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 30 - Τεύχος 6

Vol. 30 - Issue 6

Νοέμβριος - Δεκέμβριος 2021 / November - December 2021

Επετειακό Τεύχος

1821-2021 200 Χρόνια

Ελληνικός Κόσμος & Νευροεπιστήμες

Anniversary Issue

1821-2021: 200 years Greek World & Neurosciences

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 30, Τεύχος 6, Νοέμβριος - Δεκέμβριος 2021

Περιεχόμενα

Archives of Clinical Neurology

www.jneurology.gr

Volume 30:6 November - December 2021

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

OWNER

HELLENIC NEUROLOGICAL SOCIETY
10 Alkmanos str, Athens
115 28 - Greece

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

Contents

Dear colleagues and partners

Dear Readers

On the occasion of the symbolic anniversary of the two hundred years since the Revolution of 1821, the Hellenic Neurological Society (ENE) organized an online scientific event entitled "1821-2021: 200 years of the Greek World and Neurosciences", 14-16 May 2021.

With full awareness of the historically established ecumenical character of the Greek civilization and its contribution to the formation of the modern world, this event was dedicated to scientists of Greek descent who serve Neurosciences abroad as members of the so-called "Greek Diaspora". Modern Greek history is intertwined with the phenomenon of the Diaspora.

Greeks, like any other people, especially those who have experienced persecution, genocide, and struggles for freedom and self-determination, can not avoid spontaneous feelings of pride and hope with every case of prosperity and progress somewhere in the world with even a least referal to Greek identity or origin.

However, apart from the inevitable emotions due to our identity as a nation and regardless of the degree to which ties of cooperation between Metropolitan Greece and the Hellenism of the Diaspora were cultivated, science, as an important component of culture, is a field of creative relationship and mutually beneficial path. Beyond updating on scientific developments, the online event on 14-16 May 2021, was considered a clear message of such a vision in Neurology and Neuroscience in Greece, in a highly competitive and demanding global environment.

In the current issue of "Archives of Clinical Neurology", eight out of a total of twenty topics presented in this scientific event such as stroke, neuroimaging, neurogenetics and neuroimmunology, are published. We are grateful to all our invited speakers and among them, those who responded to the invitation of the journal's editorial board to submit their relevant manuscripts for publication, thus enabling us to recall some of the highlights in various areas of Neurology and Neuroscience presented in the meeting.

On behalf of the Organizing Committee

Nikolaos Grigoriadis, MD, PhD
Professor of Neurology
President of the "1821-2021: 200 years of the
Greek World and Neurosciences", 14-16 May 2021

Συντακτική Ομάδα (Editorial Board)

Συντακτική Ομάδα (Editorial Board)

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

Κεφαλαλγία

1. Χ. Αρβανίτη (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)

Συντακτική Ομάδα (Editorial Board)

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)

Συντακτική Ομάδα (Editorial Board)

Editorial Board

Editorial Board

2. Avramidis T (Red Cross Hospital, Athens, Greece)
3. Gimnopoulos K (St Luke Hospital, Thessaloniki, Greece)
4. Mitsias P (University of Crete, Heraklion, Greece & Henry Ford Hospital - Wayne State University, Detroit, USA)
5. Mitsikostas DD (National & Kapodistrian University of Athens, Athens Greece)
6. Rudolf J (Papageorgiou Hospital, Thessaloniki, Greece)
7. Vikelis M (Athens, Greece)

History of Neurology

1. Karavatos A (Aristotle University of Thessaloniki, Thessaloniki, Greece)
2. Triarchou L (University of Macedonia, Thessaloniki, Greece)

Interventional Neurology

1. Goyal N (University of Tennessee Health Sciences Center, Memphis, USA)
2. Safouris A (Metropolitan Hospital, Piraeus, Greece)
3. Sarraj A (The University of Texas McGovern Medical School, Houston, USA)

Movement Disorders

1. Arnaoutoglou M (Aristotle University of Thessaloniki, Thessaloniki, Greece)
2. Bostantjopoulou S (Aristotle University of Thessaloniki, Thessaloniki, Greece)
3. Kefalopoulou Z-M (University of Patras, Greece)
4. Konitsiotis S (University of Ioannina, Greece)
5. Politis M (University of Exeter, UK)
6. Stamelou M (University of Marburg, Germany)
7. Stefanis L (National & Kapodistrian University of Athens, Athens, Greece)

Neurogenetics

1. Dardiotis E (University of Thessaly, Larissa, Greece)
2. Hadjigeorgiou GM (University of Cyprus, Nicosia, Cyprus)
3. Kleopa K (Cyprus Institute of Neurology and Genetics, Cyprus)
4. Koutsis G (National & Kapodistrian University of Athens, Athens Greece)
5. Monos DS (University of Pennsylvania, Philadelphia, USA)
6. Xiromerisiou G (University of Thessaly, Larissa, Greece)

Neuroimmunology

1. Boziki M (Aristotle University of Thessaloniki, Thessaloniki, Greece)
2. Dardiotis E (University of Thessaly, Larissa, Greece)
3. Deretzi G (Papageorgiou Hospital, Thessaloniki, Greece)
4. Doskas T (Naval Hospital of Athens, Athens, Greece)
5. Evaggelopoulou E-M (National & Kapodistrian University of Athens, Athens, Greece)
6. Gold R (Ruhr University of Bochum, Bochum, Germany)
7. Grigoriadis N (Aristotle University of Thessaloniki, Thessaloniki, Greece)
8. Hadjigeorgiou GM (University of Cyprus, Nicosia, Cyprus)
9. Hadjivassiliou M (University of Sheffield, UK)
10. Iliopoulos I (Democritus University of Thrace, Alexandroupolis, Greece)
11. Kappos L (University of Basel, Basel, Switzerland)
12. Kilidireas K (National & Kapodistrian University of Athens, Athens, Greece)
13. Monos DS (University of Pennsylvania, Philadelphia, USA)

Editorial Board

14. Papathanassopoulos P (University of Patras, Patras, Greece)
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)

Άρθρα...

HOW WILL ACADEMIC NEUROLOGY EVOLVE IN THE NEAR FUTURE?

Panayiotis N. Varelas, MD, PhD, FAAN, FNCS

Vice President, Neurocritical Care Society

The term "*Neurology*" was first introduced by Thomas Willis in his landmark book *Cerebri Anatome* in 1664. In this book, written in Latin, "Neurologie" is referred to as "the Doctrine of Nerves" (cranial, spinal, peripheral). It did not originally include the brain and spinal cord, but later towards the end of the 18th century it acquired a broader meaning as this in the Oxford English Dictionary ("neurology: the scientific study or knowledge of the anatomy, functions and diseases of the nerves and the nervous system") [1].

Neurology as a medical specialty has passed through 3 major phases: the initial phase, where the phenomenology of the living patient was linked to the anatomical substrate, usually postmortem. This phase was started with Jean-Martin Charcot in mid-end of the 19th century, overlapped significantly with Psychiatry and continued until the second phase in 1971, when for the first time the anatomical details of the brain were visualized in vivo on computed tomography (and few years later, in 1977 on the magnetic resonance imaging). The third phase is in the current times, where through the advent of computerized data collection and analysis, we are able to decipher genetic diseases, view connectomes and develop brain-computer interphases (BCI).

The economic burden of neurological diseases is tremendous and has been estimated in 2017 to exceed $800 billion/year in the United States of America, with headache having the highest incidence and prevalence in that population [2]. This high demand for neurological services is balanced by shortage of neurologists across the Globe. Neurology faces the same challenges that other medical specialties face, which results from uncertainties in Health Care: limited access, safety, quality, and affordability. Fragmented care, lack of communication, difficult access to Neurologists, over-specialization and lack of generalists, inundation by non-Neurological patients, inability to satisfy the demand for neurological expertise (and thus "giving away" sections of Neurology), lack of patient education and participation in care, a need for a different, remote tele-neurological examination during the pandemic are few of the additional challenges that we face. Like in other specialties, neurologists and, especially, academic neurologists, must face these challenges and develop solutions to deliver services with value to their patients. I would argue that in the near future Academic Neurological Departments have to move simultaneously along 5 axes:

1. Patient Care: bring value to patients without burnout to neurologists.
2. Financial Stability: maximize the net revenue.
3. Retention, Growth and Diversity: aim at the best and for all subspecialties.
4. Education of the Next Generation: transmit the knowledge and bridge the gap.
5. Research: shape the future on a macroscale.

Patient Care: It is debatable how an academic Department can bring value to patients without burnout to neurologists, since in the USA alone in 2012 there was a 11% shortfall between supply and demand for neurologists (and this was expected to increase to 19% in 2025) [3] and at the same time Neurologists, along with emergency medicine and internal medicine physicians, had a 3-fold increased odds of burnout compared to other specialties. Moreover, 60% of neurologists report symptoms of burnout [4,5]. One solution would be to separate the 3 types of academic neurologists, clinician-educator, physician-scientist and the "triple threat" (clinician, researcher and educator) [6] into different locations, with different budgets and separate staff or develop *service lines* that reach beyond the traditional departments and encompass Neurologic, Neurosurgical, Imaging *as a continuum*, under a single umbrella [7].

Financial stability: academic Departments are not isolated from the financial pressures that modern Medicine is experiencing. Two surveys, in 2002 and 2019, by the Association of University Professors of Neurology and the American Neurologic Association showed that academic Neurology Departments spend more effort on clinical revenue-generating activities in 2019 compared with 2002 [8]. Increasing access to outpatient services via telemedicine and decreasing unnecessary demand by identifying and educat-

ing referring physicians may be tangible solutions to generate more revenue for the Departments.

Retention, Growth and Diversity: attrition is a serious problem that academic institutions face. Up to 21% of academic faculty were considering leaving Medicine because of dissatisfaction in a large survey of USA medical schools [9]. Retention and growth are therefore imperatives, and a stable financial state of any Department is a healthy springboard to achieve that. Diversity and equal payment are other problems: only 39% of all 2018 American Academy of Neurology members are women [10], only 12% of Neurology Department Chairs are held by women and there is a $37,000 gap in academic Neurology yearly compensation between men and women, the highest relative gap amongst all specialties [11].

Education of the Next Generation: there is a trend towards training residents into two separate paths, one hospital-based and another outpatient-based [7]. This may be due to different characteristics on sub-specialization the trainees seek, with neurointensivists and movement disorders specialists at the two extremes of the spectrum. By the same token, residents are paying less time educating themselves in the traditional (and to the very neurological core!) localization paradigms and more on reading images and mastering the electronic medical records, with fewer overall hours *residing* in the hospital. How this will differentiate them in the future from other health care providers (nurse practitioners and physician assistants, for example), who are cheaper compared to an academic neurologist and could equally provide tele-health services, has to be seen.

Research: although the highest percentage of research funding is still via federal entities in the USA (National Institute of Health) or pharmaceutical companies, the highest increase in compound annual research growth rate is not for those (in fact they show negative growth rates), but for medical devices and biotechnology firms [12]. How academic Neurology Departments will adjust to this type of non-bench basic research and funding is unclear, especially since there has been a plateau or decline in neuroscience research translation from bench to the patient [13]. Genetics of neurological diseases, advance neuroimaging with connectomics and networks and BCI [14, 15] seem to be the most promising fields for future academic research.

In conclusion, academic Neurology will be the core of Neurology in the near future but needs to adjust to the demands of our times. Its pillars, which will allow it to survive and thrive, will be the same: it needs to continue providing patient care with value, balance the budget, become more inclusive and diverse, train the new generation of Asclepiadae and shape the near and remote future by conducting research. It is likely that the gap between clinicians and researchers in academic Neurology will widen. The same will be true between trainees or practitioners in the inpatient and outpatient-care settings. Additional sub-sub-specializations will emerge from the Neurological Academia and spread to the rest of Neurology. Therefore, drastic changes in the organization and function of academic Departments will be required to address the internal challenges and external pressures.

Bibliography

[1] Feindel W. Thomas Willis (1621-1675)-The Founder of Neurology. Can Med Assoc J. 1962;87(6):289-96.
[2] Collaborators GUND, Feigin VL, Vos T, Alahdab F, Amit AML, Barnighausen TW, et al. Burden of Neurological Disorders Across the US From 1990-2017: A Global Burden of Disease Study. JAMA Neurol. 2021;78(2):165-76.
[3] Dall TM, Storm MV, Chakrabarti R, Drogan O, Keran CM, Donofrio PD, et al. Supply and demand analysis of the current and future US neurology workforce. Neurology. 2013;81(5):470-8.
[4] Busis NA, Shanafelt TD, Keran CM, Levin KH, Schwarz HB, Molano JR, et al. Burnout, career satisfaction, and well-being among US neurologists in 2016. Neurology. 2017;88(8):797-808.
[5] West CP, Dyrbye LN, Shanafelt TD. Physician burnout: contributors, consequences and solutions. J Intern Med. 2018;283(6):516-29.
[6] Lin DJ, Cudkowicz ME, Cho TA. Opinion and Special Articles: Challenges and opportunities in defining career identity in academic neurology. Neurology. 2018;91(14):670-2.
[7] Martin JB, Moses H, 3rd. Planning the future of neurology: crisis or opportunity. JAMA Neurol. 2015;72(2):141-2.
[8] Brey RL, Ostendorf T, Rizzo M, Sacco RL. Academic Neurology Departments: Structure, Diversity, and Financial Pressures in 2019 vs 2002. Neurology. 2021;96(10):483-90.
[9] Pololi LH, Krupat E, Civian JT, Ash AS, Brennan RT. Why are a quarter of faculty considering leaving academic medicine? A study of their perceptions of institutional culture and intentions to leave at 26 representative U.S. medical schools. Acad Med. 2012;87(7):859-69.
[10] Sacco RL. Neurology: Challenges, opportunities, and the way forward. Neurology. 2019;93(21):911-8.
[11] Hasan TF, Turnbull MT, Vatz KA, Robinson MT, Mauricio EA, Freeman WD. Burnout and attrition: Expanding the gender gap in neurology? Neurology. 2019;93(23):1002-8.
[12] Moses H, 3rd, Matheson DH, Cairns-Smith S, George BP, Palisch C, Dorsey ER. The anatomy

of medical research: US and international comparisons. JAMA. 2015;313(2):174-89.
[13] Arrowsmith J, Miller P. Trial watch: phase II and phase III attrition rates 2011-2012. Nat Rev Drug Discov. 2013;12(8):569.
[14] Kumar A, Narayanan K, Chaudhary RK, Mishra S, Kumar S, Vinoth KJ, et al. Current Perspective of Stem Cell Therapy in Neurodegenerative and Metabolic Diseases. Mol Neurobiol. 2017;54(9):7276-96.
[15] Kassubek J. The Application of Neuroimaging to Healthy and Diseased Brains: Present and Future. Front Neurol. 2017;8:61.

TARGETED DELIVERY OF THE NgR-Fc PROTEIN TO PROMOTE NEUROREPAIR IN A MODEL OF MULTIPLE SCLEROSIS

Sining Ye[1], Paschalis Theotokis[2], Danica Nheu[1], Olivia Ellen[1], Padmanabhan Ramanujam[1], Jae Young Lee[3], Michael F Azari[1], Steven Petratos[1]

[1] Department of Neuroscience, Central Clinical School, Monash University, Prahran, Victoria, Australia
[2] B', Department of Neurology, Laboratory of Experimental Neurology and Neuroimmunology, AHEPA University Hospital, Thessaloniki, Greece
[3] ToolGen Inc., Gasan Digital-Ro, Geumcheon, Seoul, Korea

Summary

Multiple sclerosis (MS) is an autoimmune-mediated inflammatory demyelinating and degenerative disease occurring in the central nervous system (CNS). There are no current therapeutics available to treat patients with progressive MS. Hence, mechanisms that govern CNS neuroprotection and repair need to be elucidated to provide novel targeted therapeutics to reverse permanent CNS damage. Our laboratory designed a novel method of delivering the NgR (310) ecto-myc-Fc fusion protein by incorporating the DNA construct into a lentiviral vector and transducing donor hematopoietic stem cells (HSCs) ex vivo, followed by their transplantation in recipient mice to target inflammatory demyelinating lesions that ensue during the MOG35-55-induced experimental autoimmune encephalomyelitis (EAE) mouse model. The aim of this study was to investigate the potential therapeutic effects of lesion-specific delivery of the NgR (310) ecto-Fc protein, following the lineage differentiation of the transplanted HSCs, demonstrating neuroprotection and neurorepair during the course of EAE.

