochondrial permeability transition, thus blocking apoptosis. Oral cyclosporine A has been investigated in five patients with subacute, unilateral LHON, but visual acuity in the first affected eye worsened, and second-eye involvement was not prevented [64].

Finally, brimonidine is an α-2 agonist, which reduces apoptosis and has a neuroprotective action on optic nerve injury. Brimonidine was used in nine patients with subacute LHON although it did not prevent involvement of the fellow eye [67].

8.3. *Oestrogens*

The fact that male sex is predominant in LHON could raise suspicions about a protective role of female sex hormones. Recent investigations indicate that estrogens reduce reactive oxygen species levels and simultaneously increase the efficiency of antioxidant enzyme superoxide dismutase. Consequently, mitochondrial oxidative phosphorylation becomes more effective [68, 69]. It remains to be seen if such intervention can be of use in LHON.

8.4. *Gene therapy*

Gene therapy is a novel therapeutic strategy in LHON, which involves intravitreal injection of a modified adeno-associated virus (AAV) vector and insertion of an unmutated MT-ND4 gene into the mitochondria of RGCs. The first effort was accomplished in 2002 by Guy et al. They transfected a synthetic ND4 subunit mutation, using an Adeno Associated Viral Vector [68]. The authors reported success in the restoration of complex I-dependent respiration, because three times as much ATP was produced by the transfected cells when compared to the mock-transfected cybrids. Further animal models using intravitreal AAV gene delivery of human ND4 have proven the safety of the injection technique and confirmed that the AAV expressed its genetic content inside the mitochondria [71-74].

The first phase 1 clinical trial in five legally blind patients with G11778A LHON showed no serious safety problems, with two of five showing significant improvement in visual acuity [75, 76]. In 2017, the RESCUE and REVERSE clinical trials (phase III), reported on patients who received a unilateral injection with GS010. GS010 is a recombinant, AAV, which contains a cDNA encoding the mitochondrial ND4 protein (rAAV2/2-ND4). This study reported a three-line increase in visual acuity (15 letters) and also showed that viral vector DNA was transferred via the optic pathways from the injected to the fellow eye [53, 77].

RESTORE is the longitudinal follow-up study of individuals who received treatment in the RESCUE and REVERSE studies. RESTORE showed that the

treatment effect of rAAV2/2-ND4 on visual acuity and vision-related quality of life reported 2 years post therapy in RESCUE and REVERSE was maintained at 3 years in the RESTORE [78].

8.5. *Mitochondrial replacement therapy*

MtDNA is transmitted maternally and so *in vitro* fertilization techniques could contribute in avoiding developing mtDNA pathogenic variants. Mitochondrial replacement with pronuclear transfer for clinical use was approved by the Human Fertilization and Embryology Authority in the United Kingdom since 2015 [79, 80]. Due to ethical and legal considerations, mitochondrial replacement therapy still remains controversial [80]. Eleven clinical trials on treatment agents in LHON have been already completed and 5 of then are active now (Table 4).

9. Genetic counseling

LHON is a maternally-inherited disease. Mothers of affected individuals have the mtDNA mutations, except for the rare occurrence of *de novo* mtDNA mutations. A female carrier of LHON-related mtDNA mutation passes the mutation to all of her children, while a male carrier of LHON-related mtDNA mutation cannot pass the mtDNA mutation to any of his children. However, genetic counseling is challenging because of the reduced penetrance characterizing the LHON-causing mtDNA pathogenic variants [81].

Similarly, prenatal investigations for heteroplasmic female LHON carriers is not very helpful, and the prenatal presence of mtDNA pathogenic variant for LHON cannot predict occurrence of disease, age of onset, or vision loss. The reason is that the mutant load in amniocytes and chorionic villi may not reflect the mutant load in other fetal cell populations, especially those programmed to mature into the RGCs [81].

10. Prognosis

The prognosis of LHON is related to the specific mutation [82, 83]. Individuals with the T14484C mutation have the higher chances of spontaneous visual recovery, which usually occurs 1-2 years after disease onset. The recovery in visual acuity is usually partial, but a few patients regain near-normal visual acuity in at least one eye, even years after the initial visual decline [82-84]. Visual field recovery is usually incomplete.

In general, permanent visual loss is usual and most people with LHON eventually qualify for registration as legally blind. Vision is typically worse than 20/200 OU, but light perception is usually preserved, and complete blindness is rare. In approximately 50% of male carriers and 90% of female carriers, blindness will not ensue during their lifetime. Finally, a younger age at onset and childhood-onset LHON have a more favorable prognosis for visual acuity [28]. A better visual acuity at the nadir and large optic disks have been also associated with higher rates of visual recovery and better visual outcome, due to less crowding of the RGC axons in the optic nerve [85, 86]. On the other hand, the presence of peripapillary telangiectasias and optic disk hyperemia have been considered as poor prognostic factors [86].

11. Conclusions

Although, the clinical and molecular diagnosis of LHON is unambiguous, management of patients with LHON remains largely supportive, including prescription of low vision aids, reconfiguration of the working environment and participation of the patient to the social services. LHON mainly affects the retinal ganglion cell layer with pronounced cell body and axonal degeneration, while sparing the photoreceptor layer. The targeted vulnerability of retinal ganglion cells layer still remains unexplained. In the future, it is hence necessary to understand the complex pathophysiology of LHON, in order to develop new therapeutic strategies. Research should focus on identifying individuals at higher risk for LHON. In the future, therapy in LHON mutation carriers may be also indicated in order to preventing disease onset.

References

[1] Wallace DC, Singh G, Lott MT, et al. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science.* 1988;242:1427-1430.

[2] Yu-Wai-Man P, Griffiths PG, Chinnery PF. Mitochondrial optic neuropathies-disease mechanisms and therapeutic strategies. *Prog Retin Eye Res.* 2011;30:81-114.

[3] Yu-Wai-Man P, Griffiths PG, Brown DT, et al. The epidemiology of Leber hereditary optic neuropathy in the North East of England. *Am J Hum Genet.* 2003;72:333-339.

[4] Yu-Wai-Man P. Therapeutic approaches to inherited optic neuropathies. *Semin Neurol*. 2015; 35:578-586.

[5] Meyerson C, Van Stavern G, McClelland C. Leber hereditary optic neuropathy: current perspectives. *Clin Ophthalmol.* 2015;9:1165-1176.

[6] Ueda K, Morizane Y, Shiraga F, et al. Nationwide epidemiological survey of Leber hereditary optic neuropathy in Japan. *J Epidemiol.* 2017;27:447-450.

[7] Shemesh A, Sood G, Margolin E. Leber Hereditary Optic. [Updated 2021 Jul 17]. In: Stat Pearls [Internet]. Treasure Island (FL): *Stat Pearls Publishing.* 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482499/.

Table 4. Active studies 2021/LHON

Study title	Goal study	Phase	Recruitment status	Study type	Actual enrollment	Allocation	Masking	Primary purpose	Actual study start Date:	Estimated study completion Date:	Locations
REFLECT Efficacy & Safety Study of Bilateral IVT Injection of GS010 in LHON Subjects Due to the ND4 Mutation for up to 1 Year	Asessment of the safety and efficacy of GS010, a gene therapy, in improving the retina functional & structural outcomes in subjects with LHON due to the G11778A ND4 mitochondrial mutation when vision loss duration is present up to one year.	3	Active, not recruiting	Interventional Genetic: GS010Drug: Placebo	90 participants	randomized	Double (Participant, Investiagtor)	treatment	March 12, 2018	June 30, 2024	USA and UK
A Single Intravitreal Injection of rAAV2-ND4 for the Treatment of Leber's Hereditary Optic Neuropathy	This study is meant to evaluate the safety and efficacy of rAAV2-ND4 treatment for Leber hereditary optic neuropathy with the G11778A mutation in mitochondrial DNA.	3, 2	Active, not recruiting	Interventional Drug: rAAV2-ND4	159 participants	N/A	None (Open Label)	treatment	December 27, 2017	January 15, 2025	China
An Open-label Dose Escalation Study of an Adeno-associated Virus Vector (scAAV2-P1ND4v2) for Gene Therapy of Leber's Hereditary Optic Neuropathy(LHON) Caused by the G11778A Mutation in Mitochondrial DNA	The primary hypothesis being tested is that there will be no toxicity resulting in loss of vision to no light perception in injected eyes.	1	Active, not recruiting	Interventional Drug: injection of scAAV2-P1ND4v2 1.18x10e9 vg (Low), Drug: injection of scAAV2-P1ND4v2 5.81 X10e9 vg (Med)	28 participants	Non-randomized	None (Open Label)	treatment	July 14, 2014	March 2023	USA

Table 4. Continuity

Study title	Goal study	Phase	Recruitment status	Study type	Actual enrollment	Allocation	Masking	Primary purpose	Actual study start Date:	Estimated study completion Date:	Locations
				Drug: injection of scAAV2-P1ND4v2 2.4 X10e10vg (High) Drug: injection of scAAV2-P1ND4v2 1.0 X10e11vg (Higher)							
Efficacy Study of Gene Therapy for The Treatment of Acute LHON Onset Within Three Months	Efficacy Study of Gene Therapy for The Treatment of Acute Leber's Hereditary Optic Neuropathy (LHON) onset within three months		Completed	Interventional Drug: rAAV2-ND4	120 participants	N/A	None (Open Label)	treatment	January 8, 2018	December 30, 2020	China
RESCUE/REVERSE Long Term follow-up	Assessment of long-term safety and efficacy of GS010, gene therapy, and quality of life in subjects with **LHON** due to the G11778A ND4 mitochondrial mutation and who were treated in the Rescue or Reverse studies	3	Active, not recruiting	Interventional Genetic:GS010 Drug: Placebo	61 participants	randomized	None (Open Label)	treatment	January 9, 2018	August 2022	USA, Europe

[8] Jurkute N, Harvey J, Yu-Wai-Man P. Treatment strategies for Leber hereditary optic neuropathy. *CurrOpin Neurol.* 2019;32:99-104.
[9] Lam BL, Feuer WJ, Abukhalil F, et al. Leber hereditary optic neuropathy gene therapy clinical trial recruitment: year 1. *Arch Ophthalmol.* 2010;128:1129-35.
[10] Klopstock T, Yu-Wai-Man P, Dimitriadis K, et al. A randomized placebo-controlled trial of idebenone in Leber's hereditary optic neuropathy. *Brain.* 2011;134:2677-86.
[11] Johns DR, Smith KH, Miller NR. Leber's hereditary optic neuropathy. Clinical manifestations of the 3460 mutation. *Arch Ophthalmol.* 1992b;110:1577-81.
[12] Harding AE, Sweeney MG, Govan GG, et al. Pedigree analysis in Leber hereditary optic neuropathy families with a pathogenic mtDNA mutation. *Am J Hum Genet.* 1995;57:77-86.
[13] Spruijt L, Kolbach DN, de Coo RF, et al. Influence of mutation type on clinical expression of Leber hereditary optic neuropathy. *Am J Ophthalmol.* 2006;141:676-82.
[14] Nikoskelainen EK. Clinical picture of LHON. *Clin Neurosci.* 1994;2:115-20.
[15] Newman NJ, Carelli V, Taiel M, et al. Visual outcomes in leber hereditary optic neuropathy patients with the m.11778G>A (MTND4) mitochondrial DNA mutation. *J Neuroophthalmol.* 2020;40:547-57.
[16] Johns DR, Heher KL, Miller NR, et al. Leber's hereditary optic neuropathy. Clinical manifestations of the 14484 mutation. *Arch Ophthalmol.* 1993;111:495-8.
[17] Yu-Wai-Man P, Chinnery PF. Leber Hereditary Optic Neuropathy. [Updated 2021 Mar 11]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. *GeneReviews®* [Internet]. Seattle (WA): University of Washington, Seattle;1993-2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1174.
[18] Kousal B, Kolarova H, Meliska M, et al. Peripapillary microcirculation in Leber hereditary optic neuropathy. *Acta Ophthalmol.* 2019;97:71-76.
[19] Ohden KL, Tang PH, Lilley CC, et al. Atypical Leber Hereditary Optic Neuropathy: 18 Year Interval Between Eyes. *J Neuroophthalmol.* 2016;36:304.
[20] Smith KH, Johns DR, Heher KL, et al. Heteroplasmy in Leber's hereditary optic neuropathy. *Arch Ophthalmol.* 1993;111:1486-90.
[21] Harding AE, Sweeney MG, Miller DH, et al. Occurrence of a multiple sclerosis-like illness in women who have a Leber's hereditary optic neuropathy mitochondrial DNA mutation. *Brain.* 1992;115:979-89.
[22] Pfeffer G, Burke A, Yu-Wai-Man P, et al. Clinical features of MS associated with Leber hereditary optic neuropathy mtDNA mutations. *Neurology.* 2013;81:2073-81.
[23] Danielson SR, Wong A, Carelli V, et al. Cells bearing mutations causing Leber's hereditary optic neuropathy are sensitized to Fas-Induced apoptosis. *J Biol Chem.* 2002;277:5810-5.
[24] Bahr T, Welburn K, Donnelly J, et al. Emerging model systems and treatment approaches for Leber's hereditary optic neuropathy: challenges and opportunities. *BiochimBiophys Acta Mol Basis Dis.* 2020;1866:165743.
[25] Hwang TJ, Karanjia R, Moraes-Filho MN, et al. Natural history of conversion of Leber's hereditary optic neuropathy: a prospective case series. *Ophthalmology* 2017;124:843-850.
[26] Macmillan C, Kirkham T, Fu K, et al. Pedigree analysis of French Canadian families with T14484C Leber's hereditary optic neuropathy. *Neurology.* 1998;50: 417-22.
[27] Yu-Wai-Man P, Votruba M, Moore AT, et al. Treatment strategies for inherited optic neuropathies: past, present and future. *Eye.* 2014;28:521-537.
[28] Majander A, Bowman R, Poulton J, et al. Childhood-onset Leber hereditary optic neuropathy. *Br J Ophthalmol.* 2017;101:1505-1509.
[29] Barboni P, Savini G, Valentino ML, et al. Leber's hereditary optic neuropathy with childhood onset. *Invest Ophthalmol Vis Sci.* 2006;47:5303-9.
[30] Thieme H, Wissinger B, Jandeck C, et al. A pedigree of Leber's hereditary optic neuropathy with visual loss in childhood, primarily in girls. *Graefes Arch Clin Exp Ophthalmol.* 1999;237:714-9.
[31] Vaphiades MS. Magnetic resonance findings in the pregeniculate visual pathways in Leber hereditary optic neuropathy. *J Neuroophthalmol.* 2011;31:194.
[32] Carelli V, Carbonelli M, de Coo IF, et al. International Consensus Statement on the clinical and therapeutic management of Leber hereditary optic neuropathy. *J Neuro-Ophthalmol.* 2017;37:371-381.
[33] Yu-Wai-Man P, Newman NJ, Carelli V, et al; LHON REALITY Study Group. Natural history of patients with Leber hereditary optic neuropathy-results from the REALITY study. *Eye* (Lond). 2021 doi: 10.1038/s41433-021-01535-9. Online ahead of print.
[34] Parisi V, Ziccardi L, Sadun F, et al. Functional Changes of Retinal Ganglion Cells and Visual Pathways in Patients with Chronic Leber's Hereditary Optic Neuropathy during One Year of Follow-up. *Ophthalmology.* 2019;126:1033-1044.
[35] Newman NJ, Biousse V, Newman SA, et al. Progression of visual field defects in leber hereditary optic neuropathy: experience of

the LHON treatment trial. *Am J Ophthalmol.* 2006;141:1061-1067.
[36] Barboni P., Carbonelli M., Savini G. et al. Natural history of Leber's hereditary optic neuropathy: longitudinal analysis of the retinal nerve fiber layer by optical coherence tomography. *Ophthalmology.* 2010;117:623-627.
[37] Huoponen K. Leber hereditary optic neuropathy: clinical and molecular genetic findings. *Neurogenetics.* 2001;3:119-25.
[38] Liu HL, Yuan JJ, Tian Z, et al. What are the characteristics and progression of visual field defects in patients with Leber hereditary optic neuropathy: a prospective single-center study in China. *BMJ* Open. 2019;9:e025307.
[39] Yuan J, Zhang Y, Liu H, et al. Clinical Observation of Patients with Leber's Hereditary Optic Neuropathy Before Gene Therapy. *Curr Gene Ther.* 2018;18:386-392.
[40] Chun BY, Kim DW, Son BJ. A Case of Atypical Leber Hereditary Optic Neuropathy With Slow, Symmetrical Progression Over Three Years. *J Neuroophthalmo.* 2018;38:421-422.
[41] Balducci N, Savini G, Cascavilla ML, et al. Macular nerve fibre and ganglion cell layer changes in acute Leber's hereditary optic neuropathy. *Br J Ophthalmol.* 2016;100:1232-7.
[42] Barboni P, Savini G, Valentino ML, et al. Retinal nerve fiber layer evaluation by optical coherence tomography in Leber's hereditary optic neuropathy. *Ophthalmology.* 2005;112:120-6.
[43] Ziccardi L, Parisi V, Giannini D, et al. Multifocal VEP provide electrophysiological evidence of predominant dysfunction of the optic nerve fibers derived from the central retina in Leber's hereditary optic neuropathy. *Graefes Arch Clin Exp Ophthalmol.* 2015;253:1591-600.
[44] Newman NJ. Treatment of Leber hereditary optic neuropathy. *Brain.* 2011;134:2447-50.
[45] Blanc C, Heran F, Habas C, et al. MRI of the Optic Nerves and Chiasm in Patients With Leber Hereditary Optic Neuropathy. *J Neuroophthalmol.* 2018;38:434-437.
[46] Küker W, Weir A, Quaghebeur G, et al. White matter changes in Leber's hereditary optic neuropathy: MRI findings. *Eur J Neurol.* 2007;14:591-3.
[47] Phillips PH, Vaphiades M, Glasier CM, et al. Chiasmal enlargement and optic nerve enhancement on magnetic resonance imaging in Leber hereditary optic neuropathy. *Arch Ophthalmol.* 2003;121:577-9.
[48] Zhao J, Zhang Q, Wang J. Magnetic Resonance Imaging Findings in the Pregeniculate Visual Pathway in Leber Hereditary Optic Neuropathy. *J Neuroophthalmol.* 2021 doi: 10.1097/WNO.0000000000001383.
[49] Carelli V, Hawlina M, Klopstock T, et al., Leber's Hereditary Optic Neuropathy - A Global Perspective, Published Online: August 8th 2019 *European Ophthalmic Review.* 2019;13(Suppl 2).
[50] Sadun AA, La Morgia C, Carelli V. Leber's hereditary optic neuropathy: new quinone therapies change the paradigm. *Expert Rev Ophthalmol.* 2012;7:251-9.
[51] Balducci N, Savini G, Cascavilla ML, et al. Macular nerve fibre and ganglion cell layer changes in acute Leber's hereditary optic neuropathy. *Br J Ophthalmol.* 2016;100:1232-1237.
[52] Kirkman MA, Yu-Wai-Man P, Korsten A, et al. Gene-environment interactions in Leber hereditary optic neuropathy. *Brain.* 2009b;132:2317-26.
[53] Yu-Wai-Man P, Newman NJ, Carelli V, et al. Bilateral visual improvement with unilateral gene therapy injection for Leber hereditary optic neuropathy. *Sci Transl Med.* 2020;12:eaaz7423.
[54] Ghelli A, Porcelli AM, Zanna C, et al. Protection against oxidant-induced apoptosis by exogenous glutathione in Leber hereditary optic neuropathy cybrids. I*nvest Ophthalmol Vis Sci.* 2008; 49:671-6.
[55] Hargreaves IP. Coenzyme Q10 as a therapy for mitochondrial disease. *Int J Biochem Cell Biol.* 2014;49:105-11.
[56] Huang CC, Kuo HC, Chu CC, et al. Rapid visual recovery after coenzyme q10 treatment of leber hereditary optic neuropathy. *J Neuroophthalmol.* 2002;22:66.
[57] Heitz FD, Erb M, Anklin C, et al. Idebenone protects against retinal damage and loss of vision in a mouse model of Leber's hereditary optic neuropathy. *PLoS One.* 2012;7:e45182.
[58] Mashima Y, Hiida Y, Oguchi Y. Remission of Leber's hereditary optic neuropathy with idebenone. *Lancet.* 1992; 340:368-9.
[59] Mashima Y, Kigasawa K, Wakakura M, et al. Do idebenone and vitamin therapy shorten the time to achieve visual recovery in Leber hereditary optic neuropathy? *J Neuroophthalmol.* 2000;20:166-70.
[60] Klopstock T, Yu-Wai-Man P, Dimitriadis K, et al. A randomized placebo-controlled trial of idebenone in Leber's hereditary optic neuropathy. *Brain.* 2011;134: 2677-86.
[61] Klopstock T, Metz G, Yu-Wai-Man P, et al. Persistence of the treatment effect of idebenone in Leber's hereditary optic neuropathy. *Brain.* 2013;136:e230.
[62] Carelli V, La Morgia C, Valentino ML, et al. Idebenone treatment in Leber's hereditary optic neuropathy. *Brain.* 2011;134:e188.
[63] Catarino CB, von Livonius B, Priglinger C, et al. Real-world clinical experience with idebenone

in the treatment of leber hereditary optic neuropathy. *J Neuroophthalmol.* 2020;40:558-65.
[64] Karaa A, Haas R, Goldstein A, et al. Randomized dose-escalation trial of elamipretide in adults with primary mitochondrial myopathy. *Neurology.* 2018;90:1212-1221.
[65] Sadun AA, Chicani CF, Ross-Cisneros FN, et al. Effect of EPI-743 on the clinical course of the mitochondrial disease Leber hereditary optic neuropathy. *Arch Neurol.* 2012;69:331-338.
[66] Leruez S, Verny C, Bonneau D, et al. Cyclosporine A does not prevent second-eye involvement in Leber's hereditary optic neuropathy. *Orphanet J Rare Dis.* 2018;13:33.
[67] Newman NJ, Biousse V, David R, et al. Prophylaxis for second eye involvement in leber hereditary optic neuropathy: an open-labeled, nonrandomized multicenter trial of topical brimonidine purite. *Am J Ophthalmol.* 2005;140:407-15.
[68] Giordano C, Montopoli M, Perli E, et al. Oestrogens ameliorate mitochondrial dysfunction in Leber's hereditary optic neuropathy. *Brain.* 2011;134:220-34.
[69] Pisano A, Preziuso C, Iommarini L, et al. Targeting estrogen receptor beta as preventive therapeutic strategy for Leber's hereditary optic neuropathy. *Hum Mol Genet.* 2015;24:6921-31.
[70] Guy J, Qi X, Pallotti F, et al. Rescue of a mitochondrial deficiency causing Leber Hereditary Optic Neuropathy. *Ann Neurol.* 2002;52:534-42.
[71] Ellouze S, Augustin S, Bouaita A, et al. Optimized allotopic expression of the human mitochondrial ND4 prevents blindness in a rat model of mitochondrial dysfunction. *Am J Hum Genet.* 2008;83:373-87.
[72] Seo BB, Thomas BB, Matsuno-Yagi A, et al. Successful amelioration of mitochondrial optic neuropathy using the yeast NDI1 gene in a rat animal model. *PLoS ONE.* 2010;5:e11472.
[73] Yu H, Koilkonda RD, Chou T-H, et al. Gene delivery to mitochondria by targeting modified adenoassociated virus suppresses Leber's hereditary optic neuropathy in a mouse model. *ProcNatlAcadSci U S A.* 2012;109:1238-47.
[74] Koilkonda RD, Yu H, Chou T-H, et al. Safety and effects of the vector for the Leber hereditary optic neuropathy gene therapy clinical trial. *JAMA Ophthalmol.* 2014;132:409-20.
[75] Feuer WJ, Schiffman JC, Davis JL, et al. Gene Therapy for Leber Hereditary Optic Neuropathy: Initial Results. *Ophthalmology.* 2016;123:558-70.
[76] Guy J, Feuer WJ, Davis JL, Porciatti V, et al.Gene Therapy for Leber Hereditary Optic Neuropathy: Low- and Medium-Dose Visual Results. *Ophthalmology.* 2017;124:1621-1634.
[77] Moster ML, Sergott RC, Newman NJ, et al; LHON study group. Cross-Sectional Analysis of Baseline Visual Parameters in Subjects Recruited Into the RESCUE and REVERSE ND4-LHON Gene Therapy Studies. *J Neuroophthalmol.* 2021;41:298-308.
[78] Biousse V, Newman NJ, Yu-Wai-Man P, et al; LHON Study Group. Long-Term Follow-Up After Unilateral Intravitreal Gene Therapy for Leber Hereditary Optic Neuropathy: The RESTORE Study. *J Neuroophthalmol.* 2021;41:309-315.
[79] Craven L, Murphy JL, Turnbull DM. Mitochondrial donation - hope for families with mitochondrial DNA disease. *Emerg Top Life Sci.* 2020;4:151-4.
[80] Newson AJ,Wilkinson S,Wrigley A. Ethical and legal issues in mitochondrial transfer. *EMBO Mol Med.* 2016;8:589-591.
[81] Yu-Wai-Man P, Votruba M, Moore AT, et al. Treatment strategies for inherited optic neuropathies: past, present and future. *Eye* (Lond). 2014;28:521-37.
[82] Stone EM, Newman NJ, Miller NR, et al. Visual recovery in patients with Leber's hereditary optic neuropathy and the 11778 mutation. *J Clin Neuroophthalmol.* 1992;12:10-4.
[83] Yamada K, Mashima Y, Kigasawa K, et al. High incidence of visual recovery among four Japanese patients with Leber's hereditary optic neuropathy with the 14484 mutation. *J Neuroophthalmol.* 1997;17:103-7.
[84] Lam BL, Feuer WJ, Schiffman JC, et al. Trial end points and natural history in patients with G11778A Leber hereditary optic neuropathy: preparation for gene therapy clinical trial. *JAMA Ophthalmol.* 2014;132:428-36.
[85] Ramos Cdo V, Bellusci C, Savini G, et al. Association of optic disc size with development and prognosis of Leber's hereditary optic neuropathy. *Invest Ophthalmol Vis Sci.* 2009;50:1666-74.
[86] Moon Y, Kim US, Han J, et al. Clinical and Optic Disc Characteristics of Patients Showing Visual Recovery in Leber Hereditary Optic Neuropathy. *J Neuroophthalmol.* 2020;40:15-21.

TUBEROUS SCLEROSIS COMPLEX: CLINICAL CHARACTERISTICS, DIAGNOSIS AND MANAGEMENT

Maria Akrioti[1], Paraskevi Keramida[2], Antigone Papavasiliou[1]

[1] Department of Pediatric Neurology, Iaso Children's Hospital
[2] Department of Pediatrics, Penteli Children's Hospital

Abstract

Tuberous Sclerosis Complex (TSC) is a rare genetic disorder caused by germline mutations in TSC1 and TSC2 genes. Loss of function genetic alterations in TSC1 and TSC2 lead to hyperactivation of the downstream mammalian target of rapamycin pathway (mTOR), which represents an important cellular circuit in the regulation of cell proliferation and survival. From a phenotypic standpoint, TSC is characterized by the development of benign hamartomatous tumors in different parts of the body, and thus a diverse clinical picture for each affected individual. The most frequently involved organ systems include the brain, the skin, the kidneys, the heart, the eyes, and the lungs. Central nervous system involvement manifests with a combination of symptoms such as seizures, impaired intellectual development, autism and behavioral problems. Accurate diagnosis is essential in implementing appropriate surveillance and treatment in patients with this disorder. The treatment is supportive and symptomatic, and requires the expertise of multiple disciplines. New treatment approaches and novel drugs, such as mTOR inhibitors, have been introduced in order to manage specific manifestations and have resulted in better outcomes and improvement of the patients' quality of life. In this review, we summarize the current data on the clinical characteristics, diagnosis and management of TSC from a neurologic perspective.

Key words: tuberous sclerosis, TSC, antiepileptic drugs, mToR inhibitors

1. INTRODUCTION

Tuberous sclerosis complex (TSC) is a genetic disease with autosomal dominant inheritance, marked by the presence of benign tumors, called "hamartomas", in various organs [1]. It is manifested simultaneously in many organs, with a special preference for the heart, the skin, the nervous, renal and pulmonary systems [2]. It affects 1 out of 6.000 to 10.000 individuals, without discrimination for gender or ethnicity [3, 4]. It represents the second most common neurocutaneous syndrome, after neurofibromatosis [5].

2. ETIOPATHOGENESIS

Molecular genetic studies [6-10] have demonstrated the involvement of two highly penetrating genes, the TSC1 gene in 9q34 chromosome and the TSC2 gene in 16p13 chromosome, coding the proteins hamartin and tuberin respectively. These proteins create a complex, responsible for cellular proliferation and proteinosynthesis, which suppresses the mammalian target of rapamycin (mTOR) pathway. More specifically, hamartin and tuberin in connection with TBC1D7, create the TSC protein complex, which uses RhebGTPase to control the function of the mTORC1. Tumor cells in tuberous sclerosis demonstrate hyperactivation of the mTORC1 signaling network. Therefore, mutations in the TSC1 and TSC2 genes result in the production of defective proteins, leading to uncontrolled cellular growth and tumor formation [11]. It should be noted that, over half of the cases are sporadic and there is no family history. This mainly concerns the TSC2 gene, the impairment of which usually leads to more serious clinical manifestations [12].

3. CLINICAL CHARACTERISTICS

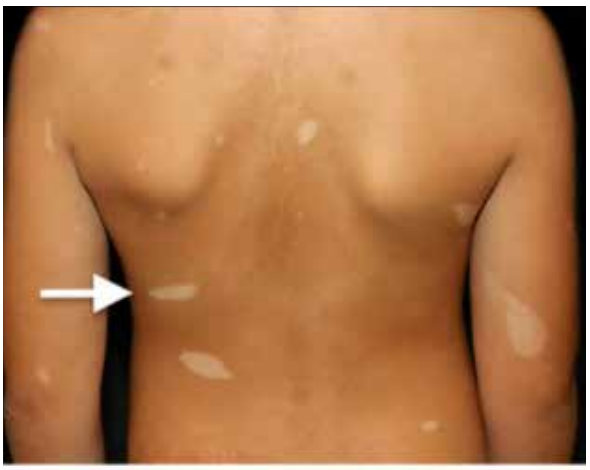
TSC is highly heterogeneous with a wide phenotypic spectrum, ranging from presentations with severe mental retardation and seizures, to affected individuals with normal intelligence and absence of epilepsy, even within the same family [12]. The most affected parts of the body are the brain and the skin with patients presenting with epileptic seizures and skin manifestations that lead patients to seek medical assistance. The most dangerous complications originate in the nervous and the renal systems and can cause death if not treated promptly [1].

3.1. Dermatologic manifestations

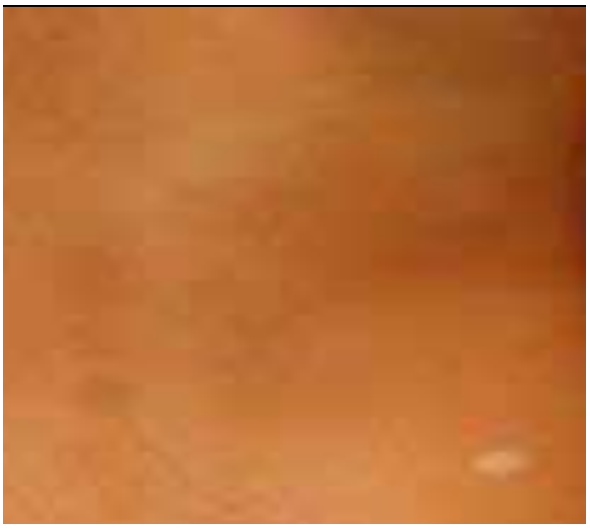
Hypomelanotic macules are found in about 90% of TSC patients [1]. Their presence consists a diagnostic criterion, if more than 3 lesions exceeding 5 mm in

Dermatologic manifestations in patients with TSC

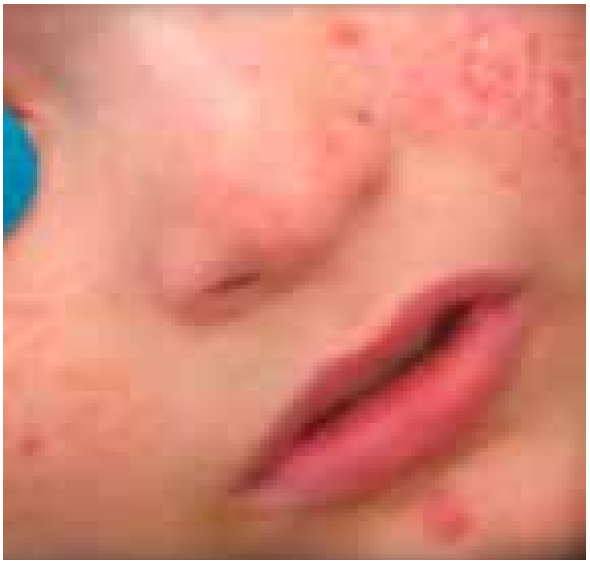
Picture 1. Hypomelanotic macules



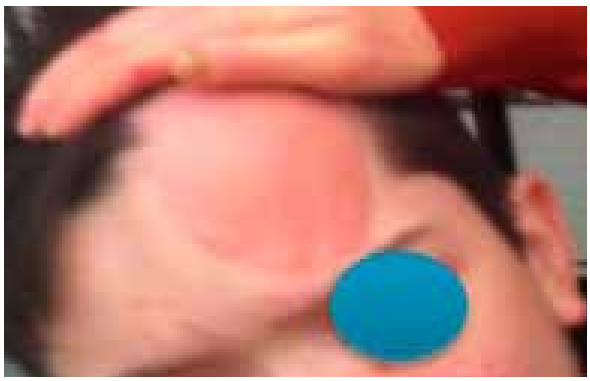
Picture 2. Hypomelanotic macules



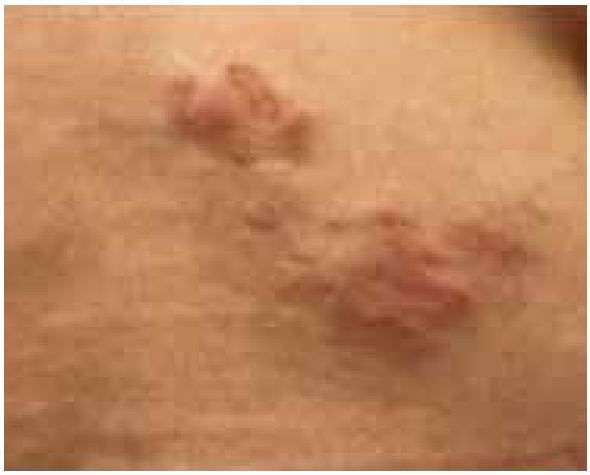
Picture 3. Facial angiofibromas



Picture 4. Fibrous cephalic plaque



Picture 5. Shagreen patch

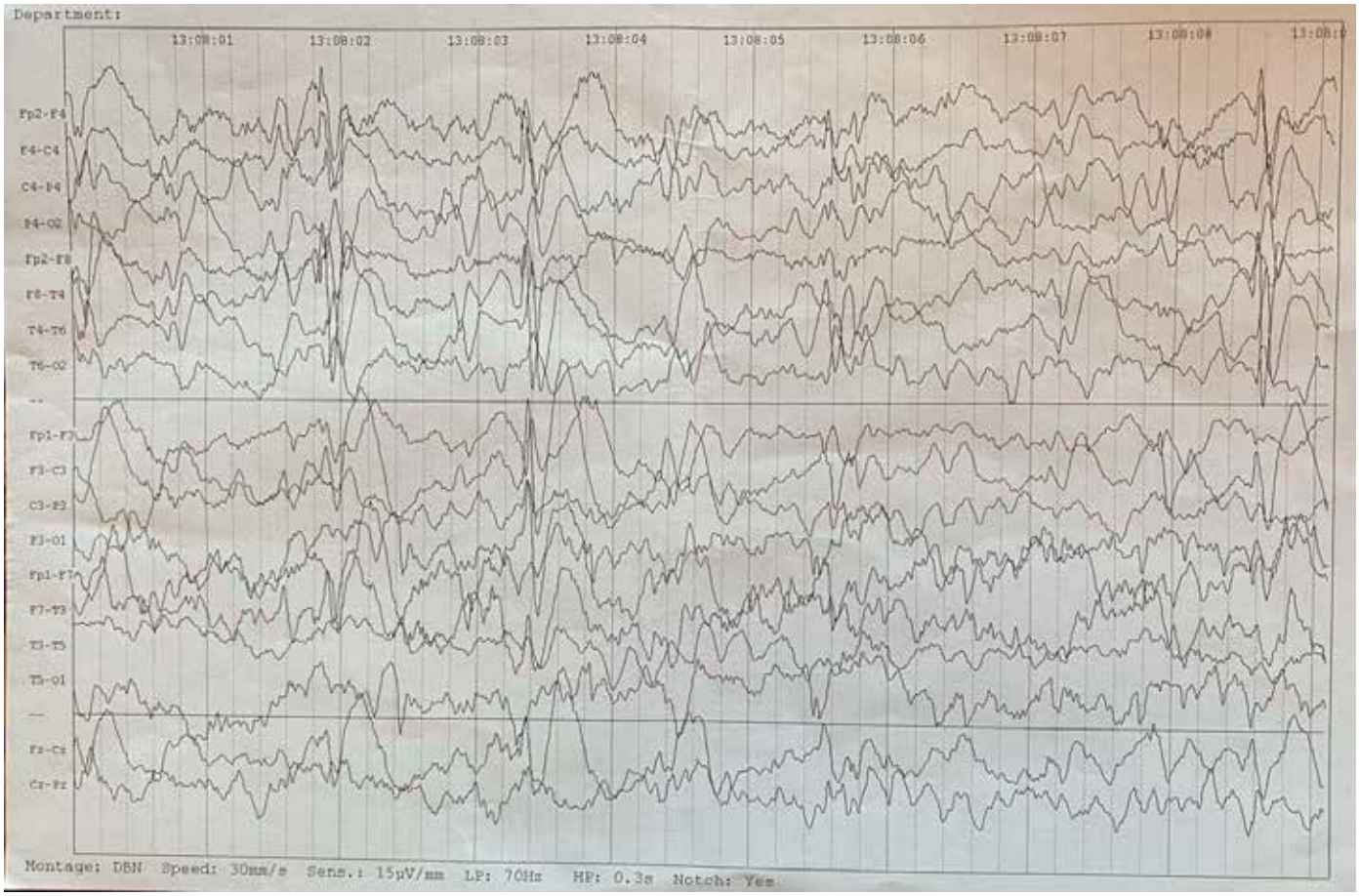


diameter are present [4]. Their shape resembles a leaf, so they are also called "ash-leaf macules". These hypomelanotic macules appear early in life, sometimes since birth, facilitating the diagnosis (Pictures 1 and 2). Over time, they usually recede and smaller lesions, "confetti-like", take their place [1]. Hypopigmented lesions, also include hypomelanotic patches of hair (poliosis) [12].

Facial angiofibromas (Picture 3) are common among young patients, around 4 years of age, in 83 to 90% of cases [1]. They look like swollen fibrous lesions, localized above blood vessels, which gives them an almost violet color. Angiofibromas are usually based on the nose and nasolabial folds, common areas of acne, from which they should be distinguished [1, 12].

An uncommon, but very specific for TSC, skin finding is the fibrous cephalic plaque (Picture 4), observed in the forehead of approximately 25% of individuals [1, 12]. In addition, clinical examination in about half of the patients with TSC reveals shagreen patches in the lumbar area (Picture 5), which are specific skin lesions with an orange peel surface [1, 12].

Picture 6. Stage 2, non-REM Sleep EEG of a 27 month-old child with TSC, post-recovery from infantile spasms. Bilateral synchronous epileptiform discharges with right hemisphere predominance



Ungual fibromas are lesions of the nails that appear later, in adulthood, at a rate of 80% [12]. They are mainly observed in women and in the toes [1].

Lesions of the oral cavity, include dental enamel pits and intraoral fibromas. Dental enamel pits can also observed in the general population, therefore they are not specific to the disease. Intraoral fibromas may be detected at the anterior gingival, oral and lips mucosa at 20 to 50% of adults with TSC [1, 12].

3.2. Neurological manifestations

The involvement of the central nervous system (CNS) is a key feature of TSC, with the typical triad of epilepsy, intellectual disability and autism spectrum disorders (ASD) [12]. More specifically, epilepsy is present in 70 to 90% of TSC cases, usually in children under the age of three [1].In about 50% of cases, it appears in infants as infantile spasms [2], described as tonic or clonic flexion or extension movements of neck, torso and limbs, with the characteristic hypsarrhythimic pattern in electroencephalographic investigation (EEG) [13]. However, every type of seizure can be part of TSC and as mentioned above with a variety of epileptiform abnormalities on EEG (Picture 6). In addition, there are cases with no neurological involvement [12]. Epilepsy indicates TSC in 10-25% of children [2].

De Vries and colleagues introduced the term "TSC-associated neuropsychiatric disorders (TAND)" in order to describe the diverse neuropsychiatric manifestations of TSC individuals [14, 15]. They also created the TAND Checklist (Table 1) in order to help the clinician detect the respective symptoms [14]. TAND involves ASD and other behavioral impairments such as aggressiveness, anxiety disorders, sleep difficulties and attention deficit hyperactivity disorder in about 40 to 50% of individuals [1]. Most TSC patients with ASD also face cognitive and learning difficulties in about 75% of cases [1]. Mental retardation is present in almost half of TSC individuals, in varying degrees of severity [12].

