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ΑΡΘΡΑ / ARTICLES

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Αλκμάνος 10, Αθήνα 115 28
Τηλ.: 210 72.47.056 - Fax: 210 72.47.556
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Editorial for the Special Issue "Treatable Genetic Neurological Diseases"

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

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

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

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

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

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Άρθρα...

BIOTINIDASE DEFICIENCY: CLINICAL CHARACTERISTICS, DIAGNOSIS AND MANAGEMENT

Filippos Karalis, MD, PhD, Georgia Deretzi, MD, PhD

Neurology Department, "Papageorgiou" General Hospital of Thessaloniki

Introduction

Biotinidase deficiency (**BTD**) is a rare neurocutaneous disorder inherited by an autosomal recessive gene in which the enzyme biotinidase is defective. Consequently, the vitamin biotin is not produced from biocytine. Clinical manifestations of patients with BTD can appear at any timepoint from infancy to adulthood. Various neurological, ophthalmological and dermatological symptoms may occur in not treated patients with BTD, such as epilepsy, ataxia, developmental delay, hearing loss, alopecia and skin rashes [1-3]. Many of the above symptoms can be alleviated if supplementation with biotin is initiated at an early stage of the disease [3, 4]. However, deficiencies of hearing and vision may persist even after the initiation of oral biotin therapy [5].

Pathogenesis

The activity of the enzyme biotinidase, which is responsible for the recycle of biotin, is reduced to a lesser or bigger extent due to mutations in the BTD (biotin cycle, figure 1) [4, 6, 7]. Patients with this defect eventually progress to suffer from biotin deficiency. Based on the biotin cycle this deficiency will finally result in impaired activity of the carboxylases which are biotin-dependent and also high quantity

Figure 1. The Biotin Cycle
Wolf B.Mol Genet Metab 2011; 104: 27-34

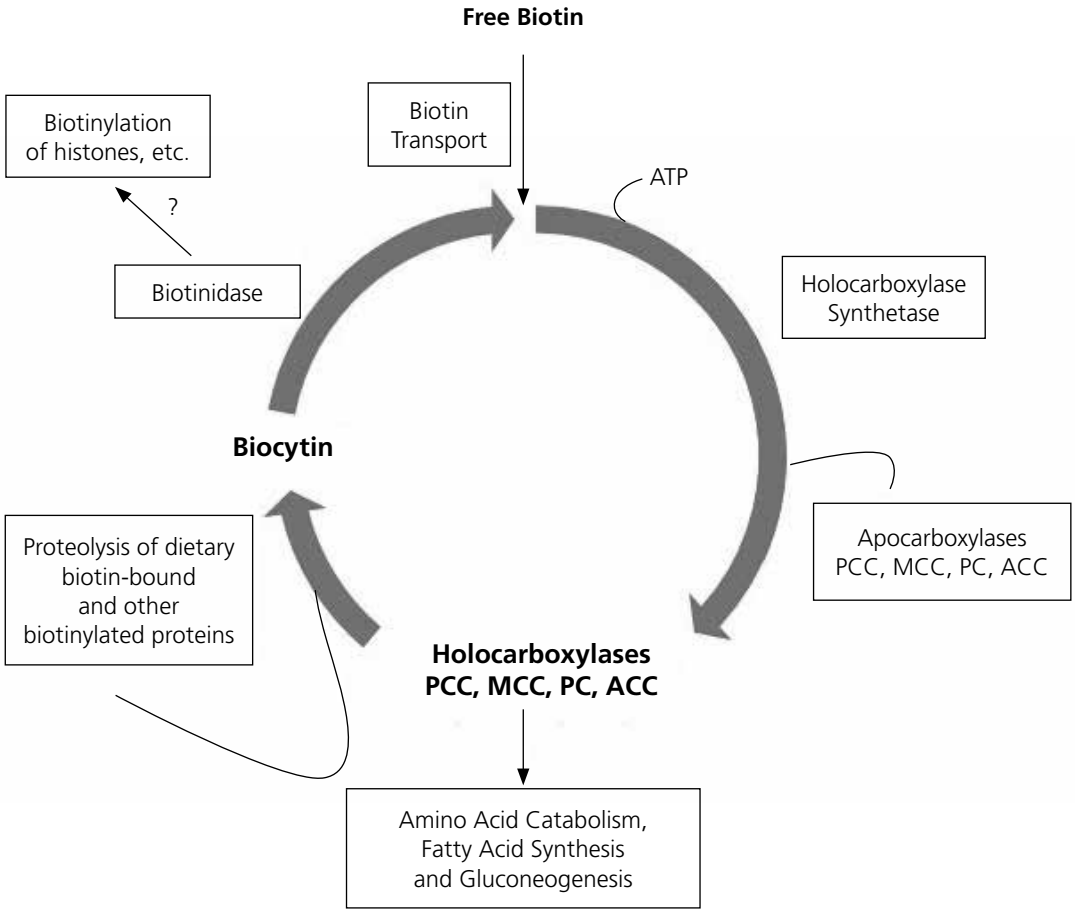

Table 1. The most frequent and occasional symptoms reported especially for profound cases of BTD

Symptom	Prevalence
Seizures	Frequent
Hypotonia	Frequent
Hearing loss	Frequent
Skin rashes (eczema)	Frequent
Hyperventilation, laryngeal stridor, and apnea	Occasional
Optic atrophy	Occasional
Developmental delay	Occasional
Hair loss (alopecia)	Occasional
Conjunctivitis	Occasional
Limb muscle weakness	Occasional
Myelopathy	Occasional
Recurrent viral infections	Occasional
Fungal infections (candidiasis)	Occasional
Ataxia	Occasional
Scotoma	Occasional
Spastic paraparesis	Occasional
Lethargy	Occasional

of related organic acids will be accumulated. These metabolic alterations could lead to ketolactic acidosis and hyperammonemia [8, 9].

Clinical characteristics

The clinical characteristics of patients with biotinidase deficiency usually vary regarding the extent of serum biotinidase activity. When biotinidase activity is reduced to 10-30% of mean normal, this condition is referred to as partial BTD, whereas when the enzyme activity is less than 10% of mean normal values these patients have profound BTD which is the more severe form of the disease [4]. If a clinician does not recognize and treats early enough this condition, its symptoms usually appear within the first few months of life, although it can also become obvious in late childhood or even in adult life [5, 10].

Most of the symptoms of BTD can be alleviated or prevented with pharmacological doses of oral biotin, specifically at a starting dose of 5-10 mg/d of free biotin, although higher daily doses may be required for some patients [9, 11]. However, if various clinical characteristics such as auditory and visual defects are observed these symptoms are usually irreversible even with biotin therapy per os [5]. If infants with profound BTD are detected with screening test and treatment is initiated soon after delivery, hearing loss can be prevented. The onset of symptoms varies from several weeks after birth to the first two years of age, although several patients may present symptoms later during life (adult forms of BTD) [4, 12].

The systems and organs of the objects with BTD that are mostly affected, especially when not treated properly are the skin, the eyes and brain functions [1-3]. Children with BTD manifest a single symptom or sometimes present with multiple neurological and dermatological findings [13, 14]. The following table summarizes the most frequent and the occasional clinical characteristics that are described in children with profound BTD (Table 1) [4, 15, 16].

Seizures are a common symptom of patients with profound BTD, although adult neurologists do not often encounter symptomatic patients, because of the early screening of the disease and the appropriate treatment [17, 18]. The most common type of seizures are generalized tonic-clonic (56%), but there have also been reported myoclonic seizures, infantile spasms, or even Ohtahara syndrome [19]. As regard to pathophysiology, it seems that due to absence of the recycling of biotin, consequently there is an accumulation of metabolites which are potentially neurotoxic and epileptogenic [3, 6, 20].

As in regard to other neurological symptoms, lately adults with profound form of BTD have been referred to physicians with symptoms like myelitis, spastic

BOX 1.

- 2-3 ml of whole blood should be obtained in a sodium or lithium heparin tube.
- Immediately or until 1 hour after collection, blood should be centrifuged to collect the serum sample and stored at -80°C until testing.
- The sample should be transported with dry ice.
- For the screening of newborns a completely dried blood spot along with the parents' samples are required.

Table 2. Laboratory values for biotinidase enzyme activity (adapted from [31])

Category	Mean activity ± SD (nmol/min/ml serum)
Normal values	7.57 ± 1.41
Heterozygotes	3.49 ± 0.72
Symptomatic individuals	0.12 ± 0.18
Newborn screening	0.19 ± 0.16
Individuals with partial BTD	1.47 ± 0.41

paraparesis or paraplegia and optic neuropathies. The above clinical findings are mimicking the symptoms of the demyelinating diseases known as neuromyelitis optica spectrum disorders (NMOSD) [21, 22]. Many cases of BTD in the literature have been reported that may be misdiagnosed as multiple sclerosis, NMOSD, myasthenia gravis, myelitis or atypical encephalitis [17, 21]. Adult cases of BTD should be considered for patients presenting with myelopathy with or without loss of vision even if there have been limited responsiveness to steroid therapy [14, 17, 22].

Many of the clinical symptoms of BTD and especially the dermatological findings are attributed to immunological dysfunction [8, 13, 23]. Sometimes symptoms can mimic conditions like primary immune deficiencies and this can disorientate the differential diagnosis [24].

Of note, several adults with profound BTD may remain asymptomatic through the course of the disease even without treatment and there is no certain explanation for this observation [25], though an any timepoint someone can develop symptoms. A possible theory is that there may be epigenetic factors that protect patients from developing symptoms [8, 26, 27]. Another probable explanation for the variations of the clinical findings in different individuals are the differences in biotinidase Km variants, which are reported in literature [28].

Patients with partial BTD usually have milder symptoms. The diagnosis of the disease may be misleading in cases of children with developmental delay and autism [2, 12, 18].

Diagnosis

The diagnosis of BTD is established by measuring biotinidase activity. Radioassay method is used to measure the enzyme activity in collected samples, especially serum or plasma. The activity can be estimated also in other tissues, like leukocytes or fibroblasts but these measurements could be less reliable [25, 29]. Sample collection should be performed according to laboratory guidance by physicians when requested. Usually 2-3 ml of whole blood should be obtained in a sodium or lithium heparin tube. Immediately or until 1 hour after collection, blood should be centrifuged to collect the serum sample and stored at -80°C until testing. The sample should be transported with dry ice, because storing samples at -20°C has been shown to diminish the biotinidase activity. For the screening of newborns a completely dried blood spot along with the parents' samples are enough to determine the laboratory diagnosis (BOX 1).

Only when testing for DNA samples shipments should be done in ambient conditions as soon as possible. The range of values from normal to several cases of BTD are shown in table 2 [7, 20, 25, 30, 31].

The enzyme analysis could show vague results regarding the asymptomatic carriers of the disease and those with partial BTD, rendering the laboratory diagnosis equivocal. In such cases and in individuals with heterozygosity genetic testing could provide better information for establishing the diagnosis [18, 26, 27, 30].

Other biochemical testing, in patients with no treatment, that could help reinforce the diagnosis may be the elevated levels of metabolic ketoacids, lactic acids and high ammonia levels [7, 8, 32].

Management

As mentioned above, initial treatment of patients

with profound BTD is with 5 mg/day supplementation of oral free biotin, but dose can be adjusted in regard to clinical outcome up to 20mg/day. All individuals presenting with symptoms of the disease tend to show clinical improvement with biotin therapy. The frequent symptom of seizure usually resolves instantly (hours or days) after treatment initiation and also skin lesions alleviate within weeks. Apart from that, other symptoms such as alopecia, ataxia, and developmental delay can improve overtime if biotin therapy per os is started in the early stages of the disease [29, 33, 34].

Treatment of partial BTD was initially controversial, but over the years, due to the appearance of patients with no treatment developing symptoms, it is strongly recommended nowadays that treatment should be applied also in cases of partial BTD [10, 16].

Screening of newborn has provided much help for the prevention of symptoms, because it has been shown that initiating treatment in yet asymptomatic children with this condition could reverse defects such as hearing loss, optic atrophy and brain dysfunction, whereas children suffering from BTD often present with these symptoms. Currently it is unclear whether patients not receiving treatment will develop symptoms or not and there is no clear prognostic factor of the disease [29, 34, 35]. From literature it is known only that patients with very low enzyme activity (<1%) appear to have high risk for presenting with symptoms of BTD.

It is strongly recommended that children with both profound and partial BTD should be evaluated for psychomotor deficits and for hearing loss periodically, and have ophthalmologic and physical testing for neurological defects, cutaneous lesions and viral or fungal infections [33]. Another parameter that should be examined is the dose of oral biotin. In a recent study, hair loss during adolescence was diminished in two girls with profound form of BTD after increasing the dosage of oral biotin [33, 35]. More data is needed in order to determine the dosage of biotin that is mandatory for children with BTD [1, 12, 23].

Oral biotin seems to have a good pharmacokinetic profile and it has not shown until now serious adverse events even when administered in high doses. Nevertheless, samples with high doses of biotin can cause incorrect results, such as false elevated levels of thyroid gland hormones (T3 and T4) and false low TSH measurements, misleading the diagnosis to hyperthyroidism. This should be considered during follow-up [36].

Conclusion

BTD is a relatively rare inherited metabolic disorder, whose early recognition can lead to successful treatment and in turn to disability prevention. Affected patients with BTD can be easily treated with supplementation of oral biotin and this treatment reverts most of the symptoms. Nowadays, newborn screening for this condition, that is applied in many countries worldwide has offered a lot to the early recognition of the disease. Apart from that, due to the reversibility of several of the symptoms the number of patients with no symptoms is rising. Early initiation of treatment in such cases is crucial for the best clinical outcome. On the contrary, the appearance of adults with BTD and various clinical characteristics designates that there are still many mechanisms to be examined and much to be learned about BTD. Based on the increasing number of patients with BTD and the detection of many mutations in the responsible gene, we expect to collect more data in the future about correlation between the genotype and the phenotype of the disease. The diagnosis of BTD should be taken into consideration by clinical pediatricians and neurologists in rare cases where differential diagnosis relates to the clinical characteristics, mainly regarding neurological and dermatological manifestations.

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TREATABLE METABOLIC EPILEPSIES. DIAGNOSIS AND TREATMENT

Sofia Markoula[1], MD, Ph.D.; Ioannis Georgiou[2], Ph.D.; Spiridon Konitsiotis[1], MD, Ph.D.

[1] Department of Neurology, Medical School, Ioannina University
[2] Department of Medical Genetics, Medical School, Ioannina University

Abstract

Over 200 different metabolic disorders are prone to epileptic seizures, as the primary clinical manifestation or with other manifestations of their clinical condition.

Most of the metabolic disorders presenting with epilepsy are diagnosed and managed in childhood. Treatable metabolic diseases causing epilepsy are a well-defined group of all metabolic disorders, which are amenable to adequate management aiming directly at the primary cause of the seizures and at preventing or minimizing seizures' complications. Treatable metabolic epilepsies are clinically important to recognize and diagnose. Although some types of metabolic epilepsy require detailed testing for the diagnosis to be made, other types may be diagnosed on the basis of the clinical assessment, as is the case of pyridoxine-dependent epilepsy, with seizures cessation after pyridoxine administration.