Key words: Nogo A, experimental autoimmune encephalomyelitis, Nogo Receptor, NgR-Fc, haematopoietic stem cells, remyelination, neurorepair, multiple sclerosis

Introduction

Multiple Sclerosis (MS) is an autoimmune disease with neurodegeneration characterized by inflammation and demyelination within the central nervous system (CNS). It impacts an individual's quality of life substantially and places a heavy burden on the public health system, with women more commonly diagnosed at the prime of their lives with onset of symptoms occurring between the ages of 20-40-year-olds [1]. The cause remains elusive, however, the presence of a heterogeneous array of symptoms involving motor, sensory, visual and autonomic systems contribute to it being the most common cause of non-traumatic neurological disability in young adults [2]. The unique symptoms arise from the development of multiple lesions across the CNS; thus, no individual may experience the exact symptoms at a specific stage of the disease course. The major effectors in the pathogenesis and sequelae of MS are infiltrating activated macrophages and endogenous microglia [3]. Due to the leaky blood-brain barrier (BBB) during active MS, monocytic-derived macrophages from the periphery may infiltrate the CNS and along with endogenous microglia, transition into a proinflammatory phenotype, actively contributing to the proinflammatory propagation of demyelination and eventually axonal damage [4]. Moreover, the activation of astrocytes and eventual dropout of mature oligodendrocytes, along with the pathological modifications of the cellular milieu all play a part in the expansion of lesion burden, promulgating neurodegenerative change over time [5, 6].

The heterogeneity of the disease poses a challenge for designing effective therapeutics that target multiple cellular and extracellular reactive changes within the brains of individuals living with MS, especially when the disease progresses. Currently, the treatments available are either immunomodulatory or immunosuppressive, limited to reducing the relapse rate for patients only. As the disease progresses, there exists no effective treatment to halt the progression towards neurodegeneration and elicit neurorepair. Limitations for effective neurorepair, have been suggested partially due to inhibitory factors in the MS lesion milieu exerted through the deposition of substantive myelin-associated inhibitory factors (MAIFs), with the most potent being the integral myelin protein, Nogo-A [7, 8]. Nogo-A, a neurite outgrowth inhibitor, is localized on the surface of oligodendrocytes and myelin sheaths [9]. It exerts

this effect by binding with high affinity to Nogo-66 receptor 1 (NgR1), which can also bind other MAIFs such as myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp) [9]. The expression of Nogo-A and NgR1 has been found upregulated in many CNS disorders, that can include MS, spinal cord injury (SCI) and brain injury, stroke, glaucoma to name a few [10]. It has been established that absence or blockage of Nogo-A may limit and protect the progression of the animal model of MS, namely experimental autoimmune encephalomyelitis (EAE) [11, 12]. Furthermore, there are suggestions that Nogo-A inhibition may shift activated macrophage and microglia from pro-inflammatory to anti-inflammatory phenotypes, promoting repair [12]. Thus, limiting the effects of Nogo-A may be a potential target to overcome the barriers to neurorepair. Designing an effective therapy that can target Nogo-A must also take into consideration that it must be able to traverse the BBB, allowing access to lesion sites within the CNS. Hence, investigations utilizing novel means of delivering antagonizing biologics such as the NgR1-Fc fusion protein may well prove to be an excellent neuroprotective or even reparative measure. The use of a NgR1-Fc fusion protein has had promising results in preclinical studies in spinal cord injury (SCI) and stroke, however, clinically effective measures of delivery across the BBB still pose a major challenge [13]. The possibility of targeting Nogo-A and its cognate receptor, NgR1, as a potential therapeutic along with hematopoietic stem cell (HSC)-based delivery methods to overcome these limitations has been investigated in our laboratory.

Blocking NgR1 signalling and how novel is the treatment?

There are several methods that can be used to block NgR1 signalling, such as humanised antibodies, fusion proteins, peptides, and pharmacological blockers [14]. Blocking of the signalling pathways can be done by blocking either the receptor (NgR1) or blocking the ligand (MAIFs). An example of blocking the receptor is the conditional deletion of NgR1 ($ngr1^{-/-}$) via the cre-lox system in EAE mice reduced axonal damage in the optic nerves of mice even in the presence of neuroinflammatory lesions [8]. In the non-human primate model of spinal cord injury (SCI), administration of NgR1-Fc increased the axon density compared to the control group [15]. On the other hand, an example of blocking the ligand is through therapeutic antibodies directed against Nogo-A in EAE rats to promote recovery and remyelination [16]. Recently the humanised anti-Nogo-A-antibody ATI355 entered a phase I clinical trial to treat SCI [17]. In a study conducted by *Tsai et al.*, giving adult rats with stroke anti-Nogo-A-antibody (11C7) ameliorates the impairment of the forelimbs [18]. In the study of optic nerve lesion, knocking down NgR1 or neutralising NogoA leads to more regeneration of the nerve but the growth rarely exceed 2 mm [19].

NgR (310) ecto-Fc fusion protein

In order to facilitate myelin debris uptake and promote remyelination, NgR (310) ecto-Fc fusion protein is constructed. The fusion protein consists of the soluble portion of NgR1 containing the ligand-binding domain, which binds to MAIF to limit the inhibition of axonal neurite outgrowth [20]. Combining this soluble portion with Fc region of immunoglobulin G (IgG) enhances the binding to activated monocytes, increasing the myelin debris clearance [21].

Delivering NgR (310) ecto-Fc fusion protein using HSCs

The delivery of fusion protein to the CNS is often hindered by the presence of a blood-brain barrier [22] but immune cells such as T cells and macrophages can pass through the BBB [23]. HSCs, as mentioned before, are capable of differentiating into these immune cells. HSCs can also be used as vehicles for drug delivery [24] and they can be modified using a lentiviral vector to express the gene of interest [25-27]. Consequently, HSCs can be used to deliver the NgR(310)ecto-Fc fusion protein to the lesion sites, which will promote the myelin debris clearance by macrophage [20].

Conclusion

The hallmarks of multiple sclerosis are inflammation in the central nervous system and demyelination of neurons, leading to axonal injury as well as neurological decline. The cause of this disease is still yet to be determined but peripheral immune cells and glial cells have been shown to contribute to the pathophysiological aspect of multiple sclerosis. Currently, there is no curative treatment for clinically diagnosed patients and the available therapies have little to no efficacy on patients with the progressive forms of the disease. Since haematopoietic stem cells are able to traverse the BBB and NgR (310) ecto-Fc fusion protein has been shown to increase the myelin debris clearance and remyelination, combining both therapies might provide a better therapeutic avenue for MS patients.

References

[1] Ahmad H, Palmer JA, Campbell JA, Mei I, Taylor B. Health Economic Impact of Multiple Sclerosis in Australia in 2017. Australia: Multiple Sclerosis Research Australia; 2017 August 2018. Contract No.: 1.

[2] Koch-Henriksen N, Sørensen PS. The changing

demographic pattern of multiple sclerosis epidemiology. The Lancet Neurology. 2010;9(5):520-32.
[3] Brück W, Porada P, Poser S, Rieckmann P, Hanefeld F, Kretzschmar HA, et al. Monocyte/macrophage differentiation in early multiple sclerosis lesions. Ann Neurol. 1995;38(5):788-96.
[4] Miron VE, Boyd A, Zhao J-W, Yuen TJ, Ruckh JM, Shadrach JL, et al. M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. Nat Neurosci. 2013;16(9):1211-8.
[5] Ponath G, Park C, Pitt D. The Role of Astrocytes in Multiple Sclerosis. Front Immunol. 2018;9:217.
[6] Wolswijk G. Oligodendrocyte survival, loss and birth in lesions of chronic-stage multiple sclerosis. Brain. 2000;123(1):105-15.
[7] Lee JY, Petratos S. Multiple Sclerosis:Does Nogo Play a Role? The Neuroscientist. 2013;19(4):394-408.
[8] Petratos S, Ozturk E, Azari MF, Kenny R, Lee JY, Magee KA, et al. Limiting multiple sclerosis related axonopathy by blocking Nogo receptor and CRMP-2 phosphorylation. Brain : a journal of neurology. 2012;135(Pt 6):1794-818.
[9] Schmandke A, Schmandke A, Schwab ME. Nogo-A: Multiple Roles in CNS Development, Maintenance, and Disease. The Neuroscientist. 2014;20(4):372-86.
[10] Schwab ME, Strittmatter SM. Nogo limits neural plasticity and recovery from injury. Curr Opin Neurobiol. 2014;27:53-60.
[11] Yang Y, Liu Y, Wei P, Peng H, Winger R, Hussain RZ, et al. Silencing Nogo-A promotes functional recovery in demyelinating disease. Annals of neurology. 2010;67(4):498-507.
[12] Alrehaili AA, Lee JY, Bakhuraysah MM, Kim MJ, Aui PM, Magee KA, et al. Nogo receptor expression in microglia/macrophages during experimental autoimmune encephalomyelitis progression. Neural Regen Res. 2018;13(5):896-907.
[13] He HW, Zhang YL, Yu BQ, Ye G, You W, So KF, et al. Soluble Nogo receptor 1 fusion protein protects neural progenitor cells in rats with ischemic stroke. Neural Regen Res. 2019;14(10):1755-64.
[14] Zörner B, Schwab ME. Anti-Nogo on the go: from animal models to a clinical trial. Ann N Y Acad Sci. 2010;1198 Suppl 1:E22-34.
[15] Wang X, Zhou T, Maynard GD, Terse PS, Cafferty WB, Kocsis JD, et al. Nogo receptor decoy promotes recovery and corticospinal growth in non-human primate spinal cord injury. Brain. 2020;143(6):1697-713.
[16] Ineichen BV, Kapitza S, Bleul C, Good N, Plattner PS, Seyedsadr MS, et al. Nogo-A antibodies enhance axonal repair and remyelination in neuroinflammatory and demyelinating pathology. Acta Neuropathol. 2017;134(3):423-40.
[17] Kucher K, Johns D, Maier D, Abel R, Badke A, Baron H, et al. First-in-Man Intrathecal Application of Neurite Growth-Promoting Anti-Nogo-A Antibodies in Acute Spinal Cord Injury. Neurorehabil Neural Repair. 2018;32(6-7):578-89.
[18] Tsai S-Y, Papadopoulos CM, Schwab ME, Kartje GL. Delayed anti-nogo-a therapy improves function after chronic stroke in adult rats. Stroke. 2011;42(1):186-90.
[19] Pernet V, Schwab ME. The role of Nogo-A in axonal plasticity, regrowth and repair. Cell Tissue Res. 2012;349(1):97-104.
[20] Kim MJ, Kang JH, Theotokis P, Grigoriadis N, Petratos S. Can We Design a Nogo Receptor-Dependent Cellular Therapy to Target MS? Cells. 2018;8(1):1.
[21] Kuhlmann T, Brück W. Immunoglobulins induce increased myelin debris clearance by mouse macrophages. Neurosci Lett. 1999;275(3):191-4.
[22] Pardridge WM. Drug transport across the blood-brain barrier. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2012;32(11):1959-72.
[23] Biffi A, De Palma M, Quattrini A, Del Carro U, Amadio S, Visigalli I, et al. Correction of metachromatic leukodystrophy in the mouse model by transplantation of genetically modified hematopoietic stem cells. J Clin Invest. 2004;113(8):1118-29.
[24] Labusca L, Herea DD, Mashayekhi K. Stem cells as delivery vehicles for regenerative medicine-challenges and perspectives. World J Stem Cells. 2018;10(5):43-56.
[25] Aiuti A, Biasco L, Scaramuzza S, Ferrua F, Cicalese MP, Baricordi C, et al. Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. Science. 2013;341(6148):1233151.
[26] Miyoshi H. Gene delivery to hematopoietic stem cells using lentiviral vectors. Methods Mol Biol. 2004;246:429-38.
[27] van Til NP, Wagemaker G. Lentiviral gene transduction of mouse and human hematopoietic stem cells. Methods Mol Biol. 2014;1185:311-9.

OPTICAL METHODS FOR INTERROGATING EPILEPTIC MECHANISMS

Joseph Lombardo PhD, Stelios M. Smirnakis MD PhD

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

Summary

A set of emerging optical imaging technologies exemplified by mesoscopic two-photon imaging with genetically targeted calcium indicators and a new generation of voltage sensitive dyes, allow us to record the activity in epileptic circuits of awake behaving animals with cell specificity at high spatio-temporal resolution, complementing classical electrophysiological approaches. These methods, in combination with transcriptomic profiling via high-throughput multiplexed error-robust fluorescence in situ hybridization (MERFISH) microscopy and with optogenetic-chemogenetic strategies for testing causality, hold great promise for dissecting the circuit mechanisms of epilepsy.

Key words: Optical methods, Circuit mechanisms of epilepsy, 2-photon imaging, transcriptomic analysis, MERFISH

Optical imaging methods have come of age in neuroscience, allowing chronic *in vivo* monitoring and manipulation of large networks of identified neurons with unprecedented yield and cell specificity. These tools are ideally suited for the study of circuit mechanisms of epilepsy. Understanding how neurons of identified type and circuit location get engaged into abnormal epileptiform patterns of activity requires the 1) study of neuronal networks across time with cell specificity and high spatio-temporal resolution, the 2) causal manipulation of circuit elements of interest, and the 3) cell-specific interrogation of molecular mechanisms. Below we focus on items 1 and 3, briefly summarizing recent work that promises to usher a new era in investigating the mechanisms underlying epileptic disorders.

The new generation of extracellular multi-electrode probes and arrays allow the monitoring of hundreds to thousands of neurons (new silicon probes [1]) or large cortical regions (MEAs) with high temporal resolution, and have successfully been used to gather valuable information about epileptic mechanisms in humans [2] and animals [1]. Silicon probes are however invasive and suffer from a selection bias, inability to accurately identify specific cell types, and poor capacity for monitoring stably the same units over time and for determining their precise localization and connectivity across the cortical circuit. Imaging methods can overcome these problems, albeit at the cost of relatively poor temporal resolution and limited access to deep brain regions. However, these problems have been recently mitigated with the advent of a new generation of *in vivo* voltage sensitive dyes whose temporal resolution is measured in milliseconds [3-5] and specialized miniscope microscopy methods capable of accessing deep nuclei for optical imaging [6, 7].

Wide-field epifluorescence microscopy with spatial resolution in the tens of micrometers and kHz frame rate has been available since the early 90's [8] and continues to yield important information. Rossi et al. recently used this method to argue that acute epileptiform activity induced by chemo-convulsant injection travels along homotypic connections to spread across cortical sensory areas extending several millimeters [9]. However this method lacks the spatial resolution needed to resolve activity arising in individual cells. In the last 2 decades, two-photon (2P) laser scanning microscopy [10] is being increasingly applied to study initiation and propagation of epileptiform activity as it affords single cell resolution at the sub-micrometer scale and can be combined with genetic labeling techniques to image specific cell types. For example, 2P imaging has been used to study genetic syndromes of epilepsy such as the mouse models of Stargazer [11] and Dravet [12], showing that desynchronization of neuronal firing is a feature of certain epileptic syndromes (see also [7]). Direct visualization of acute focal cortical seizure events induced by chemo-convulsant injections with 2P imaging, revealed that seizure events start as local neuronal ensemble hyper-synchronization that spreads in a saltatory fashion to nearby territories [13-15]. Individual excitatory neurons appear to get engaged reliably into acute epileptiform events of variable duration, with supragranular neurons

preceding infragranular ones [16, 17]. Functional connectivity analysis during interictal periods has led to the identification of epileptiform network motifs and putative cell types hypothesized to contribute significantly to the evolution of aberrant activation patterns [18,19]. Multiple other studies and applications, well beyond the scope of our brief review, are currently ongoing.

Recent advances in optical technology allow the simultaneous monitoring of exceptionally large (~5x5mm^2) cortical fields of view (mesoscopic imaging) covering multiple areas lying on the surface of the brain, imaging thousands of neurons in different layers without forgoing the micrometer resolution necessary to identify and localize individual units [20]. Deeper brain regions are also accessible for imaging using miniscope technology [6, 7]. Using this approach, Shuman et al. [7] found that CA1 place cells in mice with temporal lobe epilepsy become unstable, completely remapping their place fields across a period of a week. Importantly, miniscope technology allows imaging in freely moving animals, which is essential in epilepsy models that have low spontaneous seizure frequency. Multiple organisms from rodents to zebrafish to primates can be interrogated with these methods, yielding important mechanistic information at the pre-clinical level. Results can be compared with global EEG and fMRI measurements, which are more appropriate for studying distributed epileptic networks in human patients (see [21]). Combining these techniques with optogenetic or chemo-genetic approaches (not reviewed here) to probe causal relationships by manipulating neuronal activity in specific cell types, is a powerful approach for dissecting the circuit mechanisms of epilepsy.

The combination of new optical imaging and genetic technologies has great promise for unravelling the cellular interactions that generate epileptiform activity, that is, cause neuronal networks to become epileptogenic. Particularly promising is a recently developed high-throughput *ex vivo* mRNA hybridization microscopy method (MERFISH), capable of resolving hundreds to thousands of distinct mRNA transcripts per imaging session while preserving spatial localization, thereby making it possible to attribute in situ mRNA expression profiles to specific cell types [22-24]. Aligning 2P images obtained *in vivo* with *ex vivo* MERFISH images obtained from the same tissue, has the potential to uncover the cell-specific transcriptomic profiles that underlie the aberrant functional activity phenotype exhibited by the same cells *in vivo.* An important question that can be studied with these methods is the mechanism by which specific pharmacologic interventions succeed or fail to contain abnormal patterns of excitability that lead to seizures. In time, information gained will help identify more effective therapeutic targets for pharmaco-resistant epilepsies.