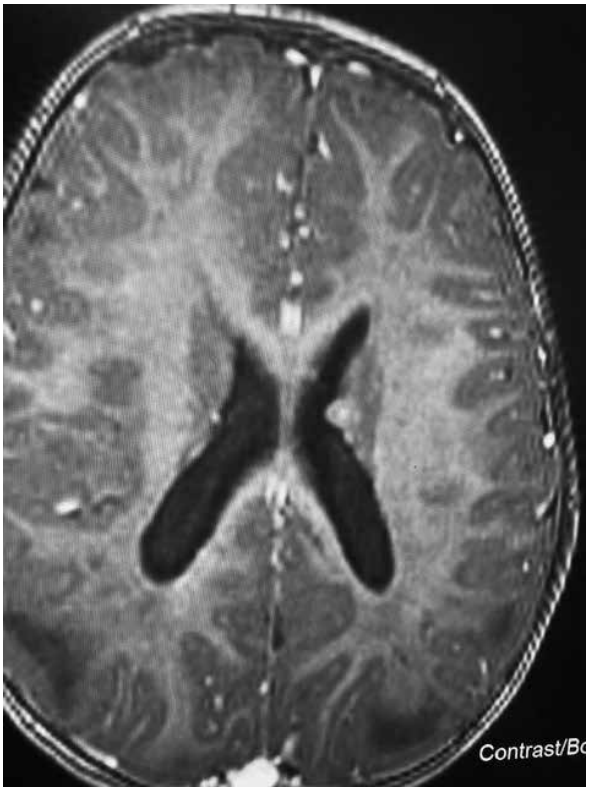
In addition, imaging methods have revealed structural abnormalities of the brain of TSC patients, including subependymal nodules (Picture 7), cortical tubers (Picture 7, 8, 9, 10) and subependymal giant cell astrocytomas (SEGAs) [1, 2, 12]. Cortical tubers are the typical imaging finding of TSC, easily de-

Table 1. TAND Checklist plan

Section	Field of study
1	Age of developmental landmarks
2	Present level of daily functionality
3	Worrying way of behaving
4	Ascertained mental health problems
5	Mental capability
6	School performance
7	Executive functions
8	Interpersonal relationships and level of self-complacence
9	Parent, carer or patient's assessment of the effect of TAND
10	Precedencies
11	Further worries
12	Doctor's/interviewer's assessment of the effect of TAND

Adapted from de Vries et al., 2015

Picture 7. 18 months of age: Subependymal nodules and cortical tubers on gadolinium-enhanced T1-weighted MR

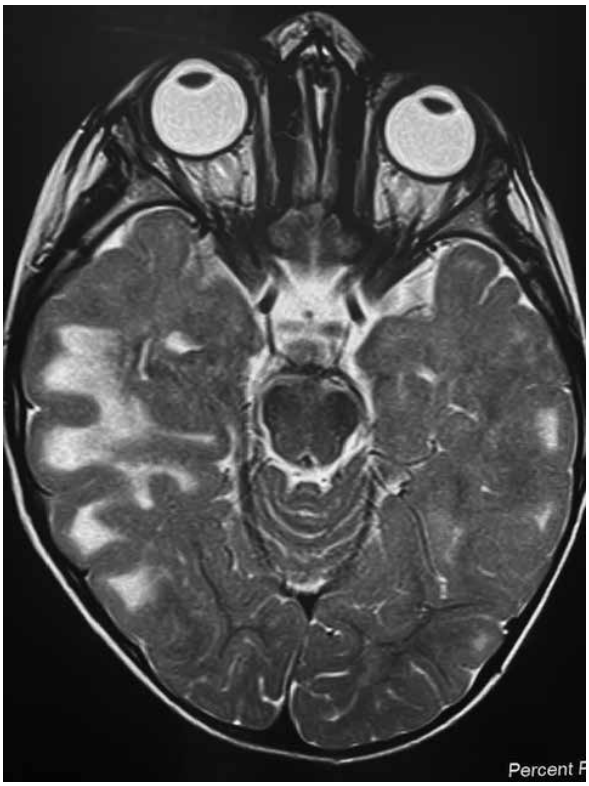


tected on magnetic resonance imaging (MRI) [12]. Approximately 80% of TSC individuals have cortical tubers in brain MRI, which do not increase in size over the years [2]. Cortical tubers are associated with the onset and severity of epilepsy [2, 12]. Subependymal nodules, calcified or not, are apparent in 80 to 90% of patients' brain MRIs, located in the wall of the lateral ventricles [1, 2, 12]. When there are more than one intraventricular subependymal nodules next to each other, the impression of a melting candle is given, known as the ''candle guttering sign'' [16]. They are often asymptomatic, but at a rate of 5 to 15% they evolve into benign SEGAs, which increase in size over time and may occlude the drainage system of the ventricles, leading to obstructive hydrocephalus [1, 12]. As a result, patients present with acute symptoms such as headache, focal neurologic signs, behavioral and mental changes and uncontrolled seizures [2, 12]. The characteristics and number of brain lesions are related to the severity of epilepsy and to the presence and severity of neuropsychiatric symptoms, and eventually determine the patient's neurologic condition [12, 15].

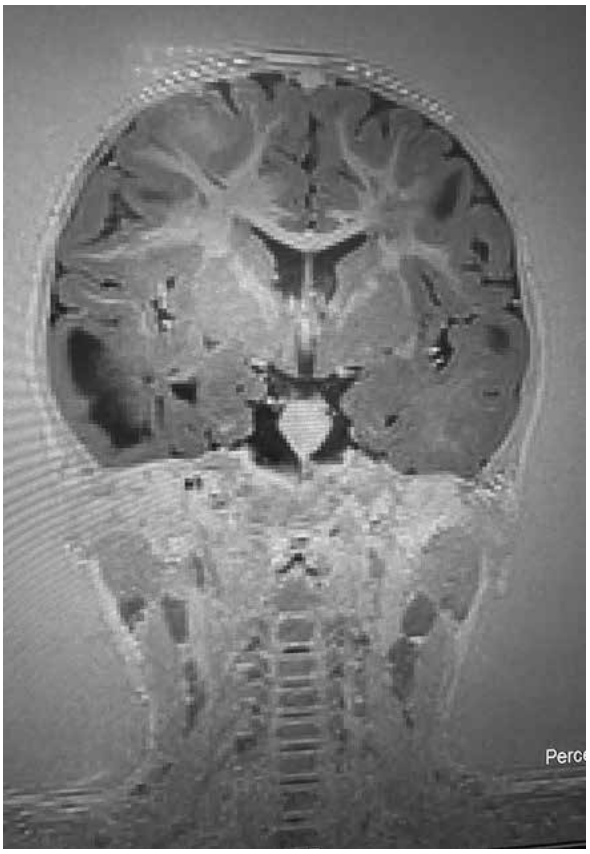
3.3. Renal manifestations

Renal involvement is mainly manifested as renal angiomyolipomas (AMLs) in 80% of TSC patients [1, 2]. In childhood and puberty, these benign tumors gradually increase in size, though they are usually asymptomatic [1]. Typically there are multiple AMLs affecting both kidneys [12]. In adults, AMLs can cause symptoms when they exceed 4 cm in diameter and can lead to death [12]. Hematuria, tumor hemorrhage, arterial hypertension and kidney failure are some of the most serious complications they can cause [1, 2, 12].

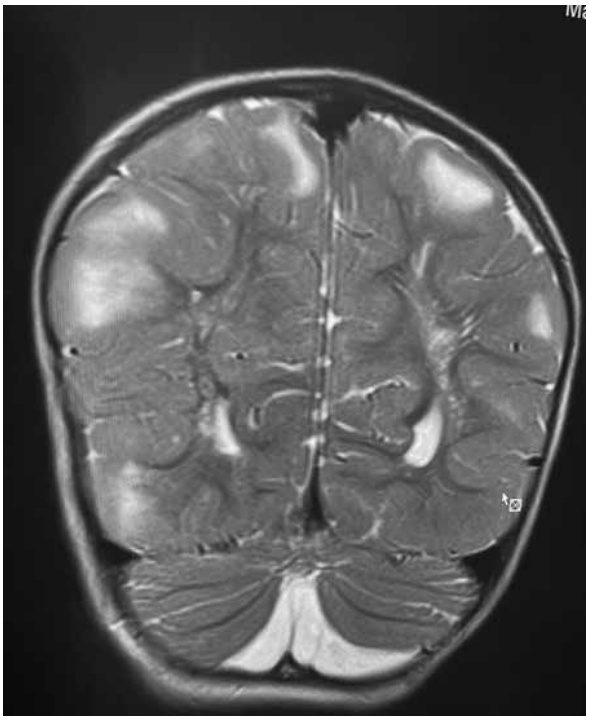
Picture 8. 18 months of age: Cortical tubers on T2-weighted MR sequence



Picture 9. 18 months of age: Cortical tubers on T2-weighted Inversion Recovery MR sequence



Picture 10. 18 months of age: Cortical tubers and white matter lesions on MR T2-weighted MR sequence



Frequently enough, AMLs are combined with renal cysts in about 45% of patients [2]. In a small portion of TSC individuals, approximately 1-2% of cases, TSC and adult polycystic kidney disease coexist, probably owing to the vicinity of the TSC2 gene and the responsible for polycystic disease gene (PKD1 gene) [12, 17].

Rarely, renal malignancy is observed in 1-2 % of TSC cases [1]. When it is not clear whether it is a benign or malignant tumor, a biopsy is necessary in order to confirm the behavior of the tumor [1].

3.4. Cardiac manifestations

Cardiac rhabdomyomas are present during ultrasound examination of the fetus in half of the cases [2, 12]. These benign tumors can be detected from the 20th week of pregnancy and there may be more than one, usually three, located in the cardiac ventricle wall [1]. Ordinarily, rhabdomyomas regress by the age of three or earlier, and they are asymptomatic [1, 2]. Depending on their dimensions, multitude and position, these tumors can cause symptoms such as heart failure, cardiac enlargement, murmurs, arrhythmias (most commonly Wolff-Parkinson-White syndrome), and even death [1, 12]. In children, enlargement of rhabdomyomas and heart block has been reported

after corticotropin and carbamazepine treatment for seizures [18, 19].

3.5. Pulmonary manifestations

Lymphangioleiomyomatosis (LAM) is the substitution of alveoli by cysts and multiplication of smooth muscle cells [1]. LAM appears almost exclusively in adult women, in approximately 40% of patients with TSC [1, 2] and remains asymptomatic before the age of 40 [2, 12]. The most frequent pulmonary symptoms involve cough, dyspnea, hemoptysis and pneumothorax [1, 2, 12]. Another pulmonary manifestation is the multifocal micronodular pneumonocyte hyperplasia (MMPH) in 60% of TSC individuals [2]. This typically affects premenopausal women and is asymptomatic when it does not coexist with LAM. It is detected on chest CT as ground-glass nodules with a maximum diameter of 10 mm [20].

3.6. Ophthalmologic manifestations

Ophthalmologic manifestations in TSC manifest as retinal astrocytic hamartomas (30-50%) [1] and retinal hypomelanotic macules (12%) [12]. Hamartomas of the retina are present during the first years of life and they rarely harm the patient's vision [1]. More than one retinal lesion can be detected in both eyes [12] and involvement of the retina usually implies impairment in the TSC2 gene [21].

3.7. Other manifestations

AMLs can be identified by MRI in different organs of the gastrointestinal and endocrine systems in about 25% of TSC individuals [2]. It is the liver of female patients which is usually affected [1].

4. DIAGNOSIS

The diagnosis of TSC is clinical and relies on a thorough examination of the patient, looking for the characteristic findings of the disease. Northrup and colleagues presented the updated 2012 international Tuberous Sclerosis Complex Diagnostic criteria (Table 2) [4]. Clinical criteria are separated into major and minor. Definite diagnosis is the result of the presence of two major criteria or one major and at least two minor criteria. Probable TSC diagnosis results from the presence of one major or at least two minor criteria [4]. Of note, although LAM and AML are included in the major criteria, they cannot confirm the diagnosis of TSC on their own, because TSC2 gene mutations, unrelated to TSC disease have been reported, which lead to LAM and AML co-existence [12]. A genetic test seeking the deactivating mutations in TSC1 and TSC2 genes can confirm the definite diagnosis. However, in 10-25% of cases, genetic tests are unable to detect the responsible mutations in patients who present with clinical manifestations of TSC. This does not exclude the TSC diagnosis when clinical suspicion is strong, because of the possibility of somatic mosaicism [4, 12]. Notably, patients with TSC1 or TSC2 mosaicism often have mild clinical features [12].

Clinical suspicion of TSC should be raised in any case of infantile spasms or other type of seizures and ASD. It should be supplemented by a thorough physical examination of all body systems (Table 3) [22]. The careful clinical evaluation helps to find the typical skin, dental and retinal lesions. Skin examination under the black light of Wood's lamp helps to detect the hypopigmented macules, scattered all over the body [2]. Fundoscopy can reveal retinal alterations connected with TSC [1, 22]. Moreover, imaging tests can identify the characteristic brain, kidney and heart lesions. Cerebral MRI has a high sensitivity in detecting the brain lesions related to TSC [12]. Subependymal nodules, especially the non-calcified ones, are obvious on T2-MRI, as high-signal areas. Calcified nodules can be detected either on CT as high-density areas or MRI. Cortical abnormalities are low-density regions on CT and can be better illustrated with MRI. SEGAs are the evolution of subependymal nodules, explaining why they are intraventricular and often calcified, better visualized on contrast- enhancement CT or MRI [12]. EEG is necessary to detect subclinical epilepsy and describe the type of seizures [1, 22]. TAND checklist detects the presence of neuropsychiatric symptoms as well [22]. Concerning heart involvement, rhabdomyomas can be detected with echocardiogram (ECHO) and the arrhythmias and conduction impairments that may ensue, with electrocardiogram (EKG). ECHO should be carried out in all patients under three years old and in the fetus, if there is suspicion of rhabdomyoma prenatally [22]. Imaging of the abdomen by ultrasound, CT or preferably MRI constitutes a diagnostic key for renal AML and renal cysts [1, 22]. Renal function can be estimated by measurement of blood pressure (BP) and glomerular filtration rate (GFR) [2, 22]. Pulmonary function should be checked in all adult women and only in symptomatic adult men via suitable pulmonary function tests and high-resolution chest computed tomography (HRCT) for the search of LAM [22]. Three-generation family history can investigate other possible TSC cases in the same family and is necessary for genetic counseling and confirmation of possible but no clinically proven cases [22].

5. MANAGEMENT

The management of patients with TSC requires a multidisciplinary approach due to the diverse nature

Table 2. Genetic and clinical diagnostic criteria of TSC

GENETIC DIAGNOSTIC CRITERIA
The detection of a pathogenic mutation in responsible TSC genes, either TSC1 or TSC2 gene is capable of making a definite diagnosis of TSC. Defined as a pathogenic mutation is a mutation that clearly impairs the function of the TSC1 or TSC2 proteins (e.g., out-of-frame indel or nonsense mutation), prevents protein synthesis (e.g., large genomic deletion), or is a missense mutation whose effect on protein function has been established. Other TSC1 or TSC2 variants with uncertain result on protein function are incapable of making a definite diagnosis of TSC. In 10-25% of TSC patients no mutation can be identified by genetic tests, so a normal result does not exclude TSC or recant the clinical diagnostic criteria to diagnose TSC.
CLINICAL DIAGNOSTIC CRITERIA
Major criteria
1. Hypomelanotic macules (≥3, ≥5mm in diameter)
2. Angiofibromas (≥3) or fibrous cephalic plaque
3. Ungual fibromas (≥2)
4. Shagreen patch
5. Multiple retinal hamartomas
6. Cortical dysplasia
7. Subependimal nobules
8. Subependymal giant cell astrocytoma
9. Cardiac rhabdomyoma
10. Lymphangioleiomyomatosis (LAM)
11. Angiomyolipomas (AML) (≥2)
Minor criteria
1."Confetti" skin lesions
2. Dental enamel pits (>3)
3. Intraoral fibroma (≥2)
4. Retinal achromic patch
5. Multiple renal cysts
6. Nonrenal hamartomas

Adapted from Nurthrup & Krueger, 2013.

of the phenotypic and clinical manifestations. Thus, the management addresses the intracranial and the extracranial manifestations of TSC.

5.1. Intracranial manifestations of TSC

The management of intracranial manifestations of TSC includes epilepsy treatment and the management of secondary CNS tumor development. Treatment can be symptomatic or it may address the inhibition of mTOR pathway. Herein, we will summarize the current data on the treatment options.

5.1.1. *TSC-associated epilepsy*

Effective management of epileptic seizures associated with tuberous sclerosis is the most important aspect in the multifaceted treatment approach of these patients. The management of epileptic seizures of TSC includes a plethora of pharmacologic and not-pharmacologic treatment modalities. Effective and timely epilepsy treatment in TSC patients is of paramount importance for the optimization of cognitive development and the improvement of quality of life of young patients [23, 24].

Vigabatrin (VGB) is the drug of choice as first line treatment for infantile spasms and/or focal epilepsy

Table 3. Assessment of possible TSC

ORGAN SYSTEM	ASSESSMENT INSTRUCTIONS
Genetics	Three-generation family history, genetic counseling
Skin, teeth, eyes	Profound clinical examination, Wood's lamp examination, fundoscopy
Brain	CT, MRI, EEG, TAND checklist
Heart	ECHO, EKG, prenatal ultrasound
Kidneys	MRI, CT, ultrasound, measurement of BP &GFR
Ists	Pulmonary function tests, HRCT

Adapted from Krueger & Northrup, 2013..

for children with TSC in their first year of age [23, 24]. VGB administration has been demonstrated to effectively control seizures in the infantile population [25-28]. Moreover, preventive treatment with VGB in infants was associated with a lower risk of clinical seizures and infantile spasms in comparison to conventional treatment [29]. The EPISTOP trial demonstrated that the administration of VGB in infants with TSC without history of seizures reduced the risk of clinical seizures, infantile spasms and drug resistant epilepsy [30]. Thus, the preventive administration of VGB may potentially alter the natural course of epileptic seizures in patients with TSC. However, in the EPISTOP trial, the preventive administration of VGB did not significantly affect the developmental delay or autism in children aged two years [30]. The PREVeNT trial [ClinicalTrials.gov identifier: NCT02849457] is a double-blind, placebo-controlled study that is currently under way and is designed to evaluate the effect of preventive VGB in infants less than 6 months of age. The results of the PREVeNT trial will provide valuable information on the potential role of preventive VGB administration in the cognitive development of patients with TSC [31].

The initial recommended dose of VGB for infantile spasms (infants and children 1 month - 2 years) is 50mg/kg/day in two daily doses. Depending on the patient's response the daily dose can be increased by 25mg/kg/day to 50mg/kg day every 3 days. The maximum dose is 150mg/kg/day [32-34]. VGB administration is generally safe. The most important side effect of VGB is retinopathy that can lead to permanent bilateral concentric visual field constriction, but the benefit-risk ratio is strongly in favor of this treatment option [23, 27, 28, 34-36]. Each patient should be examined by an ophthalmologist, with visual field testing before initiation of treatment and then this should be repeated every 3-6 months. However, in most cases (infants and children under the age of 9-10 years) the perimetry is difficult to perform, therefore there are other tests that are recommended, such as Visual Evoked Potentials (VEP), Electroretinogram (ERG) or Electro-Oculogram (EOG) [37].

If the epileptic seizures are refractory to vigabatrin, treatment with other pharmacologic and non-pharmacologic interventions should be carried out.

Pharamacologic interventions include **ACTH** (natural or synthetic) or **corticosteroids** administration as second-line therapy in children with infantile spams of TSC [22, 24]. The daily dosage of ACTH is 150 units/m^2 and the recommended dose of corticosteroids, specifically prednisolone, is 4-8 mg/kg/day or 40-60 mg/day for 14 days, followed by gradual tapering [38-41].

AEDS that enhance GABAergic transmission, such as **topiramate** and **carbamazepine**, are also used for the treatment of TSC-related seizures [24]. If the first AED is not effective, a different AED or two or more AEDS could be prescribed. There is not enough evidence to address the effectiveness of other conventional antiepileptic drugs in the treatment of seizures in patients with TSC [24].

Everolimus is a small molecular inhibitor of the mammalian target of Rapamycin (mTOR) involved in the cellural pathway that is constantly activated due to *TSC1* or *TSC2* loss of function genetic alterations [42-47]. Everolimus efficacy for refractory epilepsy due to TSC was investigated in a phase III, randomized, placebo-controlled trial (EXIST-3) [48]. In this, everolimus was demonstrated to significantly reduce seizures in patients with TSC and treatment resistant epilepsy [49]. Thus, it has received regulatory approval in the USA and in the EU for children older than two years of the age suffering from treatment resistant partial epileptic seizures [50-55]. Common side effects of its usage include mucositis, respiratory tract infections, pyrexia and pneumonitis among others. Everolimus is metabolized in the liver primarily by CYP3A4 and since antiseizure medications typically used for individuals with TSC also interact with the aforementioned enzymes the dose of everolimus

should be modified for patients with severe hepatic impairment and for patients taking concomitant medication that interacts with CYP3A4 inhibitors. Subsequently, the efficacy of everolimus for the treatment of seizures in TSC patients has also been reported in a few single-arm trials and real-world retrospective studies [50-56].

Everolimus dosage for seizure control begins at 5mg/m^2 once daily with subsequent titration in order to achieve plasma concentrations in the range of 5 ng/ml to 15ng/ml [49].

Cannabidiol is a substance derived from the *Cannabis sativa* plant. It is thought to be effective by reducing the activity of mTOR. The European Commission (EC) has approved cannabidiol as an adjunctive treatment of seizures associated with TSC in patients aged 2 years and older since April 2021 [57, 58].

Non-pharmacologic interventions for the management of treatment resistant epileptic seizures for patients with TSC consist of ketogenic diet, vagus nerve stimulation and surgery.

Ketogenic diet (KD) has been reported in experimental models to be associated with downregulation of the mTOR pathway [57-62]. By applying ketogenic diet the liver produces ketones as an alternative energy source. KD should be considered in patients (in early infancy and early childhood) with refractory seizures, who are not candidates for surgery [20, 61, 62].

Vagus nerve stimulation (VNS) –a method of application of electrical stimuli to the vagus nerve– is recommended by the consensus TSC guidelines to be considered in combination with the KD or in cases where the KD is not acceptable [23, 24]. Improvement in seizure frequency has been noted with VNS however, although the relevant data is limited, seizure freedom is quite rare [63-66].

European guidelines recommend that if the first two appropriately chosen anti-epileptic drugs fail to control seizures, a **pre-surgical evaluation** should be promptly started, to assess the possibility of **surgical resection** of the epileptic focus that is mainly responsible for the seizure symptomatology [23]. While studies have reported the benefits of epilepsy surgery, this has been underutilized. However, novel techniques are being developed, with the potential to expand the number of eligible patients, while reducing the risk of complications [67-71].

5.1.2. *Management of CNS tumors development*

The presence of a germline mutation in *TSC1* or *TSC2* genes is associated with the development of secondary CNS tumors, most commonly cortical glioneuronal hamartomas and subependymal giant cell tumors (SGCTs), also known as subependymal giant cell astrocytomas (SEGAs) [72]. Thus, individuals with known TSC must be followed up with brain MRIs every 1-3 years until the age of 25 years [22].

SEGAs can be treated with surgery or with mTOR inhibitors [22]. Treatment decision between surgery or medical treatment with everolimus must be individualized for each patient. However, in the case of a solitary and unilateral SEGA that is amenable to complete surgical resection, surgical treatment should be the treatment of choice. Importantly, due to the unique inherent genetic background of patients with TSC the risk of malignant transformation is high in the setting of radiation therapy administration. It has been reported that radiation treatment can transform SEGAs to malignant glioblastomas [73, 74]. Thus, radiation treatment should be avoided in these patients.

5.2. Extracranial manifestations of TSC

There is a wide spectrum of phenotypic manifestations ranging from skin disorders to pulmonary lymphangiomatosis [22]. Due to the diversity of these lesions a multidisciplinary management is recommended for these individuals and a regular follow-up system has to be established by nephrologists, pulmonologists, etc, in order to address the problems that arise with time. The management of organ specific alterations in the context of TSC is beyond the scope of this review.

6. CONCLUSION

TSC is a genetic disorder, affecting multiple organ systems, most predominantly the skin and the CNS, and is characterized by wide phenotypic heterogeneity even within members of the same family. High clinical suspicion followed by detailed clinical examination and genetic confirmation result in making the correct diagnosis of TSC in most of the suspected cases. Despite significant improvements in understanding the mechanisms involved in the molecular pathogenesis and subsequent pathophysiology in TSC, the management of the individuals that bear a germline mutation in *TSC1* and *TSC2* genes remains challenging and requires a multidisciplinary approach. Importantly, prompt identification and management of the CNS complications of the syndrome can be associated with significant improvements in quality of life and cognitive development for these individuals. Furthermore, the introduction of mTOR inhibitors in clinical practice has offered a new option that can alter the natural disease course and can additionally act as a significant treatment option for secondary tumor development. Finally, it is a paradigm for the development of novel treatments that are not merely symptomatic but mainly address the etiopathogenesis of the disorder.

REFERENCES

[1] Portocarrero LKL, Quental KN, Samorano LP, et al. Tuberous sclerosis complex: review based on new diagnostic criteria. *An Bras Dermatol.* 2018;93:323-331.

[2] Randle SC. Tuberous Sclerosis Complex: A Review. *Pediatr Ann.* 2017;46:e166-e171.

[3] Sadowski K, Kotulska K, Schwartz RA, et al. Systemic effects of treatment with mTOR inhibitors in tuberous sclerosis complex: a comprehensive review. *J Eur Acad Dermatol Venereol.* 2016;30:586-594.

[4] Northrup H, Krueger DA, International Tuberous Sclerosis Complex Consensus G. Tuberous sclerosis complex diagnostic criteria update: recommendations of the 2012 Iinternational Tuberous Sclerosis Complex Consensus Conference. *Pediatr Neurol.* 2013;49:243-254.

[5] Little H, Kamat D, Sivaswamy L. Common Neurocutaneous Syndromes. *Pediatr Ann.* 2015;44:496-504.

[6] Jones AC, Shyamsundar MM, Thomas MW, et al. Comprehensive mutation analysis of TSC1 and TSC2-and phenotypic correlations in 150 families with tuberous sclerosis. *Am J Hum Genet.* 1999;64:1305-1315.

[7] Au KS, Williams AT, Roach ES, et al. Genotype/phenotype correlation in 325 individuals referred for a diagnosis of tuberous sclerosis complex in the United States. *Genet Med.* 2007;9:88-100.

[8] van Slegtenhorst M, de Hoogt R, Hermans C, et al. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science.* 1997;277:805-808.

[9] European Chromosome 16 Tuberous Sclerosis C. Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell.* 1993;75:1305-1315.

[10] Magri L, Cominelli M, Cambiaghi M, et al. Timing of mTOR activation affects tuberous sclerosis complex neuropathology in mouse models. *Dis Model Mech.* 2013;6:1185-1197.

[11] Tee AR, Manning BD, Roux PP, et al. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Curr Biol.* 2003;13:1259-1268.

[12] Islam MP, Roach ES. Tuberous sclerosis complex. *Handb Clin Neurol.* 2015;132:97-109.

[13] Hancock EC, Osborne JP, Edwards SW. Treatment of infantile spasms. *Cochrane Database Syst Rev.* 2013:CD001770.

[14] de Vries PJ, Whittemore VH, Leclezio L, et al. Tuberous sclerosis associated neuropsychiatric disorders (TAND) and the TAND Checklist. *Pediatr Neurol.* 2015;52:25-35.

[15] Jambaque I, Cusmai R, Curatolo P, et al. Neuropsychological aspects of tuberous sclerosis in relation to epilepsy and MRI findings. *Dev Med Child Neurol.* 1991;33:698-705.

[16] Inoue Y, Nemoto Y, Murata R, et al. CT and MR imaging of cerebral tuberous sclerosis. *Brain Dev.* 1998;20:209-221.

[17] Kandt RS, Haines JL, Smith M, et al. Linkage of an important gene locus for tuberous sclerosis to a chromosome 16 marker for polycystic kidney disease. *Nat Genet.* 1992;2:37-41.

[18] Hishitani T, Hoshino K, Ogawa K, et al. Rapid enlargement of cardiac rhabdomyoma during corticotropin therapy for infantile spasms. *Can J Cardiol.* 1997;13:72-74.

[19] Weig SG, Pollack P. Carbamazepine-induced heart block in a child with tuberous sclerosis and cardiac rhabdomyoma: Implications for evaluation and follow-up. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society.* 1993;34:617-619.

[20] Kobashi Y, Sugiu T, Mouri K, et al. Multifocal micronodular pneumocyte hyperplasia associated with tuberous sclerosis: differentiation from multiple atypical adenomatous hyperplasia. *Jpn J Clin Oncol.* 2008;38:451-454.

[21] Aronow ME, Nakagawa JA, Gupta A, et al. Tuberous sclerosis complex: genotype/phenotype correlation of retinal findings. *Ophthalmology.* 2012;119:1917-1923.

[22] Krueger DA, Northrup H, International Tuberous Sclerosis Complex Consensus G. Tuberous sclerosis complex surveillance and management: recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference. *Pediatr Neurol.* 2013;49:255-265.

[23] Curatolo P, Jozwiak S, Nabbout R, et al. Management of epilepsy associated with tuberous sclerosis complex (TSC): clinical recommendations. *Eur J Paediatr Neurol.* 2012;16:582-586.

[24] Curatolo P, Nabbout R, Lagae L, et al. Management of epilepsy associated with tuberous sclerosis complex: Updated clinical recommendations. *Eur J Paediatr Neurol.* 2018;22:738-748.

[25] Overwater IE, Bindels-de Heus K, Rietman AB, et al. Epilepsy in children with tuberous sclerosis complex: Chance of remission and response to antiepileptic drugs. *Epilepsia.* 2015;56:1239-1245.

[26] van der Poest Clement EA, Sahin M, Peters JM. Vigabatrin for Epileptic Spasms and Tonic Seizures in Tuberous Sclerosis Complex. *J Child Neurol.* 2018;33:519-524.

[27] van der Poest Clement E, Jansen FE, Braun KPJ, et al. Update on Drug Management of Refrac-

tory Epilepsy in Tuberous Sclerosis Complex. *Paediatr Drugs.* 2020;22:73-84.
[28] Zhang B, McDaniel SS, Rensing NR, et al. Vigabatrin inhibits seizures and mTOR pathway activation in a mouse model of tuberous sclerosis complex. *PLoS One.* 2013;8:e57445.
[29] Jozwiak S, Slowinska M, Borkowska J, et al. Preventive Antiepileptic Treatment in Tuberous Sclerosis Complex: A Long-Term, Prospective Trial. *Pediatr Neurol.* 2019;101:18-25.
[30] Kotulska K, Kwiatkowski DJ, Curatolo P, et al. Prevention of Epilepsy in Infants with Tuberous Sclerosis Complex in the EPISTOP Trial. *Ann Neurol.* 2021;89:304-314.
[31] ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT02849457.
[32] Fejerman N, Cersósimo R, Caraballo R, et al. Vigabatrin as a first-choice drug in the treatment of West syndrome. *Journal of child neurology.* 2000;15:161-165.
[33] Mikati MA, Lepejian GA, Holmes GL. Medical treatment of patients with infantile spasms. *Clin Neuropharmacol.* 2002;25:61-70.
[34] Hussain SA, Tsao J, Li M, et al. Risk of vigabatrin-associated brain abnormalities on MRI in the treatment of infantile spasms is dose-dependent. *Epilepsia.* 2017;58:674-682.
[35] Rodrigues C, Chiron C, Ounissi M, et al. Pharmacokinetic evaluation of vigabatrin dose for the treatment of refractory focal seizures in children using adult and pediatric data. *Epilepsy Res.* 2019;150:38-45.
[36] Elterman RD, Shields WD, Mansfield KA, et al. Randomized trial of vigabatrin in patients with infantile spasms. *Neurology.* 2001;57:1416-1421.
[37] Hawker M, Astbury N. The ocular side effects of vigabatrin (Sabril): information and guidance for screening. *Eye.* 2008;22:1097-1098.
[38] Go CY, Mackay MT, Weiss SK, et al. Evidence-based guideline update: medical treatment of infantile spasms. Report of the Guideline Development Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology.* 2012;78:1974-1980.
[39] Thiele EA. Managing epilepsy in tuberous sclerosis complex. *J Child Neurol.* 2004;19:680-686.
[40] Nelson GR. Management of infantile spasms. *Transl Pediatr.* 2015;4:260-270.
[41] Vigevano F, Cilio MR. Vigabatrin versus ACTH as first-line treatment for infantile spasms: a randomized, prospective study. *Epilepsia.* 1997;38:1270-1274.
[42] Curatolo P. Mechanistic target of rapamycin (mTOR) in tuberous sclerosis complex-associated epilepsy. *Pediatr Neurol.* 2015;52:281-289.
[43] Crino PB. The mTOR signalling cascade: paving new roads to cure neurological disease. *Nat Rev Neurol.* 2016;12:379-392.
[44] Cardamone M, Flanagan D, Mowat D, et al. Mammalian target of rapamycin inhibitors for intractable epilepsy and subependymal giant cell astrocytomas in tuberous sclerosis complex. *J Pediatr.* 2014;164:1195-1200.
[45] Krueger DA, Wilfong AA, Holland-Bouley K, et al. Everolimus treatment of refractory epilepsy in tuberous sclerosis complex. *Ann Neurol.* 2013;74:679-687.
[46] Krueger DA, Wilfong AA, Mays M, et al. Long-term treatment of epilepsy with everolimus in tuberous sclerosis. *Neurology.* 2016;87:2408-2415.
[47] Wiegand G, May TW, Ostertag P, et al. Everolimus in tuberous sclerosis patients with intractable epilepsy: a treatment option? *Eur J Paediatr Neurol.* 2013;17:631-638.
[48] French JA, Lawson JA, Yapici Z, et al. Adjunctive everolimus therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study. *Lancet.* 2016;388:2153-2163.
[49] Curatolo P, Franz DN, Lawson JA, et al. Adjunctive everolimus for children and adolescents with treatment-refractory seizures associated with tuberous sclerosis complex: post-hoc analysis of the phase 3 EXIST-3 trial. *Lancet Child Adolesc Health.* 2018;2:495-504.
[50] Franz DN, Lawson JA, Yapici Z, et al. Everolimus for treatment-refractory seizures in TSC: Extension of a randomized controlled trial. *Neurol Clin Pract.* 2018;8:412-420.
[51] Svarrer EMM, Fischer CM, Frederiksen MG, et al. Everolimus as adjunctive treatment in tuberous sclerosis complex-associated epilepsy in children. *Dan Med J.* 2019;66.
[52] Saffari A, Brosse I, Wiemer-Kruel A, et al. Safety and efficacy of mTOR inhibitor treatment in patients with tuberous sclerosis complex under 2 years of age - a multicenter retrospective study. *Orphanet J Rare Dis.* 2019;14:96.
[53] Stockinger J, Strzelczyk A, Nemecek A, et al. Everolimus in adult tuberous sclerosis complex patients with epilepsy: Too late for success? A retrospective study. *Epilepsia.* 2021;62:785-794.
[54] Li M, Zhou Y, Chen C, et al. Efficacy and safety of mTOR inhibitors (rapamycin and its analogues) for tuberous sclerosis complex: a meta-analysis. *Orphanet J Rare Dis.* 2019;14:39.
[55] Moavero R, Coniglio A, Garaci F, et al. Is mTOR inhibition a systemic treatment for tuberous sclerosis? *Italian Journal of Pediatrics.* 2013;39:57.

[56] de Vries PJ, Franz DN, Curatolo P, et al. Measuring Health-Related Quality of Life in Tuberous Sclerosis Complex - Psychometric Evaluation of Three Instruments in Individuals With Refractory Epilepsy. *Front Pharmacol.* 2018;9:964.
[57] Kossoff EH, Thiele EA, Pfeifer HH, et al. Tuberous sclerosis complex and the ketogenic diet. *Epilepsia.* 2005;46:1684-1686.
[58] Singh A, Mettler T, Oh H, et al. The Ketogenic Diet Attenuates Both Hyperactivity in mTOR pathway and Astrogliosis Through Regulation of AMPK signaling in the Epileptic Brain. *The FASEB Journal.* 2018;32:805.811-805.811.
[59] McDaniel SS, Rensing NR, Thio LL, et al. The ketogenic diet inhibits the mammalian target of rapamycin (mTOR) pathway. *Epilepsia.* 2011;52:e7-11.
[60] Youn SE, Park S, Kim SH, et al. Long-term outcomes of ketogenic diet in patients with tuberous sclerosis complex-derived epilepsy. *Epilepsy Res.* 2020;164:106348.
[61] Park S, Lee EJ, Eom S, et al. Ketogenic Diet for the Management of Epilepsy Associated with Tuberous Sclerosis Complex in Children. *J Epilepsy Res.* 2017;7:45-49.
[62] Amin S, Kingswood JC, Bolton PF, et al. The UK guidelines for management and surveillance of Tuberous Sclerosis Complex. *QJM.* 2019;112:171-182.
[63] Elliott RE, Carlson C, Kalhorn SP, et al. Refractory epilepsy in tuberous sclerosis: vagus nerve stimulation with or without subsequent resective surgery. *Epilepsy Behav.* 2009;16:454-460.
[64] Major P, Thiele EA. Vagus nerve stimulation for intractable epilepsy in tuberous sclerosis complex. *Epilepsy Behav.* 2008;13:357-360.
[65] Parain D, Penniello MJ, Berquen P, et al. Vagal nerve stimulation in tuberous sclerosis complex patients. *Pediatr Neurol.* 2001;25:213-216.
[66] Zamponi N, Petrelli C, Passamonti C, et al. Vagus nerve stimulation for refractory epilepsy in tuberous sclerosis. *Pediatr Neurol.* 2010;43:29-34.
[67] Wu JY, Salamon N, Kirsch HE, et al. Noninvasive testing, early surgery, and seizure freedom in tuberous sclerosis complex. *Neurology.* 2010;74:392-398.
[68] Liu S, Yu T, Guan Y, et al. Resective epilepsy surgery in tuberous sclerosis complex: a nationwide multicentre retrospective study from China. *Brain.* 2020;143:570-581.
[69] Liang S, Zhang J, Yang Z, et al. Long-term outcomes of epilepsy surgery in tuberous sclerosis complex. *J Neurol.* 2017;264:1146-1154.
[70] Jansen FE, van Huffelen AC, Algra A, et al. Epilepsy surgery in tuberous sclerosis: a systematic review. *Epilepsia.* 2007;48:1477-1484.
[71] Treiber JM, Curry DJ, Weiner HL, et al. Epilepsy surgery in tuberous sclerosis complex (TSC): emerging techniques and redefinition of treatment goals. *Childs Nerv Syst.* 2020;36:2519-2525.
[72] Lopes MBS WO, et al. Tuberous sclerosis complex and subependymal giant cell astrocytoma; in Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds): World Health Organization Classification of Tumours of the Central Nervous System. Lyon, IARC Press, 2007, pp 218-221. *Nat Rev Neurol.* 12:379-392.
[73] Yates JRW. Tuberous sclerosis. *European Journal of Human Genetics.* 2006;14:1065-1073.
[74] Matsumura H, Takimoto H, Shimada N, et al. Glioblastoma following radiotherapy in a patient with tuberous sclerosis. *Neurol Med Chir (Tokyo).* 1998;38:287-291.

SHARED GENETIC PATHWAYS BETWEEN MULTIPLE SCLEROSIS AND ISCHEMIC STROKE: A REVIEW OF THE LITERATURE

Sotiria De Lorenzo-Stavropoulou, Ioanna Kapsali, Konstantina Aslanidou, Sofia Stasinou, Athina Tsimpiktsioglou, Michail Ioakeimidis, Georgios Nakkas, Dimitrios Mandras, Vasileios Gkrinias, Despoina Koutsodonti, Triantafyllos Doskas

Neurology Department, Athens Naval Hospital, 77A Vas. Sofias Avenue, Athens, Greece

Abstract

Multiple sclerosis (MS) and stroke are both neurological diseases that affect the central nervous system (CNS) and lead to long-term motor and sensory deficits and cognitive impairment. Both diseases detrimentally affect the quality of life of patients and their families. Clinical studies on patients with MS have revealed an increased incidence of any type of stroke, including ischemic stroke (IS), hemorrhagic stroke and transient ischemic attack (TIA) compared to the general population. Both MS and stroke are heterogeneous diseases that have a genetic component. As ischemic is the most frequently encountered type of stroke, the majority of available evidence on MS patients relates to IS. The increased incidence of IS in MS patients points out the need for exploration of the underlying genetic component link of both diseases. The identification of shared risk genes between the two diseases is of great importance to develop therapies that will be more effective than the currently available treatments or will be targeted at MS patients at high risk for stroke. Here, we describe the main genetic findings from genome-wide association studies that provide evidence in favour of the genetic link between MS and IS.

Key words: multiple sclerosis, stroke, ischemic stroke, genome-wide association studies

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory progressive autoimmune disease that is characterized by neuronal demyelination and leads to neurodegeneration [1]. The incidence of MS is higher in young adults, especially women. MS is attributed to genetic, immune and environmental influences under the control of epigenetic mechanisms [2-4]. Clinical evidence suggests that the incidence and prevalence of MS are higher in the patients' families compared to the general population [5]. Thus, the lifetime risk of MS in first-degree relatives of MS index cases is estimated at 3% and is 10- to 30-fold greater than the corresponding age-adjusted risk in the general population (0.1%-0.3%) [6-8]. This in turn points to the importance of genetic susceptibility for MS onset [5]. Despite the potential genetic heterogeneity, the Class II human leukocyte antigen *HLA-DRB1*15:01* allele in the HLA gene locus on chromosome 6p21 is strongly associated with a risk for MS and potentially MS severity [5, 9-11]. The pathogenesis of MS is complicated by interactions between Class II risk alleles, including *HLA-DRB1*15:01*, and environmental stimuli [12, 13].

MS is not a Mendelian disease. Based on the theory of common disease common variant (CDCV), which underlies genome-wide association studies (GWAS), common diseases in a population are attributed to several small common genetic variations that present with a high allelic frequency in the population. It can, therefore, be postulated that the inheritance of MS is associated with one locus that exerts a moderate effect (*HLA-DRB1*15:01*) and many loci with small (modest) effects) [14, 15]. The analysis of 47,351 MS cases and 68,284 healthy controls in the largest GWAS in MS up to date revealed 233 genome-wide loci that were related to MS susceptibility [8, 16]. Of these, 200 loci were located in the non-major histocompatibility complex (non-MHC) genome and had small contribution, accounting for approximately 20% of MS genetics [8, 16]. Most of the MS-associated gene variants resided either in intronic or intragenic regions, namely in enhancers or promoters of nearby genes, and affected the regulation of immune-system related genes and immune mechanisms [8, 16]. The same conclusion is applicable to findings from GWAS studies in other inflammatory autoimmune diseasesas well that are not limited to CNS [17].

The pathophysiology of autoimmune diseases, including MS, is to some extent common to stroke [18]. The inflammatory response (neuroinflamma-

tion) that underlies acute and chronic brain diseases, including IS, MS and Parkinson's disease has been the object of investigation and is considered a potential common underlying link both early on and during disease progression [18]. Based on favorable preclinical studies, the immunomodulatory drugs natalizumab and fingolimod that are used for MS have been investigated in clinical trials for IS [19-21]. According to WHO, stroke was the second leading cause of death globally in 2019, accounting for 11% of the deaths reported worldwide, and one of the leading causes of disability [22]. There are three types of stroke: ischemic stroke (IS), intracerebral hemorrhage and transient ischemic attack [23, 24]. IS accounts for the majority (70-85%) of stroke cases [23]. The risk for stroke increases with age; its incidence is, therefore, generally higher in middle-aged and elderly people [24]. The pathophysiological mechanism of IS involves endothelial dysfunction and atherosclerotic plaque formation [24]. The etiology of stroke is heterogenous [25]. Risk factors that predispose to stroke can be both modifiable and non-modifiable and include smoking, obesity, diabetes, hypertension and hypercholesterolemia [24]. Genetics, also, contribute to the risk for stroke [25, 26]. Early GWAS studies across racial groups revealed significant correlations between stroke and *ABO* blood system locus, cardioembolic stroke and variants near *PITX2* and *ZFHX3* as well as between large-vessel stroke and *HDAC9* (histone deacetylase 9) variants and the 9p21 locus, suggesting that there might be a genetic origin in the risk for stroke, regardless of geographic and racial differences [27-31]. Subsequent GWAS have identified 35 genetic loci that conferred a risk for stroke overall or predisposed to various stroke subtypes [32, 33]. Furthermore, temporal GWAS using leukocyte counts during the first 24 hours after IS have identified that the 14q24.3 locus was associated with both leucocyte counts and IS outcomes [34]. Currently, the primary FDA-approved drug for IS is intravenous alteplase. However, thrombolysis has a limited therapeutic window and many patients with IS are not eligible because of the strict criteria for alteplase administration and the unpredictable outcomes of recanalization [20]. More emphasis should, therefore, be paid to the development of neuroprotective treatments that target other mechanisms that are implicated in IS, such as inflammation and oxidative stress [35]. It is, therefore, possible that treatments that are effectively used in inflammatory diseases of the CNS, coupled with the genetic information that emerges from GWAS studies could be exploited for the treatment of the inflammatory processes that are involved in stroke.

Clinical studies have identified an increased risk and prevalence of cerebrovascular comorbidities in patients with MS after the clinical onset of the disease compared with non-MS controls [36]. The objective of the current literature review is to describe the main findings of meta-analyses of GWAS that interconnect MS and IS. IS was used rather than stroke overall was because it is the commonest type of stroke.