The etiological mechanism of seizures in metabolic disorders is very complex. Cofactor deficiency, accumulation of toxic metabolites and energy deficiency are the main causes.

Administration of supplementation, early dietary interventions or other therapy options can treat metabolic disorders and control epileptic seizures or, at least, improve outcomes.

1. Introduction

Metabolic epilepsies are caused by an underlying distinct metabolic disorder, which is in turn associated to a high risk of developing epilepsy [1]. Over 200 different metabolic disorders are clinically prone to epileptic seizures as their main manifestation or with other manifestations of a complex phenotype [2].

Underlying etiology of epileptic seizures in metabolic disorders is diverse. Cofactor deficiency is a main underlying aetiology for seizures related to metabolic diseases, as is pyridoxal phosphate responsive epilepsy and pyridoxine - dependent epilepsy. Also, when toxic metabolites, such as ammonia, accumulate the occurrence of seizures may emerge. Several metabolic diseases present with hyperammonemia, as in urea cycle metabolic disorders and in metabolic acidemias. Furthermore, seizures may be related to disturbed neurotransmission, such as glycine accumulation in glycine encephalopathy. Seizures could also be caused by energy deficiency, as in disorders underlying hypoglycemia or impaired transport of glucose in the brain. Certain metabolic diseases are related to structural malformations of the brain that may provoke seizures. One classic example being polymicrogyria that is a prominent feature in peroxisomal disorders [1].

Treatable metabolic diseases causing epilepsy are a well-defined group of all metabolic disorders, which are amenable to adequate management aiming directly at the primary cause of the seizures and at preventing or minimizing seizures' complications. Treatable metabolic epilepsies are clinically important to recognize and diagnose, since early treatment optimizes the outcome. For a better understanding of early manifestations, diagnostic procedures and treatment options, these conditions are presented in detail.

2. Algorithm concerning metabolic epilepsies

It is worth stressing that epileptic manifestations per se, are not common ground in metabolic diseases. Consequently, seizures unrelated to systemic, neurologic and/or metabolic conditions do not, likely, underly metabolic diseases [3].

Most of the metabolic disorders associated with epilepsy are diagnosed and managed in childhood. Diagnosing epilepsy, early in its course, is essential because there are metabolic disorders potentially treatable. Immediate initiation of the appropriate therapy may lead to seizure control [1].

Metabolic epilepsies should be a potential diagnosis in neonatal and infancy seizures of unknown etiology, in refractory and/or long-lasting seizures, in seizures deteriorating with AEDs, in seizures deterioration either after fasting (related to GLUT1) or after

Table 1. Clinical presentations leading to the possible diagnosis of metabolic epilepsy

Aversion or intolerance to food
Developmental delay
Hypotonia
Developmental regression
Signs of encephalopathy (irritability, restlessness, crying, vomiting)
Abnormal head circumference (micro or macrocephaly)
Facial dysmorphia
Fluctuating course of illness
Abnormalities of lens or retina (cataracts, optic nerve atrophy, retinitis pigmentosa, cherry red spot)
Organomegaly
Movement disorders
Unusual body fluid odor
Metabolic acidosis with high-anion gap and metabolic derangements
Ketonuria
Severe epilepsy in a sibling
Parental consanguinity

protein rich meals (related to urea cycle defects), in neonatal myoclonic encephalopathy, in progressive myoclonic epilepsy in adolescence and in progressive myoclonic epilepsy in young adults (Table 1) [4, 5].

A good response to AEDs does not exclude metabolic epilepsies. Patients with GLUT1 deficiency are a paradigm of favorable response to commonly used AEDs [6], and pyridoxine-dependent epilepsy patients can have moderate seizure control, even absence of seizures, after the discontinuation of pyridoxine supplementation [7].

Any type of epileptic seizures may present in metabolic epilepsies. Myoclonic seizures are the main seizures in glycine encephalopathy and other cofactor metabolic disorders, infantile spasms are the main seizures in mitochondrial disorders, in disorders with serine biosynthesis defects, in phenylketonuria and biotinidase deficiency. Patients with metabolic disorders such as deficiency of GLUT-1, deficiency of creatine, deficiency of biotinidase, ~~syndromes~~ and mitochondrial diseases present generalized tonic-clonic seizures [1].

Additional features to lead to the possible diagnosis of metabolic epilepsies [4, 5] are presented below (Table 1).

If metabolic seizures are suspected, a comprehensive laboratory workup (Table 2) and an electroencephalogram (EEG) should be performed, alongside imaging of the brain, if indicated.

First line laboratory workup is likely to uncover common metabolic causes, such as hypoglycemia, electrolyte disequilibrium, and infections of the CNS. Blood gas analysis to investigate the presence of metabolic acidosis, hyperammonemia and/or hyperlactatemia is usually part of the initial laboratory evaluation [1, 4]. Using lactate as an example, it is known that seizures can give rise to lactate levels but the levels decrease quickly after seizure is over. A suspicion for metabolic diseases should be raised when lactate elevate 1 to 2 h after a seizure [8].When diagnosis remains unclear, a second line biochemical tests are carried out, based on clinical evidence, with plasma amino acids, as well as urine acylcarnitine and organic acids. Furthermore cerebrospinal fluid (CSF) is checked for glucose, amino acids, also urine sample should be sent for purines, pyrimidines, S-sulphocysteine, and guanidinoacetate [1]. Additional testing for the metabolomic profile can be considered, as well as, genotyping for potential gene mutations and variants, and whole exome and/or genome sequencing [2] (Table 2).

Neuroimaging findings (Table 2) are usually normal or nonspecific, and include cerebellum dysplasia, neuronal dysplasia, leukodystrophy or hypomyelination, hypoplasia and agenesis of the corpus callosum [9, 10]. Certain signal abnormalities are more frequently found in certain types of metabolic disorders [9]. For example, pyridoxine-dependent epilepsy and glycine encephalopathy are associated with hypoplasia or agenesis of corpus callosum [2], glycine encephalopa-

Table 2. Laboratory, EEG and imaging workup in possible metabolic epilepsy

Initial laboratory tests
– Glucose
– Electrolytes
– Blood gases
– CNS infections
– Ammonia
– Lactate
Additional biochemical tests in plasma
– Amino acids
– Acylcarnitine profile
– α-aminoadipic semialdehyde
– Homocysteine
– S-sulfocysteine
– Ammonia
– Guanidinoacetate
CSF laboratory tests
– Glucose
– Amino acids
– Folate
– 5-methyltetrahydrofolate (MTHF)
– α-aminoadipicsemialdehyde
– Pyridoxal phosphate
Urine laboratory tests
– Organic acids
– Aminoadipicsemialdehyde
– Homocysteine
– S-sulphocysteine
– Guanidinoacetate
Metabolomic profiling
Genotyping
– PCR product sequencing
– Allele specific PCR (ARMS)
– Allele specific probes
– PCR amplification coupled with restriction enzyme analysis
– Single Strand Conformation Polymorphism
– Denaturing Gradient Gel Electrophoresis
Whole sequencing
– Exome
– Genome
EEG findings
– Generalized slowing
– Burst suppression
– Spike-wave, polyspike-wave complexes
– Status epilepticus in sleep
– Comp-like rhythm
Neuroimaging findings
– Normal
– Cerebellum dysplasia
– Neuronal dysplasia
– Leukodystrophy
– Hypomyelination
– Hemispheric hypoplasia
– Brain atrophy
– Corpus callosum hypoplasia
– Corpus callosum agenesis

Table 3. Treatable metabolic epilepsies

Cofactor metabolic disorders with epileptic seizures
– Pyridoxine dependent epilepsy
– Pyridoxal 5' - phosphate responsive epilepsy
– Early onset vitamin B6-dependent epilepsy
– Cerebral folate deficiency
– Methylene tetrahydrofolate reductase (MTHFR) deficiency
– Molybdenum cofactor deficiency type A
– Biotinidase deficiency and holocarboxylase synthetase deficiency
Epilepsy as a result of energy deficiency disorders
– Glucose transporter (GLUT-1) deficiency type 1
– Guanidinoacetate methyltransferase (GAMT) deficiency
Amino acid metabolic disorders associated with epilepsy
– Glycine encephalopathy
– Maple syrup urine disease
– Serine biosynthesis defect
– Urea cycle disorders
– Phenylketonuria

thy can be associated with delayed myelination and dilated ventricles [9], and folate deficiency are associated with hypomyelination or leukodystrophy [11].

Regarding electroencephalogram (EEG) findings, there are various EEG abnormalities (Table 2). For example, ~~a~~ patients with pyridoxine dependent and pyridoxal phosphate responsive epilepsy as well as glycine related encephalopathy may present a burst-suppression pattern in EEG while those affected by urine maple syrup disease may present a comb-like rhythm [12, 13].

The term treatable metabolic epilepsy reflects disorders in the spectrum of inborn errors of metabolism that may develop epileptic seizures, resolved with the appropriate treatment. Clinicians and investigators should investigate and diagnose these treatable but otherwise catastrophic encephalopathies.

Types of treatable metabolic epilepsy are shown in Table 3.

3. Treatable metabolic epilepsies

A. Epilepsy related to cofactor metabolic disorders

EPILEPSY DEPENDENT ON PYRIDOXINE (PDE)

PDE is an epileptic encephalopathy, inherited through the autosomal recessive (AR) way. A marked benefit to adequate therapeutic dosages of vitamin B6 coupled with non-responsive treatment with AEDs characterize the disorder [4]. Pathogenic variations in the antiquitin gene (ALDH7A1) are underlying PDE and its prevalence is 1 in 20,000 to 600,000. This epileptic encephalopathy is an example of severe but efficiently treatable disorders than clinicians do not want to miss [4, 14].

Although seizures respond to adequate therapeutic dosages of vitamin B6, PDE patients do not have B6 deficiency. PDE is manifested as a result of the deficient enzyme antiquitin in the metabolic pathway of lysine. Antiquitin is the dehydrogenase of piperideine-6-carboxylate (P6C) and a-aminoadipic semialdehyde (aAASA), and the defect results in high concentrations of αAASA and P6C, which inactivate pyridoxal-5-phosphate (PLP), a cofactor in neurotransmitter metabolism. Due to its reactive properties as a semialdehyde, αAASA also convey a pathogenetic effect, undergoing multiple reactions in the cells and interacting with diverse metabolic pathways [4]. Furthermore, pipecolic acid is a GABA modulator and its accumulation may contribute to seizure pathophysiology [15].

Presentation

PDE presents, in most cases, within the first week of life, with neonatal seizures presenting within hours of birth in 70% of affected children; atypical cases can present later, but no later than the age of 3 years [16].

Seizures of unknown etiology, mainly of myoclonic type, in the absence of any gestational complication and perinatal abnormality, unresponsive to treatment, raise flag to the suspicion for PDE. Patients with classical PDE present a spectrum of clinical features such as abnormal fetal movements or dystonic movements, respiratory distress, birth asphyxia or hypoxic-ischaemic encephalopathy, abnormal cry, irritability, vomiting, startle response, hepatomegaly, hypothermia, shock and acidosis [4].

Diagnosis

Increased αAASA (specific) and pipecolic acid (non-

specific) levels in urine, plasma and CSF, multiple folds over the higher normal values, are found, even with treatment [17, 18] (Table 4). The mutation, the age, pyridoxine treatment and possibly nutritional lysine intake have an impact on αAASA levels [4].

Urinary and plasma αAASA, together with plasma pipecolic acid are useful biomarkers to untreated as well as treated patients. Pipecolic acid is the primary biomarker used, which is complemented with αAASA determination in patients with unclear pipecolic acid results and high clinical probability to have PDE. Due to the fact that αAASA testing is limited to few laboratories worldwide it is advisable to test pipecolic acid before proceed to αAASA testing [4].

The identification of causative mutations in the antiquitin gene (ALDH7A1, chromosome 5q31) support the diagnosis. Identified mutations in the gene exons 4, 6, 9, 11 represent 60% of the pathogenic mutations reported in patients of Caucasian origin and those are the exons initially screened for mutations [19]. If sequencing is negative for point mutations, deletions may be the cause and further molecular testing is required. Point mutations may be of unknown significance and functional studies could serve to ascertain the impact of the mutation.

Treatment

The need for PDE diagnosis is inappropriate to delay the administration of pyridoxine. In cases with epileptic seizures developing within the first month of life, seizures are controlled within an hour with 50-100 mg of intravenous pyridoxine (Table 5). Seizures remain controlled with 5-30 mg/kg/d of pyridoxine given orally (Table 5) but when pyridoxine is stopped, seizures may present again [10]. When treatment is restarted, seizures are rapidly controlled [19]. In atypical (late-onset) PDE, seizure response may require up to seven days of pyridoxine therapy [10]. Pyridoxine should not exceed 500 mg daily because peripheral neuropathy may develop [20].

In some patients with unclear response to pyridoxine, initiation of folinic acid may have a beneficial effect. The mechanism underlying folinic acid responsive epilepsy is not clearly understood. The disorder is a condition similar to pyridoxine-dependent epilepsy, having comparable diagnostic laboratory markers. Regarding CSF biogenic amines, an unknown peak (peak X) is emerging [1]. In neonates, 3-5 mg/kg folinic acid daily may control the seizures [4], while folinic acid at a daily dose of 10-30 mg could be beneficial for children [2] (Table 5), although it is not well established whether long-term folinic acid may help after the stabilization of seizures. Furthermore, a high dose of folinic acid could exacerbate seizures, and the clinical benefit for this intervention should be intensively monitored and established [4].

Subsequent pregnancies have a recurrence risk of 25% and supplemental pyridoxine should be given to any pregnant with an at-risk fetus, to prevent intrauterine seizures and improve neurodevelopmental outcomes [4].

EPILEPSY DEPENDENT ON PYRIDOXAL 5´ - PHOSPHATE

Pyridoxal phosphate responsive metabolic epilepsy is an extremely rare epilepsy, inherited by the autosomal recessive disorder and only a few cases reported. This condition is due to pathogenic variations in the pyridoxamine 5'-phosphate oxidase gene (PNPO), leading to deficiency of pyridoxine phosphate oxidase, an enzyme that oxidizes both pyridoxine phosphate and pyridoxamine phosphate into pyridoxal phosphate [22]. Pyridoxal phosphate is the drastic conformation of pyridoxine [22].

Presentation

Pyridoxal phosphate responsive metabolic epilepsy is a neonatal epileptic encephalopathy with lethargy, hypotonia and refractory seizures that presents very early in life, with more than 80%of of affected neonates to present seizures in the first week of life [9]. Infantile spasms, myoclonic seizures, focal seizures, generalized tonicoclonical seizures, atonic seizures and status epilepticus may develop [23]. A range of neurological and systemic manifestations may also present, such as movement disorders and pigmentary retinopathy [24]. EEG may show, among others, electrical status epilepticus during sleep [23].

Diagnosis

The clinical diagnosis is supported by seizure control and EEG changes when pyridoxal phosphate supplementation of 50 mg is administered orally, frequently within an hour from onset (Table 5), while a percentage of neonates may have seizure cessation with pyridoxine [25].