In conclusion, now is a particularly exciting time to work in the field of epilepsy. The optical imaging and spatially resolved transcriptomic microscopy techniques outlined above, as well as other techniques not reviewed here (see [21,25,26]), will undoubtedly be harnessed in the near future to reveal in unprecedented detail the circuit mechanisms of epilepsy.

Acknowledgements

SS and JL have received support from VA Merit Awards I01 RX002981 and I01 BX005221-01.

References

[1] Jun JJ, Steinmetz NA, Siegle JH, Denman DJ, Bauza M, Barbarits B, et al. Fully integrated silicon probes for high-density recording of neural activity. Nature. 2017;551(7679):232-6.
[2] Merricks EM, Smith EH, McKhann GM, Goodman RR, Bateman LM, Emerson RG, et al. Single unit action potentials in humans and the effect of seizure activity. Brain. 2015;138(Pt 10):2891-906.
[3] Yang HH, St-Pierre F. Genetically Encoded Voltage Indicators: Opportunities and Challenges. J Neurosci. 2016;36(39):9977-89.
[4] Villette V, Chavarha M, Dimov IK, Bradley J, Pradhan L, Mathieu B, et al. Ultrafast Two-Photon Imaging of a High-Gain Voltage Indicator in Awake Behaving Mice. Cell. 2019;179(7):1590-608 e23.
[5] Chamberland S, Yang HH, Pan MM, Evans SW, Guan S, Chavarha M, et al. Fast two-photon imaging of subcellular voltage dynamics in neuronal tissue with genetically encoded indicators. Elife. 2017;6.
[6] Berdyyeva TK, Frady EP, Nassi JJ, Aluisio L, Cherkas Y, Otte S, et al. Direct Imaging of Hippocampal Epileptiform Calcium Motifs Following Kainic Acid Administration in Freely Behaving Mice. Front Neurosci. 2016;10:53.
[7] Shuman T, Aharoni D, Cai DJ, Lee CR, Chavlis S, Page-Harley L, et al. Breakdown of spatial coding and interneuron synchronization in epileptic mice. Nat Neurosci. 2020;23(2):229-38.
[8] Ratzlaff EH, Grinvald A. A tandem-lens epifluorescence macroscope: hundred-fold brightness advantage for wide-field imaging. J Neurosci Methods. 1991;36(2-3):127-37.
[9] Rossi LF, Wykes RC, Kullmann DM, Carandini M. Focal cortical seizures start as standing waves and propagate respecting homotopic connectivity. Nat Commun. 2017;8(1):217.
[10] Denk W, Strickler JH, Webb WW. Two-photon laser scanning fluorescence microscopy. Science. 1990;248(4951):73-6.

[11] Meyer J, Maheshwari A, Noebels J, Smirnakis S. Asynchronous suppression of visual cortex during absence seizures in stargazer mice. Nat Commun. 2018;9(1):1938.
[12] Tran CH, Vaiana M, Nakuci J, Somarowthu A, Goff KM, Goldstein N, et al. Interneuron Desynchronization Precedes Seizures in a Mouse Model of Dravet Syndrome. J Neurosci. 2020;40(13):2764-75.
[13] Wenzel M, Hamm JP, Peterka DS, Yuste R. Acute Focal Seizures Start As Local Synchronizations of Neuronal Ensembles. J Neurosci. 2019;39(43):8562-75.
[14] Muldoon SF, Villette V, Tressard T, Malvache A, Reichinnek S, Bartolomei F, et al. GABAergic inhibition shapes interictal dynamics in awake epileptic mice. Brain. 2015;138(Pt 10):2875-90.
[15] Feldt Muldoon S, Soltesz I, Cossart R. Spatially clustered neuronal assemblies comprise the microstructure of synchrony in chronically epileptic networks. Proc Natl Acad Sci U S A. 2013;110(9):3567-72.
[16] Wenzel M, Hamm JP, Peterka DS, Yuste R. Reliable and Elastic Propagation of Cortical Seizures In Vivo. Cell Rep. 2017;19(13):2681-93.
[17] Aeed F, Shnitzer T, Talmon R, Schiller Y. Layer- and Cell-Specific Recruitment Dynamics during Epileptic Seizures In Vivo. Ann Neurol. 2020;87(1):97-115.
[18] Sparks FT, Liao Z, Li W, Grosmark A, Soltesz I, Losonczy A. Hippocampal adult-born granule cells drive network activity in a mouse model of chronic temporal lobe epilepsy. Nat Commun. 2020;11(1):6138.
[19] Hadjiabadi D, Lovett-Barron M, Raikov IG, Sparks FT, Liao Z, Baraban SC, et al. Maximally selective single-cell target for circuit control in epilepsy models. Neuron. 2021;109(16):2556-72 e6.
[20] Sofroniew NJ, Flickinger D, King J, Svoboda K. A large field of view two-photon mesoscope with subcellular resolution for in vivo imaging. Elife. 2016;5.
[21] Wykes RC, Khoo HM, Caciagli L, Blumenfeld H, Golshani P, Kapur J, et al. WONOEP appraisal: Network concept from an imaging perspective. Epilepsia. 2019;60(7):1293-305.
[22] Moffitt JR, Hao J, Wang G, Chen KH, Babcock HP, Zhuang X. High-throughput single-cell gene-expression profiling with multiplexed error-robust fluorescence in situ hybridization. Proc Natl Acad Sci U S A. 2016;113(39):11046-51.
[23] Chen KH, Boettiger AN, Moffitt JR, Wang S, Zhuang X. RNA imaging. Spatially resolved, highly multiplexed RNA profiling in single cells. Science. 2015;348(6233):aaa6090.
[24] Moffitt JR, Bambah-Mukku D, Eichhorn SW, Vaughn E, Shekhar K, Perez JD, et al. Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic region. Science. 2018;362(6416).
[25] Mantoan Ritter L, Golshani P, Takahashi K, Dufour S, Valiante T, Kokaia M. WONOEP appraisal: optogenetic tools to suppress seizures and explore the mechanisms of epileptogenesis. Epilepsia. 2014;55(11):1693-702.
[26] Rossi LF, Kullmann DM, Wykes RC. The Enlightened Brain: Novel Imaging Methods Focus on Epileptic Networks at Multiple Scales. Front Cell Neurosci. 2018;12:82.

STROKE, IS THERE A ROLE FOR NEUROSURGERY?

Christos Tolias MD, PhD, FRCS (Engl), FRCS (SN)

Consultant Neurosurgeon, Neurovascular Surgery, Clinical Lead Neurosurgery, Clinical Lead South London Neurosurgery Network

Abstract

Brief review of the role of neurosurgery in the management of Stroke.

Key words: stroke, neurosurgery, cerebral vasular abnormalities, Moya-Moya, cerbral bypass

Stroke is a major cause of morbidity and mortality worldwide. Every year, an estimated 150,000 people in the UK have a stroke. That's one person every five minutes. Most people affected are over 65, but anyone can have a stroke, including children and even babies. A stroke is the third most common cause of death in the UK. It is also the single most common cause of severe disability. More than 250,000 people live with disabilities caused by a stroke (Stroke Association) and 1000 people under 30 have a stroke each year.

London is a city with 9 million inhabitants. Stroke remains the second biggest killer and most common cause of disability with more than 11,500 strokes reported every year, which translate to approximate 2,000 deaths. Areas with social deprivation and lower income have higher incidences of stroke, relating to diet, smoking and reduced prevention.

Traditionally Stroke was seen as a non surgical, sub acute condition, however recent advances in its management have altered this perception and are revolutionising stroke management.

The FAST campaigns and the establishment of Stroke hyper acute units strategically located around the capital have streamlined the management of the condition and allowed thrombolysis to be offered in a timely manner (within 4hours window). However the recent advent of thrombectomy have really revolutionise the management of acute stroke. The publication of eight randomised controlled trials (RCTs), including 2423 patients, reported that endovascular thrombectomy was associated with improved functional outcomes at 90 day follow-up (modified Rankin scale score 0–2, odds ratio 1.56, 95% confidence interval [CI] 1.32 to 1.85, p<0.00001). Moreover the effects of thrombectomy persisted for more than 2 years. Such results have ushered in the modern era of stroke treatment. Of course a minority of strokes are amenable to thombectomy. For example the area covered by Kings College Hospital (SE London and Kent - approximate 4,5 million people) is treating >1600 cases a year and 15-20% of these are eligible for thrombectomy.

As the procedure requires resources that were traditionally available to stroke use a major reconfiguration of services is currently on going in the UK in order to allocate the the appropriate hardware, personnel and beds to these very urgent patients.

Various paradigms have been proposed:

- 24/7 pure thrombectomy rota,
- Neurology/Cardiology involvement,
- Dually trained neurosurgeons/neurologists,
- incorporation at existing set up,
- extending the neurointerventionist cohort,
- Networks.

It is estimated that 5-7 interventionists will be needed to staff the rota. It is anticipated that 200-300 procedures / annum / per centre (including aneurysm coiling). The full service establishment has taken 4-5 years, but we now have a 24/7 service that covers thrombectomy and management of ruptured aneurysms.

And for neurosurgery? Is there a role?

I will examine five areas were neurosurgery has a significant and increasingly important role:

Patients with malignant Stroke (large MCA)

"Malignant" middle cerebral artery (MCA) stroke refers to life threatening, space occupying MCA infarctions which occur in up to 10% of all stroke patients… The mortality rate of space occupying infarctions in the MCA-territory rises up to 80% despite maximal medical treatment.

Decompressive craniotomy is a procedure proposed for the first time in 1901 by Dr Kocher for stroke and severe trauma. Its scientific basis is the Kelly-Monroe principle of the exponential increase of intracranial pressure (closed "box") as intracranial components (ie oedema or blood increase in volume, and the similar reduction of intracranial pressure when the "box" (cranium) is expanded.

During the past several years, numerous research papers have described the life-saving nature of hemi-

craniectomy for MCA-territory cerebral infarction. Most hemicraniectomy series report a reduced mortality to approximately 20%.

In three meta-analysis of patients subjected to decompressive craniotomy for stroke: DECIMAL (DEcompressive Craniectomy In MALignant middle cerebral artery infarction), DESTINY (DEcompressive Surgery for the Treatment of malignant INfarction of the middlecerebral arterY), and HAMLET, where patients were randomised within 48 h of stroke onset to surgical decompression, a reduced poor outcome (ARR 16%, -0·1 to 33) and case fatality (ARR 50%, 34 to 66) were reported.

This beneficial effect has been observed even in older individuals and the benefits have persisted for more than a couple of years. Of course controversy still remains on the usefulness of the procedure on the older populations with their significant co-morbidities as well as in patients with dominant hemisphere strokes.

Patients with Haemorrhagic Stroke

Several prospective randomised controlled trials were undertaken during the previous century, culminating in the first large trial of early surgery for spontaneous supratentorial intracerebral haemorrhage STICH the results of which were neutral. This outcome seemed to occur because some groups of patients did worse with surgery (those with deep-seated bleeds or with intraventricular haemorrhage and hydrocephalus) and some better (patients with superficial lobar haematomas without intraventricular haemorrhage). The same effect was noted in a meta-analysis of other studies: a benefit with surgery that was not significant.

The STICH II results confirmed that early surgery does not increase the rate of death or disability at 6 months and might have a small but clinically relevant survival advantage for patients with spontaneous superficial intracerebral haemorrhage without intraventricular haemorrhage.

Vascular abnormalities (Cavernomas, AVMs, DAVFs and Cerebral Aneurysms)

All the vascular abnormalities can present with a stroke type picture depending on the mode of bleeding, the area of the brain affected as well as the premorbid history. With regards to arteriovenous malformations (AVMs) in particular, one group, which is likely to comprise most Grade I and II AVMs, generally benefit from treatment. Another group, including most SIV–V AVMs, probably are best left untreated given the available data. And certainly we will find that in a significant number of patients, probably including most of those with S-M Grade III AVMs, some of the most difficult Grade II AVMs, and the easiest Grade IV AVMs, we simply do not know from the data available whether intervention should be undertaken, and what form of intervention (if any) should be offered. Important also to note that AVMs account for 30-50% of haemorrhagic strokes in children. When compared to the adult population, children suffer AVM-related haemorrhages more frequently, with some paediatric series reporting haemorrhage rates of 80–85%, resulting in mortalities up to 25%. The natural history of untreated ruptured paediatric brain AVMs is grim, with recent studies reporting mortality rates of 42.1% in this group.

Management of vascular lesions of the brain and spinal cord forms a large part of the neurosurgical workload, but specific details are beyond the scope of this brief review.

Patients with Sickle Cell Anaemia and Moya-Moya

The chronic cerebrovascular disorder known as moyamoya disease (MMD) or moyamoya syndrome (MMS) leads to the development of characteristically tortuous and friable vascular collateral network in the region of the terminal portion of the internal carotid artery. These vascular networks are prone to rupture, resulting in haemorrhagic stroke.

MMS is usually associated with: Neurofibromatosis I, Cranial therapeutic irradiation, Down syndrome, Hemoglobinopathy (Sickle), Renal artery stenosis, Ischaemic angiopathy and benign intracranial hypertension.

As many as 43% of patients with Sickle cell disease and strokes will have "moyamoya-like" collaterals on imaging studies, and patients with these findings may have a 5-fold increased risk for recurrent stroke compared with patients without these collaterals.

At present, no reliable medical treatment exists for the primary disease process causing the moyamoya vasculopathy. Current therapies are aimed at preventing symptoms and negative disease sequelae by restoring and improving blood flow to affected cerebral hemispheres. Anti-platelet and anticoagulant agents have been used to reduce the risk of ischaemic stroke with optimal management of Sickle as the main focus. Given that moyamoya preferentially affects the ICA system, surgical treatment exploits the external carotid as a source to restore blood flow to the affected cerebrum, via either a direct or indirect approach. The procedure of choice is usually the indirect approach known as Encephalo-Dura-Arterial Synangiosis (EDAS).

Patients with carotid occlusive disease refractory to maximum medical treatment (?Aspirin resistant)

Bypass surgery falls into 2 distinct categories: flow

augmentation and flow preservation. Flow augmentation aims to restore flow to hypoperfused brain territories in patients with steno-occlusive diseases. Flow preservation aims to replace the blood flow provided by a major intracranial vessel, the occlusion of which is necessary for treating an underlying disease, such as an aneurysm or a tumour.

The EC-IC bypass was a procedure developed in the 70s and was the first surgical procedure ever to be subjected to a randomised trial. This early trial and subsequent ones have failed to demonstrate a superiority of the surgical approach compared with best medical management. There remains a very small cohort of patients who may still benefit from blood flow augmentation.

Conclusion

- Stroke care is evolving and management has become acute and more invasive.
- Thrombectomy is revolutionising the management of acute stroke.
- Neurosurgical interventions have a further important role in a significant minority of properly selected patients.
- A multidisciplinary approach is imperative.

THIRTY-THREE YEARS OF TRANSLATIONAL NEUROGENETICS IN CYPRUS

Kyproula Christodoulou, PhD

Neurogenetics Department, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

Abstract

The Cyprus Institute of Neurology and Genetics (CING) is a bicommunal non-profit organisation formed in 1991. The mission of CING is to develop and provide high level medical and clinical laboratory services, develop and pursue advanced research, and provide education in the areas of Neurology, Genetics, Biomedical, Medical and related Sciences for the benefit of patients and society. The Neurogenetics Department developed translational neurogenetics alongside the global advances in genomic studies. We investigated neurological diseases prevalent in specific geographical regions of the island, such as Friedreich ataxia and many other neurogenetic disorders. Translational neurogenetics research studies in Cyprus attracted extensive funding by international and national funding bodies, produced a remarkable research output such as gene mapping and identification, and established a fruitful global network of collaborations.

Key words: Neurogenetics, Cyprus, rare neurological diseases, translational research

The first report of Friedreich ataxia (FRDA) in Cyprus was published thirty-three years ago [1] and constitutes the initiation of a fascinating journey of translational neurogenetics in Cyprus. During a study of multiple sclerosis, researchers found a cluster of FRDA patients in the neighbouring villages of Kathikas and Arodhes in Paphos. The authors estimated that 1-in-6 to 1-in-7 of the population of the villages carry the FRDA pathogenic variant. We further investigated these families with linkage analysis and Sanger sequencing and, in 1996, established the genetic diagnosis. The CING neurologists ascertained additional FRDA families. In the year 2000, it became apparent that 10 out of 11 patients originating from the district of Paphos had no evidence of origin from the above two villages. We thus focused on investigating whether the pathogenic variant spread outside the founder villages. We were successful in obtaining funding for an 18-month FRDA carrier screening programme. The programme aimed at:

1. Informing the population of the Paphos district about the disease, the mode of inheritance and available diagnostic options.
2. Collect samples from volunteers after informed and signed consent to estimate the FRDA carrier frequency in the district.
3. To offer further genetic counselling to the FRDA carriers.