ISCHEMIC STROKE IN PATIENTS WITH MULTIPLE SCLEROSIS

The meta-analysis of observational studies of various racial populations with various follow-up intervals by Hong et al. (2019) reported that both the risk and occurrence of stroke were increased in MS patients compared to the general population. IS in particular was statistically significantly more common in the MS compared to non-MS population. Additionally, the 5-year incidence of IS was 8.12/1000 person-years in people with MS and 1.48/1000 person-years in the general (non-MS) population. The incidence rate ratio of any type of stroke, including IS, hemorrhagic stroke and transient ischemic attack ranged from 2.53% to 2.85% and the incidence of IS ranged from 1.22% to 3.49% compared to non-MS individuals. Other than the common pathophysiology, common risk factors, such as obesity, and the decreased mobility that MS confers, particularly in patients with progressive forms of the disease, could account for the increased incidence of stroke in MS patients [36]. However, there is currently limited evidence on the potentially common underlying genetic component, which is increasingly investigated. Until recently, *SLC44A2* was the only common risk gene both for MS and IS. *SLC44A2* encodes solute carrier family 44, member 2 that is implicated in interleukin-enhancing binding factor 3 transcription [20, 33, 37]. The identification of shared risk genes between the two diseases has, therefore, prompted further exploration through GWAS.

GWAS IN MS AND STROKE

Li et al. (2019) performed a gene- and pathway-based meta-analysis of large-scale GWAS datasets of European/Caucasian descent to determine potential shared gene expression patterns between MS and IS. For this purpose, the large scale MS GWAS dataset from the International Multiple Sclerosis Genetics Consortium (IMSGC) derived from the Wellcome Trust Case Consortium 2 (WTCCC2) project that comprised 9,772 MS cases and 17,376 controls and the IS dataset derived from the 1000G GWAS summary results of the METASTROKE collaboration comprising 10,307 IS cases and 19,326 controls was used [33, 38]. Following identification of the significant genes for each disease ($p_{value} < 0.05$), pathway-based analysis in the following four biological pathway databases KEGG, PANTHER, REACTOME and WikiPathways as

well as GO datasets was performed [28]. The subsequent analysis revealed that MS and IS shared 9 significant ($p_{value} < 0.05$) pathways in KEGG [including the natural killer (NK) cell-mediated cytotoxicity pathway], 2 in PANTHER (the Cadherin and the Wnt signaling pathway), 14 in REACTOME [including the cell-cell communication pathway and the interferon-gamma (IFN-γ) signaling pathway), 1 in WikiPathways [the thymic stromal lymphopoietin (TSLP) signaling pathway] and 194 in GO annotations. In KEGG, the pathways could be broadly divided into six groups: immune system, environmental information processing, drug resistance and endocrine, nervous system, cancers and infectious diseases. In GO annotations the shared significant pathways concerned biological processes (85 pathways), cellular components (78 pathways) and molecular function (31 pathways). Out of all these significant shared pathways, 4 key pathways correlated with both the immune and the nervous system. These were the NK cell-mediated, the Toll-like receptor signaling (TLR), the Th1 and Th2 cell differentiation and the neurotrophin signaling pathways. The cytolytic function of NK cells is important for immune homeostasis and the regulation of immune cells of both innate and adaptive immunity. Thus, the contribution of NK cells to various autoimmune diseases, including MS, has been increasingly investigated [39-42]. The dysfunction of NK cells strongly correlates with the pathophysiological mechanisms of MS and the response of several patients to selected MS treatments [42]. One of the 3 NK subtypes, the weakly cytotoxic $CD56^{bright}$ NK cells, can acquire cytotoxic qualities and regulate immune responses via cytokine production upon stimulation [43]. Certain immunotherapies that are administered in MS, such as daclizumab and IFN-β, selectively expanded $CD56^{bright}$ NK cells that in turn correlated with decreased disease flares in MS patients [44-46]. Compared to untreated patients, NK cells from daclizumab-treated patient samples showed increased cytotoxicity toward $CD4^+$ autologous activated T cells [47]. NK cells are also important in the pathophysiology of IS [48]. The release of fractalkine by neurons in acute IS can attract lymphocytes, including NK cells in the ischemic area, and NK cells, in turn, augment neuronal death and accelerate brain infarction via the secretion of cytokines and glutamate [48]. A meta-analysis of 12 GWAS of all types of stroke revealed that the NK cell signaling pathway is the only pathway that is significantly shared by all types of stroke, including IS subtypes [28]. The TLR protein family includes pattern-recognition receptors (PRRs) that recognize microbe-specific pathogen-associated molecular patterns (PAMPs) and self-derived damaged cell-derived danger-associated molecular patterns (DAMPs) [49]. The disruption of TLR signaling is implicated in autoimmunity and inflammatory diseases, as PRRs produce immune system mediators that activate innate immune responses [49, 50]. The Wnt and the cell surface TLR2 signaling pathway are implicated in impaired remyelination in the animal model of MS (experimental autoimmune encephalomyelitis, EAE), with enhanced expression of TLR2 on oligodendrocytes in MS lesions only [50, 51]. However, the enhanced expression of TLR2 was not observed on oligodendrocytes in normal areas [50, 51]. The cell surface TLR4 can, also, promote inflammation in EAE, whereas the inflammatory response following IS was reduced in TLR4-deficient mice [52, 53]. TLR2 and TLR4 DAMP-mediated activation enhance the production of pro-inflammatory cytokines in various chronic inflammatory conditions, including autoimmune diseases [49, 53]. Furthermore, a clinical trial on IS demonstrated that the intracellular TLR7 and TLR8 correlated with poor outcomes at 3 months and infarct volume [54]. The microbiome also contributes to the regulation of innate immunity via the provision of microbial products in the systemic circulation to induce TLR2 tolerance; however, TLR2 tolerance induction is disrupted in MS patients, thus, the contribution of the microbiome in MS onset warrants further investigation [55]. Regarding the $CD4^+$ T cell differentiation into T-helper 1 and 2 (Th1 and Th2) cells in response to stimuli, through which Th1 cells are stimulated by IL-12 (interleukin-12) to produce IFN-γ and IL-2 and Th2 cells are activated by IL-4 and IL-2 to produce a range of cytokines, a shift from Th1 to Th2 cytokine production was associated with increased susceptibility to bacterial infections and conferred differential effects in infarct size in preclinical models of IS [56-58]. The susceptibility was in turn attributed to stroke-induced immunosuppression. Furthermore, the $CD4^+$ Th17 cells are also involved in the pathophysiology of autoimmune diseases via the production of IL-17[57]. Blocking IL-17 or treatment with IFN-γ in clinical trials on MS patients conferred reduction in brain MRI lesion activity or MS symptom exacerbation respectively [59, 60]. Therefore, clinical evidence supported the preclinical evidence on the effect of Th cell subsets (Th1, Th2 and Th17) in the course of MS [59, 60]. The last key shared pathway, the neurotrophin signaling pathway, is crucial for the differentiation and survival of neurons. Mammalian neurotrophins include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4 (NT-4) [61]. The neurotrophins activate the tropomyosin-related kinase (Trk) family of tyrosine kinase receptors (Trk) and p75 neurotrophin receptors (p75NTRs) [61]. The latter are involved in matrix remodelling and limit scar formation and are upregulated after tissue injury, such as after stroke [62]. Additionally, an increase of glial p75NTR expression in MS plaques, but not controls has been observed [63]. BDNF/Trk B family signaling

and TrkB-FL/TrkB-T1 balance have been exploited as targets for stroke therapies [64, 65], wheras the expression of the precursor of BDNF (pro-BDNF) was upregulated in circulating lymphocytes and infiltrated inflammatory cells both in clinical studies at the lesion sites of the brain and spinal cord of MS patients as well as in EAE [66]. Furthermore, the ciliary neurotrophic factor (CNTF) is neuroprotective in EAE and the cortex of MS patients [67, 68].

Tian et al. (2020) performed a gene-based meta-analysis of large-scale MS GWAS and IS GWAS with the aim to identify significant transcriptional changes in overlapping genes between MS and IS [20]. The MS GWAS dataset comprised 9,772 MS cases from IMSGC and 17,376 controls from the WTCCC2 and the IS dataset from METASTROKE comprised 10,307 IS cases and 19,326 controls (all of European descent). Overall 24 shared genes were identified and 5 genes (*FOXP1*, *CAMK2G*, *CLEC2D*, *LBH* and *SLC2A4RG*) with significant expression differences in the MS and IS datasets. The expression of *FOXP1* was elevated in both MS and IS datasets. *FOXP1* is located at 3p13 and encodes the forkhead box protein P1, a member of the FOX family of transcription factors [69]. *FOXP1* is essential to both immune system function and CNS development. FOXP1 is important in the early development and maturation of B cells and in the differentiation of macrophages and T cells via negative transcriptional modulation in the differentiation of $CD4^+$ follicular Th cells [69-72]. Pathological *FOXP1* upregulation impairs germinal center B cell function and distribution, thus contributing to lymphomagenesis [73]. Furthermore, FOXP1 is required for the FOXP3-mediated IL2-dependent function and responsiveness of regulatory T cells [69]. Preclinical studies have demonstrated that *FOXP1* affects the neurogenesis of neural stem cells (NSCs) via Notch signaling and triggers embryonic NSC differentiation *in vitro* [72] or modulates the neuronal migration and morphogenesis of cortical neurons during neuronal development [74]. The importance of *FOXP1* in neuronal development is evidenced by the identification of mutations or variants that are associated with several neurological disorders, such as Huntington disease [75], autism [76], and epilepsy [76, 77]. Bot et al. (2011) demonstrated that *FOXP1* is also expressed in various cells and is related to atherosclerotic plaque stability and severity through the transforming growth factor-beta (TGF-β) pathway. In the same study, *FOXP1* overexpression correlated positively with IL-2 and IL-4 levels, which may be of relevance to immunomodulatory diseases [78]. Based on this evidence it has been proposed that atherosclerosis could be effectively prevented and treated via targeting immunomodulatory pathways [79]. A strong association between *FOXP1* expression and MS may exist in large-vessel atherosclerosis, one of the major subtypes of IS, that should be explored [20]. This is further supported by *in vivo* evidence that *FOXP1* silencing delayed EAE onset and prevented mature dendritic cell-induced T-cell maturation [80]. Another gene with upregulated expression in both data sets was *CAMK2G. CAMK2G* is located at 10q22.2 and encodes the γ isoform of the calcium (Ca^{2+})/calmodulin-dependent protein kinase II (CaMKIIγ) [81, 82]. *CAMK2G* is implicated in vascular diseases and was reported as an enhancer gene for coronary artery disease in a GWAS meta-analysis [83]. Additionally, CAMK2G/CaMKIIγ enhanced neuronal survival in an experimental model of acute ischemia/reperfusion via activating protective signalling pathways [84]. Furthermore, the expression of *CAMKIIγ* in macrophages induces atherosclerotic plaque necrosis [85]. The third of the 5 genes *CLEC2D* that is located at 12p13.31 next to the NK gene complex and encodes the lectin-like transcript 1 (LLT1) was upregulated in MS but down-regulated in IS datasets [86, 87]. LLT1 has been reported as a negative ligand for CD161 receptor in humans [88] and suppressed CD161-mediated NK cell cytotoxicity [89] or affected B-cell activation in germinal centers [90]. Moreover, *LLT1* is expressed by TLR-activated cells of innate and adaptive immunity, such as dendritic cells or activated B cells [91]. Another shared gene with significant expression difference (downregulation in the MS and opposite alterations in IS datasets) was *SLC2A4RG* that is located at 20q13.33 and encodes the SLC1A4 regulator, a sodium-dependent neutral amino acid transporter [92]. *SLC2A4RG* is not solely expressed in neuronal cells and is implicated in early neuronal development; it may, thus, affect neurological diseases [93]. SLC2A4RG is also a TF that regulates the expression of *SLC2A4* [94, 95]. A large-scale GWAS provided evidence that *rs2256814/SLC2A4RG* is a novel gene with immune function that is related to MS susceptibility [37]. Additionally, Dhaouadi et al. (2014) reported that SLC2A4RG might enhance to a small extent the expression levels of cytokine TGF-β1 that has a protective effect in human atherosclerosis [96]. The last gene with altered expression in the datasets (downregulation in MS datasets and opposite alterations in IS) was the embryonic transcription cofactor *LBH* (limb-bud and heart) that is located at 2p23.1 and regulates cell development in various tissues [97-99]. The expression of *LBH* in neoplasms and the epithelium is in turn regulated by the Wnt signaling pathway. The latter is tightly regulated to preserve neurovascular functions and its disruption is involved in hemorrhagic stroke and traumatic brain injuries [100-102]. Interestingly, GWAS in 991 MS patients that experienced 2,231 relapses from a single institute in Europe identified a genetic variant of the Wnt signalling pathway (variant *rs11871306* of *WNT9B*) that was associated with relapse occurrence

in MS [103]. The alternations in expression patterns warrant further investigation but could be attributed to the heterogeneity of IS and its subtypes.

CONCLUSION

Science has made great progress in the past few decades. GWAS and meta-analyses have provided evidence of the genetic component of MS and stroke and revealed gene variants and genetic pathways that predispose to an increased risk for either of these neurological diseases. Determining and understanding the genetic correlations between MS and stroke can fill in the missing information on their interactions or their heterogeneity under the influence of environmental stimuli. The genetic component could also partly account for the increased risk and incidence for stroke in MS patients and complement the existing evidence on their pathophysiology link. A potential limitation in the generalization of the available data is that the majority of GWAS in any disease or trait, including MS or stroke, are performed on populations of European ancestry or self-reported as of European ancestry. Nevertheless, geographic and/or racial differences affect genetics, suggesting that genetic susceptibility could be subject to variation in diverse populations. Thus, further GWAS studies in other racial groups should be performed. It would, also, be interesting to perform temporal GWAS studies to determine if there is a genetic link that underlies IS onset and MS relapses. Conclusively, genetic research has provided us with new information and at the same time generated new questions and hypotheses that should be exploited further. This new information will guide us to the development of new targeted treatments that will be more effective or allow a more personalized treatment approach for MS patients who are more likely to suffer from stroke based on the evaluation of risk factors, environmental influence and genetic background.

REFERENCES

[1] Stadelmann C, Wegner C, Brück W. Inflammation, demyelination, and degeneration - Recent insights from MS pathology. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease.* 2011;1812:275-282.

[2] Celarain N, Tomas-Roig J. Aberrant DNA methylation profile exacerbates inflammation and neurodegeneration in multiple sclerosis patients. *J Neuroinflammation.* 2020;17:21.

[3] Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol.* 2015;15:545-558.

[4] Sternberg Z. Genetic, Epigenetic, and Environmental Factors Influencing Neurovisceral Integration of Cardiovascular Modulation: Focus on Multiple Sclerosis. *Neuromolecular Med.* 2016;18:16-36.

[5] Hollenbach JA, Oksenberg JR. The immunogenetics of multiple sclerosis: A comprehensive review. *J Autoimmun.* 2015;64:13-25.

[6] Compston A, Coles A. Multiple sclerosis. *The Lancet.* 2002;359:1221-1231.

[7] Sawcer S, Franklin RJ, Ban M. Multiple sclerosis genetics. *The Lancet Neurology.* 2014;13:700-709.

[8] Patsopoulos NA. Genetics of Multiple Sclerosis: An Overview and New Directions. *Cold Spring Harb Perspect Med.* 2018;8.

[9] Barcellos LF, Sawcer S, Ramsay PP, et al. Heterogeneity at the HLA-DRB1 locus and risk for multiple sclerosis. *Hum Mol Genet.* 2006;15:2813-2824.

[10] Hensiek AE, Sawcer SJ, Feakes R, et al. HLA-DR 15 is associated with female sex and younger age at diagnosis in multiple sclerosis. *J Neurol Neurosurg Psychiatry.* 2002;72:184-187.

[11] Zhang Q, Lin C-Y, Dong Q, et al. Relationship between HLA-DRB1 polymorphism and susceptibility or resistance to multiple sclerosis in Caucasians: A meta-analysis of non-family-based studies. *Autoimmun Rev.* 2011;10:474-481.

[12] Goodin DS, Khankhanian P, Gourraud PA, et al. The nature of genetic and environmental susceptibility to multiple sclerosis. *PLoS One.* 2021;16:e0246157.

[13] Moutsianas L, Jostins L, Beecham AH, et al. Class II HLA interactions modulate genetic risk for multiple sclerosis. *Nat Genet.* 2015;47:1107-1113.

[14] Bodmer W, Bonilla C. Common and rare variants in multifactorial susceptibility to common diseases. *Nat Genet.* 2008;40:695-701.

[15] O'Gorman C, Lin R, Stankovich J, et al. Modelling genetic susceptibility to multiple sclerosis with family data. *Neuroepidemiology.* 2013;40:1-12.

[16] Patsopoulos NA; (2016). 2 2016 Annual Meeting a. 200 loci complete the genetic puzzle of multiple sclerosis. *International Multiple Sclerosis Genetics Consortium.* Vancouver, Canada: American Society of Human Genetics, 2016.

[17] Farh KK, Marson A, Zhu J, et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature.* 2015;518:337-343.

[18] Vries HEd, Schwaninger M. Neuroinflammation: A common denominator for stroke, multiple sclerosis and Alzheimer's disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease.* 2016;1862:297-298.

[19] Zhu Z, Fu Y, Tian D, et al. Combination of the Immune Modulator Fingolimod With Alteplase in Acute Ischemic Stroke: A Pilot Trial. *Circulation.* 2015;132:1104-1112.

[20] Tian Z, Song Y, Yao Y, et al. Genetic Etiology Shared by Multiple Sclerosis and Ischemic Stroke. *Frontiers in genetics.* 2020;11:646.
[21] Elkins J, Veltkamp R, Montaner J, et al. Safety and efficacy of natalizumab in patients with acute ischaemic stroke (ACTION): a randomised, placebo-controlled, double-blind phase 2 trial. *The Lancet Neurology.* 2017;16:217-226.
[22] WHO. (accessed 3 Sep 2021).
[23] Smith NM, Pathansali R, Bath PM. Platelets and stroke. *Vasc Med.* 1999;4:165-172.
[24] Kuriakose D, Xiao Z. Pathophysiology and Treatment of Stroke: Present Status and Future Perspectives. *International journal of molecular sciences.* 2020;21:7609.
[25] Boehme AK, Esenwa C, Elkind MS. Stroke Risk Factors, Genetics, and Prevention. *Circulation research.* 2017;120:472-495.
[26] Bevan S, Traylor M, Adib-Samii P, et al. Genetic heritability of ischemic stroke and the contribution of previously reported candidate gene and genomewide associations. *Stroke.* 2012;43:3161-3167.
[27] Zakai NA, Judd SE, Alexander K, et al. ABO blood type and stroke risk: the REasons for Geographic And Racial Differences in Stroke Study. *J Thromb Haemost.* 2014;12:564-570.
[28] Malik R, Traylor M, Pulit SL, et al. Low-frequency and common genetic variation in ischemic stroke: The METASTROKE collaboration. *Neurology.* 2016;86:1217-1226.
[29] Traylor M, Farrall M, Holliday EG, et al. Genetic risk factors for ischaemic stroke and its subtypes (the METASTROKE collaboration): a meta-analysis of genome-wide association studies. *The Lancet Neurology.* 2012;11:951-962.
[30] Bellenguez C, Bevan S, Gschwendtner A, et al. Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke. *Nat Genet.* 2012;44:328-333.
[31] Han Z, Dong X, Zhang C, et al. Polymorphism of HDAC9 Gene Is Associated with Increased Risk of Acute Coronary Syndrome in Chinese Han Population. *BioMed Research International.* 2016;2016:3746276.
[32] Dichgans M, Pulit SL, Rosand J. Stroke genetics: discovery, biology, and clinical applications. *The Lancet Neurology.* 2019;18:587-599.
[33] Malik R, Chauhan G, Traylor M, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet.* 2018;50:524-537.
[34] Torres-Aguila NP, Carrera C, Giese A-K, et al. Genome-Wide Association Study of White Blood Cell Counts in Patients With Ischemic Stroke. *Stroke.* 2019;50:3618-3621.
[35] Chamorro Á, Dirnagl U, Urra X, et al. Neuroprotection in acute stroke: targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. *The Lancet Neurology.* 2016;15:869-881.
[36] Hong Y, Tang HR, Ma M, et al. Multiple sclerosis and stroke: a systematic review and meta-analysis. *BMC neurology.* 2019;19:139.
[37] Beecham AH, Patsopoulos NA, Xifara DK, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat Genet.* 2013;45:1353-1360.
[38] Sawcer S, Hellenthal G, Pirinen M, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature.* 2011;476:214-219.
[39] Fogel LA, Yokoyama WM, French AR. Natural killer cells in human autoimmune disorders. *Arthritis Research & Therapy.* 2013;15:216.
[40] Gross CC, Schulte-Mecklenbeck A, Wiendl H, et al. Regulatory Functions of Natural Killer Cells in Multiple Sclerosis. *Frontiers in Immunology.* 2016;7.
[41] Mimpen M, Smolders J, Hupperts R, et al. Natural killer cells in multiple sclerosis: A review. *Immunol Lett.* 2020;222:1-11.
[42] Morandi B, Bramanti P, Bonaccorsi I, et al. Role of natural killer cells in the pathogenesis and progression of multiple sclerosis. *Pharmacol Res.* 2008;57:1-5.
[43] Melsen JE, Lugthart G, Lankester AC, et al. Human Circulating and Tissue-Resident CD56bright Natural Killer Cell Populations. *Frontiers in Immunology.* 2016;7.
[44] Bielekova B, Catalfamo M, Reichert-Scrivner S, et al. Regulatory CD56(bright) natural killer cells mediate immunomodulatory effects of IL-2Ralpha-targeted therapy (daclizumab) in multiple sclerosis. *Proceedings of the National Academy of Sciences of the United States of America.* 2006;103:5941-5946.
[45] Bielekova B, Howard T, Packer AN, et al. Effect of anti-CD25 antibody daclizumab in the inhibition of inflammation and stabilization of disease progression in multiple sclerosis. *Arch Neurol.* 2009;66:483-489.
[46] Saraste M, Irjala H, Airas L. Expansion of CD56Bright natural killer cells in the peripheral blood of multiple sclerosis patients treated with interferon-beta. *Neurol Sci.* 2007;28:121-126.
[47] Jiang W, Chai NR, Maric D, et al. Unexpected role for granzyme K in CD56bright NK cell-mediated immunoregulation of multiple sclerosis. *J Immunol.* 2011;187:781-790.
[48] Gan Y, Liu Q, Wu W, et al. Ischemic neurons recruit natural killer cells that accelerate brain infarction. *Proceedings of the National Academy of Sciences of the United States of America.* 2014;111:2704-2709.

[49] Kawasaki T, Kawai T. Toll-Like Receptor Signaling Pathways. *Frontiers in Immunology.* 2014;5.
[50] Reynolds JM, Pappu BP, Peng J, et al. Toll-like receptor 2 signaling in CD4(+) T lymphocytes promotes T helper 17 responses and regulates the pathogenesis of autoimmune disease. *Immunity.* 2010;32:692-702.
[51] Hanafy KA, Sloane JA. Regulation of remyelination in multiple sclerosis. *FEBS Lett.* 2011;585:3821-3828.
[52] Reynolds JM, Martinez GJ, Chung Y, et al. Toll-like receptor 4 signaling in T cells promotes autoimmune inflammation. *Proceedings of the National Academy of Sciences.* 2012;109:13064.
[53] Caso JR, Pradillo JM, Hurtado O, et al. Toll-like receptor 4 is involved in brain damage and inflammation after experimental stroke. *Circulation.* 2007;115:1599-1608.
[54] Brea D, Blanco M, Ramos-Cabrer P, et al. Toll-like receptors 2 and 4 in ischemic stroke: outcome and therapeutic values. *Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism.* 2011;31:1424-1431.
[55] Wasko NJ, Nichols F, Clark RB. Multiple sclerosis, the microbiome, TLR2, and the hygiene hypothesis. *Autoimmun Rev.* 2020;19:102430.
[56] Prass K, Meisel C, Höflich C, et al. Stroke-induced immunodeficiency promotes spontaneous bacterial infections and is mediated by sympathetic activation reversal by poststroke T helper cell type 1-like immunostimulation. *The Journal of experimental medicine.* 2003;198:725-736.
[57] Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. *Blood.* 2008;112:1557-1569.
[58] Gu L, Xiong X, Zhang H, et al. Distinctive effects of T cell subsets in neuronal injury induced by cocultured splenocytes in vitro and by in vivo stroke in mice. *Stroke.* 2012;43:1941-1946.
[59] Havrdová E, Belova A, Goloborodko A, et al. Activity of secukinumab, an anti-IL-17A antibody, on brain lesions in RRMS: results from a randomized, proof-of-concept study. *J Neurol.* 2016;263:1287-1295.
[60] Panitch HS, Hirsch RL, Schindler J, et al. Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. *Neurology.* 1987;37:1097-1102.
[61] Reichardt LF. Neurotrophin-regulated signalling pathways. *Philosophical Transactions of the Royal Society B: Biological Sciences.* 2006;361:1545-1564.
[62] Sachs BD, Baillie GS, McCall JR, et al. p75 neurotrophin receptor regulates tissue fibrosis through inhibition of plasminogen activation via a PDE4/cAMP/PKA pathway. *The Journal of cell biology.* 2007;177:1119-1132.
[63] Dowling P, Ming X, Raval S, et al. Up-regulated p75^{NTR} neurotrophin receptor on glial cells in MS plaques. *Neurology.* 1999;53:1676.
[64] Moskowitz MA, Lo EH, Iadecola C. The science of stroke: mechanisms in search of treatments. *Neuron.* 2010;67:181-198.
[65] Vidaurre ÓG, Gascón S, Deogracias R, et al. Imbalance of neurotrophin receptor isoforms TrkB-FL/TrkB-T1 induces neuronal death in excitotoxicity. *Cell Death & Disease.* 2012;3:e256-e256.
[66] Hu ZL, Luo C, Hurtado PR, et al. Brain-derived neurotrophic factor precursor in the immune system is a novel target for treating multiple sclerosis. *Theranostics.* 2021;11:715-730.
[67] Dutta R, McDonough J, Chang A, et al. Activation of the ciliary neurotrophic factor (CNTF) signalling pathway in cortical neurons of multiple sclerosis patients. *Brain.* 2007;130:2566-2576.
[68] Kuhlmann T, Remington L, Cognet I, et al. Continued administration of ciliary neurotrophic factor protects mice from inflammatory pathology in experimental autoimmune encephalomyelitis. *Am J Pathol.* 2006;169:584-598.
[69] Konopacki C, Pritykin Y, Rubtsov Y, et al. Transcription factor Foxp1 regulates Foxp3 chromatin binding and coordinates regulatory T cell function. *Nat Immunol.* 2019;20:232-242.
[70] Wang H, Geng J, Wen X, et al. The transcription factor Foxp1 is a critical negative regulator of the differentiation of follicular helper T cells. *Nat Immunol.* 2014;15:667-675.
[71] Hu H, Wang B, Borde M, et al. Foxp1 is an essential transcriptional regulator of B cell development. *Nat Immunol.* 2006;7:819-826.
[72] Braccioli L, Vervoort SJ, Adolfs Y, et al. FOXP1 Promotes Embryonic Neural Stem Cell Differentiation by Repressing Jagged1 Expression. *Stem Cell Reports.* 2017;9:1530-1545.
[73] Sagardoy A, Martinez-Ferrandis JI, Roa S, et al. Downregulation of FOXP1 is required during germinal center B-cell function. *Blood.* 2013;121:4311-4320.
[74] Li X, Xiao J, Fröhlich H, et al. Foxp1 Regulates Cortical Radial Migration and Neuronal Morphogenesis in Developing Cerebral Cortex. *PLoS One.* 2015;10:e0127671.
[75] Tang B, Becanovic K, Desplats PA, et al. Forkhead box protein p1 is a transcriptional repressor of immune signaling in the CNS: implications for transcriptional dysregulation in Huntington disease. *Hum Mol Genet.* 2012;21:3097-3111.
[76] O'Roak BJ, Deriziotis P, Lee C, et al. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. *Nat Genet.* 2011;43:585-589.

[77] Jay K, Mitra A, Harding T, et al. Identification of a de novo FOXP1 mutation and incidental discovery of inherited genetic variants contributing to a case of autism spectrum disorder and epilepsy. *Molecular genetics & genomic medicine.* 2019;7:e00751-e00751.
[78] Bot PT, Grundmann S, Goumans MJ, et al. Forkhead box protein P1 as a downstream target of transforming growth factor-β induces collagen synthesis and correlates with a more stable plaque phenotype. *Atherosclerosis.* 2011;218:33-43.
[79] Soehnlein O, Libby P. Targeting inflammation in atherosclerosis - from experimental insights to the clinic. *Nature Reviews Drug Discovery.* 2021;20:589-610.
[80] Guo Z, Tao Y, Yin S, et al. The transcription factor Foxp1 regulates the differentiation and function of dendritic cells. *Mechanisms of Development.* 2019;158:103554.
[81] Hudmon A, Lebel E, Roy H, et al. A mechanism for Ca2+/calmodulin-dependent protein kinase II clustering at synaptic and nonsynaptic sites based on self-association. *The Journal of neuroscience: the official journal of the Society for Neuroscience.* 2005;25:6971-6983.
[82] Rokita AG, Anderson ME. New therapeutic targets in cardiology: arrhythmias and Ca2+/calmodulin-dependent kinase II (CaMKII). *Circulation.* 2012;126:2125-2139.
[83] Gong J, Qiu C, Huang D, et al. Integrative functional analysis of super enhancer SNPs for coronary artery disease. *J Hum Genet.* 2018;63:627-638.
[84] Ye J, Das S, Roy A, et al. Ischemic Injury-Induced CaMKIIδ and CaMKIIγ Confer Neuroprotection Through the NF-κB Signaling Pathway. *Mol Neurobiol.* 2019;56:2123-2136.
[85] Doran AC, Ozcan L, Cai B, et al. CAMKIIγ suppresses an efferocytosis pathway in macrophages and promotes atherosclerotic plaque necrosis. *J Clin Invest.* 2017;127:4075-4089.
[86] Yokoyama WM, Plougastel BF. Immune functions encoded by the natural killer gene complex. *Nat Rev Immunol.* 2003;3:304-316.
[87] Germain C, Bihl F, Zahn S, et al. Characterization of alternatively spliced transcript variants of CLEC2D gene. *J Biol Chem.* 2010;285:36207-36215.
[88] Rosen DB, Bettadapura J, Alsharifi M, et al. Cutting edge: lectin-like transcript-1 is a ligand for the inhibitory human NKR-P1A receptor. *J Immunol.* 2005;175:7796-7799.
[89] Aldemir H, Prod'homme V, Dumaurier MJ, et al. Cutting edge: lectin-like transcript 1 is a ligand for the CD161 receptor. *J Immunol.* 2005;175:7791-7795.
[90] Llibre A, López-Macías C, Marafioti T, et al. LLT1 and CD161 Expression in Human Germinal Centers Promotes B Cell Activation and CXCR4 Downregulation. *J Immunol.* 2016;196:2085-2094.
[91] Rosen D, Cao W, Avery D, et al. Functional Consequences of Interactions between Human NKR-P1A and Its Ligand LLT1 Expressed on Activated Dendritic Cells and B Cells. *J Immunol.* 2008;180:6508-6517.
[92] Hofmann K, Düker M, Fink T, et al. Human neutral amino acid transporter ASCT1: structure of the gene (SLC1A4) and localization to chromosome 2p13-p15. *Genomics.* 1994;24:20-26.
[93] Yang J, Li H, Hu S, et al. ACE2 correlated with immune infiltration serves as a prognostic biomarker in endometrial carcinoma and renal papillary cell carcinoma: implication for COVID-19. *Aging (Albany NY).* 2020;12:6518-6535.
[94] Oshel KM, Knight JB, Cao KT, et al. Identification of a 30-base pair regulatory element and novel DNA binding protein that regulates the human GLUT4 promoter in transgenic mice. *J Biol Chem.* 2000;275:23666-23673.
[95] Knight JB, Eyster CA, Griesel BA, et al. Regulation of the human GLUT4 gene promoter: interaction between a transcriptional activator and myocyte enhancer factor 2A. *Proceedings of the National Academy of Sciences of the United States of America.* 2003;100:14725-14730.
[96] Dhaouadi N, Li JY, Feugier P, et al. Computational identification of potential transcriptional regulators of TGF-ß1 in human atherosclerotic arteries. *Genomics.* 2014;103:357-370.
[97] Ai J, Wang Y, Tan K, et al. A human homolog of mouse Lbh gene, hLBH, expresses in heart and activates SRE and AP-1 mediated MAPK signaling pathway. *Molecular biology reports.* 2008;35:179-187.
[98] Al-Ali H, Rieger ME, Seldeen KL, et al. Biophysical characterization reveals structural disorder in the developmental transcriptional regulator LBH. *Biochem Biophys Res Commun.* 2010;391:1104-1109.
[99] Ekwall A-KH, Whitaker JW, Hammaker D, et al. The Rheumatoid Arthritis Risk Gene LBH Regulates Growth in Fibroblast-like Synoviocytes. *Arthritis & rheumatology (Hoboken, NJ).* 2015;67:1193-1202.
[100] Rieger ME, Sims AH, Coats ER, et al. The embryonic transcription cofactor LBH is a direct target of the Wnt signaling pathway in epithelial development and in aggressive basal subtype breast cancers. *Molecular and cellular biology.* 2010;30:4267-4279.

[101] Menet R, Lecordier S, ElAli A. Wnt Pathway: An Emerging Player in Vascular and Traumatic Mediated Brain Injuries. *Frontiers in physiology.* 2020;11:565667-565667.
[102] Lambert C, Cisternas P, Inestrosa NC. Role of Wnt Signaling in Central Nervous System Injury. *Mol Neurobiol.* 2016;53:2297-2311.
[103] Vandebergh M, Andlauer TFM, Zhou Y, et al. Genetic Variation in WNT9B Increases Relapse Hazard in Multiple Sclerosis. *Ann Neurol.* 2021;89:884-894.

EPISODIC ATAXIA 1 & 2: CLINICAL CHARACTERISTICS, DIAGNOSIS, AND MANAGEMENT

Martha Spilioti, Andreas Kiryttopoulos, Maria Moschou, Vasilios K. Kimiskidis

1st Department of Neurology, Aristotle University of Thessaloniki, AHEPA Hospital

Abstract

Episodic Ataxias (EAs) are autosomal-dominant inherited ion channelopathies presenting as brief paroxysmal attacks of ataxia with a wide spectrum of associated ictal and interictal neurological symptoms. Episodic Ataxia type 1, (EA1) and Episodic Ataxia type 2(EA2) are the most common forms of EAs, caused by mutations of genes altering the function of the potassium (KCNA1) and calcium (CACNA1A) channels respectively. EA1 is associated with interictal myokymia while EA2 with interictal persistent nystagmus. Moreover, patients with EA2 may present with progressive ataxia and atrophy of the cerebellar vermis. Pharmacological treatments are available for the management of EA1 and EA2.Treatment of choice for EA1 is carbamazepine whereas acetazolamide has a variable effect. In patients with EA2, acetazolamide and 4-aminopyridine seem to be helpful in decreasing the frequency of attacks. Given the genetic and phenotypic heterogeneity of episodic ataxias, next generation sequencing (NGS) could be a diagnostic tool leading to specific and more efficacious therapies.

Key words: episodic ataxia type 1, episodic ataxia type 2, genetic channelopathies, KCNA1, CACNA1A

Introduction

Episodic Ataxias (EAs) represent a group of rare neurological disorders with clinical and genetic heterogeneity. (EAs) are ion channelopathies inherited in an autosomal dominant manner but some sporadic cases have been also described. They are characterized by brief recurrent paroxysmal episodes of ataxia, with a broad spectrum of additional ictal and interictal clinical symptoms [1]. Until now, eight subtypes (EA1-EA8) have been defined by the Online Mendelian Inheritance in Man (OMIM) according to clinical and genetic characteristics. The most common types, EA1 and EA2, are channelopathies caused by mutations of genes altering the function of potassium (KCNA1) and calcium channel (CACNA1A) respectively [2, 3]. Episodic ataxias (EAs)may have also clinical and genetic overlapping with other paroxysmal disorders as familial hemiplegic migraine1 (FHM1), spinocerebellar ataxia type 6 (SCA6) and epilepsy. In this manuscript, we review the literature and focus on the clinical presentation, genetic features and management of the most frequent forms of EAs, EA1 and EA2.

Clinical and genetic features of episodic ataxia 1 and 2

Episodic ataxia type 1

The prevalence of EA1 is estimated at 1 in 500,000 [4]. EA1 can result from mutations to the potassium channel gene KCNA1, which encodes the voltage-gated potassium channel subunit Kv1.1 [5, 6]. Kv1.1 subunits are expressed in both the central and the peripheral nervous system. However, their highest level is noticed in the Purkinje cells and cerebellar interneurons where they play an important role in nerve repolarization, eventually affecting the inhibitory outputs of the cerebellum [5, 7]. In EA1, a mutation of the KCNA1 gene causes dysfunction of the potassium channel subunit Kv1.1, resulting in excessive inhibition of Purkinje cells outputs, due to hyperexcitability of interneurons [8, 9]. In a small percentage of patients with EA1 symptoms, no mutations were found in the KCNA1 gene, implying the presence of other causative genes [10]. EA1 is characterized by paroxysmal recurrent episodes of vertigo, imbalance and interictal myokymia [11, 12]. Attacks are brief and last from seconds to less than 15 minutes, although longer duration of episodes has been described [13, 14]. During episodes the patients present-cerebellar symptoms, such as incoordination, tremor, dysarthria and imbalance accompanied by diplopia, vomiting, painful body stiffness, diaphoresis and headache [5, 8, 11, 15]. The pathognomonic hallmark of EA1 is the presence of constant interictal myokymia, usually of the perioral, periocular or distal extremities muscles [4, 10, 11]. The frequency of these episodes varies from several times daily to once per month and decreases in adulthood [16]. Episodes can occur spontaneously or may be triggered by stress, physical

exercise, alcohol or caffeine intake, hunger, fever, pregnancy or menstruation in women, temperature, and kinesiogenic stimuli [10, 13]. In addition, many patients present symptoms and signs of neuromyotonia, such as muscle stiffness and twitching, reflecting the involvement of the peripheral nervous system [7]. EA1 onset is typically before the age of 20. Later in the disease course, 20% of patients will develop permanent cerebellar signs and symptoms [10]. It is worth mentioning, that EA1 may also manifest with atypical symptoms such as choreoathetosis, skeletal deformities, delayed motor development, isolated myokymia or neuromyotonia, malignant hyperthermia, cognitive dysfunction, dyspnea during episodes and carpal spasms [12, 13, 17-22]. Epilepsy is represented predominantly in Episodic Ataxia type 1. EA 1 may be associated with generalized tonic-clonic seizures as well as focal seizures. There are also reported cases of photo-sensitive epilepsy [23] and seizures with head and eyes deviation, eyelid fluttering and lip-smacking. [24].

Episodic ataxia type 2

EA2 represents the most common and well characterized subtype of episodic ataxias with an estimated prevalence of less than 1 in 100,000 [3]. Typically, disease onset is between 5 and 20 years of age, even though late-onset cases have been described [16, 25].

EA2 is caused by truncating mutations in CACNA1A gene with loss of function effect [26]. The CACNA1A gene encodes the α1 pore-forming subunit of the neuronal voltage-gated P/Q-type calcium channel [27]. The P/Q-type calcium channel is highly expressed in the cerebellum, particularly in Purkinje cells and granular layer neurons. This neuronal calcium channel has a crucial role in CNS synaptic transmission, as it is located on presynaptic nerve terminals leading to the release of neurotransmitters [28].

EA2 is characterized by paroxysmal episodes of cerebellar dysfunction presenting with incoordination, oscillopsia, vertigo, nausea, ataxia, dysarthria, and nystagmus, which is present as an ictal and interictal sign [25, 26]. Additional ictal clinical features include migraine-like symptoms, dystonia, hemiplegia, and generalized weakness. Typically, patients preserve consciousness during the episodes. EA2 is related to an increased risk of absence epilepsy between episodes [23, 29]. According to recent studies, patients with EA2 demonstrate cognitive dysfunction, with impairment in many domains, as well as psychiatric disorders, such as psychosis, autism, depression, and schizophrenia [30, 31]. Of note, some patients can present initially with paroxysmal torticollis or paroxysmal tonic upward gaze, years before the emergence of ataxic episodes [32, 33]. Episodes usually are triggered by heat, alcohol or caffeine intake, phenytoin, stress, startle, physical exercise or fever [25, 26, 32]. On the contrary, sleep or rest seem to attenuate the attacks [26, 32, 33]. The duration of the episodes ranges between hours to days, while the frequency varies from many times per week to once per year [25].

Other subtypes of episodic ataxias (EA3-EA8)

There are six other rare subtypes of inherited episodic ataxias (EA3 - EA8). These types have been observed in single families. On most occasions the responsible gene was not identified, expect for the types EA5 and EA6.

EA3 was reported in one family and is characterized by ataxia episodes of short duration with additional signs identical to those of EA1 [34]. However, in EA3 the patients present tinnitus as additional feature to the ataxia, in contrast with EA1 [34]. Episodes of ataxia in EA3 are reported as responsive to acetazolamide.

EA4, previously known as periodic vestibulocerebellar ataxia, was found in two multigenerational North Carolina families [35]. EA4 is considered a late onset episodic ataxia that occurs between third to sixth decade. Patients with EA4 share similar clinical characteristics with EA3, expect the presence of oculomotor manifestations, as gaze-evoked nystagmus and defective smooth pursuit [35]. The episodes of ataxia are not responsive to acetazolamide, as opposed to the most of EAs. It's worth mentioning, that in an autopsy of an old patient with EA4, identical neuropathological findings with SCA-6 has been identified, implying a possible role of CAG repeats in the pathophysiology of EA4. [36]. In EA5 a mutation in CACNB4 gene, that codes the β4 subunit of the voltage-dependent calcium channel, was found in three families [37, 38]. Noteworthily, the same mutation was also reported in a German family with epilepsy without symptoms of ataxia [37]. EA5 is characterized by paroxysmal attacks of ataxia similar to those described in EA2, but with later onset of symptoms [15]. In one patient with EA5 permanent cerebellar ataxia was described [38].

EA6 is due to mutations in the SLC1A3 gene. It was described in a 10-year-old boy and a Dutch family with different clinical phenotypes [39, 40].The 10-year-old patient presented with a combination of ataxia, hemiplegic migraine, and seizures, while the three family members had isolated symptoms of episodic ataxia [39-41].

EA7 was reported only in a single family with similar ictal but without the interictal features of EA2 [42]. EA7 gene locus has been identified to chromosome 19q13.

EA8 was recently identified in an Irish family with symptoms of episodic ataxia [43].The attacks were

characterized by generalized weakness, dysarthria, and unsteadiness, while some of the affected members presented myokymia, twitching around the eyes and nystagmus. Interestingly, these patients responded only to clonazepam with no benefit from acetazolamide. EA8 is considered to be related to a heterozygous mutation in UBR4 gene [43]. In addition, two unrelated cases with symptoms of episodic ataxia and mutation in UBR4 have been described [44].

Diagnosis

Diagnosis of EA1 and EA2 is mainly based on clinical presentation and molecular genetic testing for KCNA1 and CACNA1A mutations respectively [10]. EA molecular testing should be applied in the presence of paroxysmal or chronic cerebellar signs, particularly in patients with positive family history [8, 36]. In many cases the genetic test has revealed de novo mutations, so the presence of positive family history is not mandatory for the application of CACNA1A or KCNA1 sequencing [22]. EA2 should be considered also in cases with developmental delay, early onset paroxysmal dystonia, epilepsy, or epileptic encephalopathies when family history for EAs or chronic cerebellar signs coexist [32]. Furthermore, during CACNA1A sequencing all the exons must be screened for mutations. Until to date >100 mutations in CACNA1A have been reported, while in EA1 only 47 mutations [46]. In EA1, brain MRI is often normal, while in EA2 cerebellar atrophy, particularly of the vermis is evident [10, 15]. Routine nerve conduction studies in both EA1 and EA2 are normal [33]. However, EMG in patients with EA1 is characterized by the presence of myokymia and the typical findings of neuromyotonia, especially in the muscles of the hand [10, 12]. In EA2, EMG and nerve conduction studies are without significant findings, even though abnormal jitter in single fiber EMG has been described, indicating neuromuscular dysfunction [23, 47]. In addition, epileptiform EEG activity is common in EA2. However, the key clinical diagnostic features for EA1 and EA2 are mainly the interictal manifestations. EA1 presents with interictal myokymia whereas patients with EA2 exhibit interictal nystagmus (downbeat, gaze-evoked or rebound nystagmus) [4].