Low pyridoxal phosphate in CSF supports further the diagnosis. Additional findings include the diminished enzyme activities related to cofactor pyridoxal phosphate, the increase of the glycine and threonine amino acids in plasma, also the increase of the glycine, threonine and of 3- methoxytyrosine amino acids, and the decrease of 5-hydroxy indolacetic acid and homovanillic acid in CSF [1] (Table 4).

Molecular analysis of the responsible gene PNPO may establish the diagnosis. Several pathogenic variants have been identified with many missense mutations corresponding to the active arginine residues in the protein sequence [26, 27].

Table 4. Treatable metabolic epilepsies

Sample	Volume	Tube	Collection/Storage
Plasma*			
A-aminoadipic semialdehyde	2.5-5 mL	Heparin	Freeze, store at -20^{0}C
Pipecolic acid	0.5 mL	Heparin or EDTA	Freeze immediately at -20^{0}C stable for 1 month
Lactate (Lactic Acid)	>0.3mL	Fluoride-oxalate tube	Collect and send to lab on ice, test performed immediately
Folate	0.5-1 mL	Heparin	Freeze, stable 72 hours at -20^{0}C, indefinitely at -80^{0}C
Homocysteine	2.5-5 mL	Heparin	Stable for 72 hours when stored on gel at 28°C
S-sulfocysteine	2.5-5mL	Heparin	Freeze, store at -20^{0}C
Ammonia	2.5-5mL	Lithium heparin	Centrifuged and analyzed within 30 min
Glucose	>0.5ml	Lithium heparin	Samples are drawn during fasting, send immediately to lab
Guanidinoacetate	2.5-5mL	Heparin	Stored frozen at -20^{0}C for up to 7 days
Amino acids	2.5-5mL	Heparin	Samples are drawn during fasting Deproteinize and buffer, PH 2.2, freeze, storage at -20^{0}C
Acylcarnitine profile	2.5-5mL	Heparin	Freeze and store at -80^{0}C
CSF*			
A-aminoadipic semialdehyde	0.5-1 mL	Sterile vial	Freeze, stable 72 hours at -20^{0}C, indefinitely at -80^{0}C
Pyridoxal phosphate	0.5-1 mL	Sterile vial	Freeze immediately stable 72h at -20^{0}C, store indefinitely at -80^{0}C
5-methyltetrahydrofolate (MTHF)	0.5-1 mL	Sterile vial	Freeze, stable for 72 hours at -20^{0}C, indefinitely at -80^{0}C
Glucose	0.25mL	Fluoride-oxalate tube	CSF samples are drawn during fasting, send immediately to lab
Amino acids	0.2-0.5mL	Sterile vial	Samples are drawn during fasting Collect in ice, immediately to lab
Urine*			
A-aminoadipic semialdehyde	> 1mL	Sterile collection tube	Freeze, store at -20^0 C
Pipecolic acid	0.5-1mL	Sterile collection tube	Freeze immediately at -20^{0}C, stable for 1 month
Glycine	0.2mL	Sterile collection tube	Collect in ice, immediately to lab
Vanillactic acid	5-10 mL	Sterile collection tube	Random urine specimen Freeze immediately at -20^{0}C
Homocystein	5 mL	Sterile collection tube	Random urine specimen frozen at -20^{0}C within 30 min of collection
S-sulphocysteine	2-3 mL	Sterile collection tube	Random urine specimen, frozen at -20^{0}C stable for 90 days
Cystine	4 mL	Sterile collection tube	Specimen from 24-hour urine collection, freeze immediately
Organic acids	10 mL	Sterile collection tube	First morning urine before food or drink is suggested. No apples, grapes, pears, cranberries 48 hours prior to collection
Guanidinoacetate	0.5-1mL	Sterile collection tube	Freeze immediately at -20^{0}C, stable indefinitely
Phenylpyruvate	>3mL	Sterile collection tube	Freeze immediately at -20^{0}C

Presented in order of appearance in the text. Data collected from: A) https://mnglabs.labcorp.com/, B) NHS UKAS website HEFT, C) https://ltd.aruplab.com/, D) https://www.mayocliniclabs.com/, E) https://www.albertahealthservices.ca/, F) https://ltd.aruplab.com/

Table 5. Treatment of metabolic epilepsies

Pyridoxine-dependent epilepsy
50-100 mg intravenous pyridoxine, followed by of oral administration of pyridoxine at a daily dose of 5-30 mg/kg (Not exceeding 500 mg/d)
If no response to pyridoxine, oral administration of a daily dose of 3-5 mg/kg folic acid in neonates and a daily dose of 10-30 mg in children
Pyridoxal 5'-phosphate-responsive epilepsy
Oral administration of 50 mg pyridoxal phosphate, followed by 30-50 mg/kg/d
Early onset vitamin B6-dependent epilepsy
Administration of pyridoxine or pyridoxal phosphate (as previously)
Cerebral folate deficiency
Oral administration of a daily dose of 3-5 mg/kg folic acid in neonates and a daily dose of 10-30 mg in children
Methylenetetrahydrofolate reductase (MTHFR) deficiency
Oral administration of a daily dose of 100 mg/kg betain, with increments up to a daily dose of 20 g
Molybdenum cofactor deficiency type A
Intravenous cyclic pyranopterin monophosphate at a daily dose of 80-320 μg/kg, followed by a daily dose of 240 μg/kg
Biotinidase and holocarboxylase synthetase deficiency
Oral administration of 5-10 mg/d biotin
GLUT-1 deficiency
Ketogenic diet
GAMT deficiency
Oral administration of a daily dose of 400-800 mg/kg creatine and 400-800 mg/kg L-ornithine
Protein-restricted diet
Glycine encephalopathy
Sodium benzoate, dextromethorphan, felbamate, and ketamine
Branched-chain α-ketoacid dehydrogenase deficiency (Maple syrup urine disease)
BCAAs-restricted diet, and administration of a BCAA-free amino acid supplementation
Serine biosynthesis defect
Oral administration of a daily dose of 500-700 mg/kg L-serine and 200-300 mg/kg glycine for severe forms
Oral administration of 100-150 mg/kg/d L-serine for milder forms
Urea cycle disorders
Hemodialysis
Amino acids L-citrulline or L-arginine supplementation
Protein restricted diet
Liver transplantation
Phenylketonuria
Dietary therapy. Selenium, copper, magnesium, and zinc supplementation may be required
Oral administration of sapropterindihydrochloride

Treatment

Although seizures are unresponsive to AEDs in pyridoxal phosphate responsive epilepsy, they are controlled by 30-50 mg/kg/d pyridoxal phosphate [28] (Table 5). Due to the high doses of pyridoxal phosphate, hepatic function is closely inspected in children treatment, as abnormal liver function may emerge [27].

EARLY-ONSET VITAMIN B6-DEPENDENT EPILEPSY

This type of epilepsy is due to in trans pathogenic variants in both *PLPBP* gene alleles, as was recently discovered [29]. It is suggested that PLPBP affects vitamin B_6 homeostasis, by supplying PLP to apo-enzymes, and minimizes toxicity due to excessive unbound PLP [30, 31].

Presentation

Seizures in the neonates are the most characteristic feature of biallelic *PLPBP* gene mutations, although first seizure may occur later, delaying treatment with vitamin B6. Generalized tonic-clonic seizures and myoclonic seizures are predominantly observed, as well as intellectual disability is common [32].

Diagnosis

Biochemical findings are not consistent. The most common findings include elevated lactate in plasma with associated metabolic acidosis and elevated glycine in urine. Raised vanillactic acid is the most common finding in urine [29]. Molecular diagnosis is made by identifying missense, nonsense, frameshift, or splice site pathogenic PLPBP variants [29, 32] (Table 4).

Treatment

Treatment with pyridoxine or pyridoxal phosphate or both, as early as possible, is highly important (Table 5). Two inconclusive reports, one by Darin et al. states that pyridoxal phosphate is more effective than pyridoxine in controlling seizures, while in the study by Plecko et al. patients with good seizure control and normal intelligence were treated with pyridoxine [30, 33].

CEREBRAL FOLATE DEFICIENCY

Cerebral folate deficiency is rare, inherited as AR disorder and is due to defects in the folate receptor alpha gene (FOLR1) [1]. The alpha type of folate receptor is the main carrier of folate through the blood-brain barrier. Molecular defects of this carrier may lead to diminished levels of 5-methyltetrahydrofolate (MTHF) in cerebrospinal fluid. MTHF is the crucial folate metabolite and is taking part in myelin biogenesis and neurotransmitter biosynthesis [34].

Other syndromes, such as Rett and Aicardi-Goutieres, have been recognized as clinically resembling conditions of cerebral folate deficiency, as they are responding to anti-folate treatment.

Presentation

Typical features usually present from the age of 4 months to early childhood, presenting with severe developmental delay, cerebellar ataxia, spastic paraplegia, ataxia, movement disorders, irritability, sleep disturbance, progressive hearing and visual impairment, and also generalized tonic-clonic, tonic, atonic, and myoclonic seizures [2, 34]. Neuroimaging findings include cerebellar atrophy and hypomyelination or leukodystrophy [11].

Diagnosis

Diagnosis is established by abnormal levels of MTHF in the CSF, accompanied by normal levels of folates in plasma. Molecular testing of the underlying FOLR1 gene, can identify variants, which may be missense (the most common mutation), nonsense, splice mutations, or even duplication [35] (Table 4).

Treatment

Early administration of folinic acid (Table 5) can improve clinical presentation and consequently restore folate CSF levels [11, 34].

DEFICIENCY OF METHYLENETETRAHYDROFOLATE REDUCTASE

The severe form of methylenetetrahydrofolate reductase (MTHFR) deficiency is a rare (1 in 200,000) AR disorder, due to pathogenic variations of the MTHFR gene [36]. MTHFR is the reductase of 5, 10 methylene tetrahydrofolate to 5-methyltetrahydrofolate (MTHF). MTHF circulates as the abundant folate in blood, CSF and other tissues. Its deficiency increases total plasma homocysteine and reduces methionine [37]. In turn, reduced methionine leads to the deficiency of the crucial methyl donor, that takes part in multiple methylation processes, namely S-adenosylmethionine [1].

Presentation

Clinical MTHFR deficiency in infancy is characterized by lethargy, hypotonia, feeding difficulties, apnea, with seizures, including myoclonic, tonic-clonic, and infantile spasms [1, 2]. Those seizures may advance to Lennox-Gastaut syndrome [38]. Later onset forms usually present with higher MTHFR and therefore present different symptoms, such as psychiatric conditions and gait abnormalities [39] and may be asymptomatic until the late thirties [40].

Diagnosis

As expected, total homocysteine in the plasma is high, methyl donor amino acid methionine is decreased, also MTHF in CSF and blood folate may also decrease [39]. Homocysteine elevation is accompanied by homocysteinuria [38]. Conclusive evidence for this deficiency comes by the enzyme assay and alternatively by testing of the causative gene for mutations. If this particular disorder is not recognized on time, severe consequences to normal development emerge with subsequent coma, and early death [2] (Table 4).

Treatment

The treatment goal is reducing homocysteine levels through chemical conversion of homocysteine to

methionine with betaine methyltransferase [1]. The outcome can improve upon early treatment with betaine [38]. Treatment usually begins with 100 mg/kg, with increments up to 20 g/d [38] (Table 5).

MOLYDBENUM COFACTOR DEFICIENCY TYPE A

Molybdenum serves as cofactor for three different oxidases, in alphabetical order aldehyde oxidase, sulfite oxidase and xanthine oxidase. The deficiencies of the cofactor or of the sulfite oxidase, are very rare AR disorders that result in toxic concentrations of sulfites [2]. Only type A of molybdenum cofactor deficiency (with molecular defects in gene MOCS1) are treatable.

Presentation

Affected neonates have feeding disorders in a short period after birth, marked hypotonia, uncontrollable seizures and response to startle [2]. In addition, developmental delay and other clinical features, as spasticity, and lens dislocation emerge [1]. The clinical and radiological findings may resemble those of hypoxic-ischemic encephalopathy [41].

Diagnosis

Biochemical tests show reduction of plasma homocysteine and urine cystine coupled with increase in plasma and urinary S-sulfocysteine (Table 4). Diagnosis is also established by the DNA analysis of the MOCS1 gene.

Treatment

Neonates affected by molybdenum cofactor deficiency type A are administered with cyclic pyranopterin monophosphate intravenously (Table 5), which is a precursor of the molybdenum cofactor biosynthesis, is advised to be given the earliest possible before establishment of any neurologic damage [42].

BIOTINIDASE AND HOLOCARBOXYLASE SYNTHETASE DEFICIENCIES

Biotinidase and holocarboxylase synthetase deficiencies are AR disorders, induced by mutations in two genes, BTD and HLCS respectively. Biotin, which is recycled through the enzyme biotinidase, is indispensable cofactor for many carboxylases. The enzyme holocarboxylase synthetase is responsible for attracting biotin to carboxylases, subsequently activating them to function properly. Both disorders are related to multiple carboxylase deficiencies, with an estimated overall l1:60,000 incidence of biotinidase deficiencies. The later deficiency of holocarboxylase synthetase is rare with much lower incidence [43].

Presentation

Both deficiencies of biotinidase and holocarboxylase synthetase, share common clinical presentations. The deficiency of holocarboxylase synthetase presents mostly earlier, during the first 3 months, while the age of onset of biotinidase deficiency varies according to the enzyme activity, with a typical early age of presentation at 3 and a half months.

Biotinidase deficiency presents with developmental regression, ataxia, hypotonia, hearing and vision abnormalities, feeding problems, seizures, and breathing difficulties [2, 43]. Feeding problems, according to the literature, include vomiting and gagging, breathing difficulties, apnea or hyperventilation [43].

The deficiency of holocarboxylase synthetase also exhibits metabolic derangements with acidosis and hyperammonemia and also hypotonia, lethargy, vomiting, hypothermia, and tachypnea [1].Typical seizure types in this condition, include infantile spasms, generalized tonic-clonic and myoclonic seizures [1]. Both disorders are complicated by alopecia and skin rash [43]. Affected persons with biotinidase deficiency may also present neuromuscular symptoms including paresis, muscular atrophy and prominent peripheral muscle denervation [44].

Diagnosis

The typical metobolicl findings in this condition are hyperammonemia, lactic acidemia, and acidosis [1]. The most prominentl biochemical imbalances indicating the confirmation of biotinidase deficiency are the abnormal high organic acids, such as 3- methyl crotonyl glycine, propionyl glycine, 3-hydroxy isovalerate, methyl citrate, and hydroxy propionate [43] (Table 4).

The deficiency of biotinidase is established either by the assay measuring biotinidase activity or molecular testing for causative pathogenic variations in the BTD gene [43]. In contrast, only pathogenic variations corresponding to the HLCS gene, can confirm holocarboxylase synthetase deficiency [43].

Treatment

Excellent results are achieved as both deficiencies of biotinidase and holocarboxylase synthetase adequately clinically respond to administration of oral biotin [43] (Table 5).