This programme established a high frequency of FRDA carriers (1-in-12) in the overall district of Paphos [2]. It also confirmed the 1-in-7 FRDA carrier frequency in the population of Kathikas and Arodhes. These findings led to the introduction of the National Prevention Programme for Friedreich ataxia in 2010, an ongoing successful collaboration of the Ministry of Health and the CING.

Another cluster of patients with familial amyloidotic polyneuropathy (FAP) exists in Cyprus. The CING has been investigating FAP patients and their family members since 1987. All Cypriot FAP patients have a single pathogenic variant, the TTR Val30Met. We reported the prevalence and incidence of the disease at two-time points [3,4]. A dedicated clinic at CING is following up on patients. Many of them have undergone liver transplantation, and currently, Cypriot patients participate in two international clinical trials (Alnylam, since 2016 and IONIS, since 2020) through their CING neurologists.

The CING participated in several studies on gene mapping and gene identification of neuropathies through international collaborations. We mapped a distal form of spinal muscular atrophy with upper limb predominance to chromosome 7 [5]. Pathogenic variants in the Glycyl tRNA synthetase (GARS) gene are associated with this distal spinal atrophy type V and Charcot-Marie-Tooth disease type 2D [6, 7]. We mapped a novel form of distal hereditary motor neuronopathy to chromosome 9p21.1-p12 [8] that we named Jerash type dHMN (HMNJ), and recently we have reported a novel SIGMAR1 pathogenic variant that is associated with the development of this disease [9].

We participated in mapping and identifying the

Charcot-Marie-Tooth type 4B (CMT4B) gene, encoding the myotubularin-related protein-2 [10, 11] and in identifying PDXK variants that cause polyneuropathy responsive to PLP supplementation [12]. We reported several novel pathogenic variants and functional studies in CMT disease [13, 14, 15, 16, 17, 18, 19, 20, 21].

With the new era of next-generation massively parallel sequencing (NGS), we initiated studies with this new technological tool to diagnose rare neurological diseases. Initial studies of ataxias and spastic paraplegias enabled the identification of novel pathogenic variants in long-pending diagnosis patients and families. An example of applying various techniques in combination is the case of family 903 with spastic ataxia due to a GBA2 pathogenic variant [22]. Initial linkage analysis of the specific family back in the 1990s has mapped the disease in the family to the aprataxin (APTX) gene locus on chromosome 9p21.1. Sanger sequencing of the APTX gene and MLPA based investigation for APTX duplication/deletion excluded the probability of a pathogenic variant in this gene. Because the parents were third cousins, a common genetic background was suspected, and thus we performed genome-wide homozygosity mapping to identify common by descent chromosomal regions. We detected two areas with high homozygosity scores: a 3.1 Mb region on chromosome 5 that harbours eight protein-coding genes and a 6.49 Mb region on chromosome 9 with ninety-six protein-coding genes. We then performed whole-exome sequencing (WES), which helped exclude any pathogenic variant in the chromosome 5 candidate region and revealed possible pathogenic variants in three genes within the chromosome 9 candidate region. We confirmed the GBA2 pathogenic variant in this family with spastic ataxia with a segregation analysis of the five members of the family. We further performed biochemical studies of the GBA2 variant in lymphoblastoid cell lines derived from family members and healthy control individuals [23]. We recently performed transcriptomic characterisation of tissues from patients and healthy control individuals. We discovered more than 5000 differentially expressed genes. Subsequent pathway analyses reveal biological pathways implicated in spastic ataxia. This work is currently under review for publication (Kakouri A et al., under review).

We investigated several additional patients and families with rare neurological diseases using NGS based approaches. Although the hopes for reaching a molecular diagnosis were high at the initiation of this investigation, through our ten years of experience, the diagnostic yield is only above 30%. A combination of WES and transcriptomic analyses in the availability of patient material for RNA level investigation should enable a higher diagnostic yield. Thus, we are currently working in this direction. In addition, participation in multicentre studies within the framework of recently established European Reference Networks (ERNs) or any other international collaborative effort should improve diagnostic yields and improve the time to diagnose rare neurological disorders. The CING is committed to introducing cutting edge technological approaches both in the diagnostic and in the research sector for the benefit of patients and society.

References

[1] Dean G, Chamberlain S, Middleton L. Friedreich's ataxia in Kathikas-Arodhes, Cyprus. Lancet. 1988 Mar 12;1(8585):587. doi: 10.1016/s0140-6736(88)91378-5. PMID: 2894517.

[2] Zamba-Papanicolaou E, Koutsou P, Daiou C, Gaglia E, Georghiou A, Christodoulou K. High frequency of Friedreich's ataxia carriers in the Paphos district of Cyprus. Acta Myol. 2009 Jul;28(1):24-26. PMID: 19772192.

[3] Dardiotis E, Koutsou P, Papanicolaou EZ, Vonta I, Kladi A, Vassilopoulos D, Hadjigeorgiou G, Christodoulou K, Kyriakides T. Epidemiological, clinical and genetic study of familial amyloidotic polyneuropathy in Cyprus. Amyloid. 2009 Mar;16(1):32-37. PMID: 19291512.

[4] Andreou S, Panayiotou E, Michailidou K, Pirpa P, Hadjisavvas A, El Salloukh A, Barnes D, Antoniou A, Christodoulou K, Tanteles G, Kyriakides T. Epidemiology of ATTRV30M neuropathy in Cyprus and the modifier effect of complement C1q on the age of disease onset. Amyloid. 2018 Dec;25(4):220-226. doi: 10.1080/13506129.2018.1534731. Epub 2018 Dec 20. PMID: 30572722.

[5] Christodoulou K, Kyriakides T, Hristova AH, Georgiou DM, Kalaydjieva L, Yshpekova B, Ivanova T, Weber JL, Middleton LT. Mapping of a distal form of spinal muscular atrophy with upper limb predominance to chromosome 7p. Hum Mol Genet. 1995 Sep;4(9):1629-32. PMID: 8541851.

[6] Antonellis A, Ellsworth RE, Sambuughin N, Puls I, Abel A, Lee-Lin SQ, Jordanova A, Kremensky I, Christodoulou K, Middleton LT, Sivakumar K, Ionasescu V, Vance JM, Goldfarb LG, Fischbeck KH, Green ED. Glycyl tRNA Synthetase Mutations in Charcot-Marie-Tooth Disease Type 2D and Distal Spinal Muscular Atrophy Type V. Am J Hum Genet 2003 May;72(5):1293-1299. PMID: 12690580.

[7] Sivakumar K, Kyriakides T, Puls I, Nicholson GA, Funalot B, Antonellis A, Ellsworth RE, Sambuughin N, Christodoulou K, Beggs JL, Zamba-Papanicolaou E, Ionasescu V, Dalakas

MC, Green ED, Fischbeck KH, Goldfarb LG. Phenotypic spectrum of disorders associated with glycyl-tRNA synthetase mutations. Brain. 2005 Oct;128(Pt 10):2304-2314. PMID: 16014653.

[8] Christodoulou K, Zamba E, Tsingis M, Mubaidin A, Horani K, Abu-Sheik S, El-Khateeb M, Kyriacou K, Kyriakides T, Al-Qudah AK, Middleton LT. A novel form of distal hereditary motor neuronopathy maps to chromosome 9p21.1-p12. Ann Neurol 2000 Dec;48(6):877-884. PMID: 11117544.

[9] Ververis A, Dajani R, Koutsou P, Aloqaily A, Nelson-Williams C, Loring E, Arafat A, Mubaidin AF, Horany K, Bader MB, Al-Baho Y, Ali B, Muhtaseb A, DeSpenza T Jr, Al-Qudah AA, Middleton LT, Zamba-Papanicolaou E, Lifton R, Christodoulou K. Distal hereditary motor neuronopathy of the Jerash type is caused by a novel SIGMAR1 c.500A>T missense mutation. J Med Genet. 2020 Mar;57(3):178-186. doi: 10.1136/jmedgenet-2019-106108. Epub 2019 Sep 11. PMID: 31511340; PMCID: PMC7042970.

[10] Bolino A, Levy ER, Muglia M, Conforti FL, LeGuern E, Salih MA, Georgiou DM, Christodoulou RK, Hausmanowa-Petrusewicz I, Mandich P, Gambardella A, Quattrone A, Devoto M, Monaco AP. Genetic Refinement and Physical Mapping of the CMT4B Gene on Chromosome 11q22. Genomics. 2000 Jan 15;63(2):271-278. PMID: 10673338.

[11] Bolino A, Muglia M, Conforti FL, LeGuern E, Salih MA, Georgiou DM, Christodoulou K, Hausmanowa-Petrusewicz I, Mandich P, Schenone A, Gambardella A, Bono F, Quattrone A, Devoto M, Monaco AP. Charcot-Marie-Tooth type 4B is caused by mutations in the gene encoding myotubularin-related protein-2. Nat Genet. 2000 May 25(1):17-19. PMID: 10802647.

[12] Chelban V, Wilson MP, Warman Chardon J, Vandrovcova J, Zanetti MN, Zamba-Papanicolaou E, Efthymiou S, Pope S, Conte MR, Abis G, Liu YT, Tribollet E, Haridy NA, Botía JA, Ryten M, Nicolaou P, Minaidou A, Christodoulou K, Kernohan KD, Eaton A, Osmond M, Ito Y, Bourque P, Jepson JEC, Bello O, Bremner F, Cordivari C, Reilly MM, Foiani M, Heslegrave A, Zetterberg H, Heales SJR, Wood NW, Rothman JE, Boycott KM, Mills PB, Clayton PT, Houlden H; Care4Rare Canada Consortium; SYNaPS Study Group. PDXK mutations cause polyneuropathy responsive to PLP supplementation. Ann Neurol. 2019 Aug;86(2):225-240. doi:10.1002/ana.25524. PMID: 31187503.

[13] Georgiou DM, Zidar J, Korosec M, Middleton LT, Kyriakides T, Christodoulou K. A novel NF-L mutation Pro22Ser is associated with CMT2 in a large Slovenian family. Neurogenetics 2002 Oct;4(2):93-96. PMID: 12481988.

[14] Kleopa AK, Georgiou DM, Nicolaou P, Koutsou P, Papathanasiou E, Kyriakides T, Christodoulou K. A novel PMP22 mutation Ser22Phe in a family with HNPP and CMT1A phenotypes. Neurogenetics. 2004 Sep;5(3):171-175. PMID: 15205993.

[15] Georgiou DM, Nicolaou P, Chitayat D, Koutsou P, Babul-Hirji R, Vajar J, Murphy J, Christodoulou K. A novel GDAP1 mutation 439delA is associated with autosomal recessive CMT disease. Can J Neurol Sci 2006 Aug;33(3): 311-316. PMID: 17001820.

[16] Kleopa K, Zamba-Papanicolaou E, Nicolaou P, Georgiou DM, Kyriakides T, Christodoulou K. Phenotypic and cellular expression of two novel Connexin32 mutations causing CMTX, Neurology 2006 66: 396-402. PMID: 16476939.

[17] Butinar D, Starr A, Zidar J, Koutsou P, Christodoulou K. Auditory nerve is affected in one of two different point mutations of the neurofilament light (NF-L) gene. Clin Neurophysiol 2008 Feb;119(2):367-75. Epub 2007 Nov 26. PMID: 18023247.

[18] Nicolaou P, Zamba-Papanicolaou E, Koutsou P, Kleopa KA, Georghiou A, Hadjigeorgiou G, Papadimitriou A, Kyriakides T, Christodoulou K. Charcot-Marie-Tooth Disease in Cyprus: Epidemiological, Clinical and Genetic Characteristics. Neuroepidemiology 2010 Jun 23;35(3):171-177. PMID: 20571287.

[19] Nicolaou P, Cianchetti C, Minaidou A, Marrosu G, Zamba-Papanicolaou E, Middleton L, Christodoulou K. A novel LRSAM1 mutation is associated with autosomal dominant axonal Charcot-Marie-Tooth disease. Eur J Hum Genet. 2013 Feb;21(2):190-4. doi: 10.1038/ejhg.2012.146. Epub 2012 Jul 11. PMID:22781092.

[20] Minaidou A, Nicolaou P, Christodoulou K. LRSAM1 depletion affects neuroblastoma SH-SY5Y cell growth and morphology: The LRSAM1 c.2047-1G>A loss-of-function variant fails to rescue the phenotype. Cell J. 2018 Oct;20(3):340-347. doi: 10.22074/cellj.2018.5352. Epub 2018 May 15. PMID: 29845787.

[21] Minaidou A, Nicolaou P, Christodoulou K. Deregulation of LRSAM1 expression impairs the levels of TSG101, UBE2N, VPS28, MDM2 and EGFR. PLoS One. 2019 Feb 6;14(2):e0211814. doi: 10.1371/journal.pone.0211814. eCollection 2019. PMID: 30726272.

[22] Votsi C, Zamba-Papanicolaou E, Middleton LT, Pantzaris M, Christodoulou K. A novel GBA2 gene missense mutation in spastic ataxia. Ann Hum Genet. 2014 Jan;78(1):13-22. doi:

10.1111/ahg.12045. Epub 2013 Nov 20. PMID:24252062.

[23] Malekkou A, Samarani M, Drousiotou A, Votsi C, Sonnino S, Pantzaris M, Chiricozzi E, Zamba-Papanicolaou E, Aureli M, Loberto N, Christodoulou K. Biochemical characterization of the GBA2 c.1780G>C missense mutation in lymphoblastoid cells from patients with spastic ataxia. Int J Mol Sci. 2018 Oct 10;19(10). pii: E3099. doi: 10.3390/ijms19103099. PMID: 30308956.

GENETICS OF STROKE: FROM BIOLOGICAL DISCOVERIES TO CLINICAL TRANSLATION

Marios K. Georgakis, MD, PhD[1, 2, 3]

[1] *Institute for Stroke and Dementia Research, LMU University Hospital, Munich, Germany*
[2] *Center for Genomic Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*
[3] *Program of Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA, USA*

Abstract

Stroke genetics have been transformed from a field exploring causes of rare hereditary forms of cerebrovascular disease to an international dynamic and expanding arena offering key insights into stroke biology and exciting opportunities for clinical applications. Genome-wide association studies, which triggered this transformation, have already identified more than 40 genomic risk loci associated with stroke, which offer important windows into stroke pathogenesis and starting points for experimental explorations of pharmacological strategies. Furthermore, genome-wide explorations have enabled the development of polygenic risk scores, which represent a promising potential application for risk prediction in clinical populations. Utilization of genetic data further allows exploration of causal relationships between exposures and outcomes and the discovery of novel drug targets for stroke with the use of Mendelian randomization. In this review, I provide a brief overview of the major developments in the field and opportunities for applications.

Key words: genetics; genomics; stroke; cerebrovascular disease; polygenic risk prediction; Mendelian randomization

Stroke remains a leading cause of death and disability worldwide [1]. While major advances in the prevention and treatment of stroke have taken place, important gaps remain. For example, there is no specific preventive strategy for small vessel stroke and intracerebral hemorrhage, whereas neuroprotectant therapies have not met the initial expectations. As we learned from the past, understanding the biology underlying stroke pathogenesis is important to improve treatment options. Genetics were always considered anchors to fundamental biological mechanisms. But recent major advancements in available technologies have revolutionized the way we explore genetic information to discover disease mechanisms. Stroke genetics has grown from a small field exploring causes of rare hereditary forms of stroke to a dynamic and expanding arena offering insights about the pathogenesis of sporadic stroke. Genome-wide association studies (GWAS), which triggered this transformation, have offered not only unique windows into disease biology but also unexpected opportunities for clinical applications.

Gene discovery: from monogenic stroke to novel pathways in sporadic stroke

Early genetic studies found genes that underlie forms of Mendelian stroke [2] and pointed to pathways involved in stroke pathogenesis that were particularly relevant for stroke subtypes. Such examples include genes encoding proteins related to the extracellular matrix, which were associated with hereditary forms of cerebral small vessel disease (*COL4A1*, *COL4A2*, *HTRA1*, *NOTCH3*) [3]. However, beyond Mendelian stroke that is responsible for a very small proportion of stroke cases encountered in the clinic (around 1-2% of lacunar strokes and <5% in total) [4], studies in twins found a higher risk of stroke among monozygotic, as compared with dizygotic co-twins, thus suggesting a genetic component for sporadic stroke cases as well [5]. In further support of this, within-family studies showed that stroke is more common among individuals with a family history of stroke or vascular disease [6]. Before the mapping of the human genome and the subsequent development of GWASs, several studies explored candidate genes that might be associated with stroke risk. While such studies occasionally provided important insights into stroke biology, most of the described associations were not replicated in subsequent GWAS analyses [7]. The first GWASs in stroke estimated the heritability of stroke prevalence at around 40% for ischemic stroke[7] and 30% for intracerebral hemorrhage [8].