Finally, EA1 and EA2 –as paroxysmal movement disorders (PxDs)– must be also distinguished from other genetic syndromes with PxDs, mainly from kinesigenic dyskinesia and paroxysmal non kinesigenic dyskinesia [45]. EAs, as mentioned above, are characterized by episodes of cerebellar dysfunction, while PxDs present with attacks of hyperkinetic movements.

A summarized overview of clinical features and differential diagnosis between EA1 and EA2, is provided in Table 1.

Therapeutic management

Episodic ataxia type 1

Numerous drugs can improve EA1 symptoms but until now, in the absence of comparative studies and trials, no drug has been shown to be strongly effective. In addition, responses to treatment are variable. It is worth noting that the therapeutic response varies even among genotypically similar individuals [8, 10].

The pharmacological treatment of EA1 is mainly based on carbamazepine (CBZ). Occasional response has also been described in treatment with phenytoin or acetazolamide [48]. In addition to the pharmacological treatments mentioned above, the known factors that cause attacks should be avoided. Behavioral measures such as avoiding stress, sudden movements, loud noises, and caffeine can be applied to reduce the manifestations of the disease. Physical and occupational therapies are recommended to enhance mobility, improve fine motor skills and reduce the risk of complications such as contractions, scoliosis or hip dislocation [49].

CBZ is a voltage-gated sodium channel blocker that reduces synaptic transmission of excitatory stimuli by stabilizing overstimulated nerve membranes. CBZ has been used in EA1 in doses up to 1,600 mg/day, with significant improvement in symptoms as well as frequency, severity and duration of attacks. However, CBZ's initial response was not maintained in some cases [50].

The occurrence and severity of EA1 paroxysmal attacks can be alleviated by acetazolamide (ACTZ), a carbonic anhydrase inhibitor. While some patients show improvement with ACTZ, the response to treatment is only occasional. It's mechanism of action in EA1 is unclear. ACTZ exhibits a regulatory effect on intracellular pH. Therefore, ionic channels and ionic conductivity in neuronal membranes can be regulated, causing both hyperpolarization and reduced excitability, which can in turn lead to reduced attacks. Alternatively, ACTZ may decrease the excitability of GABAergic neurons as a consequence of intracellular alkalinization. The usual starting dose is 125 mg with a gradual increase up to 1000 mg maximum in 1-4 divided doses [51]. Eventually the reduced effectiveness of ACTZ and the development of adverse effects lead to the discontinuation of treatment in many patients. Long-term side effects include neuropsychiatric manifestations, hallucinations, nephrolithiasis, rash, fatigue, hyperhidrosis, and gastrointestinal disorders. Treatment with ACTZ should be avoided in people with either hepatic or renal/adrenal insufficiency [50, 52, 53].

Phenytoin is another potent regulator of Na + channels that can also reduce symptoms of ataxia and myokymia in EA1 patients. Phenytoin is usually a second-line drug for typical attacks, at a dose of

Table 1. Clinical features/Differential diagnosis of Episodic Ataxias type 1 and 2

	EA1	EA2
Gene	KCNA1	CACNA1A
Chromosome	12p13	19p13
Channel type	Potassium channel, Kv1.1	Calcium channel, Cav2.1
Inheritance	Autosomal dominant	Autosomal dominant
Age of onset	1st to 2nd decade	2nd to 3rd decade
Attack duration	Seconds to minutes	Hours to days
Frequent ictal symptoms	Vertigo	Incoordination, vertigo, dysarthria
Interictal symptoms	Myokymia	Nystagmus, late onset persistent cerebellar syndrome
Episode triggers	Stress, physical exercise, alcohol or caffeine intake, hunger, fever, pregnancy or menstruation in women, temperature and kinesiogenic stimuli.	Heat, alcohol or caffeine intake, phenytoin, stress, startle, physical exercise or fever.
Associated features	Neuromyotonia, distal weakness, epilepsy, dysarthria	Migraine, dystonia, tremor, cognitive impairment, generalized weakness, tonic upward gaze
Treatment of choice	Carbamazepine Acetazolamide (variable response)	Acetazolamide (adequate response) 4-aminopyridine Dalfampridine
Brain MRI	Usually, normal	Cerebellar vermian atrophy
EMG	Neuromyotonia, myokymic discharges	Usually normal, abnormal jitter on single fiber EMG in patients with episodic weakness

Abbreviations: MRI: Magnetic resonance imaging, EMG: Electromyography

3.7 mg/kg/day. In some cases, phenytoin but not acetazolamide has been shown to be effective for both ataxia and dyskinesias. In some cases, however, phenytoin had no therapeutic effect. Phenytoin should be used cautiously especially in younger ages, as it can cause permanent cerebral atrophy and dysfunction.

Diphenylhydantoin at doses of 150-300 mg/day led to a reasonable seizure control in a number of patients. Sulthiame is a carbonic anhydrase inhibitor that reduces the occurrence of seizures, at doses of 50-200 mg/day. However abortive attacks of a few seconds can still be observed during treatment. Paresthesias and paroxysmal carpal spasms are some of the side effects associated with sulthiame use [50, 52].

Valproic acid and Lamotrigine are sometimes used as an alternative treatment, as they lead to a reduction in seizures in some patients. Recently, some authors suggested the use of riluzole as a possible treatment option for type 1 episodic ataxia. Although there are no reports of the use of riluzole in EA1, riluzole has been used successfully in patients with cerebellar ataxia of other causes, spinocerebellar and Friedrich's, ataxia without significant side effects. Its effectiveness must be confirmed by further studies in EA1 [50, 52, 53].

Episodic Ataxia 2

For the most common subtype of EA, episodic ataxia type 2, two treatment options have been described: acetazolamide, and 4-aminopyridine (4-AP). The general therapeutic principles that apply to all episodic ataxias include physiotherapy, kinesiotherapy, occupational therapy, speech therapy and special education as well as orthotic devices for gait disturbances. Patients should be encouraged to avoid any triggers that could exacerbate the symptoms. All patients and families should receive genetic counseling.

The treatment of choice for ataxic attacks in EA2 is ACTZ with a response rate around 50-70%. Initial dose is usually 250 mg/day in two divided doses. The effective daily dose may be between 250 and 1000

Table 2. Treatment of Episodic Ataxias type 1 and 2

	Drug	Dose	Comments
EA1	Carbamazepine	up to 1,600 mg daily	Initial response not maintained in some cases.
	Acetazolamide (ACTZ)	250- 1000 mg max in divided doses	Response to treatment is only occasional
	Phenytoin	3.7 mg/kg	Second-line drug for typical attacks Caution especially in younger ages Contraindicated in EA2.
	Diphenylhydantoin	150-300 mg daily	Adequate seizure control in some patients with epilepsy.
	Sulthiame	50-200 mg daily	Abortive attacks
	Valproic acid (VPA) & Lamotrigine (LMT)	VPA: 750mg daily, LMT:75-100mg daily	Alternative treatments
	Riluzole	50 mg bid	Possible treatment. No reports in EA1
EA2	Acetazolamide (ACTZ)	250-1000 mg daily in divided doses	Treatment of choice Higher doses may be required
	4-aminopyridine (4-AP)	5 mg tid.	For non-responders or having side effects from ACTZ. Contraindicated in patients with epilepsy
	Dalfabridine	10 mg bid	Further studies are needed
	Levetiracetam	250-1500 mg daily	Reports of a good response in combination with ACTZ
	Acetyl-DL-leucine	5 gr/day	In combination with 4-AP Further studies are needed n EA2
	Benzodiazepines	Low doses	Symptomatic relief (dizziness, nausea, sleep disorders)
	Chlorozoxazone	–	Only in mice models with EA2. Studies in human patients still needed.

mg, and higher doses may be required. Acetazolamide can stop attacks by lowering the abnormally high intracellular pH by subsequently activating and deactivating the sodium and calcium channels respectively. It is worth noting that some patients have no or only transient symptomatic benefit while others discontinue acetazolamide treatment due to adverse reactions [45].

4-AP is a selective potassium channel blocker that is believed to restore Purkinje cells pacemaking in the cerebellum [49]. 4-AP can be used in patients with EA2 who are non-responders to acetazolamide treatment or discontinued therapy due to side effects. The efficacy of 4-aminopyridine has been confirmed in a randomized controlled trial in adolescents and adult patients with EA2.This study showed a significant reduction in number, severity and duration of ataxia attacks compared to the placebo group as well as a significant improvement in the quality of life of patients. The usual dosage is 5-10 mg tid. 4-AP is contraindicated in patients with epilepsy due to dose-dependent risk of seizures [54-56].

Other drugs, including dalfabridine (prolonged-release 4-aminopyridine, fabridine), have been suggested as possible treatment options with positive results at 10 mg twice daily, but further studies are needed [57].

There are also reports of a good response with the combination of levetiracetam and acetazolamide [58]. This favorable outcome is believed to be due to the inactivation of calcium channels, induced by levetiracetam [59]. A good clinical response has been also described with the combination of 4-AP with the amino-acid acetyl-DL-leucine (5 gr day). Three case series that included different types of cerebellar ataxias showed that Acetyl-DL-leucine has considerably improved cerebellar symptoms, but further studies are needed for its efficacy in EA2 [60]. In addition, the

muscle relaxant chlorozoxazone has been proposed as a potentially new treatment based on mice models with EA2, but its effectiveness remains to be studied in humans [48]. Low-dose benzodiazepines can be used for symptomatic relief, in order to minimize symptoms of severe dizziness and nausea, but also to improve sleep disorders in patients.

Conclusions

EAs are underdiagnosed neurologic disorders. A better diagnostic approach is needed for the recognition and early treatment of patients with an EA phenotype. Next generation sequencing (NGS) will represent a diagnostic tool leading to specific and efficacious therapies for this heterogeneous group of genetic disorders [48]. Given the different gene mutations that are implicated in the pathogenesis of EAs, gene therapy could be a promising therapeutic option in the future by using splicing-based strategies [46].

References

[1] Jen, J.C. and J. Wan, Episodic ataxias. *Handb Clin Neurol*, 2018. 155: p. 205-215.

[2] Giunti, P., E. Mantuano, and M. Frontali, Episodic Ataxias: Faux or Real? *International Journal of Molecular Sciences*, 2020. 21(18): p. 6472.

[3] Ophoff, R.A., et al., Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. *Cell*, 1996. 87(3): p. 543-52.

[4] Kipfer, S. and M. Strupp, The Clinical Spectrum of Autosomal-Dominant Episodic Ataxias. *Movement Disorders Clinical Practice*, 2014. 1(4): p. 285-290.

[5] Jen, J.C., et al., Primary episodic ataxias: diagnosis, pathogenesis and treatment. *Brain*, 2007. 130(Pt 10): p. 2484-93.

[6] Browne, D.L., et al., Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, KCNA1. *Nat Genet*, 1994. 8(2): p. 136-40.

[7] Choi, K.D. and J.H. Choi, Episodic Ataxias: Clinical and Genetic Features. *J Mov Disord*, 2016. 9(3): p. 129-35.

[8] D'Adamo, M.C., et al., New insights into the pathogenesis and therapeutics of episodic ataxia type 1. *Front Cell Neurosci*, 2015. 9: p. 317.

[9] Paulhus, K. and L. Ammerman, *Clinical Spectrum of KCNA1 Mutations: New Insights into Episodic Ataxia and Epilepsy Comorbidity.* 2020. 21(8).

[10] Graves, T.D., et al., Episodic ataxia type 1: clinical characterization, quality of life and genotype-phenotype correlation. *Brain*, 2014. 137(Pt 4): p. 1009-18.

[11] Danti, F.R., et al., Pediatric Paroxysmal Exercise-Induced Neurological Symptoms: Clinical Spectrum and Diagnostic Algorithm. *Front Neurol*, 2021. 12: p. 658178.

[12] Rajakulendran, S., et al., Episodic ataxia type 1: a neuronal potassium channelopathy. *Neurotherapeutics*, 2007. 4(2): p. 258-66.

[13] Brunt, E.R. and T.W. van Weerden, Familial paroxysmal kinesigenic ataxia and continuous myokymia. *Brain*, 1990. 113 (Pt 5): p. 1361-82.

[14] Lee, H.Y., et al., The gene for paroxysmal non-kinesigenic dyskinesia encodes an enzyme in a stress response pathway. *Hum Mol Genet*, 2004. 13(24): p. 3161-70.

[15] Garone, G., et al., Clinical and Genetic Overview of Paroxysmal Movement Disorders and Episodic Ataxias. *Int J Mol Sci*, 2020. 21(10).

[16] D'Adamo, M.C., et al., Novel phenotype associated with a mutation in the KCNA1(Kv1.1) gene. *Front Physiol*, 2014. 5: p. 525.

[17] Shook, S.J., et al., Novel mutation in KCNA1 causes episodic ataxia with paroxysmal dyspnea. *Muscle Nerve*, 2008. 37(3): p. 399-402.

[18] Mestre, T.A., et al., A novel KCNA1 mutation in a family with episodic ataxia and malignant hyperthermia. *Neurogenetics*, 2016. 17(4): p. 245-249.

[19] Poujois, A., et al., Chronic neuromyotonia as a phenotypic variation associated with a new mutation in the KCNA1 gene. *J Neurol*, 2006. 253(7): p. 957-9.

[20] Demos, M.K., et al., A novel KCNA1 mutation associated with global delay and persistent cerebellar dysfunction. *Mov Disord*, 2009. 24(5): p. 778-82.

[21] Eunson, L.H., et al., Clinical, genetic, and expression studies of mutations in the potassium channel gene KCNA1 reveal new phenotypic variability. Ann Neurol, 2000. 48(4): p. 647-56.

[22] Klein, A., et al., Episodic ataxia type 1 with distal weakness: a novel manifestation of a potassium channelopathy. *Neuropediatrics*, 2004. 35(2): p. 147-9.

[23] Imbrici, P., et al., Dysfunction of the brain calcium channel CaV2.1 in absence epilepsy and episodic ataxia. *Brain*, 2004. 127(Pt 12): p. 2682-92.

[24] Zuberi, S.M., et al., A novel mutation in the human voltage-gated potassium channel gene (Kv1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy. *Brain*, 1999. 122 (Pt 5): p. 817-25.

[25] Jen, J., G.W. Kim, and R.W. Baloh, Clinical spectrum of episodic ataxia type 2. *Neurology*, 2004. 62(1): p. 17-22.

[26] Rajakulendran, S., D. Kaski, and M.G. Hanna, Neuronal P/Q-type calcium channel dysfunc-

tion in inherited disorders of the CNS. *Nat Rev Neurol*, 2012. 8(2): p. 86-96.
[27] Jodice, C., et al., Episodic ataxia type 2 (EA2) and spinocerebellar ataxia type 6 (SCA6) due to CAG repeat expansion in the CACNA1A gene on chromosome 19p. *Hum Mol Genet*, 1997. 6(11): p. 1973-8.
[28] Westenbroek, R.E., et al., Immunochemical identification and subcellular distribution of the alpha 1A subunits of brain calcium channels. *J Neurosci*, 1995. 15(10): p. 6403-18.
[29] Rajakulendran, S., et al., Genetic and functional characterisation of the P/Q calcium channel in episodic ataxia with epilepsy. *J Physiol*, 2010. 588(Pt 11): p. 1905-13.
[30] Bohne, P., et al., *Cognitive deficits in episodic ataxia type 2 mouse models.* 2021. 30(19): p. 1811-1832.
[31] Indelicato, E., et al., The neuropsychiatric phenotype in CACNA1A mutations: a retrospective single center study and review of the literature. *Eur J Neurol*, 2019. 26(1): p. 66-e7.
[32] Indelicato, E. and S. Boesch, From Genotype to Phenotype: Expanding the Clinical Spectrum of CACNA1A Variants in the Era of Next Generation Sequencing. *Front Neurol*, 2021. 12: p. 639994.
[33] Tomlinson, S.E., et al., Clinical neurophysiology of the episodic ataxias: insights into ion channel dysfunction in vivo. *Clin Neurophysiol*, 2009. 120(10): p. 1768-76.
[34] Steckley, J.L., et al., An autosomal dominant disorder with episodic ataxia, vertigo, and tinnitus. *Neurology*, 2001. 57(8): p. 1499-502.
[35] Damji, K.F., et al., Periodic vestibulocerebellar ataxia, an autosomal dominant ataxia with defective smooth pursuit, is genetically distinct from other autosomal dominant ataxias. *Arch Neurol*, 1996. 53(4): p. 338-44.
[36] Merrill, M.J., et al., Neuropathology in a case of episodic ataxia type 4. *Neuropathol Appl Neurobiol*, 2016. 42(3): p. 296-300.
[37] Escayg, A., et al., Coding and noncoding variation of the human calcium-channel beta4-subunit gene CACNB4 in patients with idiopathic generalized epilepsy and episodic ataxia. *Am J Hum Genet*, 2000. 66(5): p. 1531-9.
[38] González Sánchez, M., et al., Clinical manifestations of episodic ataxia type 5. *Neurol Clin Pract*, 2019. 9(6): p. 503-504.
[39] Jen, J.C., et al., Mutation in the glutamate transporter EAAT1 causes episodic ataxia, hemiplegia, and seizures. *Neurology*, 2005. 65(4): p. 529-34.
[40] de Vries, B., et al., Episodic ataxia associated with EAAT1 mutation C186S affecting glutamate reuptake. *Arch Neurol*, 2009. 66(1): p. 97-101.
[41] Pyle, A., et al., Exome sequencing in undiagnosed inherited and sporadic ataxias. *Brain*, 2015. 138(Pt 2): p. 276-83.
[42] Kerber, K.A., et al., A new episodic ataxia syndrome with linkage to chromosome 19q13. *Arch Neurol*, 2007. 64(5): p. 749-52.
[43] Conroy, J., et al., A novel locus for episodic ataxia:UBR4 the likely candidate. *Eur J Hum Genet*, 2014. 22(4): p. 505-10.
[44] Choi, K.D., et al., Genetic Variants Associated with Episodic Ataxia in Korea. *Sci Rep*, 2017. 7(1): p. 13855.
[45] De Gusmao, C.M. and L. Silveira-Moriyama, Paroxysmal movement disorders - practical update on diagnosis and management. *Expert Rev Neurother*, 2019. 19(9): p. 807-822.
[46] Jaudon, F., et al., *Targeting Alternative Splicing as a Potential Therapy for Episodic Ataxia Type 2.* 2020. 8(9).
[47] Maselli, R.A., et al., Presynaptic failure of neuromuscular transmission and synaptic remodeling in EA2. *Neurology*, 2003. 61(12): p. 1743-8.
[48] Orsucci, D., et al., Therapy of episodic ataxias: case report and review of the literature. *Drugs Context*, 2019. 8: p. 212576.
[49] Gandini, J. and M. Manto, *The neurological update: therapies for cerebellar ataxias in 2020.* 2020. 267(4): p. 1211-1220.
[50] Lauxmann, S., et al., Therapeutic Potential of Sodium Channel Blockers as a Targeted Therapy Approach in KCNA1-Associated Episodic Ataxia and a Comprehensive Review of the Literature. *Front Neurol*, 2021. 12: p. 703970.
[51] Hasan SM, D.A.M., *Episodic Ataxia Type 1*, in GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2021., A.H. Adam MP, Pagon RA, et al., Editor. 2010 Feb 9 [Updated 2018 Nov 1].
[52] Hasan SM, D.A.M., *Episodic Ataxia Type 1*, in GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2021., A.H. Adam MP, Pagon RA, et al., Editor. 2010 Feb 9 [Updated 2018 Nov 1.
[53] Delorme, C., C. Giron, and D. Bendetowicz, *Current challenges in the pathophysiology, diagnosis, and treatment of paroxysmal movement disorders.* 2021. 21(1): p. 81-97.
[54] Strupp, M., et al., A randomized trial of 4-aminopyridine in EA2 and related familial episodic ataxias. *Neurology*, 2011. 77(3): p. 269-75.
[55] Strupp, M., et al., Treatment of episodic ataxia type 2 with the potassium channel blocker 4-aminopyridine. *Neurology*, 2004. 62(9): p. 1623-5.
[56] Strupp, M., et al., Aminopyridines for the treatment of neurologic disorders. *Neurol Clin Pract*, 2017. 7(1): p. 65-76.

[57] Muth, C., et al., Fampridine and Acetazolamide in EA2 and Related Familial EA: A Prospective Randomized Placebo-Controlled Trial. *Neurol Clin Pract*, 2021. 11(4): p. e438-e446.
[58] Na, S. and T. Kim, *Efficacy of levetiracetam in patients with episodic ataxia type 2 caused by CACNA1A mutation: three case reports.* 2021. 42(9): p. 3897-3899.
[59] Lee, H., et al., *Effectiveness of levetiracetam in an acetazolamide-unresponsive patient with episodic ataxia type 2 by a novel CACNA1A nonsense mutation.* 2017. 24(7): p. e43-e44.
[60] Penkava, J., et al., A novel pathogenic CACNA1A variant causing episodic ataxia type 2 (EA2) spectrum phenotype in four family members and a novel combined therapy. *J Neurol*, 2020. 267(Suppl 1): p. 181-184.

WILSON DISEASE: CLINICAL CHARACTERISTICS, DIAGNOSIS, AND MANAGEMENT

Antonios Provatas, Anna Tasoudi, Zisis Tsouris, Vasileios Siokas, Georgia Xiromerisiou, Efthimios Dardiotis

Laboratory of Neurogenetics, Department of Neurology, University Hospital of Larissa, Faculty of Medicine, School of Health Sciences, Larissa, Greece

Abstract

Wilson disease is a rare genetic disease, affecting multiple systems. The cause of the disease are mutations in ATP7B gene and is inherited in an autosomal recessive manner according to Mendel's laws. Mutations in ATP7B gene cause a decrease in copper secretion and consequently copper hyperdeposition in many tissues and organs. The main clinical manifestations come from the liver and the central nervous system, but a plethora of other organs may be involved. The diagnosis can be established using the Leipzig criteria, but the final diagnosis requires genetic testing. Chelation therapy is the main treatment, but secondary manifestations require specific management. Although to date there is no effective treatment, the symptoms of the disease can be treated adequately with the existing treatments and patients usually have a good quality of life.

Introduction

Wilson disease (WD) or hepatolenticular degeneration is a rare hereditary disease which affects mainly liver and central nervous system (CNS) [1] while it rarely also affects other organs or systems [2]. The disease has a clear genetic background and it is inherited following the autosomal recessive manner according to Mendel's laws [1]. The disease prevalence is approximately 30 cases per million and the presentation age has a spectrum between 3 and 60 years. It was first described by Wilson in 1912 as a progressive lesion of the lenticular nucleus and liver with the main clinical manifestation being an early-onset dystonia. Earlier, Westphal described the pseudosclerotic form of the disease and in time it was perceived that parkinsonism is a key clinical symptom of the disease [1].

Genetic

Wilson disease is a genetic disease. From its first description, this disease was considered as a familial disease. The Wilson's disease gene was mapped to chromosome 13q14.3 and the causative gene was identified as ATP7B in 1985, and contains 21 exons [3, 4]. The ATP7B gene encodes a copper transporting (6 Cu molecules), P-type transmembrane ATPase that is highly expressed in the liver and kidney. Several missense mutations, small deletion/insertion in the coding region or splice junction mutations are responsible for the clinical expression of the WD. Although mutations have been described in almost all exons, the exons 8 and 14 are the mainly affected [5] (most common mutations are the R778L and the H1069Q) [6]. Studies suggest that 90-98% of patients with WD are heterozygotes with compound heterozygous mutations [6].

Pathophysiology

The protein which is encoded by ATP7B gene plays a key role in the pathophysiology of the disease. The ATP7B protein is a copper transporting, P-type transmembrane ATPase. The normal function of ATP7B protein seems to be the incorporation of copper into cell organelles leading to the production of ceruloplasmin and secretion of copper into the bloodstream, where excessive copper is excreted to the bile [7]. As a result of mutations in the ATP7B gene, the liver is not capable of excreting copper into bile and a positive copper balance is established. That leads to the accumulation of copper firstly in the liver and then in other parts of the body, such as in the brain [7, 8].

Clinical presentation

Hepatic Features

Wilson disease mainly affects the liver. The accumulation of copper in the liver leads to degeneration and failure of the organ. Manifestations of liver disease can be divided into some distinct categories of degeneration which present significant differences among them.

Liver disease can be completely asymptomatic and is usually identified due to hepatomegaly or during the screening process because patients have discretely elevated transaminase levels in serum [9]. Some patients present with simple, acute, self-limited hepatitis-like disease with anorexia, fatigue, and abdominal pain [10]. Rarely, WD can occur as autoimmune hepatitis, with arthropathy, malaise, fatigue, and rashes. This manifestation of WD responds well to chelation therapy even if cirrhosis is present [9, 10]. Other patients present with mild to moderate degree fatty liver disease presenting abnormal liver function [10]. Moreover, serum unbound copper, in high concentrations, may cause either acute or chronic hemolysis, leading to the manifestation of hemolytic anemia. Liver disease, as well as Kayser-Fleischer rings, are likely to be present. Recurrent hemolysis further predisposes to cholelithiasis, even in children, and recurrent episodes of jaundice [9-11]. Acute hepatitis in WD is rare and when it occurs, it presents with jaundice, elevated transaminase levels, and hepatic failure with coagulopathy. Direct Coombs test-negative hemolytic anemia may coexist with acute hepatitis. Hemolysis is thought to occur secondarily to marked copper release into the bloodstream due to the acute hepatic necrosis [7, 9]. The disease typically manifests as chronic active hepatitis which slowly progresses and leads to cirrhosis and portal hypertension [9, 10].

Neurological presentation

The disease affects various structures of the CNS. It mainly affects the basal ganglia but also the cerebellum and structures of the pons. In about 20% of WD patients, the disease presents with neurological symptoms [12].

Parkinsonism

Extrapyramidal manifestations of the disease, described in 62% of patients with WD, include dysarthria, resting tremor and gait disorders [1]. Extrapyramidal symptoms in WD resemble those of idiopathic Parkinsonism with typical half-body asymmetry [1, 12].

Dystonia

Dystonia is consider as a common symptom in patients with WD (65%) [13] and its onset should raise the suspicion of the disease. It could be focal, multifocal, and even generalized, while it can evolve from focal to generalized during the course of the disease [1, 12]. Sardonic laugh is a typical clinical presentation of dystonia affecting the muscles of the head. When the laryngeal muscles are affected, it may lead to speech disorders and the characteristic dystonic dysarthria [12].

Tremor

Tremor is a prominent manifestation of the onset of WD [7]. Wilsonian tremor can occur at rest, upon assumption of posture or with action. The arms are most frequently involved but the head and the legs can also be affected. In Wilsonian tremor, asymmetry prevails. The kinetic tremor is a medium to high frequency tremor. The classical posture-induced wing-beating tremor is a lower frequency tremor concerning the upper limbs [1, 2, 12].

Dysarthria

Dysarthria is probably the most common neurologic manifestation of WD. In patients with dysarthria, dysphagia frequently coexists and usually stems from dystonic vocal cords, dystonia of other head muscles, or tremor. Ataxic dysarthria is a less common manifestation of the disease [1, 2, 10, 12].

Choreoathetosis

Chorea is a common finding in early-onset WD. Choreic movements are irregular involuntary movements that appear at rest and they are superimposed on or interrupt normal movements. Chorea symptoms range from minor movements to severe uncontrolled movements affecting muscles of the head and the arm [1, 12].

Ataxia

Ataxia has been reported in 30% of patients with WD and it presents as hypermetria of the limbs and extraocular muscles [1, 10, 12].

Other neurologic features

Moreover, in WD cognitive decline, myoclonus, and tics have also been reported [1, 10, 12].

Psychiatric features

When psychiatric manifestations are the only symptom of WD, they are usually attributed to other causes and rarely raise the suspicion of the disease. Diagnosis of WD during this period is rare. At the onset of the disease, the most common psychiatric symptoms have been reported to be personality change, incongruous behavior, irritability, and delusional thoughts [1, 10]. At the same time cognitive impairment may occur with memory impairments and executive dysfunction [1]. Many patients present with self-harm ideas especially after the diagnosis is confirmed [10].

Ophthalmologic manifestations

Copper deposits in the paralimbal area of the cor-

Table 1. Neurological manifestations of WD

Neurological symptoms	***Characteristics***	***Percentage (%)***
Parkinsonism	Dysarthria, resting tremor, gait disorders, usually symmetrical	62 %
Dystonia	Focal, multifocal, generalized, dystonic dysarthria	65 %
Tremor	Resting tremor, kinetic tremor medium to high frequency, posture tremor lower frequency	30%
Dysarthria	In patients with dystonia, salivation coexists, ataxic dysarthria (uncommon)	30%
Choreoathetosis	Young onset, range from large to fine choreic movements, commonly affects head and arm muscles	10%
Ataxia	Dysmetria in upper limbs and eyes (nystagmus)	30%
Other	Cognitive decline, myoclonus, and tics	98%

nea, known as Kayser- Fleischer (KF) rings, which are seen in almost all patients with neurologic WD. KF rings are observed in the border region where the cornea transitions to the sclera and upon treatment, the intensity of the copper deposits is reduced [1, 2]. Occasionally, sunflower cataract may be observed upon slit lamp examination.

Other manifestations

As the disease is multisystemic, several other organs and tissues may be affected. Low-molecular weight proteinuria with microscopic hematuria, and Fanconi syndrome are common kidney manifestations. Furthermore, copper accumulation in synovial membranes can cause arthritis of large joints [10, 14]. In approximately 10% of affected individuals, reduced bone mineral density with a prevalence of osteoporosis is observed [10]. Less common manifestations of WD are rhabdomyolysis of skeletal muscles, cardiac arrhythmias, pancreatitis, cardiomyopathy, and various endocrine disorders [9, 10].

Depending on the manifestations and the age of onset, various classifications of the disease have been proposed. The main classification used concerns the onset symptomatology, as it shown in Table 2 [11].

Diagnostic workup

Early-onset extrapyramidal involvement should always raise suspicion of WD. The most common screening method is the evaluation of serum ceruloplasmin levels. Ceruloplasmin is usually low, although in approximately 10% of patients it may be marginally normal or even normal [2]. However, in children lower levels of ceruloplasmin have been detected in comparison to adults [10]. Of great importance is also the evaluation of copper levels in a 24-hour urine test. In symptomatic patients copper levels in urine are always increased to a value greater than 100 mcg/day while the normal levels are below 50mcg/day [9]. Given the increased incidence, every patient should be examined for KF rings during the work-up [10]. Most patients have decreased levels of serum copper, that reflects the decreased levels of serum ceruloplasmin. Thus, the measurement of non-ceruloplasmin-bound copper is essential [15, 16]. The excess in serum non-ceruloplasmin-bound copper is indicated by the combination of low ceruloplasmin serum concentration and a normal or high serum copper concentration [10]. Although not always reliable for diagnosis, due to its high dependency on the accuracy of both the serum ceruloplasmin concentration and the serum copper concentration, high serum non-ceruloplasmin-bound copper concentrations often reflects copper overload [9, 10]. In case of hepatic impairment, indicated by abnormal liver biochemistry tests, liver biopsy is recommended. In addition to liver damage, a biopsy may also detect and quantify copper deposition [8, 9].

If the laboratory tests strongly supports WD, a genetic test should be performed to confirm the diagnosis [2, 5, 9, 10]. Genetic testing may include single gene testing, a multigene panel, and more comprehensive genomic testing [1, 10].

Magnetic resonance imaging (MRI) of the brain is characterized by high intensity T2 signal lesions on basal ganglia and white matter, as well as high intensity T1 signal lesions caused by hepatic encephalopathy [1, 12].

Finally, dopamine transporter (DaT) scan has an auxiliary role in the diagnosis of WD by revealing the decreased uptake of radiotracer in the striatum [1].

Laboratory findings alone are not sufficient for establishing the diagnosis. Therefore, the Leipzig criteria, which combine clinical, laboratory and imaging parameters, are used. According to the Leipzig criteria, more than 4 points are required in order for the WD diagnosis to be established, while an alternative diagnosis should be considered in patients with less than 4 points. Based to the criteria, genetic testing

Table 2. Classification of WD

Clinical presentation	Onset Symptomatology
No neurologic presentation	
Preclinical	Non, preclinical diagnose
Hepatic	Acute or chronic hepatic lesions / cirrhosis
Pseudo-parkinsonism (stiffness, tremor)	Slow motion, stiffness, diminution, position / energy / rest / orthostatic tremor, postural reflex disorder, dysarthria, salivation
Pseudo-sclerotic (tremor)	Position / energy / calm / orthostatic tremor, ataxia, dysarthria
Mix (chorea-athetosis non rhythmic)	Chorea, athetosis, dystonia, parkinsonism
Psychiatric	

Table 3. Leipzig criteria

Signs/Symptoms	Score
Kayser-Fleischer ring	
present	2
Absent	0
Neurological symptomatology or finding in MRI	
Severe	2
Mild	1
Absent	0
Ceruloplasmin	
Normal range (.0,2g/L)	0
0.1-0.2 g/L	1
< 0,1g/L	2
Coombs negative hemolytic anemia	
Present	1
Absent	0
Liver Copper	
> 4μmol/g2	2
0.8-4μmol/g1	1
< 0.8μmol/g	-1
Rhodamine positive granules	1
Rhodamine positive granules	1
Urine Copper excretion	
Normal	0
1-2 times ULN	1
> 2 times ULN	2
5 times ULN after penicillamine	2
Mutation analysis detected	
Both chromosomes	4
One chromosome	1
No mutations	0

Score	Classification
≥ 4	Disease
3	Possibility of disease
≥ 2	Absence of disease

and liver biopsy may not be necessary if other test results add up to 4 points at least [9].

Differential diagnosis

The differential diagnosis of WD includes a great variety of diseases with hepatic and neurological manifestations similar to those of the disease.

Concerning hepatic manifestations, the differential diagnosis includes:

- Chronic viral hepatitis.
- Primary sclerosing cholangitis.
- Non-alcoholic steatohepatitis (NASH).
  Note: patients thought to have NASH, should have WD excluded due to available treatment.
- Autoimmune hepatitis.
- *HFE*-associated hereditary hemochromatosis.
- Drug hepatotoxicity.
- Alcoholic liver disease.
- Alpha-1-antitrypsin deficiency.
- Primary biliary cirrhosis.

Concerning neurologic manifestations, the differential diagnosis includes:

- Essential tremor.
- Parkinson disease.
- Dentatorubro-pallidoluysian atrophy.
- Huntington disease.
- Hyperthyroidism.
- Dopa-responsive dystonia.
- Neurodegenerative diseases.
- Inherited forms of dystonia.
- Hereditary ataxias.
- Drug effects or toxicity.
- Niemann-Pick disease type C [2, 10].

Treatment

The purpose of the treatment in WD is on one hand the reduction of the excess of serum copper and on the other hand the symptomatic treatment.

Early initiation of treatment in asymptomatic patients could prevent the onset of several disease manifestations. The removal of excess copper is achieved with chelating agents. D-penicillamine is used as the drug of choice. It binds circulating copper, reduces the affinity of copper for proteins and releases it from tissues. It also promotes the synthesis of metallothionein that binds copper and is excreted in the urine [9]. Improvement in liver function was shown in 90% of patients with hepatic impairment, while only 55% of patients with CNS involvement were improved [1, 9, 10]. Furthermore, treatment with D penicillamine, was found to worsen pre-existing symptoms, in 10-50% of the patients, which led to the recommendation of a gradual dose increase with a starting dose of 125 mg/day [1]. The maximum daily dose is 750mg-1000mg/day.

An alternative treatment is trientine which acts like D-penicillamine. Usually, the daily dose is 900-2700mg, but recent studies suggest a bolus daily dose 15mg/KBW. Trientine is better tolerated but has been associated with worsening of the initial neurological symptoms in higher rates than D-penicillamine [1, 9, 10]. Both drugs should be administered separately from other drugs and food in order to avoid absorption disorders of the drugs [9, 10]. Zinc supplements also play an important role in the treatment of the disease. Zinc promotes the synthesis of metallothionein in the intestine and increases the excretion of copper. The daily dosage is 50mg three times a day. Co- administration with chelating agents should be avoided because its action is neutralized [1, 7, 9, 10]. Finally, tetrathiomolybdate ammonium is another therapeutic option in neurological manifestations of WD. Tetrathiomolybdate reduces copper levels by reducing its absorption from the intestine, but also promotes its binding to bile-secreting proteins [10, 17]. During treatment, monitoring of the excretion of copper in 24-h urine samples is important. Urine copper should be evaluated every 2 weeks during the first 6 weeks of treatment, and every three months in the next 12-month period [9].Consumption of foods rich in copper, such as liver, brain, chocolate, mushrooms, shellfish, and nuts should be avoided [10].

Concerning the symptomatic treatment of neurological manifestations, administration of levodopa for the treatment of extrapyramidal symptoms as well as anticholinergics for the treatment of dystonia is of great importance. Moreover, psychiatric assessment is considered necessary when psychiatric manifestations are present [1].

Follow up

Lifelong follow up of the patients with WD is important. Usually, evaluation of serum copper levels and ceruloplasmin levels at least twice per year is required for further evaluation. Moreover, liver biochemistry testing, international normalized ratio (INR), urinalysis, complete blood count, and physical examination including neurologic assessment are also of an essential need. In patients with WD under chelation therapy, monitoring with urinalysis and complete blood count are required more often. An annual evaluation of urine copper levels in 24-hour urine test samples is also required. More frequent evaluation of the above is recommended in cases of poor compliance or in dosage adjustment [1, 9, 10, 17].

Conclusion

Wilson disease is a rare and severe multisystem genetic disease with no etiologic treatment. Furthermore, the established treatment options have several limitations. However, both early diagnosis and rapid initiation of treatment are of paramount importance to avoid irreversible organ and tissue lesions as well as to avoid the accumulation of disability. Further studies promise better and effective treatment options.

References

[1] Lorincz MT. Neurologic Wilson's disease. *Ann N Y Acad Sci.* 2010;1184:173-187.
[2] Pfeiffer RF. Wilson's disease. *Handb Clin Neurol.* 2011;100:681-709.
[3] Kumar SS, Kurian G, Eapen CE, et al. Genetics of Wilson's disease: a clinical perspective. *Indian J Gastroenterol.* 2012;31:285-293.
[4] Yamaguchi Y, Heiny ME, Gitlin JD. Isolation and characterization of a human liver cDNA as a candidate gene for Wilson disease. *Biochem Biophys Res Commun.* 1993;197:271-277.
[5] Bennett J, Hahn SH. Clinical molecular diagnosis of Wilson disease. *Semin Liver Dis.* 2011;31:233-238.
[6] Stattermayer AF, Entenmann A, Gschwantler M, et al. The dilemma to diagnose Wilson disease by genetic testing alone. *Eur J Clin Invest.* 2019;49:e13147.
[7] Poujois A, Woimant F. Wilson's disease: A 2017 update. *Clin Res Hepatol Gastroenterol.* 2018;42:512-520.
[8] Shribman S, Poujois A, Bandmann O, et al. Wilson's disease: update on pathogenesis, biomarkers and treatments. *J Neurol Neurosurg Psychiatry.* 2021;92:1053-1061.
[9] Lucena-Valera A, Perez-Palacios D, Munoz-Hernandez R, et al. Wilson's disease: Revisiting an old friend. *World J Hepatol.* 2021;13:634-649.
[10] Weiss KH. Wilson Disease. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews((R)).* Seattle (WA), 1993.

[11] Hermann W. Classification and differential diagnosis of Wilson's disease. *Ann Transl Med.* 2019;7:S63.
[12] Machado A, Chien HF, Deguti MM, et al. Neurological manifestations in Wilson's disease: Report of 119 cases. *Mov Disord.* 2006;21:2192-2196.
[13] Svetel M, Kozić D, Stefanova E, et al. Dystonia in Wilson's disease. *Movement disorders: official journal of the Movement Disorder Society.* 2001;16:719-723.
[14] Grandis DJ, Nah G, Whitman IR, et al. Wilson's disease and cardiac myopathy. *The American journal of cardiology.* 2017;120:2056-2060.
[15] Macintyre G, Gutfreund KS, Martin WR, et al. Value of an enzymatic assay for the determination of serum ceruloplasmin. *J Lab Clin Med.* 2004;144:294-301.
[16] Walshe JM, Clinical Investigations Standing Committee of the Association of Clinical B. Wilson's disease: the importance of measuring serum caeruloplasmin non-immunologically. *Ann Clin Biochem.* 2003;40:115-121.
[17] Weiss KH, Thurik F, Gotthardt DN, et al. Efficacy and safety of oral chelators in treatment of patients with Wilson disease. *Clin Gastroenterol Hepatol.* 2013;11:1028-1035 e1021-1022.

NEUROFIBROMATOSIS TYPE 1: CLINICAL CHARACTERISTICS, DIAGNOSIS AND MANAGEMENT

Ioannis Tsiverdis[1], Ioannis Zaganas[1, 2]

[1] Neurogenetics Laboratory, Medical School, University of Crete, Heraklion, Crete, Greece
[2] Neurology Department, University Hospital of Crete, Heraklion, Crete, Greece

Abstract

Neurofibromatosis type 1 (*NF1*) is caused by *NF1* gene pathogenic variants that are either inherited in an autosomal dominant pattern or occur *de novo*. Both the peripheral and the central nervous system are commonly affected in *NF1*, with neurofibromas, nerve sheath tumors, and optic pathway gliomas being the most prominent features. This nervous system involvement leads to heterogeneous clinical manifestations, including affective, cognitive, and behavioral problems, visual disturbances, sensory and motor deficits, and epilepsy. Other common manifestations of *NF1* concern the skin (café-au-lait macules, axillary freckling and dermal neurofibromas), the eye (Lisch nodules) and the skeleton (scoliosis, other bone abnormalities). Diagnosis rests largely on the clinical picture, following recently revised diagnostic criteria, and often entails the use of MRIs or other imaging techniques. Lately, confirmation of the diagnosis of *NF1* is increasingly pursued through genetic testing. Management of the disease has been symptomatic and occasionally surgical, with removal of tumors that cause functional or other type of disability. Advances in the understanding of the molecular pathophysiology of the disease, including the elucidation of the mechanism of the *NF1* gene-mediated suppression of tumorigenesis, have led to the development of targeted molecular therapies. Despite the advent of these novel diagnostic and therapeutic approaches, the mainstay of management is still based on high suspicion for the disease, even in the absence of family history, to enable early detection and accurate diagnosis. This timely diagnosis of *NF1* should be followed by a multidisciplinary approach targeting the multiple facets of this challenging disease.

Key words: neurofibromatosis type 1, *NF1* gene, neurofibromas, café au lait macules, optic pathway glioma

INTRODUCTION

Neurofibromatosis type 1 (*NF1*) is one of the most common neurogenetic disorders and the most common of the neurofibromatoses, a group of disorders that also includes neurofibromatosis type 2, and schwannomatosis [1, 2]. *NF1* invariably affects the central and the peripheral nervous system, but also other organs, including the skin and the bones, with the clinical manifestations of the disease being highly heterogeneous. It is a multisystemic genetic condition with high risk of benign and malignant tumor development, leading to significant morbidity and mortality.