B. Epilepsy as a result of energy deficiency disorders

GLUCOSE TRANSPORTER 1 (GLUT1) DEFICIENCY

De Vivo et al. were first to describe, in 1991, in the literature Glut1 deficiency, also termed De Vivo disease. Pathogenic variations in the solute carrier family 2, facilitated glucose transporter member 1

(*SLC2A1*) gene underly Glut1 deficiency. In the majority of cases, these are *de novo* mutations, while in familiar cases it is inherited with an autosomal dominant pattern and rarely with an autosomal recessive pattern and a rare (1:100,000) estimated incidence [45].

The disease is characterized by the deficiency of GLUT, which is indespensable for glucose to pass the blood-brain and other barriers. When this important molecular transporter of glucose gene is affected by mutation reduces the brain main energy substrate, glucose.

Presentation

Although cases with missense mutations characterize mostly moderate to mild disease, straight phenotype-genotype correlation is not yet established. Patients diagnosed with the same pathogenic variations do not typically have the same clinical presentation [46]. This implies that confounding factors, and disease-modifying genes interplay to modify the phenotype and give rise to the complexity of this condition [46].

Developmental regression is common in this condition with refractory seizures, microcephaly and a complex movement disorder consisting of ataxia and dystonia. The most common symptom is epilepsy, which usually begins within 2 years from birth and more frequently within the first few months of life, and is resistant to traditional seizure medications [47]. Seizures may be of various types. Only 10% of cases with early GLUT-1, have absence of seizures, while in contrast only 5% of those having epilepsy with myoclonic-atonic seizures are GLUT-1 deficiency patients [47].

However, other clinical manifestations may develop later in life, such as dystonia induced by paroxysmal exertion, choreoathetosis, alternating hemiplegia, and other paroxysmal events, such as intermittent ataxia, dystonia, and migraine [47].

Diagnosis

GLUT-1 deficiency syndrome should be included in the differential diagnosis of pharmacoresistant epilepsy in the clinical practice. The mutations of SLC2A1 have been reported to be responsible for approximately 1% of all idiopathic generalized epilepsies [48]. A strong clue for the diagnosis is the presence of induced dyskinesia by paroxysmal exercise in the affected individual or in the family, which may worsen after a period of fasting. All features can relapse or even worsen with fasting. EEG findings after fasting are typically related with slow activity and coexisting multifocal or generalized high-amplitude spikes.

CSF samples in GLUT-1 deficiency is recommended to be obtained during fasting as CSF glucose and CSF-to-blood glucose ratio are expected to be low (Table 4). The diagnosis can be confirmed by looking for reduced glucose transport across the erythrocyte membrane (which carries the same glucose transporter), and molecularly, by identifying mutations of SLC2A1 gene that encodes GLUT-1 [47].

Treatment

A ketogenic diet is part of the management aiming to deviate glucose, providing in turn, ketone bodies as brain energy alternative source [1]. The efficient ketogenic management may result in adequate control on seizures, while the ameliorating effect on the patients developmental evolution is less profound [49].

GUANIDINOACETATE METHYLTRANSFERASE (GAMT) DEFICIENCY

GAMT deficiency is a rare AR condition (1 in 500,000) of creatine biosynthesis, due to mutations in the GAMT gene [11]. GAMT supports guanidinoacetate (GAA) methylation, producing creatin and consequently, GAMT deficiency results in deficiency of creatine. Creatine is an energy storage and transmission substrate, crucial for brain development, having also functional properties as a neurotransmitter and a neuromodulator [1].

Presentation

The clinical GAMT deficiency present in affected infants up to the third year [50]. GAMT deficiency causes global developmental regression and cognitive dysfunction, immediately and markedly affecting the development of speech and language ability. Additional clinical presentations are hypotonia, epilepsy, autism, hyperactivity, movement disabilities and other related problems [51, 52].

Affected children with a high probability (2/3) have seizures with generalized tonic-clonic, myoclonic, partial complex and drop attacks [52].

Diagnosis

Blood and urine exhibit elevated GAA (Table 4) and spectroscopy by magnetic resonance do not display a marked creatine peak or display a severely reduced peak. To confirm diagnosis genotyping is implemented, with more than 50 different GAMT gene pathogenic variations identified, the most common of which are missense variants [11, 53].

Treatment

GAMT is treated with high-dose (400-800 mg/kg/d) creatine administration, while GAA toxic concentrations are decreased by administration of L-ornithine

(400-800 mg/kg/d) and restriction of arginine, when a diet restricted to proteins is applied [50, 52, 54].

C. Epilepsy related to various amino acid metabolic disorders

GLYCINE ENCEPHALOPATHY

Glycine encephalopathy is an autosomal recessive metabolic disorder caused by pathogenic variations in the genes named glycine dehydrogenase (GLDC), amino methyltransferase (AMT), and glycine cleavage system protein H (GCSH), that encode the necessary subunits to form the cleavage enzyme for glycine, with an incidence of 1:60.000 [55]. The condition is characterized by the toxic accumulation of glycine. Glycine also functions as agonist to the NMDA glutamate receptor and neurotransmitter in the brainstem and spinal cord with inhibitory activity. Consequently, excitatory NMDA glutamate receptors are overstimulated causing epileptic seizures, while the inhibitory activity of glycine in the brain and the cerebrospinal cord is causing muscular dysfunction with apnea and hypotonia [1].

Presentation

Glycine excess encephalopathy is clinically classified by the age at symptoms onset in three types: neonatal, infantile and late-onset [55, 56]. The vast majority of patients fall into the neonatal category, which involves a stereotypic presentation with severe hypotonia, apnea requiring assisted ventilation, and intractable seizures, with approximately 30% of such patients dying in the neonatal period [56]. Patients with the infantile type have mild to moderate psychomotor retardation, behavioral problems, seizures, and chorea [56]. The clinical presentations of late-onset are heterogeneous with a better prognosis [57]. Myoclonic and generalized seizures in this disorder, including, are often difficult to treat [2].

Diagnosis

Glycine levels are elevated to a much greater extent in CSF than in plasma; hence, an abnormally high value for the CSF/plasma glycine ratio is observed [56] (Collection and storage conditions in Table 4).

Diagnosis can be confirmed by finding mutations, large deletions or duplications in the genes coding for GLDC, AMT, and GCSH [56].

Treatment

Severe glycine excess encephalopathy is characterized by a poor outcome even when early treatment is initiated, while milder forms usually have improved outcomes with early treatment initiatives [58]. Sodium benzoate, dextromethorphan, felbamate, and ketamine, can reduce high concentrations of plasma glycine and consequently NMDA receptor antagonism, which can assist in seizure control [1].

MAPLE SYRUP URINE DISEASE

Branched-chain α-ketoacid dehydrogenase (BCKD) deficiency, more commonly known as maple syrup urine disease (MSUD), is an AR disorder with a prevalence of 1:185,000 [59]. It is caused by trans pathogenic variations in both alleles, in each of the *BCKDHA*, *BCKDHB*, *DBT* genes that are necessary for the subunits of the mitochondrial complex that decarboxylates α-ketoacid derivatives (BCKAs) of the branched-chain amino acids (BCAAs) leucine, isoleucine and valine, leading to the accumulation of BCAAs. Amino acid leucine is neurotoxic and when accumulated is responsible for the neurological presentation of this disease [1].

Presentation

Clinical features of MSUD include maple syrup odor, irritability, feeding disorder, lethargy, apnea, opisthotonus, stereotyped movements like fencing and bicycling, and ketonuria [59]. They present during the neonatal period or later in life in milder cases. Infections, surgeries, injuries or other stressors may cause acute episodes of metabolic instability and neurologic deterioration observed at any age. During these episodes, the affected patients develop seizures, nausea, vomiting, dystonia, ataxia, decreased consciousness that may even advance to coma and subsequently death [59]. An intermittent type of MSUD is characterized by normal growth and development. However, affected children with this type of MSUD may progress to clinical features and biochemical abnormalities resembling the typical type of MSUD with catabolic stressors [59].

Diagnosis

Plasma amino acid analysis shows high levels of alloisoleucine and biochemical derangement of the normal 1:2:3 proportion of amino acids isoleucine/leucine/valine (Table 4). Diagnosis is further established based on pathogenic variations of the responsible genes BCKDHA, BCKDHB, and DBT, or alternatively by evaluating deficient enzyme activity (BCKD) in propagated leukocytes or liver biopsies [59].

Treatment

Diet has two primary measure taken, first to reduce BCAAs and second to supplement the nutrients to reduce BCAAs catabolic process [60]. Long-term treatment requires careful manipulation of calories, restriction of dietary BCAAs, and supplementation

using a BCAA-free amino acid mixture to provide non-BCAAs and other nutrients for protein synthesis [61].

SERINE BIOSYNTHESIS DEFECT

Amino acid L-serine biosynthesis by the enzyme phosphoglycerate dehydrogenase (PGDH), phosphoserine Aminotransferase (PSAT1), and phosphoserine phosphatase (PSPH). The deficiency of L-serine is caused by molecular alterations in any of these three enzymes, while phosphoglycerate dehydrogenase (PGDH) is the one with the most common defects [2]. Serine biosynthesis defects are rare, inherited in an AR mode, as a result of pathogenic variations in the corresponding genes PHGDH, PSAT1, and PSPH respectively. L-serine function as the basic molecule for the biosynthesis of more complex compounds [62]. Also L-serine and the products of its metabolism have been recognized to be essential for cell proliferation and specific functions in the central nervous system [63].

Presentation

In most patients, the onset of symptoms is present before birth, as intrauterine growth

retardation and microcephaly. Epileptic seizures develop at the first weeks to months of life. Later during the first year of life, hypertonia becomes evident, evolving into spastic tetraplegia [64].

More clinical presentations are related to hypogonadism, feeding difficulties and congenital cataracts [63].

A mild deficiency has been described infrequently in older children including absences and tonic clonic seizures [65].

Diagnosis

Aminoacid serine and, to a lesser extent, glycine is low in CSF and plasma, although they can be normal in a nonfasting state [64] (Table 4). Diagnosis can be confirmed by demonstarting the enzymes' defective activity in skin fibroblast cultures or by pathogenic variations in the three causative genes PHGDH, PSAT1, and PSPH [2].

Treatment

Administration of a higher dose of L-serine (500-700mg/kg/d) in severe forms and glycine (200-300 mg/kg/d) for infants with the severe infantile form or a lower dose of L-serine (100-150 mg/kg/d) in mild forms have a beneficial effect on seizures, irritability and spasticity [62, 65].

UREA CYCLE DISORDERS

Urea cycle disorders (UCDs) are metabolic disorders caused by molecular aberrations in the transporter enzymes collaborating in the removal of ammonia from the liver, leading to hyperammonemia. Eight disorders are considered as UCDs: N-acetylglutamate synthase deficiency (NAGSD), Carbamyl phosphatesynthetase 1 deficiency (CPS1), Ornithine transcarbamylase deficiency (OTCD), Argininosuccinatesynthetase deficiency (ASSD) (Citrullinemia), Argininosuccinatelyase deficiency (ASLD) (Argininosuccinic aciduria), Arginase deficiency (ARGD, Argininemia), Hyperornithinemia, hyperammonemia, homocitrullinuria (HHH) syndrome (or mitochondrial ornithine transporter 1 deficiency (ORNT1D) and Citrullinemia type II (mitochondrial aspartate/glutamate carrier deficiency (CITRIN).Except for ornithine transcarbamylase deficiency (OTCD) which is an X-linked UCD, the rest are inherited as autosomal recessive disorders [66]. The population incidence of urea cycle disorders is found to be 1 in 35,000 births according to screening studies [67].

Presentation

An infant with a complete defect in a urea cycle enzyme (other than ARG) commonly presents with hyperammonemia coma. If not treated, hyperammonemia coma may progress to death, because ammonia is extremely toxic in the developing brain and the CNS.

The reversibility of the toxic effects of hyperammonemia depends on how long the exposure took place, on the levels of excess ammonia in the blood and the developmental stage of the brain [67]. During neonatal life, excessive ammonia causes brain edema and results in neurological disorders including epileptic seizures, cognition decline, motor deficits and coma.

Patients with mild defects of the urea cycle can manifest hyperammonemia at any age and have a significant risk for developmental disabilities and even death. In these cases, hyperammonemia and even coma may be induced by a number of complications and interventions such as illness and surgery [66].

Diagnosis

Hyperammonemia without a high anion difference and with non-excessive plasma glucose levels is the diagnostic biomarker [67]. Other laboratory findings include, high glutamine and alanine in plasma, low plasma arginine, with the exception of arginase deficiency) and increased or decreased citrulline in plasma [68] (Table 4). Genotyping may confirm the diagnosis.

Treatment

Neonates with hyperammonemia, should be subjected to urgent treatment for the immediate reduc-

tion of ammonia. Hemodialysis as an urgent measure, is very effective and should be initiated if high levels of ammonia are observed [68]. Conventional therapy includes pharmacological intervention, so-called Nitrogen (N) scavenger therapy, supplementation with the amino acids L-citrulline or L-arginine and low-protein diet that balances N restriction with growth requirements. The only known "cure" for UCD is liver transplantation [66].

PHENYLKETONURIA/MAINKA STONE

Phenylketonuria (PKU) is a rare, inborn error of metabolism, most often caused by missense mutations in the gene encoding phenylalanine hydroxylase (PAH), which catalyzes the hydroxylation of phenylalanine (Phe) to generate tyrosine (Tyr) [69]. The incidence of PKU is about 1/15,000 [70]. High levels of Phe and its metabolites (for ex. keto acid and phenylpyruvate), cause increased oxidative stress, altered neurotransmitter metabolism and decreased cerebral protein synthesis. Low Tyr levels could have an additional role in the pathophysiology of the disorder [70].

Presentation

Signs and symptoms include delayed cognitive/motor development, epileptic seizures, a characteristic smell in urine and skin, microcephaly, hypopigmentation, hyperactivity, behavioral problems, and movement disorders. Neonates with mild forms of PKU are mostly normal as neonates and develop the clinical PKU after several months.

Affected newborns appropriately treated are less likely to develop symptoms. Still, insufficiently treated individuals may have symptoms. Neuropsychiatric characteristics include obsessive-compulsive disorders, depression and features compatible with those observed in individuals with an autism spectrum disorder.

Diagnosis

Standard newborn tests usually identify PKU at birth screening in many countries, including Greece. Commercial microarray genotyping for nucleotide variations are capable to determine predisposition for PKU. As in all AR disorders both parents should carry of a PKU pathogenic variation, in order to have a child with PKU with a probability of 25%.

Treatment

PKU is treatable by dietary intervention for a lifetime, to maintain low Phe amounts of and supportive amounts of Tyr. Life-long dietary treatment is coupled with adequate monitoring of Phe and Tyr blood levels, and assessment of the cognitive condition [70]. Deficiencies in trace elements (selenium, copper, magnesium, and zinc) also complicate the dietary intervention for PKU and supplementation may be required.