More recent GWASs including up to 71,147 cases have identified >40 risk loci for stroke risk [9, 10, 11]. The results of these studies highlight the role of specific genes in stroke pathogenesis and can be used as the starting point for follow-up functional experiments. One example is histone deacetylase 9

(HDAC9), which consistently came up as a major risk locus for multiple atherosclerotic phenotypes including large artery stroke, coronary artery disease, and peripheral artery disease and enabled functional studies in atherosclerosis-prone mice [12]. Other loci involved in stroke pathogenesis include genes implicated in the pathogenesis of major risk factors, such as hypertension (e.g. *FURIN-FES*) and hypercholesterolemia (e.g. *LDLR*) or genes that have been previously implicated in the development of major stroke causes, such as atherosclerosis, atrial fibrillation, and cerebral small vessel disease [3]. Finally, risk loci for stroke are enriched in target genes for approved treatments, such as *FGA*, encoding the target for thrombolytic agents, highlighting that the discovered risk loci may harbor targets for future drug development [10]. Until now, the largest GWASs have provided information about common variants, encountered in >0.5-1% of the population. As the datasets increase and low-cost sequencing technologies become more widely available, newer methods that also explore rare genetic variation are expected to be integrated into future studies. Preliminary analyses focused on rare variants in the exonic regions of specific genes, such as *HTRA1*, already provide important results about the pathogenesis of cerebrovascular disease [13].

Polygenic risk scores: a tool ready for clinical application?

Beyond new insights into disease biology, the results from GWAS analyses can be useful in stroke risk prediction. While individual genetic variants contribute to disease risk only minimally, by combining multiple variants with individually small effects in a so-called "polygenic risk score" (PRS; or genomic risk score), it is possible to additively quantify genetic predisposition to stroke risk [14]. Multiple novel methods have been developed that aim to combine information throughout the genome from GWASs in an optimal way, so as to maximize the predictive power of the tool [15]. For example, the predictive performance can be enhanced by combining PRSs for stroke with multiple PRSs from traits known to be involved in stroke pathogenesis, such as blood pressure, diabetes, and circulating lipids, in a so-called meta–genomic risk score. The hazard ratio obtained from such a score for stroke is 1.26 per standard deviation increment [16], whereas a score for coronary artery disease achieved a hazard ratio of 1.71 [17]. These scores consistently increase predictive power when added to models of established clinical risk factors [16, 17]. Because genetic information is present from birth and remains stable over time, PRSs can be assessed by a single genotyping effort long before traditional risk factors manifest, thus allowing early prognostication and decisions on targeted monitoring [18]. Already *post hoc* analyses from clinical trials suggest that PRSs can predict risk of stroke among patients with cardiometabolic risk factors [19]. Interestingly, among patients with atrial fibrillation, an ischemic stroke PRS can enhance the predictive performance of the CHA_2DS_2-VASc score for stroke prediction, thus opening a window for a potential clinical application in the decision-making algorithms for initiating anticoagulant treatment [19]. Very important topics remain however open before implementing PRSs: these include sex differences, which are not traditionally considered, the reproducibility of PRSs across different ancestries, the communication of PRS screening results to individuals, and the optimal management of individuals at high genetic risk [20].

Mendelian randomization: exploring causal associations with human genetic data

Another application of genetic results includes the exploration of causal relationships [21]. An instrumental variable analysis, called Mendelian randomization, makes use of genetic variants associated with a risk factor (*genetic instruments*) to investigate causal associations between the risk factor and a disease outcome [22, 23]. The emergence of large-scale GWASs enabled the discovery of multiple genetic variants that explain an increasing proportion of variance in risk factors of interest. Thus, Mendelian randomization studies may incorporate up to hundreds of genetic variants as instruments to explore associations between genetic predisposition to exposure traits and outcomes of interest. As genetic information is anchored to conception and is not influenced by other potential environmental confounders, Mendelian randomization is less prone to traditional biases in observational studies, such as confounding and reverse causation. However, a number of assumptions need to be fulfilled in order for the genetic variants to be valid instrumental variables: [23] the variants should (i) strongly be associated with and predict the risk factor of interest, (ii) only associate with the outcome through their relation to the risk factor and (iii) not relate to confounders of the exposure-outcome association. Importantly, genetic variants with so-called pleiotropic effects on potential confounders in the exposure-outcome association may not represent valid instruments [23]. Pleiotropy refers to the phenomenon where a gene or a genetic variant can influence more than one phenotypic traits and may represent a source of bias in Mendelian randomization analyses [24]. Developments in statistical methodology have offered analytical tools to test the validity of these assumptions and correct for deviations from the real effect estimates due to pleiotropy [25].

Mendelian randomization studies have offered unique insights into stroke etiology, and particularly into the etiological risk factors that underlie specific diagnostic subtypes [26]. For example, studies focused on blood pressure provided evidence for a strong association of higher genetically predicted blood pressure with all major ischemic stroke subtypes (large artery, cardioembolic, small vessel stroke) and deep intracerebral hemorrhage, but not with lobar intracerebral hemorrhage, which is traditionally associated with cerebral amyloid angiopathy [27]. Furthermore, on top of mean blood pressure, genetically predicted late-life pulse pressure, which is an indicator for arterial stiffness, is also a risk factor for ischemic stroke, and particularly large artery stroke [28]. Another interesting example includes lipid metabolism. Mendelian randomization studies confirmed a potentially causal association of LDL cholesterol levels only with large artery stroke [29], whereas for small vessel stroke, a protective effect of higher HDL cholesterol was more robust [30]. Interestingly, the inverse relationships were detected for intracerebral hemorrhage [30], further expanding on findings from *post hoc* analyses of randomized trials that lowering LDL cholesterol might be a risk factor for hemorrhagic stroke [31]. Other interesting insights include the role of type 2 diabetes [32], hyperglycemia [32], abdominal obesity [33], and smoking [34] on large artery and small vessel stroke and a rather linear association between alcohol consumption and risk of ischemic stroke [35]. As sample sizes further increase, unique opportunities will emerge for clarifying the role of traditional vascular risk factors in stroke pathogenesis, but also for discovering novel risk factors [26]. Expansions of more elegant analytical epidemiological tools to Mendelian randomization, such as multivariable and mediation analyses, will also enable a more accurate dissection of the pathways that lead to stroke [36, 37].

Leveraging genetic data for drug discovery

The drug discovery pipeline is costly and lengthy. Despite the increasing investment in drug development, only around 5% of cardiovascular disease drugs that enter phase I trials make it to market approval [38]. Historical retrospective analyses have shown that evidence of effect from human genetic studies for a candidate protein drug target increases the probability for a compound targeting this candidate to reach approval by 2- to 4-fold [39, 40]. Perhaps the example that most compellingly demonstrates this paradigm is *PCSK9*, which is the target of the recently developed proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors [41]. *PCSK9* was first described in 2003, when it was implicated in familial hypercholesterolemia [42], soon thereafter, in 2006, loss-of-function variants in *PCSK9* were associated with lower LDL levels and a lower lifetime risk or acute coronary events [43]. Already in 2017 and 2018, two large-scale phase III trials provided robust evidence that two monoclonal antibodies against PCSK9 reduced the rates of cardiovascular events on top of statins [44, 45] .

An interesting example more focused on stroke is the evidence from human genetics on the potential atheroprotective effects of anti-inflammatory drug targets. A study exploring genetic variation in the circulating levels of 41 cytokines and growth factors showed genetic variations in the circulating levels of monocyte chemoattractant protein-1 (MCP-1 or alternatively called CC-chemokine ligand-2, CCL2) to be associated with a higher risk of ischemic stroke [46], This was particularly the case for large artery stroke, but also for other atherosclerotic phenotypes, such as coronary artery disease, and myocardial infarction [46]. These results were later confirmed in prospective cohort studies [47-49] and also agree with findings from experimental atherosclerosis models that support a role of the MCP-1/CCL2 pathway in monocyte recruitment to atherosclerotic lesions [50]. Beyond MCP-1/CCL2, genetic studies also provided evidence for a potentially causal role of interleukin-6 (IL-6) signaling in large artery stroke [51]. Specifically, genetic variants within the gene encoding IL-6 receptor (*IL6R*) show strong associations with large artery stroke, abdominal aortic aneurysm, coronary artery disease, and a more favorable cardiometabolic profile [51, 52]. These data provide evidence for a causal role of IL-6 signaling in atherosclerotic cardiovascular disease. Indeed, a monoclonal antibody against IL-6 has already been tested in phase 2 trials in patients with chronic kidney disease and a history of atherosclerotic disease [53] and is currently to be tested in a phase 3 trial. Other interesting applications include phenome-wide association studies, which can reveal previously underrecognized side-effects associated with drug targets or repurposing opportunities for available drugs targeting specific drug candidates [52].

Genetics of stroke outcome might point to mechanisms related to neuroprotection

A new generation of studies aims to explore genetic determinants of outcomes after stroke. Such studies could pinpoint pathways that might serve as targets for the development of neuroprotective agents, thus addressing the high demand for such treatments. However, these efforts are in their first steps and still suffer from low power due to the small sample sizes that do not suffice for genetic discoveries [54]. Stroke outcome genetic studies are by design more challenging than studies focusing

on stroke risk. They are focused only on cases, they need to model clinical variables that strongly predict stroke outcome, such as time from stroke onset, stroke severity, and stroke etiology, and they need to balance between data availability and accuracy of outcome measures. For example, dynamic outcome measures of early neurological change, such as the change in National Institutes of Health Stroke Scale (NIHSS) from 6 hours to 24 hours after stroke have been proposed as key readouts [55] and might be better fits for genetic studies than more traditional readouts used in clinical research, such as 3-month modified Rankin scale [54]. Although still at its birth, the field of stroke outcome genetics is already growing and has provided some results about pathways of potential interest for brain injury, repair, and recovery following ischemic stroke, which demonstrate the feasibility of the approach [56-58].

Future directions and conclusions

Over the last two decades, the field of medical and population genetics in cerebrovascular disease has been growing rapidly. As a result, several opportunities for applications have emerged that could improve stroke care in the near-term future. The advancements in the field have been the result of large-scale international collaborations, such as the International Stroke Genetics Consortium, and biobanking initiatives, such as the UK Biobank, Biobank Japan, and the China-Kadoorie Biobank. The broad data sharing mentality of the field has critically boosted innovation and accelerated paths to discovery. Still, important developments are underway, which are worth mentioning. Key initiatives to integrate data from ancestries other than Europeans are expected to lead to new discoveries and to boost the performance of PRSs in risk prediction. There is a major need for diversification in genetic research, as most data come from analyses in European populations. Large benefits are also to be expected by genetic analyses of endophenotypes of cerebrovascular disease, such as MRI biomarkers of cerebral small vessel disease. The integration of other large-scale data, such as transcriptomics and proteomics, into genetic research will allow us to link the associations between genetic variants and disease risk to biochemical footprints that will enhance our understanding of disease mechanisms. Finally, important follow-up functional experiments that will enhance our understanding about the mechanisms through which identified variants influence disease risk will accelerate the translation of genetic discoveries to novel therapeutics.

References

[1] GBD 2019 Stroke Collaborators. Global, regional, and national burden of stroke and its risk factors, 1990-2019: A systematic analysis for the global burden of disease study 2019. *Lancet Neurol*. 2021;20:795-820.

[2] Tan RY, Markus HS. Monogenic causes of stroke: Now and the future. *J Neurol*. 2015;262:2601-2616.

[3] Dichgans M, Pulit SL, Rosand J. Stroke genetics: Discovery, biology, and clinical applications. *Lancet Neurol*. 2019;18:587-599.

[4] Tan RYY, Traylor M, Megy K, Duarte D, Deevi SVV, Shamardina O, Mapeta RP, Consortium NBRD, Ouwehand WH, Graf S, Downes K, Markus HS. How common are single gene mutations as a cause for lacunar stroke? A targeted gene panel study. *Neurology*. 2019;93:e2007-e2020.

[5] Bak S, Gaist D, Sindrup SH, Skytthe A, Christensen K. Genetic liability in stroke: A long-term follow-up study of danish twins. *Stroke*. 2002;33:769-774.

[6] Jerrard-Dunne P, Cloud G, Hassan A, Markus HS. Evaluating the genetic component of ischemic stroke subtypes: A family history study. *Stroke*. 2003;34:1364-1369.

[7] Bevan S, Traylor M, Adib-Samii P, Malik R, Paul NL, Jackson C, Farrall M, Rothwell PM, Sudlow C, Dichgans M, Markus HS. Genetic heritability of ischemic stroke and the contribution of previously reported candidate gene and genome-wide associations. *Stroke*. 2012;43:3161-3167.

[8] Devan WJ, Falcone GJ, Anderson CD, Jagiella JM, Schmidt H, Hansen BM, Jimenez-Conde J, Giralt-Steinhauer E, Cuadrado-Godia E, Soriano C, Ayres AM, Schwab K, Kassis SB, Valant V, Pera J, Urbanik A, Viswanathan A, Rost NS, Goldstein JN, Freudenberger P, Stogerer EM, Norrving B, Tirschwell DL, Selim M, Brown DL, Silliman SL, Worrall BB, Meschia JF, Kidwell CS, Montaner J, Fernandez-Cadenas I, Delgado P, Greenberg SM, Roquer J, Lindgren A, Slowik A, Schmidt R, Woo D, Rosand J, Biffi A, International Stroke Genetics C. Heritability estimates identify a substantial genetic contribution to risk and outcome of intracerebral hemorrhage. *Stroke*. 2013;44:1578-1583.

[9] NINDS Stroke Genetics Network (SiGN), International Stroke Genetics Consortium (ISGC). Loci associated with ischaemic stroke and its subtypes (sign): A genome-wide association study. *Lancet Neurol*. 2016;15:174-184.

[10] Malik R, Chauhan G, Traylor M, Sargurupremraj M, Okada Y, Mishra A, Rutten-Jacobs L, Giese AK, van der Laan SW, Gretarsdottir S, Anderson CD, Chong M, Adams HHH, Ago T, Almgren P, Amouyel P, Ay H, Bartz TM, Benavente OR, Bevan S, Boncoraglio GB, Brown RD, Jr., But-

terworth AS, Carrera C, Carty CL, Chasman DI, Chen WM, Cole JW, Correa A, Cotlarciuc I, Cruchaga C, Danesh J, de Bakker PIW, DeStefano AL, den Hoed M, Duan Q, Engelter ST, Falcone GJ, Gottesman RF, Grewal RP, Gudnason V, Gustafsson S, Haessler J, Harris TB, Hassan A, Havulinna AS, Heckbert SR, Holliday EG, Howard G, Hsu FC, Hyacinth HI, Ikram MA, Ingelsson E, Irvin MR, Jian X, Jimenez-Conde J, Johnson JA, Jukema JW, Kanai M, Keene KL, Kissela BM, Kleindorfer DO, Kooperberg C, Kubo M, Lange LA, Langefeld CD, Langenberg C, Launer LJ, Lee JM, Lemmens R, Leys D, Lewis CM, Lin WY, Lindgren AG, Lorentzen E, Magnusson PK, Maguire J, Manichaikul A, McArdle PF, Meschia JF, Mitchell BD, Mosley TH, Nalls MA, Ninomiya T, O'Donnell MJ, Psaty BM, Pulit SL, Rannikmae K, Reiner AP, Rexrode KM, Rice K, Rich SS, Ridker PM, Rost NS, Rothwell PM, Rotter JI, Rundek T, Sacco RL, Sakaue S, Sale MM, Salomaa V, Sapkota BR, Schmidt R, Schmidt CO, Schminke U, Sharma P, Slowik A, Sudlow CLM, Tanislav C, Tatlisumak T, Taylor KD, Thijs VNS, Thorleifsson G, Thorsteinsdottir U, Tiedt S, Trompet S, Tzourio C, van Duijn CM, Walters M, Wareham NJ, Wassertheil-Smoller S, Wilson JG, Wiggins KL, Yang Q, Yusuf S, Consortium AF, Cohorts for H, Aging Research in Genomic Epidemiology C, International Genomics of Blood Pressure C, Consortium I, Starnet, Bis JC, Pastinen T, Ruusalepp A, Schadt EE, Koplev S, Bjorkegren JLM, Codoni V, Civelek M, Smith NL, Tregouet DA, Christophersen IE, Roselli C, Lubitz SA, Ellinor PT, Tai ES, Kooner JS, Kato N, He J, van der Harst P, Elliott P, Chambers JC, Takeuchi F, Johnson AD, BioBank Japan Cooperative Hospital G, Consortium C, Consortium E-C, Consortium EP-I, International Stroke Genetics C, Consortium M, Neurology Working Group of the CC, Network NSG, Study UKYLD, Consortium M, Sanghera DK, Melander O, Jern C, Strbian D, Fernandez-Cadenas I, Longstreth WT, Jr., Rolfs A, Hata J, Woo D, Rosand J, Pare G, Hopewell JC, Saleheen D, Stefansson K, Worrall BB, Kittner SJ, Seshadri S, Fornage M, Markus HS, Howson JMM, Kamatani Y, Debette S, Dichgans M. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet*. 2018;50:524-537.