Historically, there have been accounts of patients possibly affected by *NF1* dating several centuries ago [3]. However, Friedrich Daniel von Recklinghausen was the first to give a comprehensive description of a patient and coined the term "neurofibroma" in 1882; thus the disease came to bear his name [3]. More than a century had to pass before the formulation of the first widely adopted clinical criteria for the disease in 1987 [4] and the identification of the *NF1* gene where *NF1*-causing variants reside in 1990 [5-8]. Three decades later, advances in understanding of the molecular pathophysiology of the disease have led to targeted therapeutic options. These new treatment approaches have rekindled the interest of neurologists and other specialists to *NF1*. The purpose of this review is to offer an update on the developments in the field and better equip the general neurologist and other clinicians for early detection, accurate diagnosis, and effective management of patients with *NF1*.

GENETICS AND PATHOPHYSIOLOGY

The cause of *NF1* are germline loss-of-function pathogenic variants in the *NF1* gene, a large tumor-suppressor gene residing on chromosome 17q11.2, spanning 61 exons and generating several alternatively spliced transcripts [9, 10]. The *NF1* gene encodes a large (> 2,800 amino acids long) cytoplasmic protein called neurofibromin 1. This protein is found in various tissues, with high levels observed in the nervous system. Neurofibromin acts as regulator of RAS, a signaling protein that is primarily found in an inactivated (GDP-bound) form. Its activated (GTP-

bound) form regulates multiple cellular targets and promotes cell proliferation and survival. Neurofibromin inhibits activated RAS by enhancing hydrolysis of the RAS-bound GTP [9] and this translates to decreased cell proliferation and survival. In patients with *NF1*, dysfunctional neurofibromin encoded by an *NF1* gene carrying loss-of-function pathogenic variants fails to properly modulate RAS, which becomes hyperactivated and promotes cellular proliferation and tumorigenesis. RAS effects are mediated by various signaling cascades, such as the MEK-ERK and PI3K/AKT pathways [9]. Downstream, these two pathways activate, among others, the mammalian target of rapamycin (mTOR) and the mitogen-activated protein kinase (MAPK) pathway [11, 12].

Interestingly, approximately one out of two cases of *NF1* is caused by a *de novo* mutation, namely a mutation not present in the parents [11]. The other half of *NF1* cases result from autosomal dominant inheritance of the disease trait. There are two types of *NF1*, termed generalized (or non-segmental) and segmental (or mosaic). The latter is a rare form of *NF1* that involves only part of the body, mostly unilateral, due to a genetic variant emerging during post-fertilization embryogenesis [11]. If the variant emerges at the initial stages of embryonic development, the phenotype resembles generalized neurofibromatosis [10]. Patients with segmental *NF1* display a milder phenotype, account for one-twelfth of total *NF1* patients and have a low probability of passing the pathogenic variant to their off-springs [13].

In at least some patients with single heterozygous germline pathogenic variants in one copy of the *NF1* gene, affected cells from tissue biopsies display an additional loss-of-function variant in the second *NF1* gene copy. This is compatible with the "two-hit" theory, with the "first hit" being the parental germline *NF1* gene pathogenic variant and the "second hit" brought about from an additional mutational event disrupting the other wild-type allele in a specific somatic cell. This additional mutational event could be either a *de novo* loss-of-function variant or even deletion of the entire wild-type allele, with this process termed loss of heterozygosity [9]. Since the site and time of the second hit is highly unpredictable, the "two hit" theory explains the heterogeneity of the clinical picture of *NF1*, even among relatives, and the difficulty to perform genotype-phenotype correlations. Among the few genotype-phenotype correlations described thus far is the severe phenotype, with malignant peripheral nerve sheath tumors and learning disabilities, caused by germline deletion of the entire *NF1* gene [10].

EPIDEMIOLOGY

NF1 is one of the most common neurogenetic disorders with a prevalence approximately 1 in 2,000 to 6,000 people worldwide, with varying estimates among various countries [14]. These differences in prevalence, if not attributed to different methodologies used for prevalence estimation, may relate to founder effects or factors that affect the *de novo* mutation rate in the *NF1* gene [11]. To better interpret the various prevalence rates in different countries, one should take into account that NF1 leads to an 8-25-year reduction of life expectancy compared to the general population, which is mainly due to the increased rate of malignancies in these patients [14-16].

CLINICAL MANIFESTATIONS

Patients with *NF1* display a variety of symptoms and signs, predominantly related to the skin, the nervous system, and the bones, with onset at a young age [17]. Regardless of the rich phenotypic variability of the disease that often makes accurate diagnosis challenging, there are some common clinical features shared by most patients. Based on these features, the 1987 National Institutes of Health (NIH) Consensus Development Conference formulated diagnostic criteria for *NF1* [4]. These criteria dictate that for the diagnosis of *NF1* to be justified, two or more of the following manifestations should be present: 1) six or more cafe-au-lait macules in the skin (greater than 5mm in pre-pubertal individuals or 15mm in post-pubertal individuals), 2) axillary or inguinal freckling, 3) neurofibroma (two or more of any type, or one plexiform neurofibroma), 4) optic glioma, 5) distinctive osseous lesion (such as dysplasia of the sphenoid bone), 6) two or more Lisch nodules (iris hamartomas), 7) a first-degree relative with *NF1*. Even though these criteria show rather high sensitivity and specificity, there are not always optimal, especially in children that have not developed yet several of the *NF1*-associated clinical manifestations [18]. Having this in mind and following the advances on the understanding of the disease pathogenesis and the improvement of diagnostic methods, an update on the 1987 NIH diagnostic criteria has been published in 2021 [19, 20]. This update includes 1) the presence of two or more choroidal abnormalities (defined as bright, patchy nodules imaged by optical coherence tomography or near-infrared reflectance imaging) as an alternative to the Lisch nodules, 2) the extension of the optic glioma criterion to cover all the visual pathway tumors, 3) the addition of tibial dysplasia or pseudarthrosis of a long bone on the distinctive osseous lesions criterion, 4) the modification of the family history criterion to the presence of a parent with *NF1*, 5) the inclusion of a heterozygous *NF1* gene pathogenic variant with an allele fraction of 50% in apparently unaffected tissue, such as white blood cells, as an additional criterion.

NEUROLOGICAL CLINICAL MANIFESTATIONS

Neurodevelopmental abnormalities

For decades limited attention was paid to the neurodevelopmental manifestations of *NF1*; these are increasingly recognized in recent years and are nowadays considered among the most common features of the disease [21]. Even though children with *NF1* rarely score under the 70 IQ limit defining intellectual disability, they often score at the lower end of the population norms. Also, neurodevelopmental abnormalities are common in children with *NF1* and include impairment in general cognition, cognitive processing, learning (such as in reading, writing, and mathematics), executive function and visuospatial abilities [21]. Two common forms of neurodevelopmental disorders, attention-deficit-hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) have been recognized in *NF1* patients. Specifically, for ASD, it has been rather recently recognized as a feature of *NF1*, even though clinicians have long been reporting social difficulties of children with *NF1.* Finally, patients with *NF1* are at increased risk for anxiety, depression, and sleep disturbances. How *NF1* gene pathogenic variant- related neuronal dysfunction leads to neuropsychiatric disturbances is a matter of study. Regardless of this, early recognition of neuropsychiatric symptoms in children with *NF1* is imperative for effective management.

Neurological neoplastic disease

Neurofibroma, a benign tumor emerging from the nerves sheath of peripheral nerves, is the hallmark of *NF1* (Figure 1A, 2). In histological sections of neurofibromas, fibroblasts, blood vessels, perineural cells, mast cells and Schwann cells are found [22]. Neurofibromas are categorized in different subtypes, depending on the location they arise and other features: cutaneous, subcutaneous, spinal, paraspinal, plexiform and atypical [23]. Cutaneous neurofibromas, the most common form of neurofibroma, are well defined elastic masses that derive from the nerves of the skin. Subcutaneous neurofibromas arise underneath the epidermal layer, have not well-defined texture, and are often diagnosed with palpation. With advancing age, both cutaneous and subcutaneous neurofibromas increase in size and quantity, with an accelerated rate on adolescence and pregnancy. Neurofibromas forming near the spine (paraspinal) or rarely in the spine (spinal), even though benign, can lead to scoliosis, spinal deformities, and spinal cord and root compression due to their space-occupying character [24]. Plexiform neurofibromas are complex tumors that involve multiples nerves. They commonly occur at birth and grow faster that other forms of neurofibromas. They are frequently found in the head and neck and occasionally in deep body regions, with variable manifestations, from asymptomatic presentation to severe clinical picture involving pain, disfigurement, local compression, and neurological deficits. Finally, atypical neurofibromas are characterized by hypercellularity with atypical nuclei, even though they have only few mitoses and no necrotic areas. Atypical neurofibromas and plexiform neurofibromas have a high rate of evolving into malignant peripheral nerve sheath tumor (MPNST). MPNST is a type of aggressive soft tissue sarcoma, occurring in 8-16% of patientσ with *NF1* and carrying a bad prognosis, since most patients have distal metastases at the time of diagnosis [25]. Risk factors for developing MPNST include, among others, large subcutaneous neurofibroma burden, presence of atypical neurofibroma, younger age and positive family history of MPNST [25].

Optic pathway glioma (OPG) is one of the most common tumorous manifestations of *NF1* [25, 26]. OPG is classified as astrocytoma, but it can also include oligodendrocytes, neurons, and microglia. It can form outside the orbit, everywhere in the optic nerve pathway and occasionally, it affects areas outside the optic nerve pathway, mainly the brainstem. It occurs approximately at 5-25% of patients with *NF1*, most commonly at a young age [26]. Even though OPG is characterized as a benign tumor and remains asymptomatic for years, it can in the long run affect vision and endocrine function and occasionally undergo malignant transformation, with poor outcome. Besides OPG, another, less common, CNS tumor in patients with *NF1* is brainstem glioma, including glioblastoma.

Epilepsy and other central nervous system manifestations

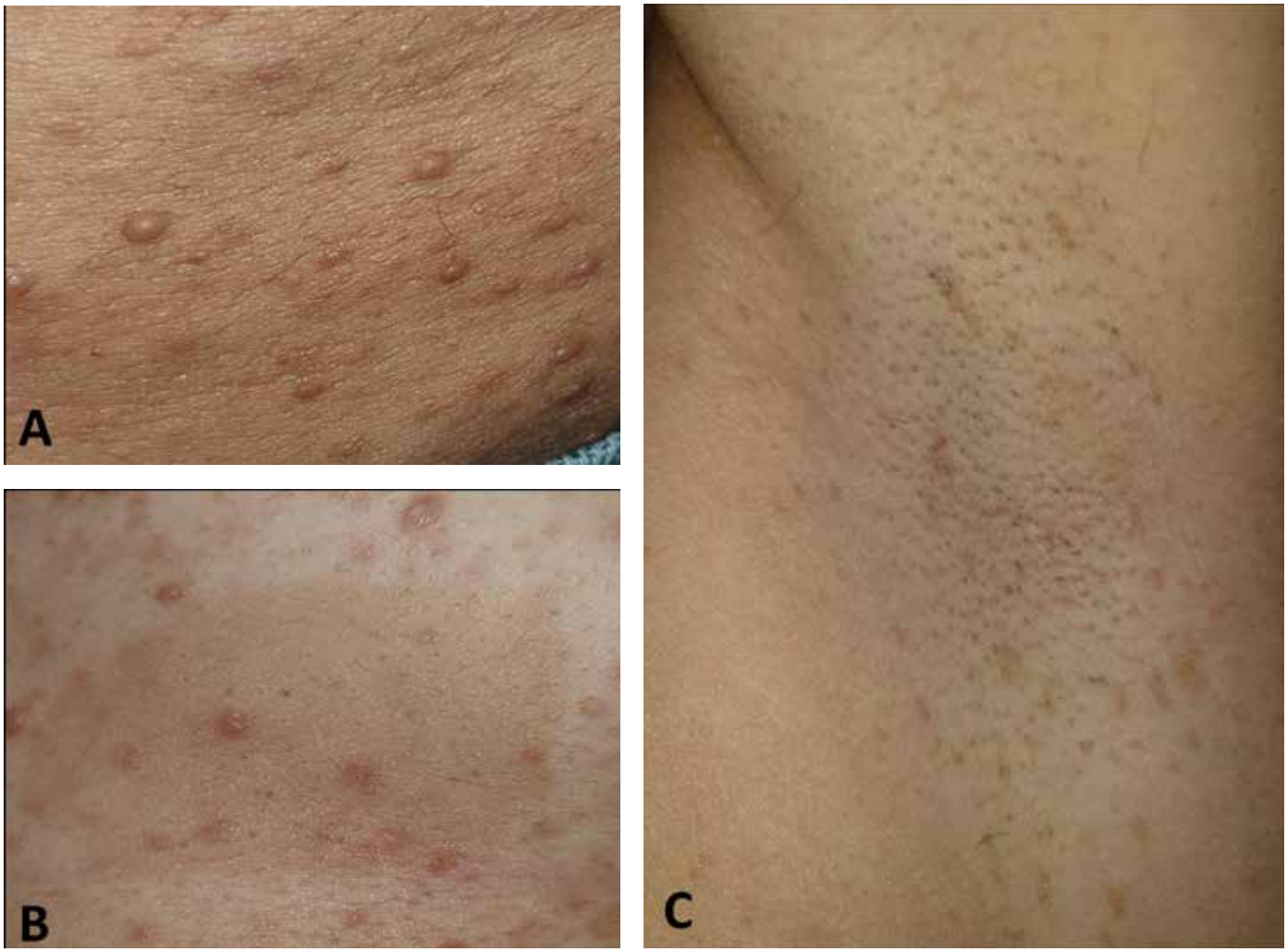
Epilepsy is commonly found in patients with *NF1* (with a prevalence of 4-14%), and includes focal epilepsy (associated with brain tumors, mesial temporal sclerosis, and cortical dysplasia) and primary generalized epilepsy [27-29]. Localization-related epilepsy in patients with *NF1* is often drug-resistant and occasionally may need epilepsy surgery for the seizures to resolve. Additionally, headache, hydrocephalus, cerebrovascular disease, and unidentified bright objects (UBOs) in MRI imaging are few of the many neurological abnormalities that have been occasionally observed in patients with *NF1* [29, 30].

NON-NEUROLOGICAL CLINICAL MANIFESTATIONS

Skin lesions

Apart from the neurofibromas described above, pigmentary abnormalities are another typical feature of *NF1* observed on the skin. The predominant skin indicator of *NF1*, the café-au-lait macules (CALMs), are frequently the first sign that clinicians and the

Figure 1. **Typical skin manifestations of *NF1***. In this figure, the skin hallmarks of NF1, namely Café au lait macule (A), neurofibromas (B) and axillary freckling (C) are shown in a 32-year-old female patient. Informed consent from the patient was obtained for publication of this image



family notes (Figure 1B, 2). These flat hyperpigmented macules with clear boundaries are observed in 95-99% of *NF1* patients and are included in the clinical criteria of *NF1* [22]. CALMs typically appear during infancy in *NF1* patients and are found all over the body except for the feet, hands, eyebrows, and scalp. It should be noted that CALMs are not always associated with *NF1*, especially if they are isolated or have irregular boundaries and non-uniform pigmentation (atypical CALMs) [31].

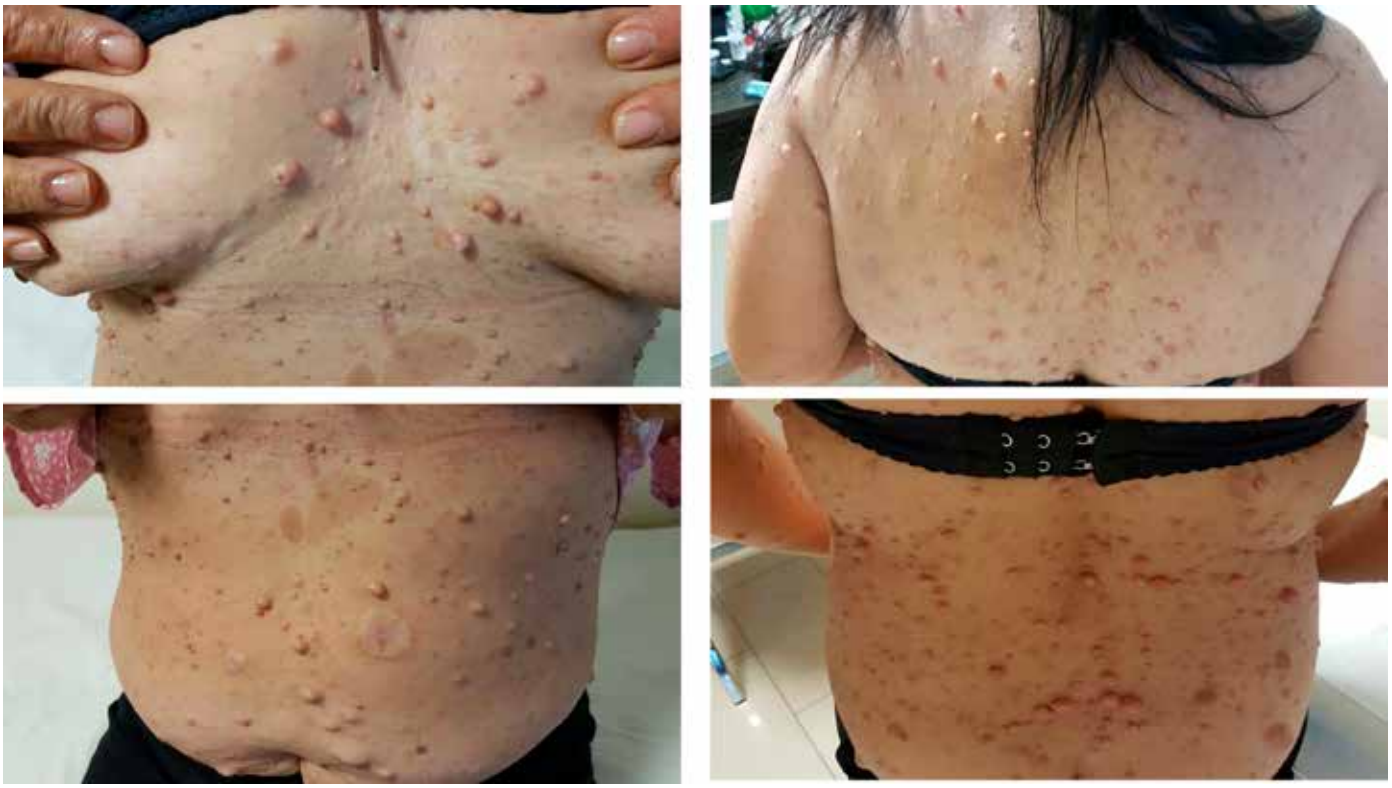
Skinfold freckling, historically called Crown sign from the physician Frank Crowe who described it in 1964, consists of pigmentary skin lesions found in most patients and included in the clinical criteria of *NF1* (Figure 1C) [22]. These skin lesions are small, light brown in color, with onset in a very young age and gradual development until adulthood. Interestingly, they are formed on the axillae or inguinal areas and not on areas exposed to sunlight [22].

In addition to neurofibromas, CALMS and skinfold freckling, patients with *NF1* display several other cutaneous signs, even though at lower frequency [22]. These additional skin manifestations include lipomas (encapsulated bulks of fat cells), nevus anemicus (pale skin area with sharp margins), nevus spilus (skin patches with multiple dark macules), juvenile xanthogranuloma (benign orange papules or nodules), vitiligo (macular skin depigmentation, an immune-mediated condition), Becker nevus (skin hamartoma with hyperpigmented, hypertrichotic lesions), poliosis circumscripta (patch of white hairs) and melanoma [18, 22].

Ocular involvement

Lisch nodules, described in 1937 by Karl Lisch, are among the most common clinical manifestations of *NF1*, along with CALMs and freckling, and are simi-

Figure 2. **Typical skin manifestations of *NF1***. The skin hallmarks of NF1, namely Café au lait macules and multiple neurofibromas are shown in the trunk of a 49-year-old female patient. Informed consent from the patient was obtained for publication of this image.



larly included in the diagnostic criteria for *NF1*. These nodules are in essence a melanocytic hamartoma of the anterior iris surface [32]. Even though prevalence is low in children, they are present in virtually all adult patients over 21 years old. They have a benign course and they usually do not cause vision or other impairment. The main clinical significance of Lisch nodules is their diagnostic value for *NF1* [26, 32]. Other less frequent ocular conditions associated with *NF1* include glaucoma, choroidal abnormalities, and, rarely, retinal involvement [26].

Bone abnormalities

Bone abnormalities in patients with *NF1* are commonly present since early childhood [33]. Osteopenia resulting from dysfunction of osteoclasts and osteoblasts, and bone dysplasia are among the main bone manifestations of *NF1* [24]. The resulting bone deformities severely affect the quality of life of these patients and commonly involve the spine, resulting in scoliosis in 10-26% individuals with *NF1*. Scoliosis in patients with *NF1* can be categorized further to dystrophic and non-dystrophic. Non-dystrophic scoliosis shares common feature with idiopathic scoliosis. In contrast, dystrophic scoliosis is characterized by short-segment and sharply angulated curves and is less frequent in *NF1* patients [24]. In addition to the spine, bone dystrophy can also affect the long bones. These skeletal abnormalities predispose to pseudarthrosis, sphenoid wing (defective orbital plate and frontal bone), congenital thoracic deformities, and congenital tibial dysplasia (bowing of the lower leg). Skeletal involvement in *NF1* often leads to neuropathic pain and neurological symptoms ranging from mild paresthesia to severe motor deficits. The low levels of vitamin D frequently found in patients with *NF1* are thought to contribute to the bone mineralization disorder and the high frequency of fractures.

Cardiovascular manifestations

Several cardiovascular abnormalities have been reported to be associated with *NF1* [34]. Hypertension is the most common one, usually characterized as essential but occasionally occurring in the context of pheochromocytoma or renal artery stenosis. Other cardiovascular problems found in patients with *NF1* are vasculopathies, such as stenotic or occlusive arteries, aneurysms, arteriovenous fistulas and moyamoya syndrome [1, 35].

Non-neurological neoplastic disease

The presence of mutated tumor-suppressor *NF1* gene, causing impairment of the function of neurofibromin, predisposes to tumorigenesis not only in the nervous system but also in other tissues. For instance, individuals with *NF1* have increased likelihood to develop leukemia compared to the general population. Types of leukemia found in these patients include juvenile myelomonocytic leukemia or chronic myelomonocytic leukemia [36, 37]. Pheochromocytoma, another neuroendocrine tumor associated with *NF1* [38], mostly presents with episodic hypertension accompanied by sweating and flushing. Occasionally, distant metastases are found at the time of the diagnosis. Breast carcinoma is more common in *NF1* patients under 50 years old compared to the general population [39], with increased frequency of triple-negative and human epidermal growth factor receptor 2 (HER2)-positive subtypes. Part of the *NF1* phenotype is the development of small, benign, tumors from the glomus bodies, called glomus tumors. Sarcomas are also found in patients with *NF1*, including rhabdomyosarcomas and gastrointestinal stromal tumors, with the latter causing abdominal pain, bleeding, and intestinal obstruction. Finally, other non-nervous tissue tumors possibly associated with *NF1* are gastrointestinal well-differentiated neuroendocrine carcinomas and malignant melanomas [23, 35].

DIAGNOSIS OF *NF1*

The diagnosis of *NF1* is largely based on clinical evaluation and use of published clinical criteria. One of the most important parts of the clinical examination of patients possibly affected with *NF1* is the evaluation of the skin, for identification of CALMs, skinfold freckling and neurofibromas. Of note, CALMs in children should be 6 or more to be considered as an *NF1* sign. In addition to the clinical examination, neurofibromas are also evaluated with magnetic resolution imaging (MRI), especially in the presence of new or deteriorating neurological symptoms, to assess possible involvement of nerves or other structures and to exclude malignant transformation [40, 41]. In the case of a plexiform neurofibroma possibly evolving to MPNST, functional MRI and positron emission tomography (PET) scans can assist in the recognition of malignant transformation [40].

Specific neurological manifestations of *NF1* may need more targeted investigation. In cases of epilepsy, electroencephalograms, and a brain MRI, preferably with contrast material administration, should be performed. Brain MRI could reveal areas of high signal in T2 sequences, called neurofibromatosis bright objects (NBOs) [42]. These NBOs are characteristic findings of *NF1* and tend to reduce with advancing age [43]. Brain MRIs are also useful to detect OPGs, tumors in the cerebral hemispheres and mesial temporal sclerosis. In addition to MRI scans, emerging evidence shows the potential of PET CT in the evaluation, monitoring and therapeutic management of *NF1* patients with tumors, especially MPNSTs. Even though imaging can identify the lesions associated with *NF1*, in several cases it cannot determine the underlying pathology. In cases of accessible lesions, MRI-guided or CT-guided biopsy is used to obtain specific histological diagnosis, the gold standard in differentiating benign from malignant tumors.

Given the increasingly recognized occurrence of *NF1*-associated neurodevelopmental abnormalities and the potential benefit of available interventions if initiated early, any sign of delay in child development regarding language and other cognitive functions or social interaction, should be detected as early as possible [21]. Parents of these patients and primary care clinicians should be aware of those manifestations and refer the child to an expert for evaluation.

Investigation of the eye manifestations of *NF1* is most often performed by an ophthalmologist, evaluating visual acuity, color vision and visual fields, along with a slit-lamp examination for anatomic assessment of the eye and identification of Lisch nodules in the iris [26]. In cases of an abnormal initial screening examination, the possibility of optic pathway glioma and other central nervous systems gliomas and tumors should be further investigated, most often with MRI of the orbits and brain. Even though most clinicians suggest performing MRI scans only when there are abnormal ocular signs or symptoms, there are some advocates of including brain and orbit MRIs in the annual monitoring of *NF1* patients.

Skeletal abnormalities, especially in children, should be assessed annually. This assessment should include evaluation for spine deformities and blood screening for vitamin D deficiency [24]. If there are indications of skeletal abnormalities, imaging of the spine and other bones with X-rays or other modalities, DEXA screening for osteoporosis and pulmonary function testing, should be performed.

In case of hypertension and other cardiovascular disorders, patients with *NF1* should receive the standard of care, but often CT angiography of renal arteries and additional tests may be needed to better characterize the cardiovascular phenotype [1, 34]. In case of drug resistant hypertension, clinicians should consider the possibility of pheochromocytoma.

Women, especially between 30 to 50 years old, should undergo regular mammogram examination and if needed breast MRI, due to the high breast cancer rate in individuals with *NF1* [25].

Genetic testing, despite not considered essential for diagnosis in the past, has gained popularity in

recent years, especially given the decreasing cost of the next generation sequencing methods. Genetic testing is especially useful to diagnose the disease in individuals that don't fulfill all the clinical criteria for *NF1* or in young children with negative family history that have not yet expressed the full *NF1* phenotype. Early diagnosis achieved through genetic testing can help guide proper management, provide better understanding of the natural history of the disease, enable genotype/phenotype correlations and provide the basis for genetic counseling [44].

MANAGEMENT OF *NF1*

Even though there is no definite cure for NF1, accumulating knowledge in recent years, based on *in vitro* and *in vivo* research in animal models, as well as results of clinical studies, has provided tools to better manage the disease and improve the quality of life of *NF1* patients.

Pigmental manifestation (CALMs, freckling) and cutaneous neurofibromas that cause severe esthetic problems, can be removed if deemed necessary by the patient and the attending physician. Subcutaneous, spinal and paraspinal neurofibromas often cause neuropathic pain and motor and sensory impairment that require medical attention and treatment by surgical resection or removal by other means such as laser or electrocautery [45]. Likewise, for plexiform neurofibromas that cause symptoms or display potential to develop to MPNSTs, surgical resection is needed. However, for neurofibromas that are close to nerves or vital organs and body structures, surgical removal can be challenging. Beyond surgical management, several medical treatment approaches including sirolimus, an inhibitor of the mTOR pathway, tipifarnib, a RAS signaling inhibitor, imatinib, a tyrosine kinase inhibitor, and interferons have been tried with mixed results [46]. However, additional hope has emerged with the promising results of selumetinib [47, 48], an inhibitor of the mitogen-activated protein kinase kinases 1 and 2 (MEK1/2). Selumetinib has recently earned FDA and EMA approval for use in patients with inoperable plexiform neurofibromas [49, 50]. Fortunately, most individuals carrying a plexiform neurofibroma don't require surgical or medical treatment, with annual monitoring being the standard of care. In case of a neurofibroma evolving to MPNST or if a MPNST is found independently from neurofibromas, aggressive management should be pursued. This includes resection of the affected area with wide surgical margins and, if needed, radiotherapy. Chemotherapy is a last resort option in cases of MPNSTs that are locally advanced or have given metastasis.

OPG, even if in most patients with *NF1* is a benign, low-grade pilocytic astrocytoma, in some cases visual problems or signs of malignant transformation can emerge. For these patients, treatment is needed in an emergency basis. Due to the complex anatomical location of OPG that hinders surgical removal, first line therapy is chemotherapy. Adjunctive radiotherapy is usually avoided due to the potential risk of local complications, secondary malignancies, and risk of neurocognitive disturbances. As in the case of plexiform neurofibromas, recent studies have shown potential benefits from the use selumetinib for OPGs [51]. For the other rare brain tumors in NF1 patients besides OPGs, surgical removal with adjunctive chemotherapy and radiotherapy can be performed, depending on the specific tumor type and location. In women with breast cancer or patients with leukemia and lymphoma, standard clinical care should be applied.

For the *NF1* associated bone abnormalities, management is dictated by the specific skeletal disorder and ideally should be offered by a specialized orthopedic surgeon [33]. Spinal and other bone abnormalities caused by bone dysplasia in children should be treated with bracing from a young age, with surgical stabilization of the spine and other bones reserved only for extreme disabling bone abnormalities. Supplementation with vitamin D could be given in cases of osteoporosis associated with vitamin D deficiency. For the less prominent manifestations of NF1, such as hypertension and other cardiovascular disorders, if they are not associated with a tumor, standard medical treatment should be provided. However, if the cause of hypertension is a pheochromocytoma, surgery is the first line option.

As in other neurogenetic disorders, there has been intensive research in the development of genetic treatments for *NF1*, that promise to change the natural history of the disease [46]. Among the techniques currently being investigated are genome editing, replacement of the mutated *NF1* gene with a full length normal *NF1* gene, and engineering of transcription activator-like effector protein to increase transcription of the wild-type *NF1* allele.

CONCLUSION

NF1 is a complex neurocutaneous hereditary disorder affecting multiple tissues and displaying large clinical heterogeneity. Despite the benign nature of most of its manifestations, *NF1* can be associated with significant morbidity and mortality. The cause of *NF1* are loss-of-function pathogenic variants in the *NF1* gene, which encodes a dysfunctional neurofibromin protein that fails to inactivate the RAS and other pathways. This failure causes unregulated cellular proliferation with multiple pathophysiological consequences, including benign and malignant tumors. Abnormalities on the skin, the eyes and the

nervous system are the cardinal clinical manifestations of the disease; however, the spine and other bones, the cardiovascular system and several other tissues are often affected. Diagnosis remains essentially clinical, following recently revised diagnostic criteria. In addition, novel diagnostic modalities, such as MRI, PET, MRI or CT-guided biopsies and improved genetic testing methods are increasingly used for faster and more accurate diagnosis. This timely diagnosis enables earlier and thus more effective initiation of specific lesion-targeted management. Furthermore, better understanding of the pathophysiology of *NF1* has led to the development of ground-breaking genetic and other molecular therapeutic approaches that hopefully will improve the quality of life of patients with *NF1.*

References

[1] Cimino PJ, Gutmann DH. Neurofibromatosis type 1. *Handb Clin Neurol.* 2018;148:799-811.

[2] Stewart DR, Korf BR, Nathanson KL, et al. Care of adults with neurofibromatosis type 1: a clinical practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med.* 2018;20:671-682.

[3] Zanca A, Zanca A. Antique Illustrations of Neurofibromatosis. *International Journal of Dermatology.* 1980;19:55-58.

[4] National Institutes of Health Consensus Development Conference Statement: neurofibromatosis. Bethesda, Md., USA, July 13-15, 1987. *Neurofibromatosis.* 1988;1:172-178.

[5] Gutmann DH, Wood DL, Collins FS. Identification of the neurofibromatosis type 1 gene product. *Proceedings of the National Academy of Sciences of the United States of America.* 1991;88:9658-9662.

[6] Wallace MR, Marchuk DA, Andersen LB, et al. Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. *Science.* 1990;249:181-186.

[7] Viskochil D, Buchberg AM, Xu G, et al. Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. *Cell.* 1990;62:187-192.

[8] Cawthon RM, Weiss R, Xu GF, et al. A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. *Cell.* 1990;62:193-201.

[9] Kiuru M, Busam KJ. The NF1 gene in tumor syndromes and melanoma. *Laboratory Investigation.* 2017;97:146-157.

[10] Shofty B, Constantini S, Ben-Shachar S. Advances in Molecular Diagnosis of Neurofibromatosis Type 1. *Seminars in Pediatric Neurology.* 2015;22:234-239.

[11] Gutmann DH, Ferner RE, Listernick RH, et al. Neurofibromatosis type 1. *Nature Reviews Disease Primers.* 2017;3:17004.

[12] Walker JA, Upadhyaya M. Emerging therapeutic targets for neurofibromatosis type 1. *Expert Opinion on Therapeutic Targets.* 2018;22:419-437.

[13] Ruggieri M, Huson SM. The clinical and diagnostic implications mosaicism in the neurofibromatoses. *Neurology.* 2001;56:1433-1443.

[14] Uusitalo E, Leppävirta J, Koffert A, et al. Incidence and Mortality of Neurofibromatosis: A Total Population Study in Finland. *Journal of Investigative Dermatology.* 2015;135:904-906.

[15] Evans DGR, O'Hara C, Wilding A, et al. Mortality in neurofibromatosis 1: in North West England: an assessment of actuarial survival in a region of the UK since 1989. *European journal of human genetics: EJHG.* 2011;19:1187-1191.

[16] Zöller M, Rembeck B, Akesson HO, et al. Life expectancy, mortality and prognostic factors in neurofibromatosis type 1. A twelve-year follow-up of an epidemiological study in Göteborg, Sweden. *Acta Derm Venereol.* 1995;75:136-140.

[17] Tabata MM, Li S, Knight P, et al. Phenotypic heterogeneity of neurofibromatosis type 1 in a large international registry. *JCI Insight.* 2020;5.

[18] Ferner RE, Huson SM, Thomas N, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. *J Med Genet.* 2007;44:81-88.

[19] Legius E, Messiaen L, Wolkenstein P, et al. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. *Genet Med.* 2021;23:1506-1513.

[20] Tadini G, Milani D, Menni F, et al. Is it time to change the neurofibromatosis 1 diagnostic criteria? *Eur J Intern Med.* 2014;25:506-510.

[21] Vogel AC, Gutmann DH, Morris SM. Neurodevelopmental disorders in children with neurofibromatosis type 1. *Developmental Medicine & Child Neurology.* 2017;59:1112-1116.

[22] Miraglia E, Moliterni E, Iacovino C, et al. Cutaneous manifestations in neurofibromatosis type 1. *Clin Ter.* 2020;171:e371-e377.

[23] Hirbe AC, Gutmann DH. Neurofibromatosis type 1: a multidisciplinary approach to care. *Lancet Neurol.* 2014;13:834-843.

[24] Shofty B, Barzilai O, Khashan M, et al. Spinal manifestations of Neurofibromatosis type 1. *Childs Nerv Syst.* 2020;36:2401-2408.

[25] Ly KI, Blakeley JO. The Diagnosis and Management of Neurofibromatosis Type 1. *Med Clin North Am.* 2019;103:1035-1054.

[26] Kinori M, Hodgson N, Zeid JL. Ophthalmic manifestations in neurofibromatosis type 1. *Surv Ophthalmol.* 2018;63:518-533.

[27] Pecoraro A, Arehart E, Gallentine W, et al. Epilepsy in neurofibromatosis type 1. *Epilepsy & Behavior.* 2017;73:137-141.
[28] Serdaroglu E, Konuskan B, Karli Oguz K, et al. Epilepsy in neurofibromatosis type 1: Diffuse cerebral dysfunction? *Epilepsy Behav.* 2019;98:6-9.
[29] Bayat M, Bayat A. Neurological manifestations of neurofibromatosis: a review. *Neurol Sci.* 2020;41:2685-2690.
[30] Créange A, Zeller J, Rostaing-Rigattieri S, et al. Neurological complications of neurofibromatosis type 1 in adulthood. *Brain.* 1999;122 (Pt 3):473-481.
[31] Bernier A, Larbrisseau A, Perreault S. Café-au-lait Macules and Neurofibromatosis Type 1: A Review of the Literature. *Pediatr Neurol.* 2016;60:24-29.e21.
[32] Karaconji T, Whist E, Jamieson RV, et al. Neurofibromatosis Type 1: Review and Update on Emerging Therapies. *Asia Pac J Ophthalmol (Phila).* 2019;8:62-72.
[33] Feldman DS, Jordan C, Fonseca L. Orthopaedic manifestations of neurofibromatosis type 1. *J Am Acad Orthop Surg.* 2010;18:346-357.
[34] Friedman JM, Arbiser J, Epstein JA, et al. Cardiovascular disease in neurofibromatosis 1: report of the NF1 Cardiovascular Task Force. *Genet Med.* 2002;4:105-111.
[35] Bergqvist C, Servy A, Valeyrie-Allanore L, et al. Neurofibromatosis 1 French national guidelines based on an extensive literature review since 1966. *Orphanet J Rare Dis.* 2020;15:37.
[36] Rosenbaum T, Wimmer K. Neurofibromatosis type 1 (NF1) and associated tumors. *Klin Padiatr.* 2014;226:309-315.
[37] Paulus S, Koronowska S, Fölster-Holst R. Association Between Juvenile Myelomonocytic Leukemia, Juvenile Xanthogranulomas and Neurofibromatosis Type 1: Case Report and Review of the Literature. *Pediatr Dermatol.* 2017;34:114-118.
[38] Lee YH, Kwon MJ, Park JH, et al. Neurofibromatosis Type 1 with the Development of Pheochromocytoma and Breast Cancer. *Intern Med.* 2020;59:1665-1669.
[39] Evans DGR, Kallionpää RA, Clementi M, et al. Breast cancer in neurofibromatosis 1: survival and risk of contralateral breast cancer in a five country cohort study. *Genet Med.* 2020;22:398-406.
[40] Ahlawat S, Blakeley JO, Langmead S, et al. Current status and recommendations for imaging in neurofibromatosis type 1, neurofibromatosis type 2, and schwannomatosis. *Skeletal Radiol.* 2020;49:199-219.
[41] Evans DGR, Salvador H, Chang VY, et al. Cancer and Central Nervous System Tumor Surveillance in Pediatric Neurofibromatosis 1. *Clin Cancer Res.* 2017;23:e46-e53.
[42] Hazan F, Gürsoy S, Unalp A, et al. Evaluation of clinical findings and neurofibromatosis type 1 bright objects on brain magnetic resonance images of 60 Turkish patients with NF1 gene variants. *Neurol Sci.* 2021;42:2045-2057.
[43] Calvez S, Levy R, Calvez R, et al. Focal Areas of High Signal Intensity in Children with Neurofibromatosis Type 1: Expected Evolution on MRI. *AJNR Am J Neuroradiol.* 2020;41:1733-1739.
[44] Radtke HB, Sebold CD, Allison C, et al. Neurofibromatosis type 1 in genetic counseling practice: recommendations of the National Society of Genetic Counselors. *J Genet Couns.* 2007;16:387-407.
[45] Wilson BN, John AM, Handler MZ, et al. Neurofibromatosis type 1: New developments in genetics and treatment. *Journal of the American Academy of Dermatology.* 2021;84:1667-1676.
[46] Feroze K, Kaliyadan F. Targeted genetic and molecular therapies in neurofibromatosis – A review of present therapeutic options and a glimpse into the future. *Indian Journal of Dermatology, Venereology and Leprology.* 2020;0.
[47] Gross AM, Wolters PL, Dombi E, et al. Selumetinib in Children with Inoperable Plexiform Neurofibromas. *New England Journal of Medicine.* 2020;382:1430-1442.
[48] Solares I, Viñal D, Morales-Conejo M, et al. Novel molecular targeted therapies for patients with neurofibromatosis type 1 with inoperable plexiform neurofibromas: a comprehensive review. *ESMO Open.* 2021;6:100223.
[49] Anderson MK, Johnson M, Thornburg L, et al. A Review of Selumetinib in the Treatment of Neurofibromatosis Type 1 – Related Plexiform Neurofibromas. *Annals of Pharmacotherapy.* 2021:10600280211046298.
[50] Mehta GU, Barone AK, Bradford D, et al. US Food and Drug Administration regulatory updates in neuro-oncology. *J Neurooncol.* 2021;153:375-381.
51] Fangusaro J, Onar-Thomas A, Poussaint TY, et al. A phase II trial of selumetinib in children with recurrent optic pathway and hypothalamic low-grade glioma without NF1: a Pediatric Brain Tumor Consortium study. *Neuro Oncol.* 2021;23:1777-1788.

CONGENITAL MYASTHENIC SYNDROMES: AN OVERVIEW OF THE CLINICAL PRESENTATION, DIAGNOSIS AND TREATMENT

Dimitra Veltsista[1], Zinovia Kefalopoulou[1], Kyriaki Kekou[2], Maria Svingou[2], Elisabeth Chroni[1]

[1] Department of Neurology, Medical School, University of Patras, Greece
[2] Laboratory of Medical Genetics, Medical School, National and Kapodistrian University of Athens, "Aghia Sophia" Children's Hospital, Athens, Greece

Abstract

Congenital myasthenic syndromes (CMS) are a diverse group of rare inherited diseases caused by mutations in various genes that encode proteins related to the function or structure of the neuromuscular junction. This is a brief review of current knowledge concerning the pathophysiology, responsive genetic defect, clinical and neurophysiological features, as well as symptomatic treatment strategies of CMS.

Key words: neuromuscular junction; muscle weakness; acetylcholine; hereditary disease

Congenital myasthenic syndromes (CMS) are a group of rare inherited diseases characterized by pathological muscle fatigue and transient or permanent weakness of facial, bulbar and limb muscles, with an onset early in life. Mutations in various genes encoding proteins related to the function or structure of neuromuscular junction domains are responsible for the different subtypes of CMS [1, 2]. CMS are more rare that myasthenia gravis, although the prevalence could be underestimated due to difficulties in diagnosis and significant variations between different ethnic and racial groups [2]. CMS prevalence has been estimated at 9.2 per 1,000,000 persons under 18 years of age [3].

The aim of the presented literature review is to provide a brief update on the genetic background, clinical phenotype and treatment options for the more common CMS.

The steps for muscle membrane activation are: 1. Formation of acetylocholine (ACh) and packaging into vesicles within the nerve terminals 2. Release of ACh via exocytosis into the synaptic cleft, when an action potential reaches the nerve ending. 3. Binding of ACh to AChR on the muscle end-plate region. 4 Breakdown of ACh by the enzyme acetylcholinesterase (AChE). Disruption at any of these steps, either due to synthesis and degradation of structural proteins or their malfunction, could lead to CMS. Neuromuscular transmission requires continuous alterations of the polarized and depolarized states of nerve and muscle fibers, and is a complex procedure involving many enzymes, ion channels and structural proteins. In order for ACh to efficiently bind to the nicotinic muscular receptors a series of consecutive elements play a part interconnecting each to the next i.e Agrin → LRP4 → DOK7 → MuSK → RAPSN → clustering of AChR. These are targets for autoimmune myasthenia as well as genetically defined CMS [4-7]. Details of the physiology are beyond the scope of this short review.

CMS are classified into 3 main categories according to the site of pathology; i.e. presynaptic, synaptic and postsynaptic syndromes. The advances in genetic analysis now allow a further subdivision based on the underlying molecular defect [8]. To date, 32 CMS subtypes have been recognized. Mutations in genes encoding the subunits of AChR (CHRNA1, CHRNB1, CHRND, or CHRNE) account for approximately half of all CMS cases. In particularly, mutations in the CHRNE gene, that code for the ε subunit, are the most commonly identified, and result in AChR deficiency or kinetic abnormalities. Mutations in RAPSN gene account for 15-20% of the all CMS cases, and COLQ and DOK7 mutations for 10-15%. CHAT (4-5%) and GFPT1 (2%) gene mutations are much less common [2, 4, 8].