Oral sapropterindi hydrochloride (common name KUVAN), is a synthetic form of BH4, which can decrease Phe in PKU patients. A 30-day trial is required to assess BH4 responders among PKU patients. Encouragingly, KUVAN supports brain well-beeing in a proportion of PKU cases [71]. An enzyme substitution treatment has recently been approved by the FDA for use in this disorder [71].

Conclusion

In newborns, infants or young children that appear normal at birth and present nonspecific manifestations such as epileptic seizures, developmental regression or delay, hypotony, lethargy, vomiting, irritability, dysmorphic features, movement disorders and organomegaly, metabolic disorders should be included in the differential diagnosis.

Treatable metabolic epilepsy is an uncommon entity and may not have an obvious vertical transition depicted in the family tree. The differential diagnosis should include treatable metabolic epilepsies in clinical phenotypes and an initial approach involves combined diagnostic and therapeutic strategies. Typical etiologies, like infection, should be examined, along with metabolic screens.

Upon the potential diagnosis of metabolic epilepsies, an emergency EEG with a pyridoxine trial (100 mg intravenously) is the appropriate management [20]. Other vitamins and cofactors can be administered, such as pyridoxal phosphate, folinic acid, and biotin, if no therapeutic response is present [2, 3]. A ketogenic diet can have a beneficial effect in children suffering from GLUT-1 deficiency.

The underlying cause of metabolic eoileosy should also be considered for the right antiepileptic drugs to be consindered. Phenobarbitone, which inhibits glucose transport, is an example of an antiepileptic drug not to be used in children with GLUT-1 deficiency [72].

Reaching an accurate diagnosis is of high importance for the right treatment and care, so as to achieve immediate cessation of seizures, and for family counseling regarding prognosis and recurrence risk [3, 20].

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VITAMIN E DEFICIENCY: CLINICAL CHARACTERISTICS, DIAGNOSIS AND MANAGEMENT

Panagiotis Stoiloudis, Athanasios-Nikolaos Terzakis, Nikoletta Smyrni, Dimitrios Parissis, Panagiotis Ioannidis

2nd Department of Neurology, AHEPA Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece

Abstract

Vitamin E is a liposoluble vitamin with a significant antioxidant role. Its deficiency can lead to neurologic symptoms in adults. Although lack of vitamin E intake through diet is considered rare, defects of metabolism of lipids and/or malabsorption might result in deficiencies. Abetalipoproteinemia (ABL), homozygous hypobetalipoproteinemia (HHBL) and ataxia with vitamin E deficiency (AVED), consist a group of the most well-characterized neurogenetic disorders associated with vitamin E deficiency. These disorders are causally related to genetic mutations and their clinical picture mainly consists of ataxia, sensory neuropathy, pyramidal signs, as well as retinopathy and gastrointestinal tract symptoms. History, clinical and detailed neurological examination, blood routine tests and genetic testing are important for their diagnosis. When clinically suspected, early diagnosis is important, because, treatment with vitamin E supplementation could prevent and sometimes improve neurological symptoms especially if given in the early stages of the disease.

Key words: vitamin E deficiency, neurogenetic disorders, ABL, HHBL, AVED

Introduction

Vitamin E is a liposoluble vitamin with significant antioxidant activity. It consists of eight isomers, classified into two groups of components, (α-, β-, γ-, δ-) tocopherols and (α-, β-, γ-, δ-) tocotrienols. A-tocopherol is the only isomer that remains unchanged once absorbed by the human organism. RRR-α-tocopherol consists the most abundant vitamin E isomer, accounting for up to 90% of the vitamin in human tissue, as well as in blood plasma [1].

Bioavailability and absorption

Vitamin E is absorbed to the human body through food or nutritional supplements. Food sources considered to be especially rich to vitamin E are edible oil from plants (sunflower, wheat, peanut), as well as cereals and butter. Absorption and transportation in the human body is a complicated procedure and depends on the level of fat that is taken with food (Fig. 1). The small intestine is responsible for its absorption, requiring acid secretion from bile. Vitamin E is mostly distributed in fat tissue as α- and γ-tocopherol due to higher dietary intake and bioavailability. More specifically, α-tocopherol is concentrated in the Golgi system and the lysosomes. Three proteins (a liver protein, a high molecular weight protein that is not only present in the liver and a protein mostly presented in liver and erythrocytes) are involved in the transportation of tocopherol to the human tissues, especially regulating α-tocopherol levels [1, 2].

Vitamin E excretion

Vitamin E and its isomers are mainly excreted through stool but also undergo enterohepatic circulation. Biliary secretion through the liver has been suggested to prevent from vitamin E toxicity after following high doses of supplements. Additionally, high content of vitamin E in stool is reported to be favorable for the gastrointestinal system, through antioxidative processes [2].

Vitamin E metabolite excretion

Carboxyethyl-hydroxychromans (CEHC) constitute a metabolite class of vitamin E which can be found in blood as well as in urine. It is hypothesized that excess α-tocopherol is metabolised to a-CEHC, which may be used as a marker of efficient vitamin E levels. Furthermore, it is reported that in patients lacking a-tocopherol transfer protein (aTTP) with extremely low plasma vitamin E, the level of a-CEHC was higher in urine than in healthy individuals. aTTP seems to be of a significant role for the secretion of liver α-tocopherol into plasma [2].

Vitamin E measurement and sampling

Recent methods have made possible to measure vitamin E levels with precision and accuracy, although in the past it was difficult due to instability and lipophilicity of its molecule. As far as it is reported, deficiency of vitamin E is considered

when concentation in plasma is below 12 μmol/L. Vitamin E levels can be measured in blood components (plasma, serum and erythrocute cells). A- and γ- tocopherol which consist isomers of vitamin E, are most precisely detected in plasma and serum. CEHC, which consist a vitamin E catabolite, can also be measured in urine (Table 1) [3-6].

It is reported that levels of vitamin E may be affected after meals or fasting, although there is no special recommendations concering fasting or not before the collection of blood samples. Vitamin E level can be measured lipid adjusted or unadjusted, thus lipid profile is recommended to be measured in plasma. Ideally, samples should be processed immediately or if analysed in a short period of time should be kept at a temperature of 4°C. Urine spot samples or 24 hour collections samples need to be adjusted for creatinine, should be kept in a temperature of 4°C and measured in a 24hour period [3].

Vitamin E and oxidative stress

In vitro studies have shown that vitamin E plays a major role against oxidative stress. It is capable of protecting against free radical oxidants, and free radical catalysed lipid peroxidation. This led to the suggestion that vitamin E and its isomers play a significant role as a liposoluble antioxidant [1, 2].

Neurodegenerative disorders

Low concentrations of vitamin E have been associated with several neurodegenerative disorders. Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, among others, may be associated with oxidative stress, where oxidative activity results to neurotoxicity and neuron cell death. Although association may possibly exist, it is under debate if deficiency of vitamin E has a clinical impact in these disorders. Thus, supplementation with vitamin E may not have beneficial results [1].

Neurogenetic disorders

Neurogenetic diseases consist a heterogeneous group causing significant disability in humans, with varying clinical features, associated with the involvement of numerous genes. Although, no specific treatment has been found for several of them, it is reported that some of the disorders may respond to vitamin supplementation. Early identification and diagnosis in early ages can be beneficial for possible treatment or prevention of the progression of these disorders [7].

Deficiency of vitamin E can lead to neurologic symptoms such as imbalance, speech disturbance and motor weakness. Although lack of vitamin E intake through diet is considered rare, defects of metabolism of lipids and/or malabsorption could result in deficiencies. AVED, ABL (Bassen-Kornzweig syndrome), and HHBL consist a group of neurogenetic diseases characterized by low levels of vitamin E where early identification and initiation of supplementation could prevent and even reverse several complications [7, 8].

A. Ataxia with vitamin E deficiency

One of the three well-characterized genetic syndromes of vitamin E deficiency is AVED. The genetic basis of this rare disease is mutations in the aTTP encoding gene. The disease has very distinct clinical

Fig. 1. Metabolic pathway of vitamin E

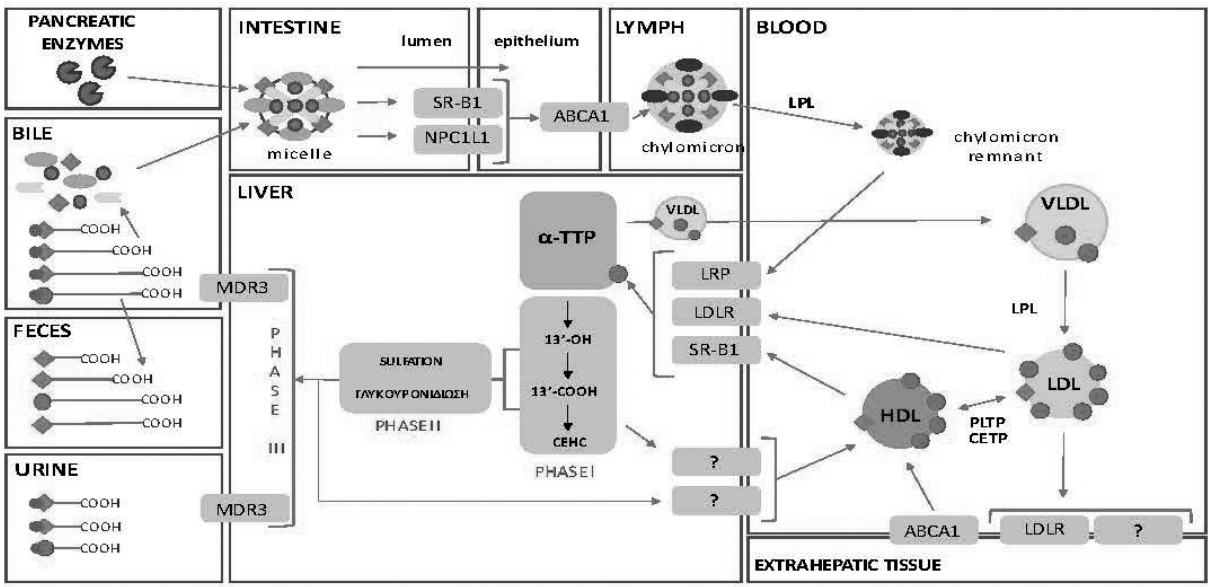


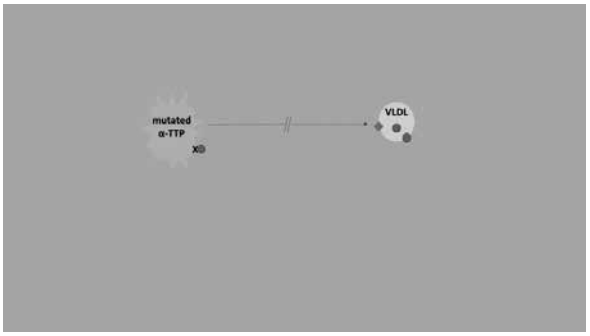
Table 1. Vitamin E metabolites and normal range in different samples

Metabolite	Normal range
α-tocopherol (plasma)	12-30 μmol/L
γ-tocopherol (plasma)	1-4 μmol/L
α-CEHC (urine)	0.5-1 (μmol/g creatinine)
γ-CEHC (urine)	0.5-3 (μmol/g creatinine)

Table 2. Clinical signs and symptoms of AVED with percentages

Clinical signs and symptoms	Percentage (%)
Absent tendon reflexes	94.7
Gait disturbance	93.4
Plantar extensor response	85.5
Posterior column spinal cord involvement	67.1
Speech disturbance/dysarthria	61.8
Head titubation	40.8
Retinitis pigmentosa	2.3
Cardiomyopathy	1.5

Fig. 2. Defective binding of vitamin E to VLDL due to mutations in aTTP



findings, yet symptoms and histopathological findings are very diverse. The mode of inheritance follows the autosomal recessive pattern [9, 10].

I. Pathophysiology

Vitamin E consists of a group of 8 fat soluble molecules that act as antioxidants. a-TTP acts as a carrier protein for RRR-a tocopherol. In its absence or malfunction, binding of vitamin E to VLDL is defective, hence low vitamin E plasma levels are detected (Fig. 2). As a result, oxidative stress occurs, and one of the most prominent tissues to be affected is the central nervous system. While the exact action of vitamin E in the central nervous system (CNS) is not understood, theories that are proposed to explain the symptoms of its low bioavailability include the increased production of cytolytic phospholipids, the exacerbation of glutamate excitotoxicity and a brain monoamine metabolism malfunction. The result of these proposed mechanisms is increased neuron apoptosis and the clinical manifestations of cerebellar ataxia and muscular degeneration [9].

II. Clinical characteristics

AVED typically manifests in the late childhood or early adulthood. Early symptoms might among others, include clumsiness, ataxic gait, low muscle tone, loss of muscle reflexes (areflexia), and impaired proprioception, clinical characteristics similar to Friedreich's ataxia. These symptoms progress with age, and are typically complicated with malfunction of other tissues, such as the heart (heart failure, left ventricular hypertrophy, cardiomyopathy), gastrointestinal tract and pancreas (diabetes mellitus) (table 2).

The most prominent symptoms are progressive cerebellar ataxia and dysarthria. Dysmetria, dysdiadochokinesia, head titubation/tremor, as well as ataxic gait are all symptoms that might appear as the disease progresses. Loss of proprioception and posterior column involvement, with a positive Romberg's sign and conservation of light and temperature touch is almost always present at an early disease stage. Areflexia of upper and lower limbs combined with muscle spasticity and a positive Babinski sign are also

Table 3. Recommended evaluation after initial diagnosis

➢ **General examination** (every 6-12 months) Growth curve if <10 years old GI tract symptoms ➢ **Neurologic examination** (every 6-12 months) Deep tendon reflexes, pyramidal signs, gait examination, speech, abnormal movements, vibratory and position sense ➢ **Brain MRI** Cerebellar degeneration and T2 hyperintensities in the periventricular and deep white matter of the hemispheres ➢ **CSF evaluation** For exclusion of other causes ➢ **Cardiac evaluation** (every 3 years) Electrocardiogam and echocardiography for detection of cardiomyopathy	➢ **Opthalmologic examination** (every 6-12 months) Detection of macular degeneration or retinitis pigmentosa Examination of visual acuity Electroretinogram ➢ **Neurophysiologic studies** Nerve conduction studies Somatosensory potentials ➢ **Genetic counseling** Genetic testing for the identification of a possible a-TTP mutation in family members ➢ **Laboratory studies (every 6-12 months)** Plasma vitamin E levels Lipid profile (total cholesterol, HDL-C, LDL-C) Liver function (AST, ALT, ALP, GGT, INR, total and direct bilirubin)

presented. Other neurological signs and symptoms consist of macular degeneration and retinitis pigmentosa, resulting in decreased visual acuity, skeletal abnormalities (pes cavus, spinal column malformations), horizontal nystagmus and eye movement dysfunction, deafness and, hyperkinesias (dystonia and fasciculations). Myocardial involvement and diabetes mellitus have been reported [9, 11].

III. Diagnosis

A wide range of para-clinical examinations need to be performed in order to set the definite diagnosis of AVED (Table 3). These consist of blood testing, brain magnetic resonance imaging (MRI), cerebrovascular fluid (CSF) evaluation, genetic testing, neurophysiologic and histopathological examination.