[11] Malik R, Rannikmae K, Traylor M, Georgakis MK, Sargurupremraj M, Markus HS, Hopewell JC, Debette S, Sudlow CLM, Dichgans M, consortium M, the International Stroke Genetics C. Genome-wide meta-analysis identifies 3 novel loci associated with stroke. *Ann Neurol*. 2018;84:934-939.

[12] Asare Y, Campbell-James TA, Bokov Y, Yu LL, Prestel M, El Bounkari O, Roth S, Megens RTA, Straub T, Thomas K, Yan G, Schneider M, Ziesch N, Tiedt S, Silvestre-Roig C, Braster Q, Huang Y, Schneider M, Malik R, Haffner C, Liesz A, Soehnlein O, Bernhagen J, Dichgans M. Histone deacetylase 9 activates ikk to regulate atherosclerotic plaque vulnerability. *Circ Res*. 2020;127:811-823.

[13] Malik R, Beaufort N, Frerich S, Gesierich B, Georgakis MK, Rannikmae K, Ferguson AC, Haffner C, Traylor M, Ehrmann M, Sudlow CLM, Dichgans M. Whole-exome sequencing reveals a role of htra1 and egfl8 in brain white matter hyperintensities. *Brain*. 2021.

[14] Choi SW, Mak TS, O'Reilly PF. Tutorial: A guide to performing polygenic risk score analyses. *Nat Protoc*. 2020;15:2759-2772.

[15] Lewis CM, Vassos E. Polygenic risk scores: From research tools to clinical instruments. *Genome Med*. 2020;12:44.

[16] Abraham G, Malik R, Yonova-Doing E, Salim A, Wang T, Danesh J, Butterworth AS, Howson JMM, Inouye M, Dichgans M. Genomic risk score offers predictive performance comparable to clinical risk factors for ischaemic stroke. *Nat Commun*. 2019;10:5819.

[17] Inouye M, Abraham G, Nelson CP, Wood AM, Sweeting MJ, Dudbridge F, Lai FY, Kaptoge S, Brozynska M, Wang T, Ye S, Webb TR, Rutter MK, Tzoulaki I, Patel RS, Loos RJF, Keavney B, Hemingway H, Thompson J, Watkins H, Deloukas P, Di Angelantonio E, Butterworth AS, Danesh J, Samani NJ, Group UKBCCCW. Genomic risk prediction of coronary artery disease in 480,000 adults: Implications for primary prevention. *J Am Coll Cardiol*. 2018;72:1883-1893.

[18] Rutten-Jacobs LC, Larsson SC, Malik R, Rannikmae K, consortium M, International Stroke Genetics C, Sudlow CL, Dichgans M, Markus HS, Traylor M. Genetic risk, incident stroke, and the benefits of adhering to a healthy lifestyle: Cohort study of 306 473 uk biobank participants. *BMJ*. 2018;363:k4168.

[19] Marston NA, Patel PN, Kamanu FK, Nordio F, Melloni GM, Roselli C, Gurmu Y, Weng LC, Bonaca MP, Giugliano RP, Scirica BM, O'Donoghue ML, Cannon CP, Anderson CD, Bhatt DL, Gabriel Steg P, Cohen M, Storey RF, Sever P, Keech AC, Raz I, Mosenzon O, Antman EM, Braunwald E, Ellinor PT, Lubitz SA, Sabatine MS, Ruff CT. Clinical application of a novel genetic risk score for ischemic stroke in patients with cardiometabolic disease. *Circulation*. 2021;143:470-478.

[20] Abraham G, Rutten-Jacobs L, Inouye M. Risk prediction using polygenic risk scores for pre-

vention of stroke and other cardiovascular diseases. *Stroke*. 2021;52:2983-2991.
[21] Smith GD, Ebrahim S. 'Mendelian randomization': Can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. 2003;32:1-22.
[22] Burgess S, Davey Smith G, Davies NM, Dudbridge F, Gill D, Glymour MM, Hartwig FP, Holmes MV, Minelli C, Relton CL, Theodoratou E. Guidelines for performing mendelian randomization investigations. *Wellcome Open Res*. 2019;4:186.
[23] Davies NM, Holmes MV, Davey Smith G. Reading mendelian randomisation studies: A guide, glossary, and checklist for clinicians. *BMJ*. 2018;362:k601.
[24] Davey Smith G, Hemani G. Mendelian randomization: Genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet*. 2014;23:R89-98.
[25] Slob EAW, Burgess S. A comparison of robust mendelian randomization methods using summary data. *Genet Epidemiol*. 2020;44:313-329.
[26] Georgakis MK, Gill D. Mendelian randomization studies in stroke: Exploration of risk factors and drug targets with human genetic data. *Stroke*. 2021;52:2992-3003.
[27] Georgakis MK, Gill D, Webb AJS, Evangelou E, Elliott P, Sudlow CLM, Dehghan A, Malik R, Tzoulaki I, Dichgans M. Genetically determined blood pressure, antihypertensive drug classes, and risk of stroke subtypes. *Neurology*. 2020;95:e353-e361.
[28] Georgakis MK, Gill D, Malik R, Protogerou AD, Webb AJS, Dichgans M. Genetically predicted blood pressure across the lifespan: Differential effects of mean and pulse pressure on stroke risk. *Hypertension*. 2020;76:953-961.
[29] Falcone GJ, Kirsch E, Acosta JN, Noche RB, Leasure A, Marini S, Chung J, Selim M, Meschia JF, Brown DL, Worrall BB, Tirschwell DL, Jagiella JM, Schmidt H, Jimenez-Conde J, Fernandez-Cadenas I, Lindgren A, Slowik A, Gill D, Holmes M, Phuah CL, Petersen NH, Matouk Md CN, Gunel M, Sansing L, Bennett D, Chen Z, Sun LL, Clarke R, Walters RG, Gill TM, Biffi A, Kathiresan S, Langefeld CD, Woo D, Rosand J, Sheth KN, Anderson CD, International Stroke Genetics C. Genetically elevated ldl associates with lower risk of intracerebral hemorrhage. *Ann Neurol*. 2020;88:56-66.
[30] Georgakis MK, Malik R, Anderson CD, Parhofer KG, Hopewell JC, Dichgans M. Genetic determinants of blood lipids and cerebral small vessel disease: Role of high-density lipoprotein cholesterol. *Brain*. 2020;143:597-610.
[31] Sanz-Cuesta BE, Saver JL. Lipid-lowering therapy and hemorrhagic stroke risk: Comparative meta-analysis of statins and pcsk9 inhibitors. *Stroke*. 2021;52:3142-3150.
[32] Georgakis MK, Harshfield EL, Malik R, Franceschini N, Langenberg C, Wareham NJ, Markus HS, Dichgans M. Diabetes mellitus, glycemic traits, and cerebrovascular disease: A mendelian randomization study. *Neurology*. 2021;96:e1732-e1742.
[33] Marini S, Merino J, Montgomery BE, Malik R, Sudlow CL, Dichgans M, Florez JC, Rosand J, Gill D, Anderson CD, International Stroke Genetics C. Mendelian randomization study of obesity and cerebrovascular disease. *Ann Neurol*. 2020;87:516-524
[34] Larsson SC, Burgess S, Michaelsson K. Smoking and stroke: A mendelian randomization study. *Ann Neurol*. 2019;86:468-471.
[35] Millwood IY, Walters RG, Mei XW, Guo Y, Yang L, Bian Z, Bennett DA, Chen Y, Dong C, Hu R, Zhou G, Yu B, Jia W, Parish S, Clarke R, Davey Smith G, Collins R, Holmes MV, Li L, Peto R, Chen Z, China Kadoorie Biobank Collaborative G. Conventional and genetic evidence on alcohol and vascular disease aetiology: A prospective study of 500 000 men and women in china. *Lancet*. 2019;393:1831-1842.
[36] Carter AR, Sanderson E, Hammerton G, Richmond RC, Davey Smith G, Heron J, Taylor AE, Davies NM, Howe LD. Mendelian randomisation for mediation analysis: Current methods and challenges for implementation. *Eur J Epidemiol*. 2021;36:465-478.
[37] Sanderson E. Multivariable mendelian randomization and mediation. *Cold Spring Harb Perspect Med*. 2021;11.
[38] Dowden H, Munro J. Trends in clinical success rates and therapeutic focus. *Nat Rev Drug Discov*. 2019;18:495-496.
[39] King EA, Davis JW, Degner JF. Are drug targets with genetic support twice as likely to be approved? Revised estimates of the impact of genetic support for drug mechanisms on the probability of drug approval. *PLoS Genet*. 2019;15:e1008489.
[40] Nelson MR, Tipney H, Painter JL, Shen J, Nicoletti P, Shen Y, Floratos A, Sham PC, Li MJ, Wang J, Cardon LR, Whittaker JC, Sanseau P. The support of human genetic evidence for approved drug indications. *Nat Genet*. 2015;47:856-860.
[41] El Khoury P, Elbitar S, Ghaleb Y, Khalil YA, Varret M, Boileau C, Abifadel M. Pcsk9 mutations in familial hypercholesterolemia: From a groundbreaking discovery to anti-pcsk9 therapies. *Curr Atheroscler Rep*. 2017;19:49.
[42] Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S,

Wickham L, Erlich D, Derre A, Villeger L, Farnier M, Beucler I, Bruckert E, Chambaz J, Chanu B, Lecerf JM, Luc G, Moulin P, Weissenbach J, Prat A, Krempf M, Junien C, Seidah NG, Boileau C. Mutations in pcsk9 cause autosomal dominant hypercholesterolemia. *Nat Genet*. 2003;34:154-156.

[43] Cohen JC, Boerwinkle E, Mosley TH, Jr., Hobbs HH. Sequence variations in pcsk9, low ldl, and protection against coronary heart disease. *N Engl J Med*. 2006;354:1264-1272.

[44] Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, Sever PS, Pedersen TR, Committee FS, Investigators. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med*. 2017;376:1713-1722.

[45] Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, Edelberg JM, Goodman SG, Hanotin C, Harrington RA, Jukema JW, Lecorps G, Mahaffey KW, Moryusef A, Pordy R, Quintero K, Roe MT, Sasiela WJ, Tamby JF, Tricoci P, White HD, Zeiher AM, Committees OO, Investigators. Alirocumab and cardiovascular outcomes after acute coronary syndrome. *N Engl J Med*. 2018;379:2097-2107.

[46] Georgakis MK, Gill D, Rannikmae K, Traylor M, Anderson CD, Lee JM, Kamatani Y, Hopewell JC, Worrall BB, Bernhagen J, Sudlow CLM, Malik R, Dichgans M. Genetically determined levels of circulating cytokines and risk of stroke. *Circulation*. 2019;139:256-268.

[47] Georgakis MK, van der Laan SW, Asare Y, Mekke JM, Haitjema S, Schoneveld AH, de Jager SCA, Nurmohamed NS, Kroon J, Stroes ESG, de Kleijn DPV, de Borst GJ, Maegdefessel L, Soehnlein O, Pasterkamp G, Dichgans M. Monocyte-chemoattractant protein-1 levels in human atherosclerotic lesions associate with plaque vulnerability. *Arterioscler Thromb Vasc Biol*. 2021;41:2038-2048.

[48] Georgakis MK, de Lemos JA, Ayers C, Wang B, Bjorkbacka H, Pana TA, Thorand B, Sun C, Fani L, Malik R, Dupuis J, Engstrom G, Orho-Melander M, Melander O, Boekholdt SM, Zierer A, Elhadad MA, Koenig W, Herder C, Hoogeveen RC, Kavousi M, Ballantyne CM, Peters A, Myint PK, Nilsson J, Benjamin EJ, Dichgans M. Association of circulating monocyte chemoattractant protein-1 levels with cardiovascular mortality: A meta-analysis of population-based studies. *JAMA Cardiol*. 2021;6:587-592.

[49] Georgakis MK, Malik R, Bjorkbacka H, Pana TA, Demissie S, Ayers C, Elhadad MA, Fornage M, Beiser AS, Benjamin EJ, Boekholdt SM, Engstrom G, Herder C, Hoogeveen RC, Koenig W, Melander O, Orho-Melander M, Schiopu A, Soderholm M, Wareham N, Ballantyne CM, Peters A, Seshadri S, Myint PK, Nilsson J, de Lemos JA, Dichgans M. Circulating monocyte chemoattractant protein-1 and risk of stroke: Meta-analysis of population-based studies involving 17 180 individuals. *Circ Res*. 2019;125:773-782.

[50] Živković L, Asare Y, Bernhagen J, Dichgans M, Georgakis MK. Ccl2/ccr2 inhibition in atherosclerosis: A meta-analysis of preclinical studies. *bioRxiv*. 2021:2021.2004.2016.439554.

[51] Georgakis MK, Malik R, Gill D, Franceschini N, Sudlow CLM, Dichgans M, Invent Consortium CIWG. Interleukin-6 signaling effects on ischemic stroke and other cardiovascular outcomes: A mendelian randomization study. *Circ Genom Precis Med*. 2020;13:e002872.

[52] Georgakis MK, Malik R, Li X, Gill D, Levin MG, Vy HMT, Judy R, Ritchie M, Verma SS, Regeneron Genetics C, Nadkarni GN, Damrauer SM, Theodoratou E, Dichgans M. Genetically downregulated interleukin-6 signaling is associated with a favorable cardiometabolic profile: A phenome-wide association study. *Circulation*. 2021;143:1177-1180.

[53] Ridker PM, Devalaraja M, Baeres FMM, Engelmann MDM, Hovingh GK, Ivkovic M, Lo L, Kling D, Pergola P, Raj D, Libby P, Davidson M, Investigators R. Il-6 inhibition with ziltivekimab in patients at high atherosclerotic risk (rescue): A double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet*. 2021;397:2060-2069.

[54] Lee JM, Fernandez-Cadenas I, Lindgren AG. Using human genetics to understand mechanisms in ischemic stroke outcome: From early brain injury to long-term recovery. *Stroke*. 2021;52:3013-3024.

[55] Heitsch L, Ibanez L, Carrera C, Binkley MM, Strbian D, Tatlisumak T, Bustamante A, Ribo M, Molina C, Davalos A, Lopez-Cancio E, Munoz-Narbona L, Soriano-Tarraga C, Giralt-Steinhauer E, Obach V, Slowik A, Pera J, Lapicka-Bodzioch K, Derbisz J, Sobrino T, Castillo J, Campos F, Rodriguez-Castro E, Arias-Rivas S, Segura T, Serrano-Heras G, Vives-Bauza C, Diaz-Navarro R, Tur S, Jimenez C, Marti-Fabregas J, Delgado-Mederos R, Arenillas J, Krupinski J, Cullell N, Torres-Aguila NP, Muino E, Carcel-Marquez J, Moniche F, Cabezas JA, Ford AL, Dhar R, Roquer J, Khatri P, Jimenez-Conde J, Fernandez-Cadenas I, Montaner J, Rosand J, Cruchaga C, Lee JM, International Stroke Genetics C. Early neurological change after ischemic stroke is associated with 90-day outcome. *Stroke*. 2021;52:132-141.

[56] Carrera C, Carcel-Marquez J, Cullell N, Torres-Aguila N, Muino E, Castillo J, Sobrino T, Campos F, Rodriguez-Castro E, Llucia-Carol L, Millan M,

Munoz-Narbona L, Lopez-Cancio E, Bustamante A, Ribo M, Alvarez-Sabin J, Jimenez-Conde J, Roquer J, Giralt-Steinhauer E, Soriano-Tarraga C, Mola-Caminal M, Vives-Bauza C, Navarro RD, Tur S, Obach V, Arenillas JF, Segura T, Serrano-Heras G, Marti-Fabregas J, Delgado-Mederos R, Freijo-Guerrero MM, Moniche F, Cabezas JA, Castellanos M, Gallego-Fabrega C, Gonzalez-Sanchez J, Krupinsky J, Strbian D, Tatlisumak T, Thijs V, Lemmens R, Slowik A, Pera J, Kittner S, Cole J, Heitsch L, Ibanez L, Cruchaga C, Lee JM, Montaner J, Fernandez-Cadenas I. Single nucleotide variations in zbtb46 are associated with post-thrombolytic parenchymal haematoma. *Brain*. 2021;144:2416-2426.