Summary of clinical manifestations:

There is high variability in distribution and severity of symptoms. Symptoms typically appear during infancy or early childhood (usually within the 1st year of life). However, milder cases manifesting in adulthood have been reported in many CMS subtypes [9]. Generalized fatigue is the cardinal symptom. Diurnal fluctuation is not so distinct, but long or short periods of relapses triggered by excessive exercise and

infection are more likely. Early onset typically results in hypotonia presenting as 'floppy infant syndrome' in the neonatal or early infantile period, with weak cry, stridor and feeding problems associated with apnea and respiratory insufficiency, which may lead to sudden death. When symptoms occur in early childhood, there is a delay in motor milestones, difficulty in running and climbing stairs, lifting objects (extensors are primarily involved) and fluctuating eye-lid ptosis. No cardiac involvement is reported in the majority of patients [10].

Clinical features vary depending on genetic subtype, more specifically:

- Axial muscle weakness is common. Particularly, limb-girdle weakness is typical for patients with COLQ, DOCK7, GFPT1.
- Respiratory insufficiency: COLQ, CHRNE.
- Episodic apnea: CHAT, COLQ, RAPSN.
- Facial bulbar weakness: COLQ (isolated vocal cord paralysis, facial diplegia)
- Ocular: eye-lid ptosis is very common. Other ophthalmokinetic muscles are less affected.
- Fluctuations and relapses: in all CMS. Relapses triggers by fever, excitement.
- CHAT with onset in infancy may later show improvement during childhood.
- Scoliosis: CHRNE.

The differential diagnosis mainly includes muscular dystrophies and congenital myopathies [6]. Muscle atrophy, such as tongue atrophy in DOK7, associated with needle electromyography (EMG) evidence of myopathy makes the diagnosis even more challenging. When weakness is restricted to the ocular muscles CMS can be confused with mitochondrial myopathy of chronic progressive external ophthalmoplegia.

The most common syndromes will be briefly described:

I. Pre-synaptic CMS

Eight proteins are involved in presynaptic CMS, which include SLC5A7, **CHAT**, SLC18A3, SNAP25, VAMP1, SYB1, SYT2, and MUNC13-1. Defects in these proteins cause defective choline uptake in nerve endings, abnormalities in synthesis and recycling of acetylcholine, and impairment of synaptic vesicles exocytosis [2].

Responsible gene: **CHAT** that encodes the cholin acetyl transferase, responsible for the resynthesis of ACh.

Phenotype: Eye-lid ptosis, generalized fatigability and recurrent episodic apneas which might lead to cerebral hypoxia. Onset of symptoms at birth or rarely in child- or adulthood. Possible requirement of respiratory support and permanent proximal muscle weakness

Neurophysiology: Prolonged RNS at low frequency or alternative 5-10 min of isometric exercise may unmask significant decrement [11].

Treatment: Acetylcholinesterase inhibitors (AChEIs) are mildly effective. Supplementary treatment with 3,4-DAP and salbutamol or ephedrine have been recommended as 2nd and 3rd line therapy respectively [9].

II. SYNAPTIC CMS

Four CMS are due to malfunction of synaptic proteins, including **COLQ**, LAMB2, LAMA5, and COL13A1.

Responsible gene: **COLQ** encodes a functional protein crucial for anchoring AChE to the basal lamina

Phenotype: Diverse symptomatology ranging from mild fatigue to severe weakness and loss of ambulation or respiratory failure. Proximal limb muscles are predominately affected while ocular are often spared. Usually weakness of axial muscles (limb-girdle muscular dystrophy-type) is severe and early death can occur. Relapses are reported. Isolated vocal cord paralysis, facial diplegia have been reported as sole initial symptoms [2].

Neurophysiology: Double muscle response to single nerve stimulus is seen [2].

Treatment: salbutamol or ephedrine, avoidance of pyridostigmine [5, 12].

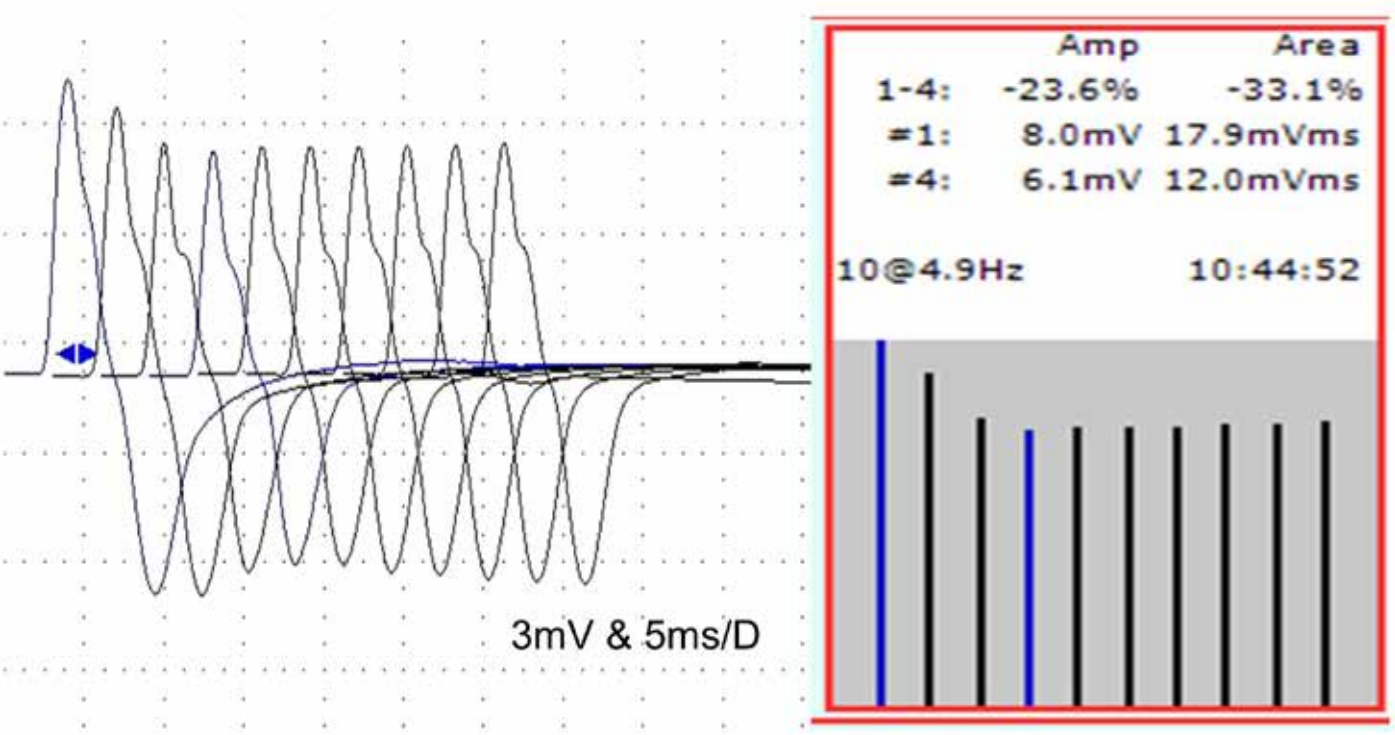
III. Post-synaptic CMS

Mutations in genes encoding post-synaptic proteins are responsible for 15 CMS, including CHRNA1, CHRNB1, CHRND, **CHRNE**, CHRNG**,DOK7**, MUSK, MYO9A, AGRN, LRP4, PREP1, SCN4A,**RAPSN**, PLEC, and SLC25A1 [2].

The majority of CMS belong to this category. These mutations are associated with primary deficiencies of the AChR, kinetic abnormalities of the AChR, or defects within the AchR-clustering pathway.

Responsible gene: **CHRNE** encodes the ε subunit of the AChR. Various recessive mutations i.e missense, nonsense, or splice site and promoter region mutations in all five AChR subunits have been identified. CHRNE variants affecting AChRε subunit are estimated to account for over 50% of CMSs related to AChR deficiency in humans [13]. The most common variant, CHRNE:c.1267delG (also known as ε1267delG), has been detected in many populations whereby resulting to a frequency of ~0.000128 in GnomAD. This frame shift alteration abolishes the normal stop codon in the last exon and gives rise to a different and extended nonfunctional protein, where the last 51 amino acids are replaced and 12 more are added (ClinVar entry ID: > 243031). The variant is mostly detected in European Gypsy patients presenting with symptoms of a myasthenic syndrome [14]. A survey of CHRNEc.1267delG in a large cohort of patients from the Roma populations within the Greek territory (unpublished data) revealed homozygosity of

Figure 1. Repetitive stimulation at 10Hz of the ulnar nerve and recording from abductor digiti minimi muscle: reduction >20% of compound muscle action potential amplitude and area



the variant in 25 (11 females, 14 males) patients of 0-49 years. Given that Roma population extents to ~175.000 subjects in Greece a prevalence of about 1/7000 Roma habitants is indicated. All patients presented with symptoms within the first years of life, of which the most prominent were blepharoptosis and swallowing difficulties.

***Phenotype**:* Variety in distribution and severity of symptoms

Weakness of ocular muscles present at birth or generalized fatigue and respiratory failure, with some patients experiencing delays or inability to achieve ambulation.

Neurophysiology: RNS could be abnormal and S-F EMG shows increased jitter. Due to long existing manifestations, classical EMG reveals myopathic changes of motor unit potentials (MUPs). Some patients may show repetitive CMAPs

Treatment: Initiation with AChEI, but in some cases it might fail or worsen the symptoms. Salbutamol or ephedrine can be effective. Combination of salbutamol with fluoxetine has been reported to be beneficial [2, 9].

Responsible gene: **RAPSN** encodes rapsyn a postsynaptic membrane protein that anchors the nicotinic AchR to the motor endplate and also binds to β-dystroglycan. It is necessary for clustering AchR

Phenotype: Fluctuating ptosis, bulbar symptoms and mild axial weakness. Relapses can occur, particularly precipitated by infections. Additional features are arthrogryposis multiplex congenita, contractures and hyperlordosis [2, 10].

Treatment: AchEI is favorable but outcomes can be improved by adding 3,4 DAP. General anesthesia can worsen the weakness.

Responsible gene: **DOK7** encodes protein responsible for activation of MuSK. Abnormal protein causes a default in AChR clustering

Phenotype: LGMD like pattern of muscle weakness or gait disturbance, occasionally mechanical ventilation. Ptosis but rarely ophathalmoparesis, severe relapses, vocal paralysis, tongue atrophy and feeding difficulties which may require PEG [2, 10].

Treatment: AchEI are usually ineffective and may even worsen clinical manifestations Ephedrine (initially 25 mg/dand increased to 75-100 mg/d) seems to be an effective. Alternatively, salbutamol may be successfully given.

Two well-described syndromes related to kinetics of AChR are: 1. Fast-channel (FCCMS) caused by unusual short time of AchR subunits able to interact with AChE. Depending on the mutation for AChR subunits, which is often loss-of function, increase rate of channel closing or reduced rate of channel opening responsible for this syndrome. Symptoms typically appear in infancy or early childhood.

Treatment: FCCMS responds to pyridostigmine or the addition of 3,4-DAP.

2. Slow-channel (SCCMS) is characterized by prolonged channel opening and hyperexcitability of the muscle fibers, which is usually caused by a gain-of function mutation in gene of AChR subunits. In terms of inheritance, unlike the majority of AChR deficiency syndromes, which had a autosomal recessive type, SCCMS follow an autosomal dominant inheritance. The clinical onset of SCCS is variable with the patients usually presenting symptoms after adolescent, although cases with severe symptoms in early life and leading consecutively to permanent disability might occur. Typically, weakness affects the cervical, scapular, wrist, and finger extensor zones [2]. Additionally symptoms involve the ocular, pharyngeal, proximal limb and respiratory muscles [15].

DEFECT IN GLYCOSYLATION OF POST-SYNAPTIC PROTEINS

Currently, mutations in five genes are known that are involved in protein glycosylation and may be associated with CMS. These genes include ALG2, ALG14, DPAGT1, **GFPT1**, and GMPPB.

Responsible gene: **GFPT1** encodes the enzyme that controls the flux of glucose for the glycosylation of proteins and lipids

Phenotype: LGMD like weakness fatigability and milder involvement of facial and bulbar muscles.

Treatment: Most patients respond beneficially to AChEI and in some patients the effect is significant [2].

Neurophysiology

Tests for neuromuscular junction have similar findings with those of the auto-immune pre and post synaptic disorders:

1. Standard procedure of repetitive nerve stimulation might show amplitude decrement, but normal findings does not exclude the diagnosis. Prolonged exercise or long stimulation at slow frequency (5 trains of 1 min at 3Hz separated by 5sec rest) might be necessary to reveal amplitude reduction. The recovery period in acetylcholinesterase deficiency (CHAT mutations) is long from 5 to 15 minutes [11]. In SCCMS and COLQ, a rate-dependent response is expected where the amplitude decrement is enhanced with high stimulation frequency [6]. Occasionally, such a pattern was also recorded in RAPSN syndrome [3]. A response similar to that seen in L-E syndrome i.e. more than 60% increase after 10 sec of exercise is indicative of presynaptic CMS.

2. Single-fiber EMG required prolonged axonal stimulation or voluntary contraction in order to demonstrate increased jitter and blocking [16].

3. Double muscle response to single nerve stimulus is seen in CMS caused by synaptic or post-synaptic disorders i.e. CHRNE, COLQ, SCCMS [17]. After discharges occurred by single stimulus, in small hand and foot muscles, and are abolished by fast (>0.5 Hz) repetitive stimulation.

4. Concentric needle EMG, particularly in chronic cases, demonstrates short duration, polyphasic MUAPs (similar to myopathic), described with the term "endplate myopathy" which can be reversible. For this reason mild CK increase is seen [3].

Treatment

Although CMS are genetic diseases and corrections of the underlying gene defects are not yet possible, most CMS subtypes are susceptible to pharmacotherapy [2, 4, 8, 10].

Available medication:

AChEI (pyridostigmine) inhibits degradation of ACh and is the most frequently used medication, although it can cause deterioration particularly in CMS presenting with excessive amount of ACh. In adults the daily dosage can reach 500mg in 4-6 divided doses. Gastrointestinal symptoms are the most common reported side-effects. Cholinergic crisis due to depolarized block, that may occur with high doses of pyridostigmine in patients with auto-immune myasthenia gravis, has not been reported in CMS [8]. Prophylactic administration or increased dose of pyridostigmine is recommended in cases of infection together with antibiotics to prevent the occurrence of episodic apnea and respiratory insufficiency [2].

3,4-Diaminopyridine (3,4-DAP) is the next most common medication after AChEIs. It is administrated as monotherapy or in combination with AChEI. 3,4-DAP acts as a potassium channel blocker that prolongs the opening of calcium channels and thus the duration of the presynaptic action potential resulting in enhancement of Ach quantal release from nerve terminal. It has also been proposed that 3,4-DAP has an effect on postsynaptic potassium channels but the mechanism is less clear [4]. For adults, initiation with tablets 5mg twice dayly, titrated at weekly intervals by 5mg up to a dosage between 15 and 80mg daily divided in 3 to 4 doses [8]. A limited number of case reports appeared in the literature regarding the administration of 3,4-DAP in CMS and several in patients with Lambert-Eaton syndrome. No interaction was reported with this drug when given together with pyridostigmine. Serum half-life is 20 min to 2 hours [18]. The most serious side-effect is epileptic seizures particularly at high dose (90-100mg/day). Tendency for epilepsy or electroencephalographic abnormalities should be excluded in children prior to drug administration. Other common side-effects are paresthesias in distal limbs and perioral, myoclonia, supraventricular tachycardia, epigastric distress [8, 18].

Salbutamol, known as albuterol in the United States, and ephedrine are β2 adrenergic receptor

agonists with a beneficial effect as a first-line treatment in some CMS, while in other has been used supplementary to pyridostigmine and 3,4-DAP [4]. Their mechanism of action is poorly understood; it has been hypothesized that these drugs backup agrin complex, stabilizing endplate structure and stimulate intracellular potassium uptake [4, 9]. There may be a significant delay of several months prior to achievement of clinical benefit. Salbutamol, which is more commonly prescribed nowadays, is given at a daily dosage of 8-12 mg in 2-3 divided doses. It is necessary to monitor patients for side-effects including restlessness and insomnia, tachyarrhythmia, hypertension and hypokalemia [8].

Two drugs with different indications are reported to provide clinical and neurophysiological improvement in some patients with SCCMS. Fluoxetine is a selective serotonin reuptake inhibitor and quinidine an antiarrhythmic agent. Both are long-lived drugs that blocks nicotinic AchRs in nerve and muscle at the open state and thus shorten the duration of the pathologically prolonged synaptic current and suppress the depolarization block [4, 8]. Fluoxetine is more oftenly prescribed compared to quinidine and their side effects during chronic administration should be considered. Fluoxetine is started at 10 mg/d and titrated up to 80 mg/d. Beneficial effect in CHRNE but deterioration in RAPSN [2]. Quinidine sulfate was administered per os. at a dose of 200 mg three times daily with possible increase to 900mg/day. Attention should be focused on severe cases to exclude respiratory insufficiency as a side effect, possibly due to excessive blockage of neuromuscular transmission in respiratory muscles [19].

Treatment depends on the subtype. Certain drugs which have a favorable response in some CMS type may have no effect or more over a negative effect in others [9].

There are two main groups in terms of treatment approach:

The era of rapidly evolution of neurogenetics leads the way for the discovery of CMS related new genes and their role in neuromuscular junction as well as the muscle fibers and the nervous system as a whole, justifying the variability of manifestations in these patients. The optimum treatment would require genetic diagnosis and the conduction of well-designed, randomized control, clinical trials. The latter is goal that is far from being achieved due to the underdiagnosis and rarity of the disease. For the time, the awareness of CMS as a potential diagnosis of cases with early onset weakness is the first step. The referral to specialized center with a multidiscipline approach in patients' monitoring is the second step.

In brief, one should be aware that CMS:

1. Typically manifest very early in life, but may do so in adulthood
2. Present no characteristic or pathognomonic symptoms
3. Fluctuate in severity, showing stability or even improvement during motor development in childhood and periods of remission
4. In most cases have usually a negative family history, since the majority are autosomal recessive diseases
5. Require high degree of suspicion due to difficult to diagnosis, mainly via specific neurophysiological testing

References

[1] Engel AG, Shen XM, Selcen D, et al. Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment. *Lancet Neurol.* 2015;14:420-434.
[2] Finsterer J. Congenital myasthenic syndromes. *Orphanet J Rare Dis.* 2019;14:57.
[3] Iyadurai SJP. Congenital Myasthenic Syndromes. *Neurologic Clinics.* 2020;38:541-552.
[4] Iyadurai SJP. Congenital Myasthenic Syndrome

I. Patients who benefit from increase of acetylcholine levels

Treatment line	CHAT	AChR deficiency	FCCMS	RAPSN*	GFPT1
1st: pyridostigmine	+	+	+	+	+
2nd: add 3,4 DAP	+	+	+	+	+
3rd: add salbutamol or ephedrine		+ (CHRNE)	+	+	+

* fluoxetine reported to cause symptoms worsening in some patients

II. Patients who do not improve or worsen following pyridostigmine and 3, 4 DAP administration, since they further increase the already prolonged action of Ach on the receptors

Treatment line	COLQ	DOC7	SCCMS
1st:	salbutamol or ephedrine		Fluoxetine
2nd:	add 3,4 DAP		Quinidine

Treatment Heterogeneity makes diagnosis and treatment challenging. *Practical Neurology* 2019.
[5] Lee M, Beeson D, Palace J. Therapeutic strategies for congenital myasthenic syndromes. *Ann N Y Acad Sci.* 2018;1412:129-136.
[6] Finlayson S, Beeson D, Palace J. Congenital myasthenic syndromes: an update. *Pract Neurol.* 2013;13:80-91.
[7] Rodríguez Cruz PM, Cossins J, Beeson D, et al. The Neuromuscular Junction in Health and Disease: Molecular Mechanisms Governing Synaptic Formation and Homeostasis. *Front Mol Neurosci.* 2020;13:610964.
[8] Farmakidis C, Pasnoor M, Barohn RJ, et al. Congenital Myasthenic Syndromes: a Clinical and Treatment Approach. *Curr Treat Options Neurol.* 2018;20:36.
[9] Thompson R, Bonne G, Missier P, et al. Targeted therapies for congenital myasthenic syndromes: systematic review and steps towards a treatabolome. *Emerg Top Life Sci.* 2019;3:19-37.
[10] Ciafaloni E. Myasthenia Gravis and Congenital Myasthenic Syndromes. *Continuum (Minneap Minn).* 2019;25:1767-1784.
[11] Juel VC. Evaluation of neuromuscular junction disorders in the electromyography laboratory. *Neurol Clin.* 2012;30:621-639.
[12] Laforgia N, De Cosmo L, Palumbo O, et al. The First Case of Congenital Myasthenic Syndrome Caused by a Large Homozygous Deletion in the C-Terminal Region of COLQ (Collagen Like Tail Subunit of Asymmetric Acetylcholinesterase) Protein. *Genes (Basel).* 2020;11.
[13] Abicht A, Dusl M, Gallenmüller C, et al. Congenital myasthenic syndromes: achievements and limitations of phenotype-guided gene-after-gene sequencing in diagnostic practice: a study of 680 patients. *Hum Mutat.* 2012;33:1474-1484.
[14] Morar B, Gresham D, Angelicheva D, et al. Mutation history of the roma/gypsies. *American journal of human genetics.* 2004;75:596-609.
[15] Otero-Cruz JD, Báez-Pagán CA, Dorna-Pérez L, et al. Decoding pathogenesis of slow-channel congenital myasthenic syndromes using recombinant expression and mice models. *P R Health Sci J.* 2010;29:4-17.
[16] Stålberg EV, Trontelj J, Sanders DB. *Single Fiber EMG*, third ed., Edshagen Publishing House, Fiskebäkskil, Sweden, 2010.
[17] Howard JF, Jr. Electrodiagnosis of disorders of neuromuscular transmission. *Phys Med Rehabil Clin N Am.* 2013;24:169-192.
[18] Lindquist S, Stangel M. Update on treatment options for Lambert-Eaton myasthenic syndrome: focus on use of amifampridine. *Neuropsychiatr Dis Treat.* 2011;7:341-349.
[19] Harper CM, Engel AG. Quinidine sulfate therapy for the slow-channel congenital myasthenic syndrome. *Ann Neurol.* 1998;43:480-484.

PHARMACOGENOMICS IN NEUROLOGY

Styliani-Aggeliki Sintila, Christos Bakirtzis, Ioannis Nikolaidis, Natalia Konstantinidou, Evangelia Kesidou, Paschalis Theotokis, Nikolaos Grigoriadis, Marina Boziki

2nd Neurological University Department, Aristotle University of Thessaloniki, AHEPA University General Hospital, Thessaloniki, Greece

Abstract

Precision medicine is an emerging medical approach which aims to individualize therapies in patients with complex, multifactorial disease in order to increase drug effectiveness and prevent adverse drug reactions. Among high-throughput -'omic technologies (genomics, proteomics, metabolomics), pharmacogenomics investigates the application of genomics to personalize drug selection, according to the patient's genetic traits. Genetic variations influence the pharmacokinetic and pharmacodynamic profile of many therapies in different fields in neurology, such as immune-mediated disease, neurodegenerative disease and ischemic stroke. Until now, available clinically useful pharmacogenomic biomarker does not exist to distinguish between responders and non-responders regarding MS treatments. In patients with stroke who receive clopidogrel, CYP2C19 testing in clinical practice has not been established yet. In Parkinson's disease, MTHFR gene mutations may be correlated with higher incidence of hyperhomocysteinemia due to L-dopa treatment. Finally, apolipoprotein E (APOE) gene has been linked with Alzheimer's disease pathogenesis and is regarded as a reference gene in several pharmacogenetic studies. In the era of precision medicine, educating clinicians on pharmacogenomics may assist with the implementation of genetic information in the clinical practice, thus enhancing genetically-guided treatment decisions.

Key words: precision medicine; omic technologies; pharmacogenomics; genetic variations; CYP2C19 testing; apolipoprotein E

Introduction

Precision medicine is an emerging medical approach according to which the patients' genetic profile, lifestyle and environment are taken into consideration, in order to provide personalized treatment [1]. The potential of precision medicine is seemingly unlimited as scientists from multiple fields use high-throughput 'omic technologies to improve patient outcomes. -'Omic technologies (genomics, proteomics, metabolomics etc.) generate a large quantity of data, thus offering a molecular fingerprinting of a patient, aiming to assist with and/or guide clinical decisions [2]. One of the main developing applications of this novel approach is pharmacogenomics.

Response to a drug may be variable among patients, related both to pharmacokinetic (phases of absorption, distribution, metabolism) or pharmacodynamic (drug's mode of action) factors, and this variability may also depend on environmental and genetic factors [3]. Pharmacogenomics investigates the application of genomics into personalized drug selection, aiming to increase drug effectiveness and prevent adverse drug reactions [4]. In this respect, pharmacogenomic analysis may adjust drug selection according to the patient's genetic traits [5]. Pharmacogenomics have been developed within a short time over the last 50 years, upon progress in human genome sequencing, as it was first assumed that genetics might affect drug response phenotypes [6]. It became clear that deviation in drug response could be partly explained by the effects of genetic inheritance. Over the last twenty years, the Human Genome Project was brought to completion allowing for a robust evolution in pharmacogenomics, especially facilitated through the development of techniques such as Next Generation Sequencing (NGS) and genome-wide association studies (GWAS) [4]. Up to now, international scientific associations have developed and approved guidelines concerning several drug-gene interactions that are accessible at no cost as an on-line source (www.pharmgkb.org) [7].

Pharmacogenomics in Neurology

Substantial therapeutic progress has been achieved in various fields in neurology, such as neuroimmunological disease, neurodegenerative disease, ischemic stroke and epilepsy which can, however, be linked with potentially severe adverse events and high financial cost. Pharmacogenomics thus addresses an increasing need to individualize therapeutic choices and to maximize the benefits against risks [3].

Pharmacogenomics in Multiple Sclerosis

Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease of the central nervous system (CNS). It is classified in three types, the relapsing remitting form (RRMS) (80-85% of patients) which may evolve into secondary progressive form (SPMS) and the primary progressive MS (PPMS) (10-15% of patients) [8]. According to current evidence, 30-50% of patients are non-responders to first-line therapies and inter-individual genetic variability may, at least in part, contribute to this heterogeneity [9]. Until now, available clinically useful pharmacogenomic biomarker does not exist in order to timely distinguish between responders and non-responders regarding MS treatments [8].

Interferon-beta (IFNb) is a widely prescribed immunomodulatory treatment for MS. IFNb binds to specific receptors on the surface of the immune system cells inhibiting the synthesis of inflammatory cytokines and increase the production of anti-inflammatory ones [10]. Several gene studies investigated the association of genetic variants with response to IFNb, yielding inconclusive results [11-14]. A few recent whole-genome association studies (GWAS) investigated the association between IFNb treatment response and genetic variability with inconsistent findings, without verifying previously conducted candidate-gene studies [11, 12, 13, 14] reviewed in (8). Regarding the development of neutralizing antibodies (NAbs) against IFNb, their use as an early pharmacogenetic biomarker is limited and it seems to account for resistance towards IFNb treatment in a minority of patients [15].

Glatiramer acetate (GA) is the first non-interferon approved treatment for RRMS. It acts on innate and acquired immune system and it has been linked with a shift in the T-effector phenotype from pro-inflammatory (T-helper 1 and 17 cells) to anti-inflammatory (regulatory T cells and T-helper 2 cells) [8]. Human-leucocyte antigen (HLA) class I/II polymorphisms are positively associated with response to treatment with GA, more specifically, the HLA DRB1 * 1501 [16, 17]. Two single nucleotide polymorphisms (SNPs), rs71878 in the T-cell receptor beta (TCRB) gene and rs2275235 in the cathepsin S (CTSS) gene were significant associated with GA treatment in one study [18]. Moreover, one GWAS study on GA treatment response demonstrated significant associations between GA treatment response and the ensuing genes: ZAK (rs139890339), UVRAG (rs80191572), MBP (rs1789084) and HLA-DQB2 (rs28724893), [18, 19].

Mitoxantrone, a cytotoxic agent that inhibits DNA repair, acts on macrophages B cells and T cells, and suppresses their proliferation as well as pro-inflammatory cytokine production [20]. Two pharmacogenomics studies provided conflicting results [21, 22].

Natalizumab, a humanized monoclonal antibody, prevents the entry of lymphocytes into the CNS [20]. To our knowledge, one pharmacogenetic study that has been conducted reported that the wild-type genotype or heterozygous presence of a polymorphism for NQO1 or GSTP1 gene is possibly related to beneficial clinical outcomes upon treatment with natalizumab [23].

Siponimod, a particular sphingosine-1-phosphate (S1P) receptor (S1P1 and S1P5) inhibitor blocks the egress of lymphocytes from lymphoid system cells and thus it mitigates the entry of T-lymphocytes into the CNS. Siponimod has been studied in phase II and phase III trials in RRMS and SPMS, respectively. Siponimod's metabolism is susceptible to variability in cytochrome P450 (CYP) activity among individuals, involving mainly the CYP2C9 and CYP3A4 enzymes. Hence, genetic testing is required before treatment [24].

Several other disease-modifying treatments for MS, such as dimethyl fumarate, teriflunomide or fingolimod do not exhibit known variable pharmacogenomic associations to clinical outcome [8].

Pharmacogenomics and Stroke

Genetic variations influence the pharmacokinetic and pharmacodynamics profile of several therapies for primary and secondary stroke prevention [25].

Aspirin is considered to be the most commonly prescribed antiplatelet therapy for stroke prevention (primary and secondary). Aspirin irreversibly inhibits COX (cyclooxygenase)-1 and thromboxane A2 production. Aspirin resistance has been associated with several genetic variants, most well studied being the PlA1/A2 of the GPIIIa (glycoprotein IIIa) gene and the COX-I polymorphisms [26-28]. However, the results are inconsistent and more extensive randomized controlled trials (RCTs) are required in order to reach safe conclusions.

Clopidogrel is an antiplatelet agent able to diminish the risk of recurrent ischemic stroke. For its antiplatelet action, it requires conversion to an active metabolite by cytochrome P-450 (CYP) enzymes. The majority of genetic studies have focused on the hepatic CYP2C19 enzyme. A reduced-function mutation in at least one allele of this enzyme (CYP2C19 * 2 or CYP2C19 * 3) is related to 33% reduction of plasma concentration of the active metabolite compared to the wild type genotype [29] and an increased risk of vascular events [30]. In contrast, gain-of-function allele (CYP2C19 * 17) is related to higher levels of active metabolite of clopidogrel and equivalent risk of bleeding [31]. However, in a meta-analysis including 4 placebo-controlled RCTs, the loss-of-function mutation did not affect the risk of vascular events or bleeding [32]. Due to the uncer-

tainty in the advantages of treating patients in the context of their CYP2C19 carrier status and taking into consideration that other therapeutic agents, such as ticagrelor and prasugrel may be considered apart from clopidogrel, CYP2C19 testing in clinical practice has not been established [33]. Up to date, no RCTs have estimated the efficacy of CYP2C19 testing in patients with ischemic stroke. More large-scale, well-designed trials are needed [34].

Statins are of great importance for the prevention and treatment of atherosclerotic cardiovascular disease. They act through inhibition of HMG-CoA reductase. However, some patients do not respond favorably and a number of them present with side-effects, most commonly statin-associated muscle disorder [35]. Of the plethora of candidate gene studies and GWASs, the SLCO1B1 521C genetic variant is, at present, the only clinically applicable pharmacogenetic test concerning toxicity from statins. The SLCO1B1 gene expresses a transport protein found in liver cells and this polymorphism seems to associate with myopathy following the use of simvastatin [36]. Furthermore, taking into consideration that lovastatin, atorvastatin, and simvastatin are metabolized mainly by cytochrome P450 3A enzymes, the US Food and Drug Administration (FDA) warns medical doctors about the risk of simvastatin muscle toxicity linked with concurrent use of CYP3A-inhibiting agents, such as clarithromycin, fluoxetine and omeprazole. Nevertheless, studies investigating the possible relation between CYP3A polymorphisms and the risk of statin side effects present inconsistent results. Therefore, routine CYP3A testing is not recommended at present [36].

Regarding the use of *anticoagulants* in patients with atrial fibrillation, numerous studies have focused on the pharmacogenetics of vitamin K antagonists (VKAs), particularly warfarin.

Warfarin is mainly metabolized in liver by the microsomal enzyme CYP2C9 and inhibits vitamin K metabolism targeting the vitamin K epoxide reductase complex subunit 1 (VKORC1) enzyme [37]. Additionally, the CYP4F2 gene encodes a vitamin K oxidase [38]. VKORC1, CYP2C9 and CYP4F2 polymorphisms are the genetic variants that have been studied the most [34]. Carriers of rare mutations in the protein-coding region of the VKORC1 gene [VKORC1:c.76G > A (Ala26 → Thr), VKORC1:c.76G > A (Ala26 → Thr), VKORC1: c.84C > T (Val29 → Leu), VKORC1: c. 85G > T (Val29 → Leu), VKORC1: c.107A > G (Asp36 → Gly), VKORC1: c.155C > G (Ser52 → Trp), VKORC1: c.167C>T (Ser56 → Phe), VKORC1: c.176G > T (Trp59 → Leu), VKORC1: c.177G > T (Trp59 → Cys), VKORC1: c.196G>A(Val66 → Met), VKORC1: c.197T > G (Val66 → Gly), VKORC1: c.212G > C (Gly71 → Ala), VKORC1: c.230A > G (Asn77 → Ser), VKORC1: c.229A > T (Asn77 → Tyr), VKORC1: c.368T > A (Ile123 → Asn), VKORC1: c.415T > C(Tyr139 → His)], are associated with oral anticoagulant resistance and higher dosage requirement, exhibiting a greater risk of unfavorable ischemic events" [39, 40]. Instead, carriers of the more common rs9923231 (VKORC1) variant require a lower dose of oral anticoagulant (39). A loss-of-function CYP2C9 mutation has been linked with reduction in warfarin metabolism and puts carriers at increased risk for bleeding [41]. CYP4F2 variant carriers require an increased warfarin dose [38]. Based on the results of recent trials, it is still not certain whether the integration of pharmacogenetic testing in those receiving warfarin is clinically effective and improves patient management [34].

Regarding non-vitamin K antagonist oral anticoagulants (NOACs) so far, a single GWAS has been conducted to examine the influence of genetics on dabigatran pharmacokinetic. It was based on participants from the RE-LY trial (dabigatran versus warfarin) [42] and revealed three single nucleotide polymorphisms (SNPs) (2 in the CES1 gene and 1 in the ABCB1 gene) that are associated with the fluctuation in plasma levels of dabigatran [43]. To our knowledge, no GWASs have been conducted to examine the impact of genetic variability on treatment with other NOACs such as rivaroxaban, apixaban and edoxaban. Large-scale studies are lacking; therefore, recommendations cannot be made for NOACs yet [44].

Pharmacogenomics in neurodegenerative disorders

More than 50 different neurodegenerative disorders (NDDs) can affect humans worldwide. Alzheimer's disease (AD) and Parkinson's disease (PD) are among the most common and account for high cost for the society [45].

Parkinson's disease (PD) is on top of the neurodegenerative movement disorders and the second most common neurodegenerative disease nowadays [46, 47]. PD is pathologically characterized by the intracellular aggregation of α-synuclein and the loss of dopaminergic neurons [48]. The cornerstone of pharmacologic therapy is dopamine replacement with L-dopa in combination with dopamine receptor agonists, monoamine oxidase (MAO) inhibitors or catechol-O-methyltransferase (COMT) inhibitors [49]. There is a significant degree of difference in drug response which is linked to the subtypes of the disease and the patients' genetic variability. Unfortunately, despite the advances of pharmacogenomics, there are currently no guidelines in the daily medical practice of treating PD taking into account pharmacogenomics. Moreover, a search in the pharmacogenomics knowledge-base (pharmaGKB) retrieves only

ten clinical annotations most of which are associated with low level of evidence [50].

Levodopa (L-dopa), combined with dopa decarboxylase inhibitors, augments the availability of dopamine in the CNS. The COMT enzyme is involved in levodopa metabolism. Most studies have focused on the COMT gene polymorphisms but their results are conflicting, thus limiting their potential for clinical application [50]. SNPs of the genes involved in the mTOR pathway are linked with either increased or reduced chance of treatment-induced motor symptoms, nevertheless larger cohort studies are required [50].

Hyperhomocysteinemia and impulse control disorder (ICD) are well known complications of dopaminergic treatment with *L-dopa or dopamine receptor agonists (DA).* They are associated with genetic factors. MTHFR gene mutations may increase the incidence of hyperhomocysteinemia during L-dopa treatment and this effect may be attenuated by co-treatment with COMT inhibitors [51]. For younger patients who initiate therapy with *dopamine receptor agonists (DA)*, polymorphisms in Dopamine receptor 1 (DRD1), Opioid Receptor Kappa 1 (OPRK1), Opioid Receptor Mu 1 (OPRM1) and COMT genes were linked with a high risk of ICD [52]. An DRD3 mutation was also related to increased incidence of ICD during L-dopa therapy [53].

Regarding the pharmacogenomic properties of *COMT* and *MAO inhibitors* sufficient evidence for clinical recommendations is lacking [50].

In relation to the etiology of PD, genetics play a role both in the multifactorial sporadic form of the disease, as well as in the single-gene, rare inherited forms of PD [46]. Most studied single gene mutations implicate genes encoding α-Synuclein (SNCA), Leucine-rich repeat kinase 2 (LRRK2), parkin RBR E3 ubiquitin-protein ligase (PRKN), vacuolar protein sorting-associated protein 35 (VPS35), PTEN-induced putative kinase 1 (PINK1), glucocerebrosidase (GBA) and oncogene DJ-1 [54]. Published studies investigating levodopa treatment in patients with LRRK2, SNCA and GBA genes mutations resulted in inconsistent data [50]. PRKN, PINK1 and DJ1 gene mutations were linked with a steady L-dopa response at lower dose, but also with early treatment-induced motor symptoms (dyskinesias and dystonia) [50]. In this way, the clinical phenotype of early treatment-induced motor symptoms may draw suspicion of these mutations and guide genetic testing before expected.

Alzheimer's disease (AD) is considered to be the most common neurodegenerative disease and the dominant form of dementia (>50%) [55]. Genomic defects, epigenetic changes and multiple environmental factors precipitate pathogenic cascades leading to dementia.

Three acetylcholinesterase inhibitors (AChEIs), donepezil, galantamine and rivastigmine have been approved for the treatment of AD. Memantine, an N-Methyl-D-Aspartate (NMDA) receptor antagonist, was approved by the FDA in 2003 [56] and, recently, aducanumab was approved by the US FDA [57]. Most pharmacogenomics studies on AD focus on these drugs. Furthermore, apolipoprotein E (APOE) gene polymorphisms contribute to the pathogenesis of AD and it is regarded as the reference gene in the majority of pharmacogenetic studies [55].

Donepezil, the most frequently prescribed AChEI, is a major substrate of CYP2D6, CYP3A4, acetylcholinesterase (ACHE) and UGTs (glucuronosyltransferase family polypepetides). Carriers of the APOE-4 seem to be poor responders to donepezil, whereas APOE-3 carriers seem to respond most optimally. Moreover, CYP2D6 normal metabolizers are optimal responders to donepezil, whereas CYP2D6-poor metabolizers are also poor responders to donepezil [55]. Carriers of the coomon CYP2D6 rs1080985 variant are poor donepezil responders [58]. Donepezil is not recommended for APOE-ε4/Butyrylcholinesterase K (BCHE-K *) carriers who present with an earlier disease onset and a hastened cognitive decline [59].

Rivastigmine is an inhibitor of both acetylcholinesterase (ACHE) and butyrylcholinesterase (BCHE) [60]. APOE, amyloid beta precursor protein (APP), choline acetyltransferase (CHAT), ACHE, BCHE, cholinergic receptor nicotinic alpha 4 (neuronal) (CHRNA4), cholinergic receptor, nicotinic, beta 2 (neuronal) (CHRNB2), and microtubule associated protein tau (MAPT) variants affect rivastigmine both pharmacokinetically and pharmacodynamically. Moreover, patients carrying the BChE K-variant (rs1803274) show poor clinical response to rivastigmine [55].

Memantine is an NMDA receptor antagonist. APOE, presenilin 1 (PSEN1), and MAPT variants may have an effect on the role of memantine in AD. The co-administration of CYP2B6 substrates may decrease the metabolism of the memantine by 65% [55].

Aducanumab is a monoclonal antibody targeting the N-terminus of the amyloid beta peptide (Aß). It is administered at monthly intravenous infusions [57]. According to recommendations of an Expert Panel, aducanumab is indicated for patients diagnosed with early AD. Administration of aducanumab has been associated with an increased rate of amyloid-related imaging abnormalities (ARIA) either with brain effusion or hemorrhage. These 2 types of ARIA present more common in APOE ε 4 (APOE-4) polymorphism carriers and may be more severe in APOE-4 homozygotes [61]. However, the prescription does not strictly require APOE genotyping. Moreover, aducanumab dosing scheme and monitoring instructions do not differ between APOE ε 4 carriers and non-carriers [57]. However, an informative discussion with the patient and the care partner is recommended and APOE genotyping may be sought prior to aducanumab

initiation. In case of the presence of APOE4 polymorphism, the clinician should discuss the increased likelihood for ARIA [57].

Pharmacogenomics in epilepsy

There is significant variation in the response to antiepileptic treatment in terms of seizure control and adverse reactions in people suffering from epilepsy [62]. Genetic factors contribute a lot to this variation [63].

The advances in the field of the genetics of the epilepsies provide the base for a new era in the treatment of epilepsy according to precision medicine [64].

However, guidelines on clinical management of individual epileptic patients are lacking.

Genetic factors and response to AEDs

Most antiepileptic drugs (AEDs) are metabolised by the cytochrome P450 (CYP) family. Allelic variants of some of these enzymes encode isoforms which differ in activity leading to altered serum AED concentrations.

An example to this variability are polymorphisms in CYP2C9 and CYP2C19 genes [65]. The phenytoin metabolism at a rate of 90% is mediated by CYP2C9. Carriers of CYP2C9 alleles, which encode enzymes with lessened activity metabolize *phenytoin* more slowly and carry an increased risk of concentration-dependent neurotoxicity. CYP2C9 * 3 (rs1057910(C)) and CYP2C9 * 2 (rs1799853) polymorphisms are the best recorded [66, 67].

A GWAS of cases with cutaneous adverse reactions being on *phenytoin* found out that CYP2C9 * 3 (rs1057910) polymorphism is significantly associated with these adverse events [68]. Nevertheless, testing for CYP2C9 genetic variants is not routine practice.

Studies in Asian populations found that CYP2C19 polymorphisms are associated with the serum concentration of the active metabolite of *clobazam*, N-clobazam, with clinical effectiveness [64].

Regarding *valproate* (VPA), only 15-20% of its dose is metabolized by CYP enzymes. The main enzyme is CYP2C9 and to a lesser extent CYP2A6 and CYP2B6 [69].

Population study in Japan found that CYP2C19 genotypes are responsible for some of the adverse reactions after treatment of epileptic patients with *zonisamide* [70].

CYP3A4 is considered as the main enzyme responsible for the carbamazepine metabolism. Although CYP3A4 has very known polymorphisms these are not frequent enough to although to cause significant inter-individual variability in vivo [71].

A study in Han people with epilepsy discovered that sodium voltage-gated channel alpha subunit 1 (SCN1A), ATP Binding Cassette Subfamily C Member 2 (ABCC2) and UDP Glucuronosyltransferase Family 2 Member B7 (UGT2B7) genetic polymorphisms are related with *oxcarbazepine* maintenance doses [72].