Endorsement of the disease is low plasma vitamin E (α-tocopherol < 12μmol/L) levels in the setting of normal blood cholesterol, lipoprotein and triglycerides levels, and an absence of intestinal causes of malabsorption. Concerning neuroimaging, brain MRI is the preferred method, and with cerebellar degeneration and T2 hyperintensities in the periventricular and deep white matter of the hemispheres appearing in more than half of the cases. CSF testing is usually normal and performed for the exclusion of other causes. Somatosensory evoked potentials (SEPs) and electrophysiological examination show either pure sensory, pure motor or mixed motor and sensory neuropathy and increased conduction time between C1 level of the spinal cord and the cortex. Histopathologically, increased loss of Punkinje cells, demyelination and dying back type degeneration of the posterior spinal cord columns, axonal spheroids and lipofuscin accumulation have been reported. Finally, genetic testing also helps in setting the diagnosis of AVED, by detection of mutations in the aTTP encoding gene [9, 11].

IV. Differential diagnosis

Differential diagnosis is vast and consists of diseases mimicking symptoms of AVED, most prominent of which is Freidreich's Ataxia (Table 4). Exclusion of malabsorption causes of low plasma vitamin E like cholostatic liver disease, IBD, short bowel syndrome, cystic fibrosis, is necessary. Charcot Marie Tooth disease, Refsum's disease, other ataxias as well as cerebrotendinous xanthomatosis might manifest with symptoms resembling AVED [9, 11].

V. Management

The most effective treatment of AVED consists of lifelong high doses of vitamin E supplementation (800-1500mg/day). Early diagnosis and treatment is crucial in order to stop the disease from progressing. Reversion of ataxia and head tremor has been reported, but posterior column involvement is usually permanent once it has been established. Presymptomatic patients typically do not exhibit symptoms if treated early. Genetic counseling and surveillance for relatives of people with AVED is highly advised [9-11].

B. Abetaliproteinemia

ABL or else Bassen-Kornzweig syndrome is a rare disease of lipid metabolism due to a genetic mutation in the microsomal triglyceride transfer protein (MTP) encoding gene located on chromosome 4q23 inherited with an autosomal recessive manner. Until recently, more than 20 mutations in the MTP gene have been reported in English, Irish, Japanese, American, French and Italian families. MTP is required for

Table 4. Differential diagnosis between AVED and Friedreich's ataxia

Clinical features	AVED	Friedreich's ataxia
Head tremor	++	Rare
Dystonia	++	–
Low visual aquity	++	Rare
Diabetes mellitus type I	+	++
Plantar extensor response	+	++
Retinitis pigmentosa	+	–
Peripheral neuropathy	+	++
Cavus foot	Rare	++
Disorder of cardiac conduction	Rare	++
Cardiomyopathy	+	++
Muscle atrophy	–	++

++: present, +: sometimes present, –: generally absent

the concentration and secretion of apolipoprotein B (apoB) in the gastrointestinal system (liver and intestine). It is responsible for the transportation of triglyceride, cholesteryl ester, and phospholipid molecules between phospholipid surfaces. Genetic defects lead to low levels of apoB, resulting in chronic fat malabsorption. Thus, plasma levels of triglyceride, cholesterol, and of liposoluble vitamins A, K, and especially E are low. Patients with ABL develop gastrointestinal and neurological symptoms, most commonly ataxia and retinitis pigmentosa. Treatment by vitamin supplementation mainly with vitamin E has to be initiated early [9, 12].

I. Pathophysiology

MTP is crucial for the concentration of very low density lipoproteins (VLDL) and the secretion of apoB from the liver. It transfers triglycerides to the apoB polypeptide chain, allowing the assembly and secretion of lipoproteins to synthesize VLDL in hepatocytes, and chylomicrons in enterocytes. Mutations in the MTP gene cause apoB to not be properly lipidated, leading to a significant decrease in plasma levels of cholesterol, triglycerides and apoB-containing lipoproteins. Therefore, synthesis of chylomicrons and VLDL, carrying fat-soluble vitamins in blood, is impaired, leading to a malfunction of the transportation of these vitamins to the peripheral tissue [7, 9].

II. Clinical characteristics

Initial symptoms affecting the gastrointestinal system occur since the first months of life, consisting of fat malabsorption symptoms including nausea, vomiting, diarrhea, steatorrhea and inability to gain weight. Fat intolerance is not unusual. These symptoms may resolve if patients follow a strict fat-free diet.

Patients may also be asymptomatic until the disease is diagnosed through routine blood tests detecting low levels of cholesterol and triglycerides. On this stage, neurological symptoms may not be revealed until the third or fourth decade of life, where a neurological examination may reveal a positive Romberg's test and diminished deep tendon reflexes. Visual loss may sometimes occur [9].

If undiagnosed and untreated, ABL can manifest with neurological signs and symptoms of ataxia and sensory neuropathy at the first or second decade of life. Neurological examination often initially reveals absent deep tendon reflexes following sensory loss mostly in the lower limbs as well as cerebellar signs including ataxic gait, dysarthria and dysmetria. Additionally, upper motor signs involving Babinski sign and low limb weakness can be observed. Skeletal deformities such as pes cavus, pes equinovarus, and kyphoscoliosis can be encountered as well [7, 9].

Pathologic eye manifestations mainly include retinitis pigmentosa and macular degeneration. Vitamin E deficiency plays a major role in the retinopathy according to the presence of lipofuscin pigment in the retina. Visual loss especially at night can also be a presenting symptom due to a concominant vitamin A deficiency [7-9, 12].

Other manifestations can include anemia due to malabsorption, and bleeding diathesis because of vitamin K deficiency. Cardiac manifestations have also been reported, with congestive cardiac failure being observed in one patient [9].

III. Diagnosis

Clinical diagnosis is based on a history of malabsorption syndromes in infancy, lipid profile, peripheral blood smear, and abnormal findings from the neurological examination [8].

Routine blood tests involving serum cholesterol and serum lipid electrophoresis are necessary. Characteristically, absence of apoB, reduced cholesterol levels (<500mg/L) and very low triglyceride levels (<100mg/ dl) are profound. Furthermore, levels of vitamin E and A are usually reduced.

Serum transaminases may be elevated and this is usually associated with liver steatosis and mild hepatomegaly.

Acanthocytosis in peripheral blood smear is usually observed. Abnormal star-shaped erythrocytes can be detected, reflecting the abnormal synthesis of the cell membrane due to defects of the plasma lipoproteins [9, 12].

Neurophysiologic studies with visual evoked potentials often reveal normal amplitude and increased latency in less than half of the patients. SEPs (especially N18 and P22) might show delayed cortical or dorsal column dysfunction [13].

Evidence of sensory axonal neuropathy is established through sensory nerve conduction tests revealing reduction of sensory action potential amplitudes.

Gastrointestinal evaluation is required in order to exclude other chronic malabsorption syndromes. 72-hour fecal fat excretion is abnormal with the percentage of coefficient of excretion higher than normal. Endoscopy of the gastrointestinal tract reveals discoloration of the duodenum and white colour of the intestinal mucosa. Vacuolization of villus cells may be found in biopsy.

Genetic testing by sequencing of the MTP gene provides a definitive diagnosis. Over 30 mutations in MTP have been identified [7-9].

IV. Management

Dietary modification with a diet with low fat, especially enriched with long chain fatty acids, in order to improve steatorrhea is suggested as a treatment. Additionally, high dose supplementation with oral vitamin E is suggested and has been associated with better clinical improvement. Oral vitamin E should be given in doses ranging from 2400 to 12000IU per day. Monitoring of vitamin E level in the serum is helpful to assess the effectiveness of treatment. Parenteral treatment has also been proposed and may be more effective. Moreover, treatment with daily doses of vitamin A and D should be considered [8, 12, 14-16].

C. Homozygous hypobetalipoproteinemia

HHBL consists a genetic disorder in which patients are homozygous or heterozygous for mutations in the apoB gene. Clinical features and management are similar to ABL. In many cases, fatty liver can be the only clinical manifestation [8, 16, 17].

Conclusion

In summary, AVED, ABL and HHBL consist genetic disorders associated with vitamin E deficiency, causing severe neurological manifestations if remained undiagnosed and untreated. Clinical suspicion, early diagnosis and supplementation with high doses of liposoluble vitamins can protect against neurological and systemic complications.

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THIAMINE DEFICIENCY: A REVIEW OF ACQUIRED AND GENETIC CAUSES

Vaios Samaras, MD; Ioanna Koutroulou, MD, PhD; Nikolaos Grigoriadis, MD, PhD; Theodoros Karapanayiotides, MD, PhD

2nd Department of Neurology, Aristotle University of Thessaloniki, School of Medicine, Faculty of Health Sciences, AHEPA University Hospital, Thessaloniki

1. Introduction

Thiamine, also known as vitamin B1, is a water soluble essential micronutrient. It belongs to the B vitamin complex and plays a vital role in many biochemical processes of the human body [1]. Thiamine functions as a coenzyme for a number of enzymatic reactions mostly involved in antioxidant production, in nucleic acid biosynthesis, and especially in energy production. Since these processes are of utmost importance for the nervous system, thiamine deficiency often presents with neurologic manifestations.

Thiamine biosynthesis occurs in bacteria, fungi and plants, but is not possible for mammalian cells. The only source of thiamine for mammals is dietary, with pork, poultry,beef, cerials, eggs, fish and nuts being rich in thiamine [2]. The human body stores are limited to approximately 30mg of thiamine, with daily requirements depending on carbohydrate and calorie consumption [3-5]. Symptoms of thiamine deficiency develop approximately 4-6 weeks after inadequate thiamine intake [6]. The recommended adult daily dose is 1.2 mg for men and 1.1 mg for women [4].

Thiamine deficiency disorders were first described by Carl Wernicke in 1881. Wernicke described a neuropsychiatric disorder characterized by the triad of opthalmoplegia, ataxia, and confusion [7]. Later, Korsakoff described a series of patients with memory loss and in 1897, Murawieff proposed that the two entities, described by Wernicke and Korsakoff, shared a common etiology: thiamine deficiency [8, 9]. In the 1880s, the work of Christiann Eijkman led to the discovery of the cause of beriberi. He observed that a diet comprising of processed rice led to symptoms of peripheral neuropathy in chickens and that an "antineuritic vitamin" contained in the pericarpium of the rice can treat this disorder. This discovery won him the Nobel prize for Medicine or Physiology in 1929 [10].

Thiamine deficiency has a wide variety of manifestations, ranging from neurologic to cardiovascular. In adults, Wernicke encephalopathy is the most common manifestation of thiamine deficiency, potentially leading to permanent sequelae if left untreated. In childhood, genetic disorders of thiamine absorption, transfer and utilization can present with complex phenotypes. The identification of both acquired and genetic disorders is important, and prompt treatment with thiamine supplementation leads to symptom reversal.

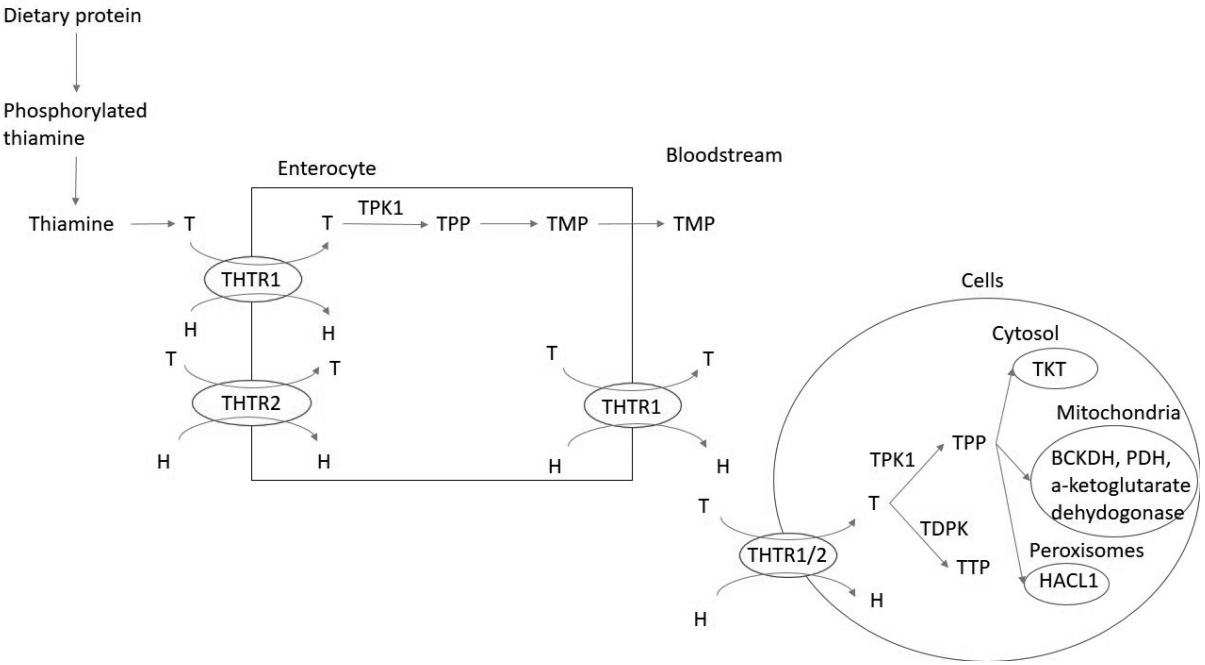
2. Epidemiology

Thiamine deficiency is considered a rare disorder in healthy individuals in food-secure settings. Although dietary thiamine is abundant in developed countries, diets containing mainly processed foods are increasingly prevalent, leading to thiamine deficiency [11]. Industrial food processing, leading to thiamine destruction, coupled with a diet high in carbohydrates can deplete thiamine and lead to a phenomenon known as high calorie malnutrition. Accordingly, in a recent study, individuals considered for bariatric surgery were found to be nutrient- and in many cases thiamine-deficient [12]. Thus, thiamine deficiency is more prevalent than commonly thought. To prevent this, in many high-income countries, some food supplies are fortified with thiamine [13].

In developing countries where low-thiamine food such as processed grains are the staples, thiamine deficiency is a common concern [14, 15]. Thiamine deficiency can also occur after consuming food with thiamine antagonists or thiamine metabolizing enzymes, like betel nut, raw fish and tea. Food scarcity is also a cause of thiamine deficiency in populations displaced due to war, famine or natural disasters [16, 17]. Worldwide, in regions of Asia, Africa, and the Americas, food scarcity, food preference, inequities in the nutrient content of food, and inadequate fortification of food supplies are causes of thiamine deficiency [17].

Infants, especially exclusively breastfed infants of thiamine-deficient mothers, are at the highest risk of thiamine deficiency [18]. Pregnancy and lactation increase thiamine needs and can lead to asymptomatic maternal thiamine deficiency, often manifesting as beriberi (described below) in breastfed infants [19].