[57] Mola-Caminal M, Carrera C, Soriano-Tarraga C, Giralt-Steinhauer E, Diaz-Navarro RM, Tur S, Jimenez C, Medina-Dols A, Cullell N, Torres-Aguila NP, Muino E, Rodriguez-Campello A, Ois A, Cuadrado-Godia E, Vivanco-Hidalgo RM, Hernandez-Guillamon M, Sole M, Delgado P, Bustamante A, Garcia-Berrocoso T, Mendioroz M, Castellanos M, Serena J, Marti-Fabregas J, Segura T, Serrano-Heras G, Obach V, Ribo M, Molina CA, Alvarez-Sabin J, Palomeras E, Freijo M, Font MA, Rosand J, Rost NS, Gallego-Fabrega C, Lee JM, Heitsch L, Ibanez L, Cruchaga C, Phuah CL, Lemmens R, Thijs V, Lindgren A, Maguire J, Rannikmae K, Sudlow CL, Jern C, Stanne TM, Lorentzen E, Munoz-Narbona L, Davalos A, Lopez-Cancio E, Worrall BB, Woo D, Kittner SJ, Mitchell BD, Montaner J, Roquer J, Krupinski J, Estivill X, Rabionet R, Vives-Bauza C, Fernandez-Cadenas I, Jimenez-Conde J. Patj low frequency variants are associated with worse ischemic stroke functional outcome. *Circ Res*. 2019;124:114-120.

[58] Soderholm M, Pedersen A, Lorentzen E, Stanne TM, Bevan S, Olsson M, Cole JW, Fernandez-Cadenas I, Hankey GJ, Jimenez-Conde J, Jood K, Lee JM, Lemmens R, Levi C, Mitchell BD, Norrving B, Rannikmae K, Rost NS, Rosand J, Rothwell PM, Scott R, Strbian D, Sturm JW, Sudlow C, Traylor M, Thijs V, Tatlisumak T, Woo D, Worrall BB, Maguire JM, Lindgren A, Jern C, International Stroke Genetics Consortium tN-SC, the Genetics of Ischaemic Stroke Functional Outcome N. Genome-wide association meta-analysis of functional outcome after ischemic stroke. *Neurology*. 2019;92:e1271-e1283.

MACHINE LEARNING IN NEUROIMAGING: APPLICATIONS TO BRAIN AGING, AD, SCHIZOPHRENIA, AND BRAIN CANCER

Christos Davatzikos, Ph.D.

Professor of Radiology, Director, Center for Biomedical Image Computing and Analytics, University of Pennsylvania, U.S.A.

Abstract/Summary

Quantitative and computational methods have increasingly provided insights in many neuroscience problems. Amongst them, AI and machine learning have relatively recently emerged as very promising avenues for knowledge discovery, especially in the era of complex, big and diverse data. Herein, applications of machine learning in neuroimaging are discussed, with emphasis on aging and Alzheimer's Disease (AD), schizophrenia, and the most aggressive brain cancer, namely glioblastoma (GBM). In particular, machine learning is shown to produce highly sensitive and specific imaging signatures of brain change during early preclinical stages of AD, as well as to identify neuroanatomically distinct subtypes of schizophrenia. Finally, machine learning is shown to produce imaging signatures that predict patient outcome. These representative results highlight the potential of machine learning in neuroimaging as means to derive sensitive and specific biomarkers, and to reduce complex and diverse data into a small number of dimensions capturing different aspects of the neurobiology of brain diseases.

In the past 30 years we have experienced an exponential growth of various neuroimaging methods, which capture complex aspects of structure, function and connectivity of the human brain, in healthy as well as in diseased states. Quantitative analysis ways have progressed in parallel, responding to the complexity of this data and the richness of the information that can be derived from them. Among these methods, machine learning has emerged as a promising tool for extracting imaging signatures that contribute to personalized precision diagnostics and prognostication [1-6]. Although machine learning has often been viewed as a way to automate tasks that currently require a great deal of human effort (e.g. precise segmentation of anatomical structures or detection of lesions), its greatest potential lies in "seeing" in the data what humans are unable to see, thereby leading to knowledge discovery.

Imaging patterns can be quite complex. For example, no brain region offers sufficient sensitivity and specificity in detecting AD, schizophrenia, and most other brain diseases, despite the fact that numerous studies have associated them with changes in brain volumes, cortical thickness, brain connectivity and function. The main premise of machine learning is that the proper integration of many such "weak predictors" forms strong and highly sensitive and specific imaging signature which can serve as biomarkers of disease and offer personalized prognostications.

This talk discussed two such MR imaging signatures reported in AD [7] and schizophrenia [3], which are identified on an individual basis with promising accuracy. Perhaps most importantly, the former was also found to progressively increase relatively more rapidly in individuals with normal cognition who later progressed to mild cognitive impairment (MCI) [1], thereby potentially offering an early biomarker of AD during stages in which pharmacological and lifestyle interventions might be most effective. In GBM, machine learning derived imaging signatures have been found to improve our predictions of patient outcome [8], thereby offering additional information that can influence patient management, targeted recruitment into clinical trials, as well as more effective evaluation of treatment effects via comparisons to personalized estimated of outcome, rather than to generic population-based medians.

A notorious limitation of machine learning methods has been their often poor generalization and reproducibility in new patients and scans. This weakness is not necessarily fundamental for these methods, but rather emerges from the oftentimes poor application of these approaches to biomedical data. Insufficient training is among the most prominent challenges, as the sheer dimensionality and complexity of various types of neuroimaging data would

normally necessitate training on tens of thousands of scans in order to sample the variability of brain structure and function, as well as to access the diversity of various imaging acquisition protocols and scanner characteristics. Recent work on the formation of international consortia bringing together thousands, or tens of thousands of datasets have offered promise that sufficiently ample and diverse training and validation will be soon possible, which will propel machine learning methods into routine clinical use. Several such consortia were described on studies of brain aging [9, 10], schizophrenia [11, 12], and GBM [13].

As the availability of very large and diverse neuroimaging and clinical datasets increases, additional problems that were previously inaccessible can now be addressed. One such important problem is that of heterogeneity of brain diseases: perhaps seeking an imaging signature of AD or schizophrenia is mundane, since both of these diseases are highly heterogeneous. Recent work has developed advanced semi-supervised machine learning methods, which simultaneously seek to estimate disease subtypes and establish respective imaging signatures [14-16]. Application of these methods to schizophrenia identified two neuroanatomically distinct subtypes/dimensions of schizophrenia, which also showed differences in schizophrenia-related polygenic risk scores. This suggests that diseases that are clinically categorized as unique entities might have quite distinct neuropathological underpinnings, and potentially different response to various treatments. A similar recent study in MCI and AD uncovered 4 dimensions of structural brain change [16], and two progression pathways. Although one of them appeared to be aligned with typical AD-like patterns of atrophy, the second one was more associated to global patterns of brain atrophy potentially related to small vessel ischemic disease and other comorbid pathologies that accelerate the process of brain aging and dementia. Similar work in GBM has identified distinct imaging subtypes of GBM, with differences in patient survival and in molecular characteristics of the tumor [17].

These and many other studies of similar flavor are setting the foundation for more precise definition of neurologic and neuropsychiatric diseases, based on underlying neurobiological signatures, in part derived from imaging. Importantly, such methods are gradually establishing a dimensional view of brain pathologies, with various dimensions informed by neurobiological signatures derived from a variety of biomarkers, including imaging. Eventually, categorizations of a patient into a single and specific disease might become practice of the past, and replaced by placement of a patient in a brain coordinate system spanning the heterogeneity of normal and abnormal brain structure and function. Numerous prior studies can offer contextual information about the clinical implications of a patient being in a particular location on this brain chart (e.g. implications about response to certain treatments). Machine learning methods applied to neuroimaging data gradually and systematically build such dimensions and contextual knowledge.

References

[1] C. Davatzikos, F. Xu, Y. An, Y. Fan, and S. M. Resnick, "Longitudinal progression of Alzheimer's-like patterns of atrophy in normal older adults: the SPARE-AD index", *Brain: a journal of neurology*, vol. 132, no. Pt 8, pp. 2026-35, Aug 2009, doi: 10.1093/brain/awp091.

[2] C. Davatzikos *et al.*, "Classifying spatial patterns of brain activity with machine learning methods: application to lie detection", *NeuroImage*, vol. 28, no. 3, pp. 663-8, Nov 15 2005, doi: 10.1016/j.neuroimage.2005.08.009.

[3] C. Davatzikos *et al.*, "Whole-brain morphometric study of schizophrenia revealing a spatially complex set of focal abnormalities" (in English), *Archives of general psychiatry*, vol. 62, no. 11, pp. 1218-1227, Nov 2005. [Online]. Available: <Go to ISI>://WOS:000233050900005.

[4] S. Kloppel *et al.*, "Automatic classification of MR scans in Alzheimer's disease", *Brain: a journal of neurology*, vol. 131, no. Pt 3, pp. 681-689, March 2008. [Online]. Available: http://brain.oxfordjournals.org/cgi/reprint/awm319v1.

[5] N. Koutsouleris *et al.*, "Prediction Models of Functional Outcomes for Individuals in the Clinical High-Risk State for Psychosis or With Recent-Onset Depression: A Multimodal, Multisite Machine Learning Analysis", *Jama Psychiat*, vol. 75, no. 11, pp. 1156-1172, Nov 1 2018, doi: 10.1001/jamapsychiatry.2018.2165.

[6] N. Koutsouleris *et al.*, "Use of neuroanatomical pattern classification to identify subjects in at-risk mental States of psychosis and predict disease transition", (in eng), *Archives of general psychiatry*, vol. 66, no. 7, pp. 700-712, Jul 2009. [Online]. Available: http://archpsyc.ama-assn.org/cgi/reprint/66/7/700. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19581561.

[7] X. Da *et al.*, "Integration and relative value of biomarkers for prediction of MCI to AD progression: spatial patterns of brain atrophy, cognitive scores, APOE genotype and CSF biomarkers", *NeuroImage. Clinical*, vol. 4, pp. 164-73, 2014, doi: 10.1016/j.nicl.2013.11.010.

[8] L. Macyszyn *et al.*, "Imaging patterns predict patient survival and molecular subtype in glio-

blastoma via machine learning techniques", *Neuro-oncology*, vol. 18, no. 3, pp. 417-25, Mar 2016, doi: 10.1093/neuonc/nov127.

[9] M. Habes *et al.*, "The Brain Chart of Aging: Machine-learning analytics reveals links between brain aging, white matter disease, amyloid burden, and cognition in the iSTAGING consortium of 10,216 harmonized MR scans", *Alzheimer's & dementia : the journal of the Alzheimer's Association*, vol. 17, no. 1, pp. 89-102, Jan 2021, doi: 10.1002/alz.12178.

[10] R. Pomponio *et al.*, "Harmonization of large MRI datasets for the analysis of brain imaging patterns throughout the lifespan", *NeuroImage*, vol. 208, p. 116450, 2020.

[11] G. B. Chand *et al.*, "Two distinct neuroanatomical subtypes of schizophrenia revealed using machine learning", *Brain: a journal of neurology*, vol. 143, no. 3, pp. 1027-1038, Mar 1 2020, doi: 10.1093/brain/awaa025.

[12] M. Rozycki *et al.*, "Multisite Machine Learning Analysis Provides a Robust Structural Imaging Signature of Schizophrenia Detectable Across Diverse Patient Populations and Within Individuals", *Schizophrenia bulletin*, vol. 44, 11/24 2017, doi: 10.1093/schbul/sbx137.

[13] C. Davatzikos *et al.*, "AI-based prognostic imaging biomarkers for precision neuro-oncology: the ReSPOND consortium", *Neuro-oncology*, vol. 22, no. 6, pp. 886-888, Jun 9 2020, doi: 10.1093/neuonc/noaa045.

[14] A. Dong *et al.*, "Heterogeneity of neuroanatomical patterns in prodromal Alzheimer's disease: links to cognition, progression and biomarkers," (in eng), *Brain: a journal of neurology*, vol. 140, no. 3, pp. 735-747, Mar 01 2017, doi: 10.1093/brain/aww319.

[15] E. Varol, A. Sotiras, C. Davatzikos, and I. Alzheimer's Disease Neuroimaging ,"HYDRA: Revealing heterogeneity of imaging and genetic patterns through a multiple max-margin discriminative analysis framework" (in Eng), *NeuroImage*, vol. 145, no. Pt B, pp. 346-364, Jan 15 2017, doi: 10.1016/j.neuroimage.2016.02.041.

[16] Zhijian Yang, Junhao Wen, and C. Davatzikos, "Smile-GANs: Semi-supervised clustering via GANs for dissecting brain disease heterogeneity from medical images", *Arxiv*, vol. arxiv.org/abs/2006.15255, 2020.

[17] S. Rathore *et al.*, "Radiomic MRI signature reveals three distinct subtypes of glioblastoma with different clinical and molecular characteristics, offering prognostic value beyond IDH1", *Nature Scientific Reports*, vol. 8, no. 1, p. 5087, 2018/03/23 2018, doi: 10.1038/s41598-018-22739-2.

ECHOGENIC PATTERNS IN TRANSCRANIAL SONOGRAPHY

Daniel Richter MD[1], Christos Krogias MD[1, 2]

[1] Department of Neurology, St. Josef-Hospital Bochum, Ruhr University Bochum, Germany
[2] Medical Faculty, Ruhr University Bochum, Germany

Abstract

This short review will summarize the major echogenic patterns in transcranial sonography (TCS) for the diagnostic workup of Parkinson's disease (PD). PD is a primary neurodegenerative disorder caused by a loss of dopaminergic cells in the substantia nigra (SN). In addition to the dopamine deficiency, changes in other neurotransmitter systems are present, including alterations in the serotonergic system [1]. The definitive cause of PD is unknown. The disease is characterized by a slowly progressive disease course with bradykinesia as leading motor symptom, but also non-motor symptoms such as depression are common and can precede motor manifestations. The TCS analysis of different brain regions has been proven helpful in the diagnostic workup of PD, including differential diagnostic, non-motor diagnostic, and risk stratification of the healthy elderly population.

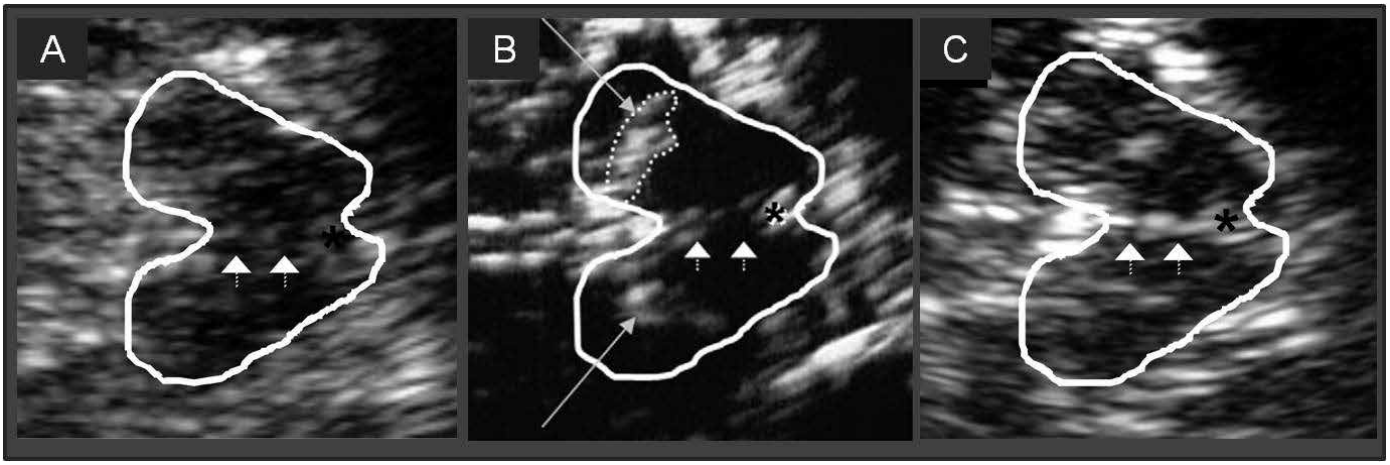
Key words: ultrasound, sonography, substantia nigra, lentiform nucleus

Substania nigra hyperechogenicity

The most prominent echogenic pattern in Parkinson's disease (PD) is a substantia nigra (SN, figure 1) hyperechogenicity that is assessed through the transtemporal bone window. The SN is displayed in the axial mesencephalic examination plane of transcranial sonography (TCS). A hyperechogenicity of the SN is defined by a planimetric measurement that shows an enlarged echogenic signal of the SN [2].