Human leukocyte antigen (HLA) alleles and AEDs side effects

Genetic polymorphisms, especially in certain human leukocyte antigen (HLA) alleles, have also been linked with the risk of idiosyncratic adverse reactions to AEDs.

HLA-B * 15:02 allele has been reported to be strongly associated with the Stevens-Johnson syndrome in Han Chinese people on treatment with *carbamazepine* [73].

Guidelines recommend that patients of South Asian origin be tested for HLA-B * 15:02 allele carriage before treatment with carbamazepine and carriers of this allele optimally avoid carbamazepine [74, 75].

Association has also been found between HLA-B * 15:02 allele and the risk of Stevens-Johnson syndrome in patients treated with *phenytoin* [76], *oxcarbazepine* [77] and *lamotrigine* [76].

HLA-A * 31:01 is another allele that has been linked with elevated risk of cutaneous adverse reactions, such as maculopapular exanthema or blistering, in European and Japanese patients treated with *carbamazepine* [78, 79]. However, its testing in routine practice has recently been regarded as cost-effective.

A summary of all the findings is presented in Table 1.

Conclusions

With the progress in precision medicine, Neurology has entered a new era in relation to several therapeutic approaches. Among '-omic technologies, pharmacogenomics plays an important role as it may enable drug selection considering the patient's genetic profile.

As healthcare shifts from a traditional pathway toward precision medicine, standardized pharmacogenomic education for clinicians becomes necessary. Recently, therapeutic agents have been developed in the context of pharmacogenomic biomarkers related to their safety and efficacy. In the era of precision medicine, educating clinicians on pharmacogenomics may assist with the implementation of genetic information in the clinical practice, thus enhancing genetically-guided treatment decisions.

Abbreviation List

ACHE:	Acetylcholinesterase
AD:	Alzheimer's disease
APOE:	Apolipoprotein E
APP:	Amyloid Beta Precursor Protein
ARIA:	amyloid-related imaging abnormality
BCHE:	butyrylcholinesterase
CHAT:	choline acetyltransferase

Table 1. Clinical effects associated with specific gene and genetic variants according to neurological disease and medication

Disease	Treatment	Polymorphism / Genes	Clinical effects	References
MS	*IFNβ*		candidate gene studies: inconclusive results; GWAS: inconsistent findings; NAbs against IFNb: resistance towards IFNb treatment	[11-15, 80-84]
	GA	HLA DRB1 * 1501	positively associated with GA treatment response	[16, 17]
		rs71878 in TCRB gene	significantly associated with GA treatment	[18]
		rs2275235 in CTSS gene	significantly associated with GA treatment	[18]
		UVRAG (rs80191572)	significantly associated with GA treatment	[18, 19]
		HLA-DQB2 (rs28724893)	significantly associated with GA treatment	[18, 19]
		MBP (rs1789084)	significantly associated with GA treatment	[18, 19]
		ZAK (rs139890339)	significantly associated with GA treatment	[18, 19]
	Mitoxantrone		conflicting results	[21, 22]
	Natalizumab	wild-type genotype or heterozygous presence of one polymorphism for NQO1 or GSTP1	possibly related to beneficial clinical outcomes upon treatment with natalizumab	[23]
	Siponimod	CYP2C9; CYP3A4	affect Siponimod's metabolism	[24]
Stroke	*Aspirin*	PlA1/A2 of the GPIIIa gene	associated with aspirin resistance; inconsistent results; more large-scale RCTs required	[26-28]
		COX-I polymorphisms	associated with aspirin resistance; inconsistent results; more large-scale RCTs required	[26-28]
	Clopidogrel	reduced-function mutation in CYP2C19 * 2 or CYP2C19 * 3	33% reduction of plasma exposure to the active metabolite compared to the wild type genotype and increased risk of vascular events (?); more large-scale trials needed	[29, 30, 32, 34]
		gain-of-function allele CYP2C19 * 17	increased levels of clopidogrel active metabolite and increased risk of bleeding; more large-scale trials needed	[31, 34]
	Statins	SLCO1B1 521C genetic variant	associated with myopathy following the use of simvastatin; the only clinically relevant pharmacogenetic test concerning statin toxicity	[36]
		CYP3A polymorphisms	studies on its possible effects on the risk of statin side effects: inconsistent results	[85]
	VKAs and Warfarin	VKORC1 mutation	resistance and increased risk of unfavorable ischemic events	[39, 40]
		loss-of-function CYP2C9 mutation	reduction in warfarin metabolism and increased risk of bleeding	[41]
		CYP4F2 variant	increased warfarin dose required	[38]
	NOACs	3 SNPs (2 in CES1 gene and 1 in ABCB1 gene)	associated with the variability in plasma levels of dabigatran; large-scale studies needed	[43]
PD	*L-dopa*	COMT polymorphisms	conflicting results	[86-95]
		SNPs of mTOR pathway-related genes	either increased or reduced risk for treatment-induced dyskinesias; larger cohort studies required	[96-108]
		MTHFR mutations	may increase the incidence of hyperhomocysteinemia	[51]
		DRD3 mutation	increased incidence of ICD	[53]
		LRRK2 gene mutations	inconsistent data	[109-112]

Table 1. Continuity

Disease	Treatment	Polymorphism / Genes	Clinical effects	References
PD		GBA gene mutations	inconsistent data	[113-116]
		SNCA mutations	inconsistent data	[117]
		PRKN mutations	steady L-dopa response at lower dose; early treatment-induced motor symptoms (dyskinesias and dystonia)	[118-120]
		PINK1 mutations	steady L-dopa response at lower dose; early treatment-induced motor symptoms (dyskinesias and dystonia)	[118]
		DJ1 mutations	steady L-dopa response at lower dose; early treatment-induced motor symptoms (dyskinesias and dystonia)	[121-123]
	DA	DRD1	high prediction rate of ICD	[52]
		OPRK1	high prediction rate of ICD	[52]
		OPRM1	high prediction rate of ICD	[52]
		polymorphisms in COMT genes	high prediction rate of ICD	[52]
	COMT inhibitors		insufficient evidence for clinical recommendations	[124-128]
	MAO inhibitors		insufficient evidence for clinical recommendations	[129]
AD	*Donepezil*	APOE-4	poor responders	[55]
		APOE-3	optimal responders	[55]
		CYP2D6 (rs1080985)	poor responders	[58]
		APOE-ε4/BCHE-K *	donepezil not recommended	[59]
	Rivastigmine	BChE K (rs1803274)	poor clinical response	[55]
		APOE	affect pharmacokinetics and pharmacodynamics of rivastigmine	[55]
		APP	affect pharmacokinetics and pharmacodynamics of rivastigmine	[55]
		CHAT	affect pharmacokinetics and pharmacodynamics of rivastigmine	[55]
		ACHE	affect pharmacokinetics and pharmacodynamics of rivastigmine	[55]
		BCHE	affect pharmacokinetics and pharmacodynamics of rivastigmine	[55]
		CHRNA4	affect pharmacokinetics and pharmacodynamics of rivastigmine	[55]
		CHRNB2	affect pharmacokinetics and pharmacodynamics of rivastigmine	[55]
		MAPT	affect pharmacokinetics and pharmacodynamics of rivastigmine	[55]
	Memantine	APOE	may influence the effect of memantine in AD	[55]
		PSEN1	may influence the effect of memantine in AD	[55]
		MAPT	may influence the effect of memantine in AD	[61]
	Aducanumab	APOE-4	increased rate of ARIA; more severe in homozygotes	[61]
Epilepsy	*Phenytoin*	CYP2C9 * 2 (rs1799853)	slower metabolism of phenytoin, concentration-dependent neurotoxicity	[66, 67]
		CYP2C9 * 3 (rs1057910(C))	slower metabolism of phenytoin, concentration-dependent neurotoxicity	[66, 67]
		CYP2C9 * 3 (rs1057910)	cutaneous adverse reactions	[68]
		HLA-B * 15:02 allele	risk of Stevens-Johnson syndrome	[76]

Table 1. Continuity

Disease	Treatment	Polymorphism / Genes	Clinical effects	References
Epilepsy	*Clobazam*	CYP2C19	Asian populations, associated with the serum concentration and clinical effectiveness	[64]
	Valproate	CYP2C9	metabolism of valproate	[69]
		CYP2A6	metabolism of valproate	[69]
		CYP2B6	metabolism of valproate	[69]
	Zonisamide	CYP2C19	adverse reactions	[70]
	Carbamazepine	CYP3A4	infreqent polymorphisms, insignificant inter-individual variability *in vivo*	[71]
		HLA-B * 15:02 allele	Stevens-Johnson syndrome in Han Chinese people	[73]
		HLA-A * 31:01 allele	increased risk of cutaneous adverse reactions in European and Japanese patients	[78, 79]
	Oxcarbazepine	SCN1A	related with oxcarbazepine maintenance doses	[72]
		ABCC2	related with oxcarbazepine maintenance doses	[72]
		UGT2B7	related with oxcarbazepine maintenance doses	[72]
		HLA-B * 15:02 allele	risk of Stevens-Johnson syndrome	[77]
	Lamotrigine	HLA-B * 15:02 allele	risk of Stevens-Johnson syndrome	[76]

MS: Multiple Sclerosis; **IFNβ**: interferon-β; **GWAS**: Genome-Wide Association Study; **Nabs**: neutralizing antibodies; **GA**: glatiramer acetate; **HLA**: Human Leukocyte Antigens; **MBP**: myelin basic protein; **ZAK**: zipper containing kinase AZK; **CYP**: cytochrome; **RCTs**: randomization - controlled studies; **COX**: cyclooxygenase; **VKAs**: vitamin K antagonists; **NOACs**: novel oral anticoagulants; **SNPs**: single-nucleotide polymorphism; **mTOR**: mammalian target of rapamycin; **MTHFR:** methylenetetrahydrofolate reductase; **DRD:** Dopamine Receptor D; **ICD**: idiopathic cervical dystonia; **LRRK**: Leucine-rich repeat kinase; **GBA**: Glucosylceramidase Beta; **SNCA**: Synuclein Alpha; **PRKN**: Parkin RBR E3 Ubiquitin Protein Ligase; **PINK**: PTEN Induced Kinase; **DJ1**: Protein DJ-1; **DA:** dopamine agonist; **OPRK**: Opioid Receptor Kappa; **OPRM**: Opioid Receptor Mu; **COMT**: catechol-O-methyltransferase; **MAO**: monoamine oxidase; **AD:** Alzheimer's disease; **BCHE**: butyrylcholinesterase; **APOE**: Apolipoprotein E; **APP**: Amyloid Beta Precursor Protein; **CHAT:** choline acetyltransferase; **ACHE**: Acetylcholinesterase; **CHRNA4**: Cholinergic Receptor Nicotinic Alpha 4 Subunit; **CHRNB2**: Cholinergic Receptor Nicotinic Beta 2 Subunit; **MAPT**: Microtubule Associated Protein Tau; **PSEN**: Presenilin; **ARIA**: amyloid-related imaging abnormality

CHRNA4: Cholinergic Receptor Nicotinic Alpha 4 Subunit
CHRNB2: Cholinergic Receptor Nicotinic Beta 2 Subunit
COMT: catechol-O-methyltransferase
COX: cyclooxygenase
CYP: cytochrome
DA: dopamine agonist
DJ1: Protein DJ-1
DRD: Dopamine Receptor D
GA: glatiramer acetate
GBA: Glucosylceramidase Beta
GWAS: Genome-Wide Association Study
HLA: Human Leukocyte Antigens
ICD: idiopathic cervical dystonia
IFNβ: interferon-β
LRRK: Leucine-rich repeat kinase
MAO: monoamine oxidase
MAPT: Microtubule Associated Protein Tau
MBP: myelin basic protein
MS: Multiple Sclerosis
MTHFR: methylenetetrahydrofolate reductase
mTOR: mammalian target of rapamycin
Nabs: neutralizing antibodies
NOACs: novel oral anticoagulants
OPRK: Opioid Receptor Kappa
OPRM: Opioid Receptor Mu
PINK: PTEN Induced Kinase
PRKN: Parkin RBR E3 Ubiquitin Protein Ligase
PSEN: Presenilin
RCTs: randomization - controlled studies
SNCA: Synuclein Alpha
SNPs: single-nucleotide polymorphism
VKAs: vitamin K antagonists
ZAK: zipper containing kinase AZK

References

[1] Ta R, Cayabyab MA, Coloso R. Precision medicine: a call for increased pharmacogenomic education. *Per Med.* 2019;16:233-245.

[2] McCue ME, McCoy AM. The Scope of Big Data in One Medicine: Unprecedented Opportunities and Challenges. *Front Vet Sci.* 2017;4:194.
[3] Chan A, Pirmohamed M, Comabella M. Pharmacogenomics in neurology: current state and future steps. *Ann Neurol.* 2011;70:684-697.
[4] Weinshilboum RM, Wang L. Pharmacogenomics: Precision Medicine and Drug Response. *Mayo Clin Proc.* 2017;92:1711-1722.
[5] Cecchin E, Stocco G. Pharmacogenomics and Personalized Medicine. *Genes (Basel).* 2020;11.
[6] Motulsky AG. Drug reactions enzymes, and biochemical genetics. *J Am Med Assoc.* 1957;165:835-837.
[7] Relling MV, Klein TE, Gammal RS, et al. The Clinical Pharmacogenetics Implementation Consortium: 10 Years Later. *Clin Pharmacol Ther.* 2020;107:171-175.
[8] Hocevar K, Ristic S, Peterlin B. Pharmacogenomics of Multiple Sclerosis: A Systematic Review. *Front Neurol.* 2019;10:134.
[9] Rio J, Nos C, Tintore M, et al. Defining the response to interferon-beta in relapsing-remitting multiple sclerosis patients. *Ann Neurol.* 2006;59:344-352.
[10] McGraw CA, Lublin FD. Interferon beta and glatiramer acetate therapy. *Neurotherapeutics.* 2013;10:2-18.
[11] Cunningham S, Graham C, Hutchinson M, et al. Pharmacogenomics of responsiveness to interferon IFN-beta treatment in multiple sclerosis: a genetic screen of 100 type I interferon-inducible genes. *Clin Pharmacol Ther.* 2005;78:635-646.
[12] Martinez A, de las Heras V, Mas Fontao A, et al. An IFNG polymorphism is associated with interferon-beta response in Spanish MS patients. *J Neuroimmunol.* 2006;173:196-199.
[13] O'Doherty C, Favorov A, Heggarty S, et al. Genetic polymorphisms, their allele combinations and IFN-beta treatment response in Irish multiple sclerosis patients. *Pharmacogenomics.* 2009;10:1177-1186.
[14] Wergeland S, Beiske A, Nyland H, et al. IL-10 promoter haplotype influence on interferon treatment response in multiple sclerosis. *Eur J Neurol.* 2005;12:171-175.
[15] Hartung HP, Freedman MS, Polman CH, et al. Interferon beta-1b-neutralizing antibodies 5 years after clinically isolated syndrome. *Neurology.* 2011;77:835-843.
[16] Fusco C, Andreone V, Coppola G, et al. HLA-DRB1*1501 and response to copolymer-1 therapy in relapsing-remitting multiple sclerosis. *Neurology.* 2001;57:1976-1979.
[17] Gross R, Healy BC, Cepok S, et al. Population structure and HLA DRB1 1501 in the response of subjects with multiple sclerosis to first-line treatments. *J Neuroimmunol.* 2011;233:168-174.
[18] Grossman I, Avidan N, Singer C, et al. Pharmacogenetics of glatiramer acetate therapy for multiple sclerosis reveals drug-response markers. *Pharmacogenet Genomics.* 2007;17:657-666.
[19] Ross CJ, Towfic F, Shankar J, et al. A pharmacogenetic signature of high response to Copaxone in late-phase clinical-trial cohorts of multiple sclerosis. *Genome Med.* 2017;9:50.
[20] Auricchio F, Scavone C, Cimmaruta D, et al. Drugs approved for the treatment of multiple sclerosis: review of their safety profile. *Expert Opin Drug Saf.* 2017;16:1359-1371.
[21] Cotte S, von Ahsen N, Kruse N, et al. ABC-transporter gene-polymorphisms are potential pharmacogenetic markers for mitoxantrone response in multiple sclerosis. *Brain.* 2009;132:2517-2530.
[22] Grey Nee Cotte S, Salmen Nee Stroet A, von Ahsen N, et al. Lack of efficacy of mitoxantrone in primary progressive Multiple Sclerosis irrespective of pharmacogenetic factors: a multicenter, retrospective analysis. *J Neuroimmunol.* 2015;278:277-279.
[23] Alexoudi A, Zachaki S, Stavropoulou C, et al. Possible Implication of GSTP1 and NQO1 Polymorphisms on Natalizumab Response in Multiple Sclerosis. *Ann Clin Lab Sci.* 2016;46:586-591.
[24] Siponimod for multiple sclerosis. *Aust Prescr.* 2021;44:69-70.
[25] Meschia JF. Pharmacogenetics and stroke. *Stroke.* 2009;40:3641-3645.
[26] Goodman T, Ferro A, Sharma P. Pharmacogenetics of aspirin resistance: a comprehensive systematic review. *Br J Clin Pharmacol.* 2008;66:222-232.
[27] Clappers N, van Oijen MG, Sundaresan S, et al. The C50T polymorphism of the cyclooxygenase-1 gene and the risk of thrombotic events during low-dose therapy with acetyl salicylic acid. *Thromb Haemost.* 2008;100:70-75.
[28] Maree AO, Curtin RJ, Chubb A, et al. Cyclooxygenase-1 haplotype modulates platelet response to aspirin. *J Thromb Haemost.* 2005;3:2340-2345.
[29] Mega JL, Close SL, Wiviott SD, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med.* 2009;360:354-362.
[30] Mega JL, Simon T, Collet JP, et al. Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. *JAMA.* 2010;304:1821-1830.
[31] Sibbing D, Koch W, Gebhard D, et al. Cytochrome 2C19*17 allelic variant, platelet aggre-

gation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. *Circulation.* 2010;121:512-518.
[32] Holmes MV, Perel P, Shah T, et al. CYP2C19 genotype, clopidogrel metabolism, platelet function, and cardiovascular events: a systematic review and meta-analysis. *JAMA.* 2011;306:2704-2714.
[33] Pare G, Eikelboom JW, Sibbing D, et al. Testing should not be done in all patients treated with clopidogrel who are undergoing percutaneous coronary intervention. *Circ Cardiovasc Interv.* 2011;4:514-521; discussion 521.
[34] Ross S, Pare G. Pharmacogenetics of Stroke. *Stroke.* 2018;49:2541-2548.
[35] Leusink M, Onland-Moret NC, de Bakker PI, et al. Seventeen years of statin pharmacogenetics: a systematic review. *Pharmacogenomics.* 2016;17:163-180.
[36] Kitzmiller JP, Mikulik EB, Dauki AM, et al. Pharmacogenomics of statins: understanding susceptibility to adverse effects. *Pharmgenomics Pers Med.* 2016;9:97-106.
[37] Johnson JA, Gong L, Whirl-Carrillo M, et al. Clinical Pharmacogenetics Implementation Consortium Guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. *Clin Pharmacol Ther.* 2011;90:625-629.
[38] McDonald MG, Rieder MJ, Nakano M, et al. CYP4F2 is a vitamin K1 oxidase: An explanation for altered warfarin dose in carriers of the V433M variant. *Mol Pharmacol.* 2009;75:1337-1346.
[39] Rieder MJ, Reiner AP, Gage BF, et al. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. *N Engl J Med.* 2005;352:2285-2293.
[40] Watzka M, Geisen C, Bevans CG, et al. Thirteen novel VKORC1 mutations associated with oral anticoagulant resistance: insights into improved patient diagnosis and treatment. *J Thromb Haemost.* 2011;9:109-118.
[41] Limdi NA, Arnett DK, Goldstein JA, et al. Influence of CYP2C9 and VKORC1 on warfarin dose, anticoagulation attainment and maintenance among European-Americans and African-Americans. *Pharmacogenomics.* 2008;9:511-526.
[42] Connolly SJ, Ezekowitz MD, Yusuf S, et al. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med.* 2009;361:1139-1151.
[43] Pare G, Eriksson N, Lehr T, et al. Genetic determinants of dabigatran plasma levels and their relation to bleeding. *Circulation.* 2013;127:1404-1412.
[44] Kampouraki E, Kamali F. Pharmacogenetics of anticoagulants used for stroke prevention in patients with atrial fibrillation. *Expert Opin Drug Metab Toxicol.* 2019;15:449-458.
[45] Cacabelos R. Pharmacogenomics of Alzheimer's and Parkinson's diseases. *Neurosci Lett.* 2020;726:133807.
[46] Sardi SP, Cedarbaum JM, Brundin P. Targeted Therapies for Parkinson's Disease: From Genetics to the Clinic. *Mov Disord.* 2018;33:684-696.
[47] Poewe W, Seppi K, Tanner CM, et al. Parkinson disease. *Nat Rev Dis Primers.* 2017;3:17013.
[48] Bloem BR, Okun MS, Klein C. Parkinson's disease. *Lancet.* 2021;397:2284-2303.
[49] Ciccacci C, Borgiani P. Pharmacogenomics in Parkinson's disease: which perspective for developing a personalized medicine? *Neural Regen Res.* 2019;14:75-76.
[50] Vuletic V, Racki V, Papic E, et al. A Systematic Review of Parkinson's Disease Pharmacogenomics: Is There Time for Translation into the Clinics? *Int J Mol Sci.* 2021;22.
[51] Zoccolella S, Lamberti P, Armenise E, et al. Plasma homocysteine levels in Parkinson's disease: role of antiparkinsonian medications. *Parkinsonism Relat Disord.* 2005;11:131-133.
[52] McDonell KE, van Wouwe NC, Harrison MB, et al. Taq1A polymorphism and medication effects on inhibitory action control in Parkinson disease. *Brain Behav.* 2018;8:e01008.
[53] Castro-Martinez XH, Garcia-Ruiz PJ, Martinez-Garcia C, et al. Behavioral addictions in early-onset Parkinson disease are associated with DRD3 variants. *Parkinsonism Relat Disord.* 2018;49:100-103.
[54] Masellis M, Collinson S, Freeman N, et al. Dopamine D2 receptor gene variants and response to rasagiline in early Parkinson's disease: a pharmacogenetic study. *Brain.* 2016;139:2050-2062.
[55] Cacabelos R. Pharmacogenomics of Cognitive Dysfunction and Neuropsychiatric Disorders in Dementia. *Int J Mol Sci.* 2020;21.
[56] Li DD, Zhang YH, Zhang W, et al. Meta-Analysis of Randomized Controlled Trials on the Efficacy and Safety of Donepezil, Galantamine, Rivastigmine, and Memantine for the Treatment of Alzheimer's Disease. *Front Neurosci.* 2019;13:472.
[57] Cummings J, Aisen P, Apostolova LG, et al. Aducanumab: Appropriate Use Recommendations. *J Prev Alzheimers Dis.* 2021;8:398-410.
[58] Birks JS, Harvey RJ. Donepezil for dementia due to Alzheimer's disease. *Cochrane Database Syst Rev.* 2018;6:CD001190.
[59] De Beaumont L, Pelleieux S, Lamarre-Theroux L, et al. Butyrylcholinesterase K and Apolipoprotein E-varepsilon4 Reduce the Age of Onset of Alzheimer's Disease, Accelerate Cognitive Decline, and Modulate Donepezil Response in Mild Cognitively Impaired Subjects. *J Alzheimers Dis.* 2016;54:913-922.
[60] Birks JS, Chong LY, Grimley Evans J. Rivastigmine

for Alzheimer's disease. *Cochrane Database Syst Rev.* 2015;9:CD001191.
[61] VandeVrede L, Gibbs DM, Koestler M, et al. Symptomatic amyloid-related imaging abnormalities in an APOE epsilon4/epsilon4 patient treated with aducanumab. *Alzheimers Dement (Amst).* 2020;12:e12101.
[62] Moshe SL, Perucca E, Ryvlin P, et al. Epilepsy: new advances. *Lancet.* 2015;385:884-898.
[63] Franco V, Perucca E. The pharmacogenomics of epilepsy. *Expert Rev Neurother.* 2015;15:1161-1170.
[64] Balestrini S, Sisodiya SM. Pharmacogenomics in epilepsy. *Neurosci Lett.* 2018;667:27-39.
[65] Lopez-Garcia MA, Feria-Romero IA, Fernando-Serrano H, et al. Genetic polymorphisms associated with antiepileptic metabolism. *Front Biosci (Elite Ed).* 2014;6:377-386.
[66] Lee CR, Goldstein JA, Pieper JA. Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. *Pharmacogenetics.* 2002;12:251-263.
[67] Depondt C, Godard P, Espel RS, et al. A candidate gene study of antiepileptic drug tolerability and efficacy identifies an association of CYP2C9 variants with phenytoin toxicity. *Eur J Neurol.* 2011;18:1159-1164.
[68] Chung WH, Chang WC, Lee YS, et al. Genetic variants associated with phenytoin-related severe cutaneous adverse reactions. *JAMA.* 2014;312:525-534.
[69] Kiang TK, Ho PC, Anari MR, et al. Contribution of CYP2C9, CYP2A6, and CYP2B6 to valproic acid metabolism in hepatic microsomes from individuals with the CYP2C9*1/*1 genotype. *Toxicol Sci.* 2006;94:261-271.
[70] Okada Y, Seo T, Ishitsu T, et al. Population estimation regarding the effects of cytochrome P450 2C19 and 3A5 polymorphisms on zonisamide clearance. *Ther Drug Monit.* 2008;30:540-543.
[71] Klein K, Thomas M, Winter S, et al. PPARA: a novel genetic determinant of CYP3A4 in vitro and in vivo. *Clin Pharmacol Ther.* 2012;91:1044-1052.
[72] Ma CL, Wu XY, Jiao Z, et al. SCN1A, ABCC2 and UGT2B7 gene polymorphisms in association with individualized oxcarbazepine therapy. *Pharmacogenomics.* 2015;16:347-360.
[73] Chung WH, Hung SI, Hong HS, et al. Medical genetics: a marker for Stevens-Johnson syndrome. *Nature.* 2004;428:486.
[74] Ferrell PB, Jr., McLeod HL. Carbamazepine, HLA-B*1502 and risk of Stevens-Johnson syndrome and toxic epidermal necrolysis: US FDA recommendations. *Pharmacogenomics.* 2008;9:1543-1546.
[75] Amstutz U, Shear NH, Rieder MJ, et al. Recommendations for HLA-B*15:02 and HLA-A*31:01 genetic testing to reduce the risk of carbamazepine-induced hypersensitivity reactions. *Epilepsia.* 2014;55:496-506.
[76] Cheung YK, Cheng SH, Chan EJ, et al. HLA-B alleles associated with severe cutaneous reactions to antiepileptic drugs in Han Chinese. *Epilepsia.* 2013;54:1307-1314.
[77] Hung SI, Chung WH, Liu ZS, et al. Common risk allele in aromatic antiepileptic-drug induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Han Chinese. *Pharmacogenomics.* 2010;11:349-356.
[78] Ozeki T, Mushiroda T, Yowang A, et al. Genome-wide association study identifies HLA-A*3101 allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population. *Hum Mol Genet.* 2011;20:1034-1041.
[79] McCormack M, Alfirevic A, Bourgeois S, et al. HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. *N Engl J Med.* 2011;364:1134-1143.
[80] Byun E, Caillier SJ, Montalban X, et al. Genome-wide pharmacogenomic analysis of the response to interferon beta therapy in multiple sclerosis. *Arch Neurol.* 2008;65:337-344.
[81] Comabella M, Craig DW, Morcillo-Suarez C, et al. Genome-wide scan of 500,000 single-nucleotide polymorphisms among responders and nonresponders to interferon beta therapy in multiple sclerosis. *Arch Neurol.* 2009;66:972-978.
[82] Esposito F, Sorosina M, Ottoboni L, et al. A pharmacogenetic study implicates SLC9a9 in multiple sclerosis disease activity. *Ann Neurol.* 2015;78:115-127.
[83] Mahurkar S, Moldovan M, Suppiah V, et al. Response to interferon-beta treatment in multiple sclerosis patients: a genome-wide association study. *Pharmacogenomics J.* 2017;17:312-318.
[84] Clarelli F, Liberatore G, Sorosina M, et al. Pharmacogenetic study of long-term response to interferon-beta treatment in multiple sclerosis. *Pharmacogenomics J.* 2017;17:84-91.
[85] Administration USFD. FDA drug safety communication: new restrictions, contraindications, and dose limitations for Zocor (simvastatin) to reduce the risk of muscle injury. In: Administration USFD, ed. *US FDA Safety Announcement 06-08-2011.* Washington, DC: FDA; 2011, 2011.
[86] Bialecka M, Kurzawski M, Klodowska-Duda G, et al. The association of functional catechol-O-methyltransferase haplotypes with risk of Parkinson's disease, levodopa treatment response,

and complications. *Pharmacogenet Genomics.* 2008;18:815-821.
[87] Cheshire P, Bertram K, Ling H, et al. Influence of single nucleotide polymorphisms in COMT, MAO-A and BDNF genes on dyskinesias and levodopa use in Parkinson's disease. *Neurodegener Dis.* 2014;13:24-28.
[88] Bialecka M, Drozdzik M, Klodowska-Duda G, et al. The effect of monoamine oxidase B (MAOB) and catechol-O-methyltransferase (COMT) polymorphisms on levodopa therapy in patients with sporadic Parkinson's disease. *Acta Neurol Scand.* 2004;110:260-266.
[89] Contin M, Martinelli P, Mochi M, et al. Genetic polymorphism of catechol-O-methyltransferase and levodopa pharmacokinetic-pharmacodynamic pattern in patients with Parkinson's disease. *Mov Disord.* 2005;20:734-739.
[90] Sampaio TF, Dos Santos EUD, de Lima GDC, et al. MAO-B and COMT Genetic Variations Associated With Levodopa Treatment Response in Patients With Parkinson's Disease. *J Clin Pharmacol.* 2018;58:920-926.
[91] Lee MS, Lyoo CH, Ulmanen I, et al. Genotypes of catechol-O-methyltransferase and response to levodopa treatment in patients with Parkinson's disease. *Neurosci Lett.* 2001;298:131-134.
[92] Becker ML, Visser LE, van Schaik RH, et al. OCT1 polymorphism is associated with response and survival time in anti-Parkinsonian drug users. *Neurogenetics.* 2011;12:79-82.
[93] Altmann V, Schumacher-Schuh AF, Rieck M, et al. Influence of genetic, biological and pharmacological factors on levodopa dose in Parkinson's disease. *Pharmacogenomics.* 2016;17:481-488.
[94] de Lau LM, Verbaan D, Marinus J, et al. Catechol-O-methyltransferase Val158Met and the risk of dyskinesias in Parkinson's disease. *Mov Disord.* 2012;27:132-135.
[95] Watanabe M, Harada S, Nakamura T, et al. Association between catechol-O-methyltransferase gene polymorphisms and wearing-off and dyskinesia in Parkinson's disease. *Neuropsychobiology.* 2003;48:190-193.
[96] Martin-Flores N, Fernandez-Santiago R, Antonelli F, et al. MTOR Pathway-Based Discovery of Genetic Susceptibility to L-DOPA-Induced Dyskinesia in Parkinson's Disease Patients. *Mol Neurobiol.* 2019;56:2092-2100.
[97] Foltynie T, Cheeran B, Williams-Gray CH, et al. BDNF val66met influences time to onset of levodopa induced dyskinesia in Parkinson's disease. *J Neurol Neurosurg Psychiatry.* 2009;80:141-144.
[98] Dos Santos EUD, Sampaio TF, Tenorio Dos Santos AD, et al. The influence of SLC6A3 and DRD2 polymorphisms on levodopa-therapy in patients with sporadic Parkinson's disease. *J Pharm Pharmacol.* 2019;71:206-212.
[99] Rieck M, Schumacher-Schuh AF, Altmann V, et al. DRD2 haplotype is associated with dyskinesia induced by levodopa therapy in Parkinson's disease patients. *Pharmacogenomics.* 2012;13:1701-1710.
[100] Strong JA, Dalvi A, Revilla FJ, et al. Genotype and smoking history affect risk of levodopa-induced dyskinesias in Parkinson's disease. *Mov Disord.* 2006;21:654-659.
[101] Zappia M, Annesi G, Nicoletti G, et al. Sex differences in clinical and genetic determinants of levodopa peak-dose dyskinesias in Parkinson disease: an exploratory study. *Arch Neurol.* 2005;62:601-605.
[102] Oliveri RL, Annesi G, Zappia M, et al. Dopamine D2 receptor gene polymorphism and the risk of levodopa-induced dyskinesias in PD. *Neurology.* 1999;53:1425-1430.
[103] Lee JY, Cho J, Lee EK, et al. Differential genetic susceptibility in diphasic and peak-dose dyskinesias in Parkinson's disease. *Mov Disord.* 2011;26:73-79.
[104] Kaiser R, Hofer A, Grapengiesser A, et al. L-dopa-induced adverse effects in PD and dopamine transporter gene polymorphism. *Neurology.* 2003;60:1750-1755.
[105] Wang J, Liu ZL, Chen B. Association study of dopamine D2, D3 receptor gene polymorphisms with motor fluctuations in PD. *Neurology.* 2001;56:1757-1759.
[106] Paus S, Gadow F, Knapp M, et al. Motor complications in patients form the German Competence Network on Parkinson's disease and the DRD3 Ser9Gly polymorphism. *Mov Disord.* 2009;24:1080-1084.
[107] Schumacher-Schuh AF, Altmann V, Rieck M, et al. Association of common genetic variants of HOMER1 gene with levodopa adverse effects in Parkinson's disease patients. *Pharmacogenomics J.* 2014;14:289-294.
[108] Purcaro C, Vanacore N, Moret F, et al. DAT gene polymorphisms (rs28363170, rs393795) and levodopa-induced dyskinesias in Parkinson's disease. *Neurosci Lett.* 2019;690:83-88.
[109] Corvol JC, Poewe W. Pharmacogenetics of Parkinson's Disease in Clinical Practice. *Mov Disord Clin Pract.* 2017;4:173-180.
[110] Yue Z. LRRK2 in Parkinson's disease: in vivo models and approaches for understanding pathogenic roles. *FEBS J.* 2009;276:6445-6454.
[111] Shu L, Zhang Y, Pan H, et al. Clinical Heterogeneity Among LRRK2 Variants in Parkinson's Disease: A Meta-Analysis. *Front Aging Neurosci.* 2018;10:283.

[112] Yahalom G, Kaplan N, Vituri A, et al. Dyskinesias in patients with Parkinson's disease: effect of the leucine-rich repeat kinase 2 (LRRK2) G2019S mutation. *Parkinsonism Relat Disord.* 2012;18:1039-1041.
[113] Silveira CRA, MacKinley J, Coleman K, et al. Ambroxol as a novel disease-modifying treatment for Parkinson's disease dementia: protocol for a single-centre, randomized, double-blind, placebo-controlled trial. *BMC Neurol. 2*019;19:20.
[114] Alcalay RN, Levy OA, Waters CC, et al. Glucocerebrosidase activity in Parkinson's disease with and without GBA mutations. *Brain.* 2015;138:2648-2658.
[115] Lesage S, Anheim M, Condroyer C, et al. Large-scale screening of the Gaucher's disease-related glucocerebrosidase gene in Europeans with Parkinson's disease. *Hum Mol Genet.* 2011;20:202-210.
[116] Avenali M, Blandini F, Cerri S. Glucocerebrosidase Defects as a Major Risk Factor for Parkinson's Disease. *Front Aging Neurosci.* 2020;12:97.
[117] N. A, A. A. The Relationship between Alpha-Synuclein (SNCA) Gene Polymorphisms and Development Risk of Parkinson's Disease. In: Andrei Surguchov I, ed. *Synucleins - Biochemistry and Role in Diseases*: IntechOpen, 2019.
[118] Zhang Y, Sun QY, Zhao YW, et al. Effect of GBA Mutations on Phenotype of Parkinson's Disease: A Study on Chinese Population and a Meta-Analysis. *Parkinsons Dis.* 2015;2015:916971.
[119] Kasten M, Hartmann C, Hampf J, et al. Genotype-Phenotype Relations for the Parkinson's Disease Genes Parkin, PINK1, DJ1: MDSGene Systematic Review. *Mov Disord.* 2018;33:730-741.
[120] Sassone J, Valtorta F, Ciammola A. Early Dyskinesias in Parkinson's Disease Patients With Parkin Mutation: A Primary Corticostriatal Synaptopathy? *Front Neurosci.* 2019;13:273.
[121] Repici M, Giorgini F. DJ-1 in Parkinson's Disease: Clinical Insights and Therapeutic Perspectives. *J Clin Med.* 2019;8.
[122] Kuhl M. https://www.michaeljfox.org/news/next-line-parkinsons-therapies-prkn-and-pink1 (accessed 10/01/2022 2022).
[123] Rahman AA, Morrison BE. Contributions of VPS35 Mutations to Parkinson's Disease. *Neuroscience.* 2019;401:1-10.
[124] Bonifacio MJ, Palma PN, Almeida L, et al. Catechol-O-methyltransferase and its inhibitors in Parkinson's disease. *CNS Drug Rev.* 2007;13:352-379.
[125] Liu W, Ramirez J, Gamazon ER, et al. Genetic factors affecting gene transcription and catalytic activity of UDP-glucuronosyltransferases in human liver. *Hum Mol Genet.* 2014;23:5558-5569.
[126] Yamanaka H, Nakajima M, Katoh M, et al. A novel polymorphism in the promoter region of human UGT1A9 gene (UGT1A9*22) and its effects on the transcriptional activity. *Pharmacogenetics.* 2004;14:329-332.
[127] Martignoni E, Cosentino M, Ferrari M, et al. Two patients with COMT inhibitor-induced hepatic dysfunction and UGT1A9 genetic polymorphism. *Neurology.* 2005;65:1820-1822.
[128] Kaplowitz N. Idiosyncratic drug hepatotoxicity. *Nat Rev Drug Discov.* 2005;4:489-499.
[129] Guay DR. Rasagiline (TVP-1012): a new selective monoamine oxidase inhibitor for Parkinson's disease. *Am J Geriatr Pharmacother.* 2006;4:330-346.

SPINAL MUSCULAR ATROPHY: CLINICAL CHARACTERISTICS, GENETICS AND MANAGEMENT

Dimitra Papadimitriou, MD, PhD

Henry Dunant Hospital Center, Director, 1st Neurology Department

Spinal Muscular Atrophy

Spinal Muscular Atrophy (SMA) is a neurodegenerative disease, following the autosomal receive mode of inheritance and it is characterized by degeneration of anterior horn Motor Neurons (alpha MNs) in the lower spinal cord, leading to progressive symmetrical muscular weakness and atrophy. The worldwide incidence of SMA is approximately 1-2 in 11.000 [1] and the prevalence is 1-2 per 100.000. The carrier frequency ranging from 1:91 in African Americans to 1:35 in Caucasians and 1:54 in United States [2, 3] could be slightly different based on the ethnic group [3, 4]. Werdnig and Hoffman in the early 1890s were the first to describe a mild phenotype of SMA; but only in the early 1900s did Beevor describe for the first time a severe form of SMA with the involvement of the respiratory system due to intercostal muscle weakness [5, 6]. SMA is caused by loss of the Survival Motor Neuron 1 (*SMN1*) gene on chromosome 5q (in 95% of SMA cases) which encodes for the *SMN* protein [7]. Interestingly, in humans on the same chromosome there is a second centromeric form of *SMN1*, called *SMN2*, which translates approximately 10% of the functional *SMN* protein and can be present in different copy numbers [7], which is relevant for the clinical manifestation as *SMN2* copy number significantly correlates with disease severity (see below).

Clinical Picture

SMA pathology is heterogeneous, with a wide range of phenotypic spectrum. It is classified into five different types according to the age of onset and maximum function (Table 1). Briefly, the most severe types are SMA 0 (Congenital SMA) and SMA type I (Werdnig Hoffmann disease). Regarding SMA type 0, the onset is before birth (in utero) and death occurs immediately after or within two weeks from birth. In SMA type I, children never achieve the ability to sit independently and they present also fasciculations of the tongue. Oculomotor and mimic muscles seem not to be involved; but in cases with cardiogenesis, defects have been reported [8]. Of further note, cognitive and sensory functions are not compromised. If not treated or unsupported, death usually occurs before reaching the second year of life [9]. In contrast to SMA1 patients, SMA type II patients are able to sit but unable to walk independently and may survive into adulthood - though with shorter life expectancy. SMA type III and type IV are the mildest forms. In particular, SMA type III patients are ambulant, even though some of them might lose autonomous de-ambulation over time. Meanwhile SMA type IV is considered an "adult-onset" form of SMA, with age-of-onset typically occurring after 30 years and ranging from normal motor function to mild motor impairment and preserved life expectancy. All SMA patients lack the *SMN1* gene, therefore the amount of *SMN* protein produced depends entirely on the number of *SMN2* copies that each individual carries. The broad phenotypic spectrum associated with *SMN1* deficiency is driven by the number of the *SMN2* gene copies (see Table 1).

SMA can also be classified into 4 groups based on the severity of the disease and on the number of *SMN2* copies carried by patients [10].

SMN Gene And Protein

SMN1 is a telomeric gene comprised of eight exons: denoted 1, 2a, 2b, 3, 4, 5, 6 and 7. This gene is located on the chromosome 5 in a complex region (5q13.2) characterized by the duplication of an approximate 500 kb element containing several genes [7].

The *SMN1* gene encodes a 294 amino acid RNA-binding protein (38 kDa), which is ubiquitously expressed in all the human body, with higher levels detected in the spinal cord, brain, kidney and liver [7, 11]. Immunohistological staining of *SMN* protein revealed its localization in the nucleus, in the shape of foci, and diffuse staining in the cytoplasm [12]. The dot-like structures formed by *SMN*, similar and adjacent to coiled bodies (CBs or Cajal bodies), are called "Gemini of coiled bodies", simplified as Gems [11]. Gems are a dynamic nuclear structure and their number, like the expression level of *SMN* protein, is directly correlated to the SMA phenotype [11, 13].

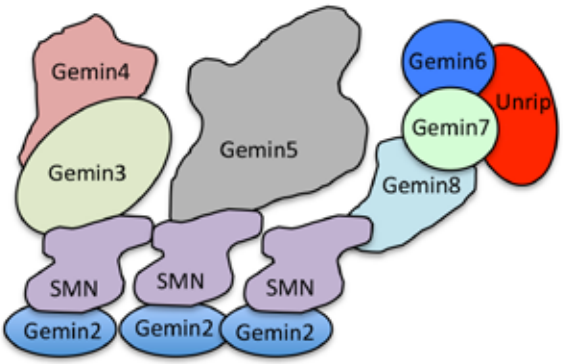
Table 1. SMA phenotype classification

Phenotype	Age at Onset	Life span	Motor Milestones	SMN2 Copies
Healthy Carrier	/	Normal	Normal	0
SMA 0 (Congenital SMA)	Prenatal	<6 months	None Achieved	1
SMA I (Werdnig-Hoffmann disease)	< 6 months	Most often ≤ 2 years, but may live longer	Sit with support only	2
SMA II (Dubowitz disease)	6-18 months	70% alive at age 25	Independent sitting when placed	3-4
SMA III (Kugelberg-Welander disease)	> 18months	Normal	Independent ambulation	3-4
SMA IV	Adulthood	Normal	Normal	4-8

SMN is composed by five domains: (i) N-Terminal-Gemin 2 domain, (ii) Tudor domain, (iii) basic-lysine-rich domain, (iv) the YG box domain, and (v) C-terminal proline-rich domain [14] (Fig. 1). In particular, the basic/lysine-rich region, encoded by exon 2, has been demonstrated to interact with *SMN*-Interacting Protein 1 (SIP1), also known as Gemin2, RNAs and other proteins, such as p53, both *in vitro* and *in vivo* [15-17]. The Tudor domain is another important region present on *SMN*. It is a highly conserved motif codified by exon 3 that binds to the terminal glycine and symmetrical dimethylated arginine-rich tails of Sm ribonucleoproteins, facilitating the assembly of the spliceosomes [18-20]. Moreover, it is responsible for the interaction with other proteins, such as Fused-in-Sarcoma (FUS), Histone 3 and Fragile X Mental Retardation Protein (FMRP) RNA polymerase II 21. Mutations in this domain are often found in SMA patients [18-21].