Fig. 1. Thiamine metabolism



T: thiamine, **H**: hydrogen ions, **TPK1**: thiamine pyrophosphokinase, **TPP**: thiamine pyrophosphate, **TMP**: thiamine monophosphate, **THTR1**: thiamine/H+ antiporter 1, **THTR2**: thiamine/H+ antiporter 2, **TDPK**: thiamine diphosphokinase, **TKT**: transketolase, **BCKDH**: branched-chain α-ketoacid dehydrogenase (BCKDH), **PDH**: pyruvate dehydrogenase, **HACL1**: 2-hydroxyacyl-CoA lyase 1

Other populations at risk include alcoholics, patients after bariatric surgery or suffering from bulimia or anorexia nervosa, critically ill or cancer patients, and patients with HIV/AIDS, gastrointestinal disorders or receiving total parenteral nutrition, diuretics or renal replacement therapy [20-26].

However, despite a thiamine rich diet, deficiency may result from genetic abnormalities in thiamine absorption, transport and cellular handling [27]. These disorders are known as thiamine metabolism dysfunction syndromes.

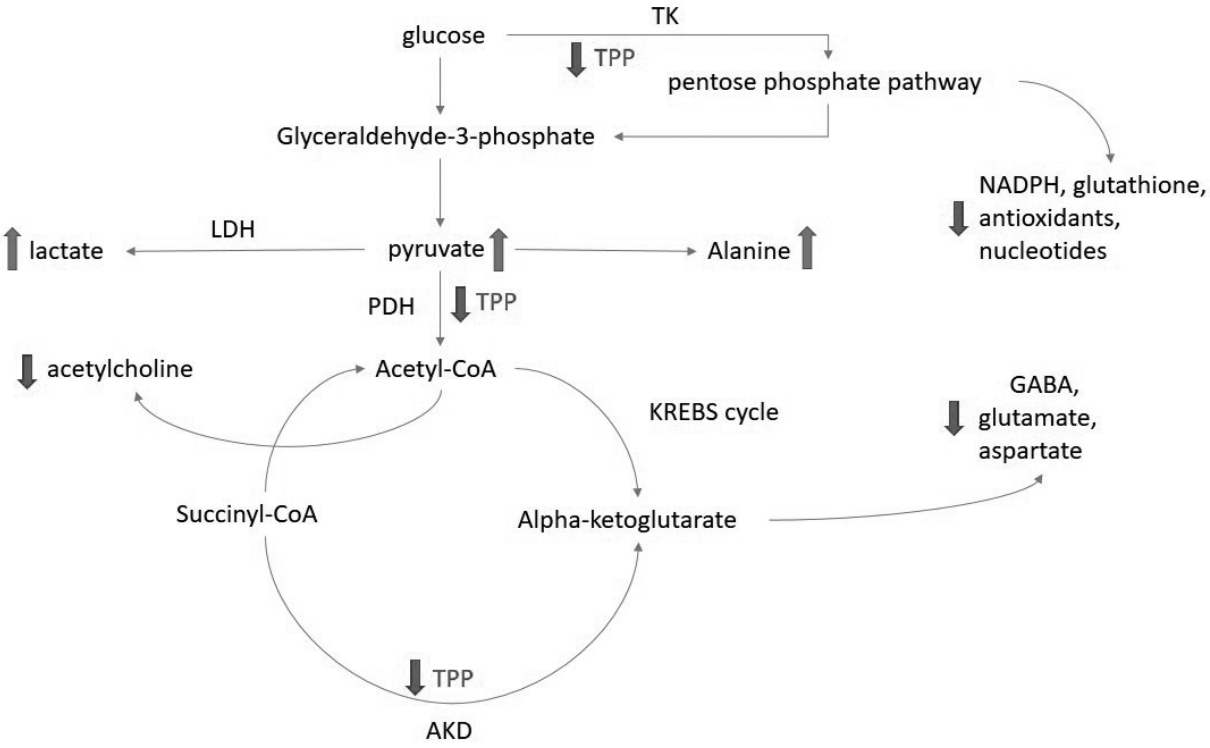
3. Thiamine metabolism

Thiamine is found in mammals either as free thiamine, or in phosphorylated forms. These are thiamine monophosphate (TMP), thiamine diphosphate (TDP) also known as thiamine pyrophosphate (TPP), and thiamine triphosphate (TTP) [11]. TPP is the active form of thiamine and accounts for 80% of the total body stores [28]. Multiple enzyme complexes in the cytosol (pentose phosphate pathway - transketolase), mitochondria (pyruvate dehydrogenase, oxoglutarate dehydrogenase, branched-chain alpha-ketoacid dehydrogenase) and peroxisomes (2-hydroxyacyl-CoA lyase)depend on the presence of TPP [29-31] (Figure 1)

Thiamine absorption mainly occurs in the jejunum [32]. Dietary proteins are hydrolyzed in the digestive tract, releasing phosphorylated thiamine. Unphosphorylated free thiamine is then released by alkaline phosphatases [33] There are two thiamine absorption mechanisms in the small intestine. The first one, passive diffusion, is dependent on high thiamine concentration, whereas the second functions at lower thiamine concentrations. When thiamine concentration is lower than 1μM, thiamine absorption depends on an energy-dependent saturable transporter (thiamine/H+ antiporter, also known as THTR1) [32]. After entering the enterocytes, thiamine is converted first to TPP by thiamine pyrophosphokinase (TPK1) and then to TMP. Thiamine then reaches the bloodstream either in the form of TMP by an energy-dependent transport system, or as free thiamine through the THTR1 transporter [34]. Once in the bloodstream, thiamine enters most cells via two transporters, THTR1 and THTR2 [35, 36]. In blood, it circulates primarily in red-blood cells and leukocytes, and is delivered to organs with high metabolic demands such as the skeletal and heart muscles, pancreas, liver and brain. In cells TPP is once more synthesized from thiamine by TPK1 or by thiamine diphosphokinase (Figure 1).

In a state of thiamine deficiency, all the aforementioned metabolic pathways are affected, albeit not equally. Accordingly, all tissues are not equally affected, with the brain and especially the cerebellum, mammillary bodies, thalamus, hypothalamus,

Fig. 2. Biochemical basis of thiamine deficiency



LDH: lactate dehydrogonase, **PDH**: pyruvate dehydrogonase, **TPP**: thiamine pyrophosphate, **TK**: transketolase, **AKD**: alpha-ketoglutarate dehydrogenase.
Modified from Calderón-Ospina CA, Nava-Mesa MO. B Vitamins in the nervous system: Current knowledge of the biochemical modes of action and synergies of thiamine, pyridoxine, and cobalamin. CNS *Neurosci Ther.* 2020;26(1):5-13.

and brainstem being more vulnerable [4, 37]. It seems that the α-ketoglutarate dehydrogenase pathway is the most sensitive to thiamine deficiency, and its dysfunction can quickly lead to reduced ATP synthesis, oxidative damage, and, ultimately, cell death [38].

4. Pathophysiology of thiamine deficiency

As stated above, thiamine deficiency leads to a cellular state of reduced energy synthesis and increased oxidative stress [39]. In this state, pyruvate, the main product of glycolysis, cannot be transformed to acetyl-CoA, due to low pyruvate dehydrogenase activity. Pyruvate, thus cannot enter the Krebs cycle, and is preferentialy transaminated to alanine or transformed to lactate via lactate dehydrogenase (Figure 2). This leads to elevated lactate and organic acid levels in CSF, urine, and blood in thiamine deficient patients. Tissues with high energy demand, such as the nervous system and the heart, are highly dependent on glucose metabolism and mitochondrial function. In a state of thiamine depletion, dysfunction of oxidative phosphorylation and the consequent impaired energy productionhave been associated with cytotoxicity, cytotoxic edema and cell death resulting in several neurologic and neurodegenerative conditions [40, 41], major psychiatric illnesses [42, 43], and heart failure.

Specific brain regions show pronounced sensitivity based on their metabolism, and symptoms in patients with thiamine deficiency are a direct result of the affected structures. In Wernicke encephalopathy, ocular symptoms are the product of brainstem lesions affecting the pons and midbrain [44, 45]. Ataxia and gait difficulties arise when the cerebellar vermis is damaged. Lesions in these areas reflect high metabolic demands. Pathology examinations of the brain show bilateral symmetric hemorrhagic/necrotic lesions, mainly affecting the aforementioned areas [46].

In the condition known as 'dry beriberi' (beriberi meaning extreme weakness in the Sinhalese language), lesions firstly occur in distal peripheral nerves [47, 48]. An axonal pattern of degeneration with preservation of small myelinated and unmyelinated axons is observed [47]. Degeneration of neurons in the spinal cord, mainly of the anterior horn cells and posterior ganglion cells, is also present. In wet beriberi, the heart is dilated, and cardiac myocytes show edema, fragmentation and vacuolization.

Table 1. Symptoms and syndromes associated with thiamine deficiency

	Adult	Child
General symptoms	nausea – abdominal discomfort, apathy, fatigue, sleep disturbance	anorexia, colics, vomiting, ataxia, obtundation
Clinical syndromes		
Infantile beriberi		loud piercing cry, vomiting, constipation, peripheral edema, tachycardia, agitation, nystagmus, obtundation
Adult beriberi	Wet: peripheral edema, tachycardia, dyspnea	
	Dry: lower limb paresthesias, calf pain, muscle cramps, foot drop, lower limb weakness	
Wernicke encephalopathy	opthalmopllegia, ataxia, confusion	
Korsakoff syndrome	amnesia (episodic memory), confabulations, apathy, irritability	

4. Clinical presentation

Thiamine deficiency has a variable presentation in children and adults (Table 1). In children, a broad range of neurologic symptoms can be present early in the course of the disease, such as anorexia, vomiting, colic pain, irritability, muscle pains, ataxia and reduced level of consciousness [49]. In adults, thiamine deficiency presents with atypical symptoms in early stages, such as nausea, apathy, fatigue, sleep disturbance, and abdominal discomfort [38]. Later, if the deficiency is not corrected, classical constellations of symptoms specific for thiamine deficiency arise. These can be classified into two categories, beriberi and Wernicke encephalopathy.

4.1. *Beriberi*

There are two types of this disorder, depending on the age of the patient affected, infantile beriberi and adult beriberi.

4.2.1. *Infantile beriberi*

Infantile beriberi presents in the age between two and three months and mainly affects breastfed infants of mothers with thiamine deficiency [50].The presenting symptom is often a characteristic "loud piercing cry" and gastrointestinal dysfunction with colics, vomiting and constipation [49]. Later signs of congestive heart failure appear with edema, tachycardia, dyspnea, cyanosis and pulmonary hypertension [51, 52]. A fulminant form of infantile beriberi is known as Shoshin syndrome and presents with severe decompensated heart failure and type B lactic acidosis [53]. In older infants, four to seven months-old, the presentation differs and neurologic symptoms, such as agitation, an aphonic (soundless) cry, nystagmus, altered consciousness, and seizures can be present [49]. This ''aphonic'' form later evolves into heart failure. Treatment with parenteral thiamine is curative, with most patients responding to doses of 100-150mg of thiamine within days [49].

4.1.2. *Adult beriberi*

Adult beriberi is classically described as having two phenotypes, dry beriberi characterized by peripheral neuropathy, and wet beriberi characterized by heart failure with or without peripheral neuropathy. In most patients, a clear phenotype cannot be determined and mixed presentations are more common [54].

Thiamine deficiency with neurologic symptoms is termed dry beriberi. It occurs most often in patients with low caloric and thiamine intake and relative inactivity. Most patients present with a distal symmetric sensorimotor neuropathy, with reduced deep tendon reflexes [55]. Symptoms of dry beriberi begin with paresthesias of the lower limbs, a burning sensation of the feet, calf pain and muscle cramps. Loss of vibration sense can be present, as well as calf tenderness and proximal weakness. Later, loss of ankle and knee jerks, toe and foot drop and loss of vibration and position sense ensue. The arms follow after the lower limbs are completely affected [38]. The occurrence of cerebral symptoms is most common in severe thiamine deficiency associated with alcoholism and is termed Wernicke encephalopathy.

Cardiovascular involvement in thiamine deficient patients is termed wet beriberi [56]. Wet beriberi is initially characterized by a high cardiac output state, due to peripheral vasodilation. Neurohormonal changes lead to water and salt retention, fluid overload and peripheral edema [57]. Increased heart workload leads to overuse injury and symptoms of

decompensated heart failure. If thiamine deficiency is corrected without cardiac function optimization, a state of low output heart failure can occur. Thiamine administration in this state leads to reversal of vasoconstriction and increased cardiac workload.

4.2. *Wernicke encephalopathy & Korsakoff syndrome*

Wernicke encephalopathy (WE) and Korsakoff syndrome (KS) represent different stages of thiamine deficiency. Wernicke encephalopathy is an acute and treatable disorder, whereas Korsakoff syndrome is chronic and irreversible.

4.2.1. *Wernicke encephalopathy*

WE is an acute neurophychiatric disorder presenting with the characteristic clinical triad of opthalmoplegia, ataxia, and confusion [58]. Most patients present with delirium and the presence of all three symptoms in a patient is rare; inonly 16% of patients [58-60]. The initial symptoms of WE are non-specific, such as frequent headaches, upper gastrointestinal symptoms, mood changes, and fatigue. In younger patients with WE after gastric surgery, the disorder presents with sensory motor symptoms, mainly ataxia, and the classic triad is much more common than classically described (54% vs 16%) [61].

Ocular movement abnormalities are the hallmark of Wernicke encephalopathy. Nystagmus is especially characteristic, followed by sluggish pupillary light reaction, anisocoria and gaze palsies [62, 63]. The nystagmus in patients with WE is usually of the horizontal gaze-evoked type [64], initially brief and non-sustained, later sustained without deficits in gaze-holding, and finally accompanied by gaze-holding failure. The next most common ocular abnormality is bilateral abducens palsy, followed by conjugate gaze palsy, usually horizontal and less commonly vertical [65, 66]. Complete opthalmoplegia is the last common stage of all ocular motor abnormalities in WE and, while considered a core sign, it is rare [67]. Other ocular abnormalities have also been described, such as gaze-holding failure/impairment of the vestibule-ocular reflex [68], primary position upbeat or downbeat nystagmus [62], light-near dissociation, anisocoria and blepharoptosis [69].

Gait ataxia is another common manifestation of WE. The stance of patients is broad-based, with the gait difficulty ranging from mild to gross inability to walk. The gait ataxia of WE is the consequence of combined lesions in the cerebellum, the vestibular apparatus, and the ensuing polyneuropathy [70]. As in dry beriberi, peripheral neuropathy of the symmetrical sensorimotor type involving the lower extremities can develop over time.

Symptoms of alcohol withdrawal are also common among patients with WE. Hyperactive delirium can be present and is attributed to both thiamine deficiency and alcohol withdrawal. Severe depression of consciousness can also be present in a minority of patients and in these patients, the recognition of thiamine deficiency is crucial. Concurrent hypothermia and hypotension are warning signs of thiamine deficiency [38].