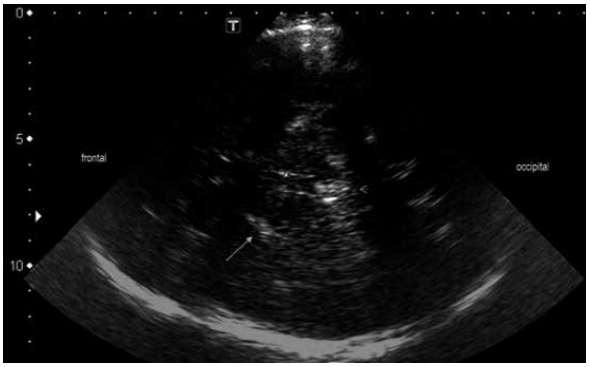
In 1995, Becker et al. published the first study on SN hyperechogenicity in PD [3]. In this first study, 40% of the examined PD patients had a hyperechogenic SN in the TCS examination. In comparison, none of the healthy controls in this study showed an SN hyperechogenicity. In 2001, Berg et al. demonstrated that approximately 90% of the patients with PD have a hyperechogenic SN. Together with an improvement of the TCS image resolution, these

Figure 1. Substantia nigra and brainstem raphe



Legend: The butterfly-shaped midbrain is outlined for better visualization. The asterisk indicates the aqueduct. Arrowheads indicate the brainstem raphe. The long arrows in (B) mark the hyperechogenic enlarged area of Substania nigra. Raphe grading (A-C): (A) Raphe structure not visible, grade 0, pathologic finding. (B) Echogenic line of the raphe is interrupted, grade 1, pathologic finding. (C) Normal echogenicity, grade 2, normal finding.

Figure 2. Nucleus lentiformis hyperechogenicity



Legend: Large arrow displays the hyperechogenicity of the right nucleus lentiformis. Small arrow marks the pineal gland. The distance between the two small crosses defines the third ventricle diameter

results were reproduced by other research groups, proofing the SN hyperechogenicity as a reliable and valid marker for PD and the differential diagnosis of extrapyramidal movement disorders [4, 5, 6, 7]. The enlarged echogenic signal of substantia nigra represents a characteristic hallmark of PD today, with a prevalence in newly diagnosed PD patients of about 80-90% [8, 9]. Therefore, TCS is increasingly applied for the differential diagnosis of PD and essential tremor (ET), where an SN hyperechogenicity is detectable in about 8-16%. The prevalence of an SN hyperechogenicity in the healthy population is even lower [10, 11, 12]. The echogenic area of the SN is routinely evaluated in a single axial mesencephalic examination plane, but it can also be depicted in a coronal examination plane, revealing a good sensitivity of 90.3% and a specificity of 96.9% to distinguish PD from healthy controls and patients with essential tremor, respectively [13].

The extent of the motor symptoms is not correlated with the sonographically measured area size of the SN [14]. Longitudinal studies have demonstrated that the presence of an SN hyperechogenicity in elderly healthy subjects is a factor that increases the risk by approximately 17 times to develop PD within three years [15]. Therefore, the presence of an SN hyperechogenicity has been included in the research criteria for prodromal Parkinson's syndrome [16].

The histopathological correlate of the hyperechogenic SN is still unknown, but it is assumed to indicate an increased amount of iron bound to proteins that differ from ferritin [17]. However, the extent of hyperechogenicity is currently not seen as correlating to progressive neurodegeneration in the SN [9].

Nucleus lentiformis hyperechogenicity

As the SN hyperechogenicity allows a clear distinction between PD, healthy controls, or patients with essential tremor, the discrimination between idiopathic and atypical parkinsonian syndromes (aPS) is insufficient [18]. A meta-analysis by Shafieesabet et al. found a prevalence of SN hyperechogenicity in 84% of PD patients and 28% of aPS patients [19].

Besides the SN, several other structures in the brain have been examined by TCS in extrapyramidal movement disorders [6, 20]. A hyperechogenicity of the nucleus lentiformis (LN, figure 2) was found to appear more frequently in patients with aPS, especially in patients with the parkinsonian phenotype of multiple system atrophy (MSA-P) or in patients with progressive supranuclear palsy (PSP) [21]. Thus, LN hyperechogenicity has been considered as a promising echogenic pattern of aPS. The LN is investigated in TCS using the diencephalic axial examination plane. A meta-analysis examining the frequency of LN hyperechogenicity in PD and aPS demonstrated a prevalence of 76% (95% CI: 0.62-0.88) in aPS compared to 16% (95% CI: 0.10-0.23) in PD [22].

So far, no studies are investigating the cellular and extracellular changes in PD patients with LN hyperechogenicity. However, an increase in the tissue iron level could cause the hyperechogenic alterations of the LN visualized in TCS. Apart from that, Walter et al. conducted a tissue metal analysis in autopsy brains of 11 patients with Wilson's disease (WD), in which the LN hyperechogenicity is a common ultrasound finding [23]. Diagnosis of WD was confirmed for all of these WD cases after an autopsy, and they all showed an LN hyperechogenicity in TCS. The authors found a clear correlation between the LN hyperechogenicity and the putaminal concentration of copper but not iron.

Raphe hypoechogenicity

Besides its value in diagnosing and discriminating Parkinsonian syndromes, TCS is also a valuable tool for investigating non-motor features. Depression and apathy frequently appear in PD patients and can represent early non-motor symptoms [24-26]. Several studies have shown that many PD patients are affected by depression, even in the prodromal state of disease [27].

Brainstem raphe (BR) alterations in TCS have been associated with depression in PD patients, underlining an involvement of the serotonergic system in this non-motor feature of PD [7, 28, 29]. Different from the enlarged echogenic area of the SN, a reduced echogenic signal of the BR is thought to visualize changes in the serotonergic system [7]. The echogenic pattern of BR is assessed by the axial mesencephalic examination plane and classified semi-quantitatively on a three-point scale (figure 1): 0 = raphe structure not visible, 1 = slight and interrupted echogenic ra-

phe structure, 2 = normal echogenicity (echogenicity of raphe structure is not interrupted). Alteration of the BR can also be depicted in the coronal examination plane and have also been associated with apathy in PD [29].

Until now, BR alterations in PD have been investigated in a cross-sectional study approach. Future studies should investigate its structural correlate and the predictive value of BR hypoechogenicity for patients with PD, which might enable the identification of a subgroup of PD patients at higher risk of suffering from or developing depression or apathy.

Conclusion

The assessment of echogenic patterns in PD covers a broad spectrum of diagnostic questions, which the non-invasive TCS technique can quickly assess [30]. Nevertheless, TCS requires a sufficient transtemporal bone window lacking in 5-40% of patients depending on age, sex, and geographic origin [9]. Furthermore, the reliability of the findings depends on a high-quality ultrasound system and the investigator's qualification. Future efforts should further develop this method and achieve its full potential in diagnosing PD and other neurological diseases.

References

[1] Huot P, Fox SH, Brotchie JM. The serotonergic system in Parkinson's disease. Prog Neurobiol. 2011 Oct;95(2):163-212. doi: 10.1016/j.pneurobio.2011.08.004. Epub 2011 Aug 22. PMID: 21878363.

[2] Walter U, Behnke S, Eyding J, Niehaus L, Postert T, Seidel G, Berg D. Transcranial brain parenchyma sonography in movement disorders: state of the art. Ultrasound Med Biol. 2007 Jan;33(1):15-25. doi: 10.1016/j.ultrasmedbio.2006.07.021. PMID: 17189043.

[3] Becker G, Seufert J, Bogdahn U, Reichmann H, Reiners K. Degeneration of substantia nigra in chronic Parkinson's disease visualized by transcranial color-coded real-time sonography. Neurology. 1995 Jan;45(1):182-4. doi: 10.1212/wnl.45.1.182. PMID: 7824114.

[4] Berg D, Siefker C, Becker G. Echogenicity of the substantia nigra in Parkinson's disease and its relation to clinical findings. J Neurol. 2001 Aug;248(8):684-9. doi: 10.1007/s004150170114. PMID: 11569897.

[5] Walter U, Wittstock M, Benecke R, Dressler D. Substantia nigra echogenicity is normal in non-extrapyramidal cerebral disorders but increased in Parkinson's disease. J Neural Transm (Vienna). 2002 Feb;109(2):191-6. doi: 10.1007/s007020200015. PMID: 12075859.

[6] Berg D, Godau J, Walter U. Transcranial sonography in movement disorders. Lancet Neurol. 2008 Nov;7(11):1044-55. doi: 10.1016/S1474-4422(08)70239-4. PMID: 18940694.

[7] Krogias C, Walter U. Transcranial Sonography Findings in Depression in Association With Psychiatric and Neurologic Diseases: A Review. J Neuroimaging. 2016 May;26(3):257-63. doi: 10.1111/jon.12328. Epub 2016 Jan 19. PMID: 27119431.

[8] Gaenslen A, Unmuth B, Godau J, Liepelt I, Di Santo A, Schweitzer KJ, Gasser T, Machulla HJ, Reimold M, Marek K, Berg D. The specificity and sensitivity of transcranial ultrasound in the differential diagnosis of Parkinson's disease: a prospective blinded study. Lancet Neurol. 2008 May;7(5):417-24. doi: 10.1016/S1474-4422(08)70067-X. Epub 2008 Apr 3. PMID: 18394965.

[9] Walter U, Školoudík D. Transcranial sonography (TCS) of brain parenchyma in movement disorders: quality standards, diagnostic applications and novel technologies. Ultraschall Med. 2014 Aug;35(4):322-31. doi: 10.1055/s-0033-1356415. Epub 2014 Apr 24. PMID: 24764215.

[10] Budisic M, Trkanjec Z, Bosnjak J, Lovrencic-Huzjan A, Vukovic V, Demarin V. Distinguishing Parkinson's disease and essential tremor with transcranial sonography. Acta Neurol Scand. 2009 Jan;119(1):17-21. doi: 10.1111/j.1600-0404.2008.01056.x. Epub 2008 Jun 10. PMID: 18549415.

[11] Krogias C, Hoffmann K, Eyding J, Scheele D, Norra C, Gold R, Juckel G, Assion HJ. Evaluation of basal ganglia, brainstem raphe and ventricles in bipolar disorder by transcranial sonography. Psychiatry Res. 2011 Nov 30;194(2):190-7. doi: 10.1016/j.pscychresns.2011.04.002. Epub 2011 Sep 29. PMID: 21958513.

[12] Stockner H, Wurster I. Transcranial sonography in essential tremor. Int Rev Neurobiol. 2010;90:189-97. doi: 10.1016/S0074-7742(10)90014-7. PMID: 20692503.

[13] Richter D, Woitalla D, Muhlack S, Gold R, Tönges L, Krogias C. Coronal Transcranial Sonography and M-Mode Tremor Frequency Determination in Parkinson's Disease and Essential Tremor. J Neuroimaging. 2017 Sep;27(5):524-530. doi: 10.1111/jon.12441. Epub 2017 Apr 20. PMID: 28426143.

[14] Jesus-Ribeiro J, Sargento-Freitas J, Sousa M, Silva F, Freire A, Januário C. Substantia nigra hyperechogenicity does not correlate with motor features in Parkinson's disease. J Neurol Sci. 2016 May 15;364:9-11. doi: 10.1016/j.jns.2016.03.002. Epub 2016 Mar 2. PMID: 27084206.

[15] Berg D. Substantia nigra hyperechogenicity is a

risk marker of Parkinson's disease: yes. J Neural Transm (Vienna). 2011 Apr;118(4):613-9. doi: 10.1007/s00702-010-0565-6. Epub 2011 Jan 5. PMID: 21207077.

[16] Heinzel S, Berg D, Gasser T, Chen H, Yao C, Postuma RB; MDS Task Force on the Definition of Parkinson's Disease. Update of the MDS research criteria for prodromal Parkinson's disease. Mov Disord. 2019 Oct;34(10):1464-1470. doi: 10.1002/mds.27802. Epub 2019 Aug 14. PMID: 31412427.

[17] Berg D, Roggendorf W, Schröder U, Klein R, Tatschner T, Benz P, Tucha O, Preier M, Lange KW, Reiners K, Gerlach M, Becker G. Echogenicity of the substantia nigra: association with increased iron content and marker for susceptibility to nigrostriatal injury. Arch Neurol. 2002 Jun;59(6):999-1005. doi: 10.1001/archneur.59.6.999. PMID: 12056937.

[18] Tao A, Chen G, Deng Y, Xu R. Accuracy of Transcranial Sonography of the Substantia Nigra for Detection of Parkinson's Disease: A Systematic Review and Meta-analysis. Ultrasound Med Biol. 2019 Mar;45(3):628-641. doi: 10.1016/j.ultrasmedbio.2018.11.010. Epub 2019 Jan 3. PMID: 30612821.

[19] Shafieesabet A, Fereshtehnejad SM, Shafieesabet A, Delbari A, Baradaran HR, Postuma RB, Lökk J. Hyperechogenicity of substantia nigra for differential diagnosis of Parkinson's disease: A meta-analysis. Parkinsonism Relat Disord. 2017 Sep;42:1-11. doi: 10.1016/j.parkreldis.2017.06.006. Epub 2017 Jun 15. PMID: 28647434.

[20] Krogias C, Eyding J, Postert T. Transcranial sonography in Huntington's disease. Int Rev Neurobiol. 2010;90:237-57. doi: 10.1016/S0074-7742(10)90017-2. PMID: 20692506.

[21] Behnke S, Berg D, Naumann M, Becker G. Differentiation of Parkinson's disease and atypical parkinsonian syndromes by transcranial ultrasound. J Neurol Neurosurg Psychiatry. 2005 Mar;76(3):423-5. doi: 10.1136/jnnp.2004.049221. PMID: 15716540; PMCID: PMC1739539.

[22] Richter D, Katsanos AH, Schroeder C, Tsivgoulis G, Paraskevas GP, Müller T, Alexandrov AV, Gold R, Tönges L, Krogias C. Lentiform Nucleus Hyperechogenicity in Parkinsonian Syndromes: A Systematic Review and Meta-Analysis with Consideration of Molecular Pathology. Cells. 2019 Dec 18;9(1):2. doi: 10.3390/cells9010002. PMID: 31861253; PMCID: PMC7016776.

[23] Walter U, Skowrońska M, Litwin T, Szpak GM, Jabłonka-Salach K, Skoloudík D, Bulska E, Członkowska A. Lenticular nucleus hyperechogenicity in Wilson's disease reflects local copper, but not iron accumulation. J Neural Transm (Vienna). 2014 Oct;121(10):1273-9. doi: 10.1007/s00702-014-1184-4. Epub 2014 Mar 11. PMID: 24615184.

[24] Aarsland D, Kramberger MG. Neuropsychiatric Symptoms in Parkinson's Disease. J Parkinsons Dis. 2015;5(3):659-67. doi: 10.3233/JPD-150604. PMID: 26406147.

[25] Reijnders JS, Ehrt U, Weber WE, Aarsland D, Leentjens AF. A systematic review of prevalence studies of depression in Parkinson's disease. Mov Disord. 2008 Jan 30;23(2):183-9; quiz 313. doi: 10.1002/mds.21803. PMID: 17987654.

[26] Pedersen KF, Larsen JP, Alves G, Aarsland D. Prevalence and clinical correlates of apathy in Parkinson's disease: a community-based study. Parkinsonism Relat Disord. 2009 May;15(4):295-9. doi: 10.1016/j.parkreldis.2008.07.006. Epub 2008 Sep 17. PMID: 18801696.

[27] Gaenslen A, Wurster I, Brockmann K, Huber H, Godau J, Faust B, Lerche S, Eschweiler GW, Maetzler W, Berg D. Prodromal features for Parkinson's disease-baseline data from the TREND study. Eur J Neurol. 2014 May;21(5):766-72. doi: 10.1111/ene.12382. Epub 2014 Feb 24. PMID: 24612314.

[28] Walter U, Hoeppner J, Prudente-Morrissey L, Horowski S, Herpertz SC, Benecke R. Parkinson's disease-like midbrain sonography abnormalities are frequent in depressive disorders. Brain. 2007 Jul;130(Pt 7):1799-807. doi: 10.1093/brain/awm017. Epub 2007 Feb 28. PMID: 17329323.

[29] Richter D, Woitalla D, Muhlack S, Gold R, Tönges L, Krogias C. Brainstem Raphe Alterations in TCS: A Biomarker for Depression and Apathy in Parkinson's Disease Patients. Front Neurol. 2018 Aug 7;9:645. doi: 10.3389/fneur.2018.00645. PMID: 30131761; PMCID: PMC6090021.

[30] Κρόγιας Χ, Κερασνούδης Α. Η εφαρμογή της διακρανιακής υπερηχογραφίας του εγκεφαλικού παρεγχύματος στη διαφορική διαγνωστική των εξωπυραμιδικών νοσημάτων. *Νευρολογία* 2013;22(5):35-45.

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

2021-2022

- **16-19 Δεκεμβρίου 2021: 8ο Πανελλήνιο Συνέδριο Ελληνικής Ακαδημίας Νευροανοσολογίας, Υβριδικό**, Θεσσαλονίκη
- **24-27 Φεβρουαρίου 2022: WCN 2022, Kuala Lumpur**, Malaysia
- **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
- **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