The other domains of *SMN* are YG box (tyrosine-glycine-rich region), which interacts with zinc-finger protein (ZPR1), amphipathic helix protein SIN3A (transcription co-repressor) and Gemin3 [14], and the proline rich domain that interacts with Profilins, a family of proteins involved in actin dynamics [22]. It has been reported that *SMN* protein, through the YG domain, is able to oligomerize with itself and that the loss of exon 7 decreases the efficiency and stability of self-oligomerization [16, 23] (Fig. 2).

Fig. 1. Schematic representation of *SMN* mRNA and corresponding *SMN* protein binding domains



SMN1 possess four domains which allowed the binding to other proteins or with itself. Lysine-rich domain, Proline-rich domain and YG box domain bind to other *SMN1*, Tudor domain allows the binding to Sm and Lysin-erich domain binds to Gemin2 protein

Fig. 2. Schematic representation of SMN complex. SMN complex is composed by nine proteins, SMN, Gemin family from 2-7 and Unirip

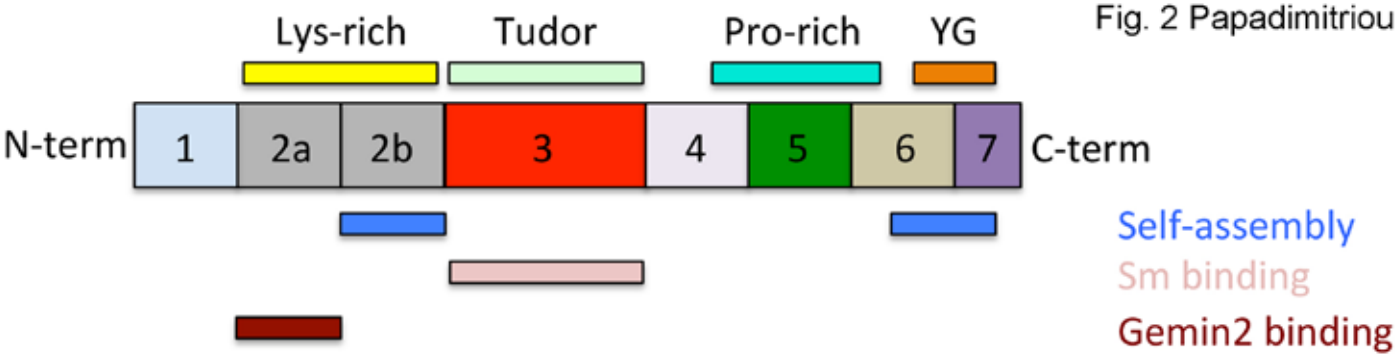
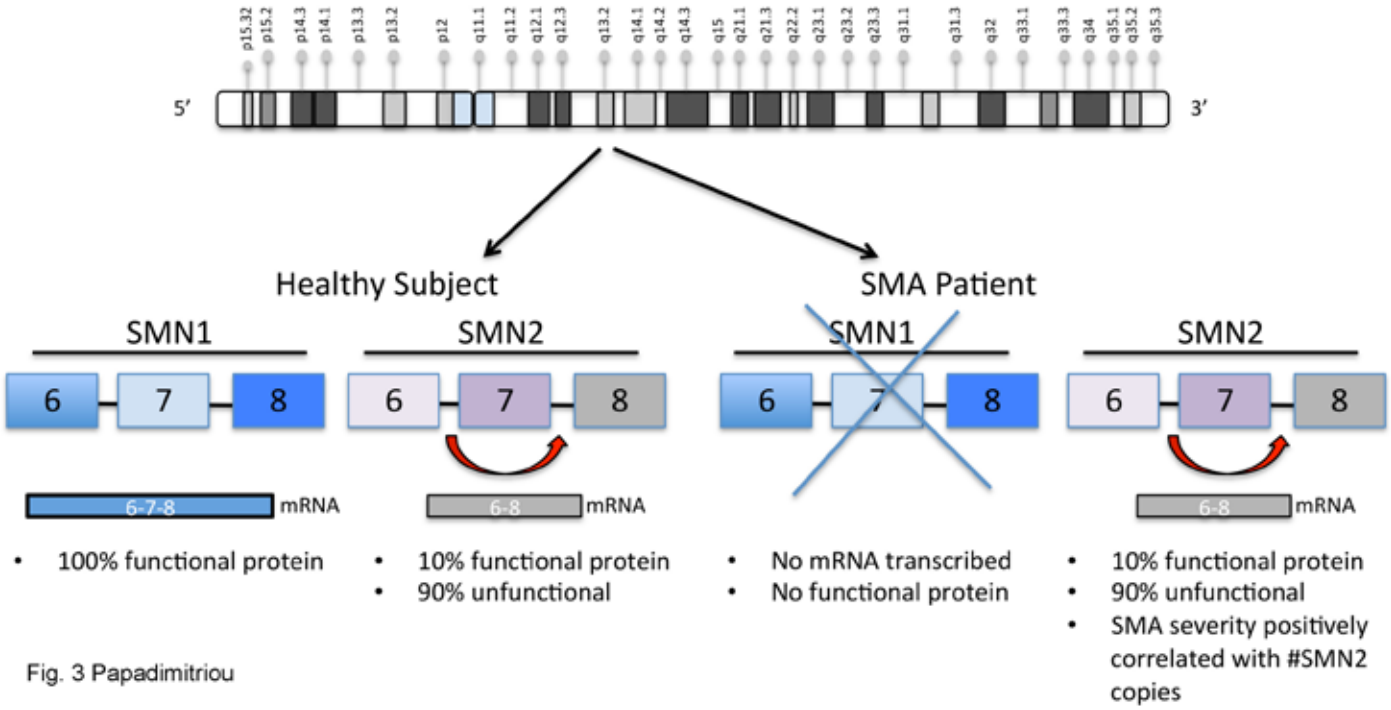


Fig. 3. Schematic representation of *SMN1* and *SMN2* in healthy and affected subjects



SMN (also referred to as Gemin1) can also be recruited in a group of protein called *SMN* complex [24-28]. This complex is composed by members of the Gemin family of proteins, in particular from Gemin2 (formerly SIP1), Gemin3/DP103 (a DEAD-box RNA helicase), Gemin4, Gemin5/p175 (a WD repeat protein), Gemin6, Gemin7 and UNRIP (UNR-interacting protein) [29]. In the middle of the complex there are *SMN*, Gemin7 and Gemin8, while the other components are bound to the complex through different interactions [30] (Fig. 2).

On the same chromosome, the *SMN2* gene shares more than 99% nucleotide identity with *SMN1*; indeed, the sequence differs only in five nucleotides (two exonic and three intronic) [31]. Nevertheless, a single point mutation (840C ≥ T substitution) in the last codon (exon 7) of *SMN2* modifies its splicing, resulting in a truncated and unstable form of *SMN* protein lacking 16 amino acids and the carboxyl terminus. The *SMN2* gene encodes for an *SMN* protein also called *SMN*Δ7 – as the 7th exon is missing in this case. In most of the cases (90-95%) *SMN*Δ7 protein is functionally defective, and is rapidly degraded (two-fold shorter half-life compared to the full-length), while only 5-10% is a functional full-length (FL) protein [16] (Fig. 3). Healthy subjects express 100% FL-*SMN* protein as a result of *SMN1* translational process and 10% functional FL-*SMN* protein as a result of *SMN2* translation. SMA subjects lack both *SMN1* alleles and consequently *SMN2* plays a pivotal role in the definition of the phenotype. Indeed, several genotype/phenotype analyses confirmed an inversed correlation between the phenotypic appearance of SMA and the *SMN2* copy numbers (e.g. higher *SMN2* copy number correlates with a milder SMA phenotype [32]).

The *SMN* protein is implicated in a few different functions, such as RNA metabolism (pre-mRNA splicing, transcription through its interaction with CTD of pol II, translation and stress granule (SG) formation), signal transduction, intracellular trafficking (endocytosis, cytoskeleton), and DNA recombination/repair [14]. In particular, with respect to RNA metabolism, the *SMN* complex is important not only for pre-mRNA splicing, but it is also involved as a chaperon in maturation, assembly and function of spliceosomal small nuclear ribonucleoproteins (snRNPs) U1, U2, U4, and U5, which are the core component of the spliceosomal complex [33-35]. Spliceosomal snRNPs are U-rich snRNPs composed by seven Sm proteins (B/B', D1, D2, D3, E, F, and G) that remove introns from pre-RNA. Their biogenesis is a multi-step process that begins in the nucleus, translocates to the cytoplasm, and then back to the nucleus. It starts with transcription, regulated by RNA polymerase II (pol II), of pre-snRNAs that are co-transcriptionally processed at their 5'-end, with the inclusion of 7- methyl guanosine (m7G) cap and cleaved at their 3'-end. Through an export complex, pre-snRNAs are transported in the cytoplasm where they are further processed by *SMN* complex and heptameric Sm ring. These protein interactions facilitate the hypermethylation, 3'-end trimming,

and subsequently the snRNPs translocate together into the nucleus; here the complex dissociates and *SMN* transiently localizes in Cajal Bodies (CBs), where snRNAs undergo to full maturation [14, 36].

Another important role played by *SMN* is the trafficking of mRNAs along the axon and in the growth cone of primary MNs [37]. Indeed, it has been shown that *SMN* modulates the localization of β-actin within the growth cones [38]. In particular, *SMN* has been found to colocalize with profilin 2a, which is an actin-binding protein [39]. Moreover, *SMN* complex seems to interact also with candidate plasticity gene 15 (Cpg15). Indeed, it has been showed that the depletion of *SMN* causes Cpg15 and β-actin reduction in distal axons [40]. In a recent study, Rage and colleagues performed a genome-wide study in MN-like cells (NSC-34) of *SMN*-associated RNAs. The authors identified over 200 mRNAs associated with *SMN*, of which 30% localized to the axon in an *SMN*-dependent manner [41]. Further evidence concerning *SMN* and mRNA interaction in the axons, is provided by Fallini and colleagues; in fact, they present a potential non-canonical function of *SMN* in axons through the binding of the *SMN* Tudor domain to a neuronal-specific mRNA-binding protein, HuD, which has a role in neuronal development and plasticity [42]. Results showed that impairment in *SMN* expression caused the reduction of HuD protein levels in the axons, and this decrease could impair the axonal localization and the interaction of mRNAs with mRNA-binding proteins like HuD, KSRP, and hnRNP-R/Q resulting in a defective subcellular localization of transcripts likely necessary for MNs maintenance. They also demonstrated that *SMN* is required for axonal localization of poly(A) mRNA-containing granules. Although these data suggest a possible involvement of *SMN* in mRNA transport, it has not yet been demonstrated if mRNA trafficking impairment in SMA is due to *SMN* deficiency, or whether the MN degeneration is caused by the damaged transport of specific transcripts.

There are several mechanisms that regulate *SMN* protein levels, such as post-translational modifications (sumoylation, ubiquitination and phosphorylation), *SMN* mRNA stabilization, genomic and sequence integrity, transcription regulation, transregulatory splicing factors and subcellular localization. Moreover, other external factors could also be involved in *SMN* expression regulation, such as oxidative stress, hypoxia and starvation [43]. While *SMN* and *SMN*Δ7 proteins are expressed ubiquitously, and there is evidence of dysfunction in other systems [e.g. 8], the reason(s) why MN's exhibit greater vulnerability in SMA patients remains to be determined.

Therapeutics

In the past decade the majority of therapeutic approaches to rescue the pathological and clinical phenotype in SMA have been focusing on *SMN* protein level restoration. This can be achieved either through administration of a functional *SMN1* gene, or by acting on *SMN2* protein levels. Currently there are three commercially available treatments for SMA in Europe: Nusinersen, Risdiplan and Zolgensma, described in detail below.

SMN2 Targeting Approaches

As mentioned above, *SMN2* gene differs from *SMN1* by only one base change and *SMN2* is present in all SMA patients in different copy numbers. Compounds that target *SMN2* could potentially benefit the SMA phenotype through stabilization of *SMN2* mRNA or protein, by increasing *SMN2* transcription or enhancing exon 7 inclusion [44].

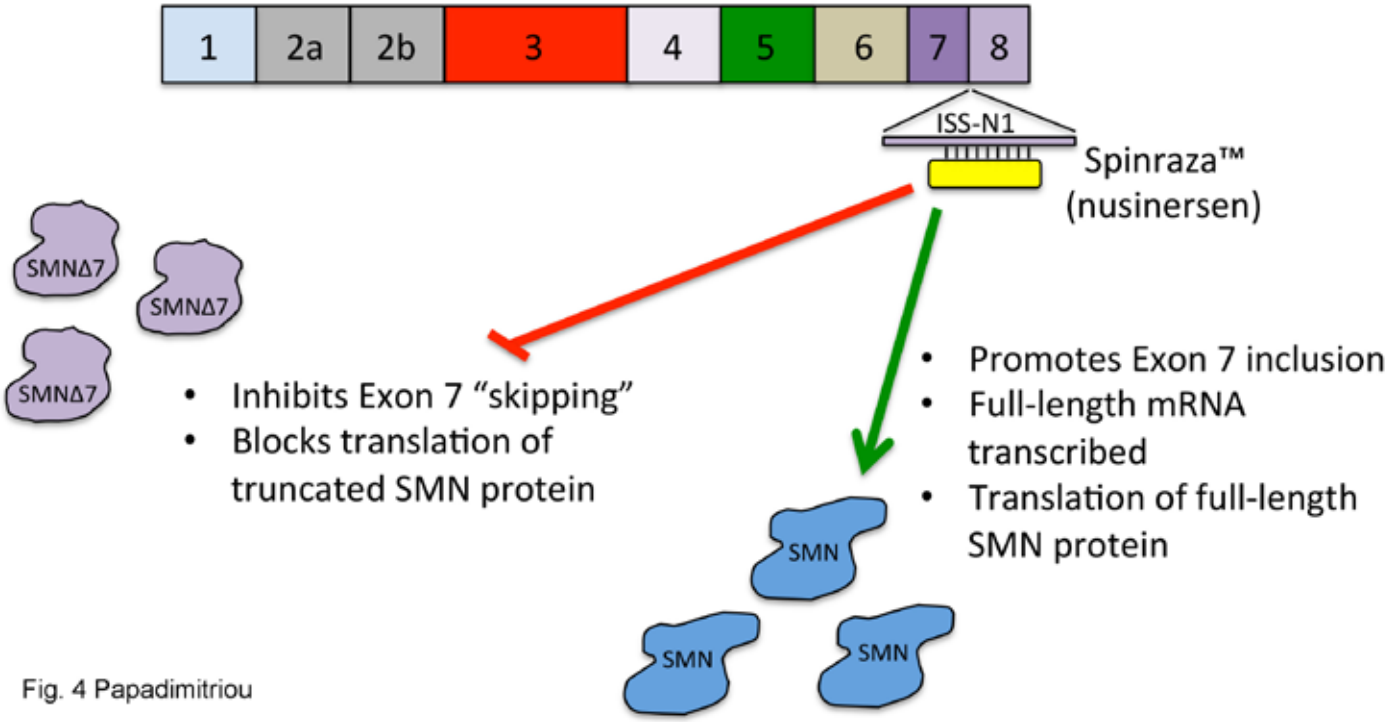
NUSINERSEN

In SMA patients, as previously described, around 90% of the produced *SMN2* protein lacks exon 7, resulting in a non-functional and unstable protein. The use of antisense oligonucleotides (ASOs) has been shown to restore, reduce or modify protein expression. This specific approach is engineered to specifically bind to the cis-acting splicing regulatory motif, promoting the exon 7 inclusion in *SMN2* [45] (Fig 4). In particular, the inclusion of exon 7 is modulated by two intronic splicing enhancers, both located on intron 7, and two intronic splicing silencer sequences (ISSs), in intron 6 and 7 (ISS-N1) [46]. Deletion of ISS-N1 within intron 7 leads to a significantly enhanced incorporation of exon 7 in *SMN2* minigenes [47].

Nusinersen, marketeded as Spinraza®, is the first FDA (FDA, 2016) and EMA-approved (EMA 2017) drug for SMA treatment based on ASO technology. Indeed, hybridization of Nusinersen to ISS-N1, causes rearrangements of DNA structure masking site where the splicing machinery binds (hnRNP A1/A2) promoting the inclusion of the exon 7 in *SMN2*, and therefore resulting in the production of FL-*SMN* protein [14] (Fig. 4).The schedule of the treatment consists of four loading doses; the first to the third should be delivered at 14-day intervals, while the fourth dose should be administrated 30 days following the third. It is further recommended that a maintenance dose be administrated once every 4 months lifelong (FDA, 2016).

The phase I and II clinical trials of Nusinersen for children with SMA type 2 and 3 [48, 49] were promising, followed by three phase III studies (ENDEAR, CHERISH, & NURTURE). The ENDEAR study, included 121 infants (younger than 7 months of age) with SMA type 1 who underwent: a) repeated intrathecal administration of Nusinersen; or b) a sham-infusion. The group that received Nusinersen demonstrated

Fig. 4. Schematic representation of Nusinersen's mechanism of action



Nusinersen binds the ISS-N1 sequence on *SMN2* gene and modulate its splicing, leading to the production of a functional FL-*SMN2* protein

significantly prolonged survival or delayed need for permanent ventilation compared to the sham-control group [50]. Fifty-one percent of the Nusinersen group reached the criteria of "motor-milestone-responders" (achievement of motor milestones in HINE-2 scale; *Hammersmith Infant Neurological Examination*) compared to 0% in the sham-control group. Even though the motor development of the ASO treated group significantly modified the disease's natural course, the critical milestone of independent sitting was achieved only by a minority of patients (6/73) at one year of treatment [50]. The CHERISH trial, included 126 older children with a median age of 4 years with SMA type 2. As with the ENDEAR study, the Nusinersen group exhibited a significant gain in motor function (mean increase of 4.0 points in the HFMSE scale); while the sham control group deteriorated slightly (mean *decrease* of 1.9 points in the HFMSE scale) [51]. The results were so encouraging after an interim analysis, that both studies were terminated prematurely and all participants were switched to the treatment group. Subsequently the NURTURE study included 25 pre-symptomatic infants, 15 of them carrying 2 *SMN2* copies and 10 of them with 3 copies, all under 6 weeks of age. Interestingly, the ability to sit independently was acquired by all 25 patients while 22 out of 25 patients achieved independent walking [52, 53]. After completion of these studies, Nusinersen was approved in December 2016 by the FDA and in May 2017 by the EMA. The schedule of the treatment consists of four loading doses. The first, second, and third doses should be delivered at 14-day intervals, while the fourth dose should be administrated 30 days following the third. It was further recommended that a maintenance dose be administrated once every 4 months for the remainder of the patients life.

RISDIPLAM

Risdiplam (Evrysdi) is a small molecule (RG7916) developed by Roche. This compound is a pyridazine with high binding affinity to exons 5 and 7. The mechanism of action of this compound is rooted in its ability to modify the splicing pattern at these exons and ultimately increase the amount of functional *SMN*-protein. It is an orally administered compound that can cross the blood-brain barrier; and leads to an elevation of FL-*SMN* levels both in the CNS and in peripheral tissues [54].

Originally in the FIREFISH-study, 21 infants with a diagnosis of SMA type 1 between 1 to 7 months of age received either Risdiplam in a low-dose (Part 1, n = 4) primary aiming to assess safety, or in a high dose (Part 2, n = 17) to assess efficacy [like independent sitting after 1 year (12 months) of treatment]. 33% of infants (n =7/21), and 41% of those infants in the higher dose group in Part 2 (n = 7/17)

obtained independent sitting after a median time of 14.8 months of treatment. No safety issues were reported. The SUNFISH-study SMA type 2 and 3 older patients receiving RG7916. Like the FIREFISH trial, this study addressed dose-finding in Part 1 and efficacy in part 2 in a double-blind, placebo-controlled design. In this trial 58% of the patients demonstrated an improvement of at least 3 points according to the Motor Function Measure-32 (MFM32) scale [55].

The JEWELFISH trial is an ongoing open-label study involving all types of SMA non-naïve patients and previously treated with therapies targeting to *SMN*, olesoxime, or gene therapy, and subsequently given Risdiplam with a wide age range from 6 months to 60 years. The study aimed to assess mostly pharmacokinetics and pharmacodynamics data to determine if prior treatment affected the response to Risdiplam. To date, there are 174 patients enrolled. So far the adverse effect profile is very consistent with what was reported in the SUNFISH trial. There was a 2-fold elevation in *SMN* protein levels reported. The most recent data announced at the MDA [Muscular Dystrophy Association] [56] conference showed that the increase in the *SMN* protein was higher in those who had the lowest levels at baseline: patients with SMA type 1. Particularly encouraging have been the preliminary results of the RAINBOWFISH-study. In this trial, pre-symptomatic infants with SMA were treated with Risdiplam for at least one year. The preliminary results of this ongoing trials showed that treated infants gained the ability to sit, stand and walk. This trial is also currently ongoing.

SMN1 Gene Replacement

The primary goal in gene therapy approaches for the treatment of SMA is to directly target the dysfunctional *SMN1* gene. The adaptation of Adeno-associated viruses (AAV) into gene therapy vectors have opened up a new field for the development of therapeutic strategy for diseases of the CNS. These viruses can cross the BBB and serotype 9 in particular, has a particular tropism for CNS and MNs [57, 58].

It has been demonstrated that a single intravenous injection in neonatal mice of self-complementary AAV9 (scAAV9) can transduce around 60% of lumbar MNs and it is also able to transduce brain neurons, dorsal root ganglia, astrocytes both in the spinal cord and in the brain, as well as cardiac and skeletal muscle tissue. The persistence of the virus in these tissues was up to 5 months [59].

One approach, developed by AveXis, takes advantage of scAAV9 encoding wild type *hSMN1*. This new drug, called Zolgensma, is currently produced and marketed in a partnership with Novartis, was approved in May 2019, by the FDA for children with SMA under 2 years of age. Murine studies had previously showed significant changes in the disease's course [60, 61].

ZOLGENSMA

Fifteen infants with SMA type 1 carrying 2 copies of *SMN2* and less than 8 months of age were included in the first Zolgensma (AVXS-101) clinical trial [62]. All the participants received a single intravenous administration of either high- (n = 12) or low-(n = 3) dose of AVXS-101. The high dose group showed a significant improvement in Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) scores, with 11 participants attaining scores >40 points – a milestone rarely achieved in the natural history of SMA-1. During the follow-up period, 9 of the 12 participants receiving Zolgensma in high-dose could sit for >30 seconds, without support. When compared to a control cohort with a natural disease progression improvement of motor function and survival motor and achievement of milestones by AVXS-101 therapy were largely confirmed [63]. Moreover, the phase-3 STR1VE study involved 22 participants with the type 1 of SMA and age under the 6 months at the time of administration, who achieved independent sitting for 30 s or longer at the 18 month of age study visit while 3 of them presented serious side effects (one with hydrocephalus, and two with increased hepatic aminotransferases) [64].

While the route of administration for infants undergoing gene therapy involves *systemic* intravenous injections regardless of the target, for older patients intrathecal application might result a more efficient technique in terms of motor neuron transduction [65]. Initial trials comparing intrathecal to iv gene therapy in pigs and mice have shown improved gene expression [66, 67]. Moreover, the STRONG trial examines the effects of intrathecal administration of Zolgensma in patients with SMA type 2 (under the age of 6 years).

Children in which a titre of antibodies against the viral vector was detected, were excluded from the study. Two of the main concerns are: a) efficacy decline, indeed, a repeated injection would not be feasible due to the formation of the antibodies against scAAV9; and b) the deficiency of *SMN* in peripheral tissue, which could reveal a previously hidden non-cell autonomous mechanism and phenotype. In fact, AAV9 has a high affinity for post-mitotic cells such as MNs, but not for highly proliferative cells like muscle cells; which are also affected in SMA [68]. Although gene therapy seems to be the most promising, results are consistent with oligonucleotide therapy, confirming that the therapeutic window is very narrow, and that it is very important the timely intervention for best therapeutic improvement.

Upregulation of muscle function

As an alternative approach to the therapeutic approaches that target cell autonomous mechanisms in MNs in SMA, there is a parallel treatment strategy that specifically targets skeletal muscle function. The most studied and clinically advanced compounds are Myostatin-inhibitors and Fast Skeletal Muscle Troponin Activators (FSTA). During development, myostatin, which is primarily expressed in skeletal muscle, inhibits muscle over-growth. Administration of the myostatin-inhibitor SRK-015 in SMA-mice led to improved muscle function [69], while the safety profile of SRK-015 in humans is being evaluated in a phase II clinical study. Another example of a compound targeting muscle is Reldesemtiv (CK-2127107), which is a FSTA-class compound which leads to improved contraction of skeletal muscle fibers by slowing the release of calcium from the troponin complex [69]. Its use as a therapeutic for SMA was assessed in 70 participants that had been diagnosed with SMA type 2-4. While the final results of this trial are pending, a mild improvement (that reached the statistically significant threshold), in a motor performance test (six-minute walk test; 6MWT) after both 4 or 8 weeks of treatment, revealed after the interim analyses.

Conclusion

With a deep pipeline for SMA therapeutics, and currently three FDA and EMA approved treatments for the disease, the era of SMA therapeutics has completely changed over the past years achieving a fundamentally altered natural course of a fatal disease. New therapeutic options, such as those targeting splicing events or other gene therapy approaches, shed light on the clinical management of a so far untreatable disease; which could also open up a number of possibilities for other genetic disorders. As the treatment window, particularly in diseases like SMA, is critical to achieve maximum therapeutic effects, it is imperative for early diagnosis and treatment initiation; dictating the need for a broader prenatal/newborn *SMN* screening.

Additional therapeutic approaches at advanced stages of clinical development are currently being evaluated and are likely to provide additional treatment options for people suffering from SMA. The widening of the therapeutic interventions should increase, on one hand, the level of awareness; and on the other hand, the focus on interdisciplinary clinical management. Despite widening new drug treatment options, there is still a lifelong disease burden to consider. Upon approval, the data concerning safety and long-term effects of the novel compounds are limited, therefore additional collection and analyses of real-world data are indispensable for more meaningful and long-lasting effects.

References

[1] Verhaart IEC, Robertson A, Wilson IJ, et al. Prevalence, incidence and carrier frequency of 5q-linked spinal muscular atrophy A literature review. *Orphanet Journal of Rare Diseases.* 2017; 12(1):124.

[2] Prior TW. Spinal muscular atrophy: newborn and carrier screening. *Obstet Gynecol Clin North Am.* 2010; 37, 23-36.

[3] Sugarman EA, Nagan N, Zhu H, et al. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72 400 specimens. *Eur J Hum Genet.* 2012; 20, 27-32.

[4] Hendrickson BC, Donohoe C, Akmaev VR, et al. Differences in *SMN1* allele frequencies among ethnic groups within North America. *J Med Genet.* 2009; 46(9):641-4.

[5] Beevor CE. A case of congenital spinal muscular atrophy (family type), and a case of hæmorrhage into the spinal cord at birth, giving similar symptoms. *Brain.* 1902; 25, 85-108.

[6] Werdnig G. Zwei frühinfantile hereditäre Fälle von progressiver Muskelatrophie unter dem Bilde der Dystrophie, aber anf neurotischer Grundlage. *Arch Psychiatr Nervenkr.* 1891; 22, 437-480.

[7] Lefebvre S, Burglen L, Reboullet S, et al. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell.* 1995; 80, 155-65.

[8] Rudnik-Schöneborn S, Heller R, Berg C, et al. Congenital heart disease is a feature of severe infantile spinal muscular atrophy. *J Med Genet.* 2008; 45, 635-638.

[9] Govoni A, Gagliardi D, Comi GP, et al. Time Is Motor Neuron: Therapeutic Window and Its Correlation with Pathogenetic Mechanisms in Spinal Muscular Atrophy. *Mol Neurobiology.* 2018; 55, 6307-6318.

[10] Kolb SJ, Kissel JT. Spinal Muscular Atrophy: A Timely Review. *Arch Neurol.* 2011; 68.

[11] Liu Q, Fischer U, Wang F, et al. The spinal muscular atrophy disease gene product, *SMN*, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. *Cell.* 1997; 90, 1013-1021.

[12] Sun Y, Grimmler M, Schwarzer V, et al. Molecular and functional analysis of intragenic *SMN1* mutations in patients with spinal muscular atrophy. *Hum Mutat.* 2005; 25, 64-71.

[13] Coovert DD, Le TT, McAndrew PE, et al. The survival motor neuron protein in spinal muscular atrophy. *Hum Mol Genet.* 1997; 6, 1205-1214.

[14] Singh NN, Howell MD, Androphy E, et al. How the discovery of ISS-N1 led to the first medi-

cal therapy forspinal muscular atrophy. *Gene Therapy.* 2017; 24, 520-526.
[15] Young PJ, Jensen K, Burger T, et al. Minute virus of mice NS1 interacts with the *SMN* protein, and they colocalize in novel nuclear bodies induced by parvovirus infection. *J Virol.* 2002; 76, 3892-904.
[16] Lorson CL, Hahnen E, Androphy E, et al. A single nucleotide in the *SMN* gene regulates splicing and is responsible for spinal muscular atrophy. *Proc Natl Acad Sci.* 1999; 96(11):6307-11.
[17] Bertrandy S, Burlet P, Clermont O, et al. The RNA-binding properties of *SMN*: Deletion analysis of the zebrafish orthologue defines domains conserved in evolution. *Hum Mol Genet.* 1999; 8, 775-782.
[18] Selenko P, Sprangers R, Stier G, et al. *SMN* tudor domain structure and its interaction with the Sm proteins. *Nat Struct Biol.* 2001; 8, 27-31.
[19] Bühler D, Raker V, Lührmann R, et al. Essential role for the tudor domain of *SMN* in spliceosomal U snRNP assembly: Implications for spinal muscular atrophy. *Hum Mol Genet.* 1999; 8, 2351-2357.
[20] Cuscó I, Barceló MJ, Del Río, et al. Detection of novel mutations in the *SMN* Tudor domain in type I SMA patients. *Neurology*. 2004; 63, 146-149.
[21] Sanchez G, Dury AY, Murray LM, et al. A novel function for the survival motoneuron protein as a translational regulator. *Hum Mol Genet.* 2013; 22, 668-684.
[22] Carlsson L, Nyström LE, Sundkvist I, et al. Actin polymerizability is influenced by profilin, a low molecular weight protein in non-muscle cells. *J Mol Biol.* 1977; 115, 465-483.
[23] Talbot K, Rodrigues NR, Ignatius J, et al. Gene conversion at the *SMN* locus in autosomal recessive spinal muscular atrophy does not predict a mild phenotype. *Neuromuscul Disord.* 1997; 7, 198-201.
[24] Carissimi C, Baccon J, Straccia M, et al. Unrip is a component of *SMN* complexes active in snRNP assembly. *FEBS Lett.* 2005; 579, 2348-2354.
[25] Carissimi C, Saieva L, Baccon J, et al. Gemin8 is a novel component of the survival motor neuron complex and functions in small nuclear ribonucleoprotein assembly. *J Biol Chem.* 2006; 281, 8126-34.
[26] Charroux B, Pellizzoni L, Perkinson RA, et al. Gemin4: A novel component of the *SMN* complex that is found in both gems and nucleoli. *J Cell Biol.* 2000; 148, 1177-86.
[27] Charroux B, Pellizzoni L, Perkinson RA, et al. Gemin3: A novel DEAD box protein that interacts with *SMN*, the spinal muscular atrophy gene product, and is a component of gems. *J Cell Biol.* 1999; 147, 1181-1193.
[28] Workman E, Kalda C, Patel A, et al. Gemin5 Binds to the Survival Motor Neuron mRNA to Regulate *SMN* Expression. *J Biol Chem.* 2015; 290(25): 15662-15669.
[29] Gubitz AK, Feng W, Dreyfuss G. The *SMN* complex. *Exp Cell Res.* 2004; 296(1):51-6.
[30] Otter S, Grimmler M, Neuenkirchen N, et al. A comprehensive interaction map of the human survival of motor neuron (*SMN*) complex. *J Biol Chem.* 2007; 282, 5825-33.
[31] Biros I, Forrest S. Spinal muscular atrophy: Untangling the knot? *Journal of Medical Genetics.* 1999; 36(1):1-8.
[32] Mailman MD, Heinz JW, Papp AC, et al. Molecular analysis of spinal muscular atrophy and modification of the phenotype by *SMN2. Genet Med.* 2002; 4(1):20-6.
[33] Battle DJ, Kasim M, Yong J, et al. The *SMN* complex: An assembly machine for RNPs. in *Cold Spring Harbor Symposia on Quantitative Biology.* 2006; 71:313-20.
[34] Meister G, Fischer U. Assisted RNP assembly: *SMN* and PRMT5 complexes cooperate in the formation of spliceosomal UsnRNPs. *EMBO J.* 2002; 21, 5853-5863.
[35] Pellizzoni L, Kataoka N, Charroux B. et al. A novel function for *SMN*, the spinal muscular atrophy disease gene product, in pre-mRNA splicing. *Cell*. 1998; 95, 615-24.
[36] Lanfranco M, Vassallo N, Cauchi RJ. Spinal Muscular Atrophy: From Defective Chaperoning of snRNP Assembly to Neuromuscular Dysfunction. *Front Mol Biosci.* 2017; 4, 41.
[37] Chaytow H, Huang YT, Gillingwater, et al. The role of survival motor neuron protein (*SMN*) in protein homeostasis. *Cell and Molec Life Sci.* 2018; 75, 3877-3894.
[38] Rossoll W, Jablonka S, Andreassi C, et al. *SMN*, the spinal muscular atrophydetermining gene product, modulates axon growth and localization of β-actin mRNA in growth cones of motoneurons. *J Cell Biol.* 2003; 163, 801-812.
[39] Sharma A, Lambrechts A, Hao LT, et al. A role for complexes of survival of motor neurons (*SMN*) protein with gemins and profilin in neurite-like cytoplasmic extensions of cultured nerve cells. *Exp Cell Res.* 2005; 309, 185-197.
[40] Akten B, Kye MJ, Hao LT, et al. Interaction of survival of motor neuron (*SMN*) and HuD proteins with mRNA cpg15 rescues motor neuron axonal deficits. *Proc Natl Acad Sci USA.* 2011; 108, 10337-42.
[41] Rage F, Boulisfane N, Rihan K, et al. Genome-wide identification of mRNAs associated with the protein *SMN* whose depletion decreases

their axonal localization. *RNA.* 2013; 19, 1755-1766.
[42] Fallini C, Zhang H, Su Y, et al. The Survival of Motor Neuron (*SMN*) protein interacts with the mRNA-binding protein HuD and regulates localization of poly(A) mRNA in primary motor neuron axons. *J Neurosci.* 2011; 31, 3914-3925.
[43] Sumner CJ. Molecular Mechanisms of Spinal Muscular Atrophy. *J Child Neurol.* 2007; 22, 979-989.
[44] Calder AN, Androphy EJ, Hodgetts KJ. Small Molecules in Development for the Treatment of Spinal Muscular Atrophy. *J Med Chem.* 2016; 59(22):10067-10083.
[45] Zanetta C, Riboldi J, Nizzardo M, et al. Molecular, genetic and stem cell-mediated therapeutic strategies for spinal muscular atrophy (SMA). *J Cell Mol Med.* 2014; 18, 187-196.
[46] Gladman JT, Chandler DS. Intron 7 conserved sequence elements regulate the splicing of the *SMN* genes. *Hum Genet.* 2009; 126, 833-41.
[47] Singh NK, Singh NN, Androphy EJ, et al. Splicing of a Critical Exon of Human Survival Motor Neuron Is Regulated by a Unique Silencer Element Located in the Last Intron. *Mol Cell Biol* 2006; 26(4):1333-46.
[48] Chiriboga CA, Swoboda KJ, Darras BT, et al. Results from a phase 1 study of nusinersen (ISIS-*SMN*(Rx)) in children with spinal muscular atrophy. *Neurology.* 2016;86:890-7.
[49] Finkel RS, Chiriboga CA, Vajsar J, et al. Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. *Lancet Lond Engl.* 2016;388:3017-26.
[50] Finkel RS, Mercuri E, Darras BT, et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. *N Engl J Med.* 2017;377:1723-32.
[51] Mercuri E, Darras BT, Chiriboga CA, et al. Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy. *N Engl J Med.* 2018;378:625-35.
[52] Bertini E, Hwu W-L, Reyna SP, et al. Efficacy and safety of nusinersen in infants with presymptomatic spinal muscular atrophy (SMA): Interim results from the NURTURE study. *Eur J Paediatr Neurol.* 2017;21:e14.
[53] De Vivo DC, Bertini E, Swoboda KJ, et al. Nusinersen initiated in infants during the presymptomatic stage of spinal muscular atrophy: Interim efficacy and safety results from the Phase 2 NURTURE study. *Neuromuscul Disord.* 2019; 29(11):842-856.
[54] Poirier A, Weetall M, Heinig K, et al. Risdiplam distributes and increases *SMN* protein in both the central nervous system and peripheral organs. *Pharmacol Res Perspect.* 2018;6:e00447.
[55] Media release ROCHE 2020 https://www.roche.com/media/releases/med-cor-2020-06-12.htm.
[56] 2021 MDA Clinical and Scientific Conference.
[57] Duque S, Joussemet B, Riviere C, et al. Intravenous administration of selfcomplementary AAV9 enables transgene delivery to adult motor neurons. *Mol Ther.* 2009; 17(7):1187-96.
[58] Foust KD, Nurre E, Montgomery CL, et al. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. *Nat Biotechnol.* 2009; 27(1):59-65.
[59] Dayton RD, Wang DB, Klein RL. The advent of AAV9 expands applications for brain and spinal cord gene delivery. *Expert Opin Biol Ther.* 2012;12:757-66.
[60] Valori CF, Ning K, Wyles M, at al. Systemic delivery of scAAV9 expressing *SMN* prolongs survival in a model of spinal muscular atrophy. *Sci Transl Med.* 2010;2:35ra42.
[61] Foust KD, Wang X, McGovern VL, et al. Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of *SMN. Nat Biotechnol.* 2010;28:271-4.
[62] Mendell JR, Al-Zaidy S, Shell R, et al. Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy. *N Engl J Med.* 2017;377:1713-22.
[63] Al-Zaidy SA, Kolb SJ, Lowes L, et al. AVXS-101 (Onasemnogene Abeparvovec) for SMA1: Comparative Study with a Prospective Natural History Cohort. *J Neuromuscul Dis.* 2019; 6(3):307-317.
[64] Day W J, Finkel S R, Chiriboga A C et al. Onasemnogene abeparvovec gene therapy for symptomatic infantile-onset spinal muscular atrophy in patients with two copies of *SMN2* (STR1VE): an open-label, single-arm, multicentre, phase 3 trial. *Lancet Neurol.* 2021 Apr;20(4):284-293.
[65] Hardcastle N, Boulis NM, Federici T. AAV gene delivery to the spinal cord: Serotypes, methods, candidate diseases, and clinical trials. *Expert Opin Biol Ther.* 2018;18:293-307.
[66] Federici T, Taub JS, Baum GR, et al. Robust spinal motor neuron transduction following intrathecal delivery of AAV9 in pigs. *Gene Ther.* 2012;19:852-9.
[67] Snyder BR, Gray SJ, Quach ET, et al. Comparison of adeno-associated viral vector serotypes for spinal cord and motor neuron gene delivery. *Hum Gene Ther.* 2011;22:1129-35.
[68] Simone C, Ramirez A, Bucchia M, et al. Is Spinal Muscular Atrophy a disease of the motor neurons only: pathogenesis and therapeutic implications? *Cell Mol Life Sci.* 2016; 73(5): 1003-1020.
[69] Long KK, O'Shea KM, Khairallah RJ, et al. Spe-

cific inhibition of myostatin activation is beneficial in mouse models of SMA therapy. *Hum Mol Genet.* 2019;28:1076-89.

[70] Andrews JA, Miller TM, Vijayakumar V, et al. CK-2127107 amplifies skeletal muscle response to nerve activation in humans. *Muscle Nerve.* 2018;57:729-34.

Εκπαιδευτικές Δράσεις της ΕΝΕ

ΕΚΠΑΙΔΕΥΤΙΚΟ ΠΡΟΓΡΑΜΜΑ ΕΛΛΗΝΙΚΗΣ ΝΕΥΡΟΛΟΓΙΚΗΣ ΕΤΑΙΡΕΙΑΣ ΓΙΑ ΤΟ ΑΚΑΔΗΜΑΪΚΟ ΕΤΟΣ 2021-2022

- **20 Νοεμβρίου 2021: «Βιοδείκτες στη διάγνωση των Ανοϊκών Συνδρόμων»**, Webinar
- **4 Δεκεμβρίου 2021: «Ιατρική βασισμένη στην τεκμηρίωση: Από τη θεωρία στην πράξη»**, Ημερίδα, Αθήνα
- **29-30 Ιανουαρίου 2022: «Γενετικός Έλεγχος στα Νευρολογικά Νοσήματα-Θεραπεύσιμα Νευρογενετικά Νοσήματα»**, Διημερίδα, Αθήνα
- **26-27 Φεβρουαρίου 2022: «Κεφαλαλγίες»**, Διημερίδα, Ηράκλειο Κρήτης
- **19-20 Μαρτίου 2022: «Απομυελίνωση και όψιμη ηλικία»**, Διημερίδα, Θεσσαλονίκη
- **9 Απριλίου 2022: «Νεότερες διαγνωστικές και θεραπευτικές εξελίξεις στο χώρο των Νευρομυϊκών Νοσημάτων»**, Μονοήμερο Σεμινάριο, Πάτρα
- **14 Μαΐου 2022: «Πρακτική διαχείριση των ασθενών με Άνοια στην καθημερινότητα»**, Μονοήμερο Σεμινάριο, Αθήνα
- **16-19 Ιουνίου 2022: 33ο Πανελλήνιο Συνέδριο Νευρολογίας**, Ηράκλειο Κρήτη

Ενημερωτικές Σελίδες...

Συνέδρια - Ημερίδες - Συμπόσια - Επιστημονικές εκδηλώσεις

2022

- **13-18 Μαρτίου 2022: XVII World Congress of Neurosurgery WFNS**, Bogota, Colombia
- **24-27 Μαρτίου 2022: 16th World Congress on Controversies in Neurology (CONy)**, London Uk
- **2-8 Απριλίου 2022: AAN Annual Meeting**, Seatle, UK
- **4-6 Μαΐου 2022: 8th European Stroke Organisation Conference (ESOC)**, Lyon, France
- **5-8 Μαΐου 2022: 34ο Πανελλήνιο Συνέδριο Γενικής/Οικογενειακής Ιατρικής**, Σύρος
- **4 Ιουνίου 2022: Σπάνια Νευρολογικά Νοσήματα, Ημερίδα Α΄ Νευρολογικής Κλινικής**, διαδικτυακή
- **16-19 Ιουνίου 2022: 33ο Πανελλήνιο Συνέδριο Νευρολογίας**, Ηράκλειο Κρήτης
- **25-28 Ιουνίου 2022: 8th Congress of the European Academy of Neurology**, Vienna, Austria
- **9-13 Ιουλίου 2022: 14th European Epilepsy Congress**, Geneva, Switzerland
- **8 Σεπτεμβρίου 2022: Νευρολογικές παθήσεις στην ΠΦΥ διεπιστημονικές προσεγγίσεις, Ε.ΚΟ.ΓΕΝ.ΙΑ.**, Πόρτο Χέλι