The clinical presentation, as described, is commonly non-specific, making the diagnosis extremely difficult. The combination of opthalmoplegia, ataxia, and alteration of consciousness may even mimic posterior circulation ischemia [71]. WE should be considered in every patient with long term malnutrition, bariatric surgery or chronic alcohol abuse, and episodes of confusion and altered mental status. In many cases symptoms of alcohol toxicity overlap with WE, confounding the clinical presentation.

There are no specific laboratory tests and the diagnosis remains clinical and based on the physician's suspicion [72]. However, erythrocyte transketolase level and blood thiamine concentration may be used. The latter is not a valid method, since the blood concentration of thiamine represents only a minimal fraction of the total body stores. Urinary excretion is also a poor indication of thiamine stores, being dependent upon preceding thiamine ingestion. In contrast, erythrocyte transketolase activity is a sensitive assay. The method used involves an estimation of the *in vitro* activity of erythrocyte transketolase before and after thiamine addition [72]. Increase in the measured activity of this enzyme upon addition of thiamine is a sensitive sign of deficiency [73, 74]. Samples of whole blood, washed erythrocytes in EDTA or lithium heparin-containing tubes or hemolysates can be used and stored at 4 degrees C for 24h. If immediate analysis is not possible, the various samples can be kept at lower temperatures (-20 or -70 degrees) for longer periods of time. Samples of washed erythrocytes from fasting patients are preferred [75]. Pyruvate concentrations, in whole blood or plasma, have also been used as a marker of thiamine status. Elevated concentrations of pyruvate are indicative of thiamine deficiency, but the many false positives of this assay make it difficult to use in a clinical setting. Waiting for a laboratory diagnosis is not a valid option and it is imperative to initiate treatment upon suspicion of WE [38]. The response to treatment can in most cases confirm the diagnosis [73, 74].

Lumbar puncture and cerebrospinal fluid examination is sometimes performed in patients with symptoms of Wernicke encephalopathy, mainly to rule out an infectious etiology. CSF shows normal or slightly elevated protein content without pleocytosis. A case series in 2008 showed that the CSF of patients with acute WE can show elevated total tau, decreasing

after the acute stage of the disease and reflecting acute neuronal damage [76].

Magnetic resonance imaging (MRI) is usually performed in patients with symptoms of WE. While helpful in ruling out alternative diagnoses, MRI is not adequately sensitive to exclude WE. On MR imaging, patients with WE usually have evidence of symmetric T2/FLAIR hyperintensities in the dorsmedial thalami, the tectal plate, the periventricular area of the third ventricle, the periaqueductal grey matter, the floor of the fourth ventricle, and the mammillary bodies [71, 77-79]. Enhancement of the mammillary bodies after gadolinium administration is also common [80]. FLAIR weighted imaging shows signs of both vasogenic and cytotoxic edema, with DWI showing high signal with concurrent ADC hypointensity [77]. Non-alcoholic patients may have different or atypical findings and MR imaging, similar to metronidazole induced encephalopathy [78]. In this patient group, infratentorial lesions are more common, with abducens, facial, vestibular, and hypoglossal nerve nuclei and cerebellum signal-intensity alterations [78].

Electroencephalography is not particularly useful in the diagnosis of WE. EEG changes parallel the severity of the encephalopathy. Diffuse background slowing, followed by low-voltage theta and delta activity predominantly over the fronto-temporal brain regions are commonly present. Seizures and epileptiform activity is rare in adults [81].

The clinical diagnosis of WE is based on the criteria proposed by Caine et al. in 1997. If two of the following are present a diagnosis of WE can be considered: 1. history of dietary deficiency 2. oculomotor abnormalities 3. cerebellar dysfunction 4. memory impairment/altered mental state. These criteria have a sensitivity of 85% and a specificity of 100% [72]. In non-alcoholic patients the sensitivity and specificity of these criteria is not known, and a high-index of suspicion for thiamine deficiency and Wernicke-Korsakoff syndrome needs to be maintained in vulnerable populations.

4.2.2. *Korsakoff syndrome*

KS is a late neuropsychiatric manifestation of WE. It is characterized by an abnormal mental state, in which episodic memory is affected out of proportion to other cognitive functions [82, 83]. KS is a residual, largely irreversible syndrome in patients who suffered WE and did not receive treatment with thiamine supplementation [84]. In a small subset of patients, an acute episode of WE is not recognized, but characteristic WE lesions are present in their brains [85]. KS is much more common in alcohol-associated WE, reflecting the neurotoxic effects of alcohol [86].

While KS is primarily considered a disorder of memory, most patients present with a constellation of cognitive and behavioral symptoms. The severe memory impairment of KS relates to declarative memory. Episodic memory, related to events and semantic memory, related to facts are both affected, with the anterograde memory system is affected more than the retrograde one [87]. In some patients, remote memory is also affected and remote memory loss can extend back many years [88]. Executive dysfunction is also present in patients with KS, with defective planning, concept shifting, response generation and awareness of cognitive dysfunction [89, 90]. Confabulations, false memories that fill gaps in memory, are characteristic of KS [91]. Apathy, irritability, euphoria and affective instability are also considered common in KS. MRI studies of patients with KS can be normal or reveal grey and white matter atrophy, especially of the medial thalami and mammillary bodies [92].

5. Genetic associations

Genetic susceptibility in the development of symptoms of thiamine deficiency has been widely hypothesized. This hypothesis is triggered by the fact that Europeans with thiamine deficiency most commonly develop Wernicke-Korsakoff syndrome, while Asians develop symptoms of beriberi. The potential role of genes SLC19A2 and SLC19A3 has been reported, but more studies are needed to confirm this association [93].

Mutations in genes involved in thiamine metabolism result in symptoms of thiamine deficiency, despite a thiamine rich diet. These inborn errors of metabolism, caused by mutations in genes encoding proteins implicated in thiamine transport and metabolism lead to syndromes knows as thiamine metabolism dysfunction syndromes (Table 2). Currently, there are four genes implicated, SLC19A2, SLC19A3, SLC25A19, and TPK1. These gene defects produce five distinct phenotypes, termed thiamine dysfunction syndromes 1 through 5 [27].

5.1. *Thiamine metabolism dysfunction syndrome 1*

This syndrome, also known as thiamine responsive megaloblastic anemia (TRMA) or Roger's syndrome, is caused by mutations in SLC19A2. Symptoms develop in homozygotes or compound heterozygotes. SLC19A2 is a gene located in chromosome 1 and encodes thiamine transporter 1 (TTR-1), a high-affinity saturable thiamine transporter [94]. Roger's syndrome is a rare disorder, with almost all cases found in children of consanguineous partners [95].

TRMA can manifest anytime between infancy and adolescence. Affected individuals present witha clinical triad of megaloblastic anemia, non-autoimmune diabetes mellitus and sensorineural deafness [96]. Additionally, patients may experience a multitude

Table 2. Inborn errors of thiamine metabolism (thiamine metabolism dysfunction syndromes)

Thiamine metabolism dysfunction syndrome	Other terms	Gene	Age at onset	General symptoms	Neurologic symptoms
1	Roger's syndrome or thiamine responsive megaloblastic anemia	SLC19A2	6 weeks to adolescence	megaloblastic anemia, diabetes mellitus, short stature, retinopathy	Sensorineural deafness, seizures, ataxia, neurodevelopmental delay, recurrent strokes
2	Biotin- or thiamine responsive encephalopathy type	SLC19A3	infancy to adulthood		Leigh syndrome: failure to thrive, respiratory distress, seizures, lethargy
					Childhood encephalopathy: confusion, dysarthria, dysphagia
					Wernicke-like encephalopathy: seizures, ataxia, nystagmus, opthamloplegia
3	Microcephaly Amish type	SLC25A19	infancy	congenital microcephaly, global developmental delay	Episodic encephalopathy with lactic acidosis, seizures
4	Bilateral striatal degeneration and progressive polyneuropathy	SLC25A19	20 months to 6,5 years-old		Episodic encephalopathy, distal weakness, dystonia, dysphagia, seizures
5	Episodic encephalopathy	TPK1	1 month to 4,5 years		Episodic encephalopathy, ataxia, dystonia, dysarthria, ataxia, seizures, opthalmoplegia

of neurologic, hematologic, endocrinologic, ophthalmologic and gastrointestinal symptoms [97]. At onset, all three characteristic findings may not be present, confounding diagnostic efforts [98]. Most patients present with diabetes and anemia in the neonatal period, requiring insulin and multiple blood transfusions. Hearing loss usually follows and requires hearing aids or cochlear implants [99].

Neurological symptoms associated with TRMA include focal or generalized epilepsy, ataxia, cognitive disorder, stroke-like episodes, spastic quadriplegia, and cerebral and optic nerve atrophy [97, 100]. Patients can present with recurrent strokes, either ischemic or hemorrhagic, usually involving the MCA territory [101, 102].

Currently, the first line treatment for TRMA is pharmacological doses of thiamine (25-75mg/d). Thiamine supplementation leads to improvements in anemia and glycemic control [99, 101]. Many children manage to discontinue insulin treatment after thiamine supplementation, but this responsivity decreases after puberty. Adults may become insulin and transfusion dependent. Hearing loss, short stature and strokes cannot be prevented by thiamine supplementation [101].

5.2. *Thiamine metabolism dysfunction syndrome 2 (biotin- or thiamine-responsive encephalopathy type)*

Thiamine metabolism dysfunction syndrome 2 is also known as biotin- or thiamine-responsive encephalopathy. It is an autosomal recessive disease caused by mutations in the SLC19A3 gene, encoding the thiamine transporter type 2 (TTR-2) protein [103, 104]. This entity was formerly known as biotin responsive basal ganglia disease and it can present with three distinct phenotypes [104]. SLC19A3 mutations most commonly present as early infantile Leigh syndrome. Infants with this phenotype present with encephalopathy, failure to thrive, feeding difficulties, lactic acidosis and respiratory distress

[105]. MRI of the brains of these patients shows either lesions limited to the basal ganglia and the perirolandic area, or diffuse brain edema and T2 hyperintensities of the cerebellum, cerebral white matter, thalami, basal ganglia and brainstem [106, 107]. Classic childhood encephalopathy is another distinct phenotype, presenting in children with initially normal psychomotor development. Mean age at onset is 3-7 years and most patients present with encephalopathy triggered by infection, trauma, vaccination or other stress. Confusion, dysarthria, and dysphagia predominate, with occasional central facial palsy or external ophthalmoplegia. If left untreated, this disorder progresses to severe cogwheel rigidity, dystonia, seizure, quadriparesis, and even death [103, 108, 109]. On the other spectrum of SLC19A3 gene mutation phenotypes lies adult onset Wernicke-like encephalopathy [110]. This disorder is characterized by acute onset of classical WE symptoms, as well as seizures, and by MRI abnormalities with diffuse leukoencephalopathy [108, 110].

Thiamine metabolism dysfunction syndrome 2 is considered a treatable disease if suspected early and biotin and thiamine supplementation starts promptly. Thiamine supplementation in these patients normalizes the metabolic abnormalities in serum (lactic acid) and urine (organic acids) by restoring thiamine levels in the cell and in the CSF [29, 30]. In some cases, radiological abnormalities are also significantly reduced by thiamine supplementation, while residual atrophy and necrosis persist in most patients [109, 111]. Biotin supplementation, on the other hand, is controversial. A recent study showed that combination therapy with biotin and thiamine was not superior to thiamine monotherapy regarding the recurrence rate, neurological sequela, or radiological abnormalities [112], as opposed to the initial description [103].

Prognosis depends on the timing of treatment initiation [27]. Infantile Leigh syndrome has a poor prognosis despite timely vitamin supplementation [107]. In the childhood and adult form, thiamine supplementation has a rapid effect on symptom control and prevents symptom recurrence [27].

5.3. *Thiamine metabolism dysfunction syndrome 3 (Microcephaly Amish type) and thiamine metabolism dysfunction syndrome 4 (bilateral striatal degeneration and progressive polyneuropathy type)*

There are two clinical phenotypes associated with SLC25A19 mutations, Amish lethal microcephaly (MCPHA) and bilateral striatal degeneration and progressive polyneuropathy [27, 113]. SLC25A19 is a gene encoding the thiamine mitochondrial carrier. The phenotype can be predicted by the mutation affecting the SLC25A19 gene. There are three missense mutations implicated, C.530G > C, which leads only to MCPHA presentations, while C.373G > A and C.580T > C4 lead to striatal necrosis and progressive polyneuropathy [27, 113, 114].

Amish lethal microcephaly is a severe autosomal recessive disorder, characterized by severe microcephaly, global developmental delay, alpha-ketoglutaric aciduria, lactic acidosis, CNS malformations (callosal dysgenesis, spinal dysraphia and lissencephaly) and encephalopathy [115, 116]. All patients reported had Amish origins and were homozygous for the same mutation (p.G177A) [117]. MRI abnormalities are present with hypoplasia of the brainstem, cerebellum and corpus callosum, as well as lissencephaly [116].

On the other hand, thiamine metabolism dysfunction syndrome 4 is a rare autosomal recessive disease, with only 5 patients reported. These patients presented with encephalopathy triggered by febrile illness, later developing polyneuropathy [27, 113]. Lactic acidosis is common in both disorders, especially during acute disease [115, 116]. Radiological studies show striatal degeneration, with bilateral symmetrical hyperintensities involving the putamen and caudate nuclei with sparing of the globus pallidum [113].

Treatment of both disorders depends on thiamine supplementation. Amish lethal encephalopathy has a poor prognosis despite treatment [116]. A mitochondrial cocktail, consisting of thiamine, co-enzyme Q10, carnitine vitamin E, vitamin C, vitamin K and riboflavin, at the same time as a high fat diet have been shown to reduce metabolic abnormalities in patients with Amish lethal microcephaly, increasing weight gain [116]. This high fat diet with concurrent low carbohydrate ingestion supports energy production through fatty acid β-oxidation and prevents lactic acidosis. Patients with thiamine metabolism dysfunction syndrome 4 treated with high dose thiamine have a better prognosis, with some improvement in weakness and prevention of further polyneuropathy progression [27, 113].

5.4. *Thiamine metabolism dysfunction syndrome 5 (episodic encephalopathy type)*

Thiamine metabolism dysfunction syndrome 5 is caused by TPK1 gene mutations, encoding thiamine phosphokinase 1 [118]. TPK1 plays an important role in thiamine metabolism, as it catalyzes the first step of the sub-pathway that synthesizes thiamine pyrophosphate from thiamine. TPK1 gene mutations lead to a defective TPK1 protein, that is either unstable or presents reduced thiamine binding, and reduced or in some patients increased enzymatic activity [119].

Patients with thiamine pyrophosphokinase deficiency present with episodic encephalopathy associated with infections or metabolic decompensation

Table 3. Treatment of thiamine deficiency disorders

Syndrome		Treatment	
Beriberi	Dry	mild: 10-20mg/day for 2 weeks severe: 20-30mg/day for 2 weeks	
	Wet	100mg/day for 2-3 weeks *heart failure treatment must precede thiamine supplementation	
Wernicke encephalopathy		Alcoholic	Non-Alcoholic
		500mg t.i.d. intravenously for 3 days followed by 250mg t.i.d. for 3 days, oral supplementation indefinitely	100-200mg/day intravenously
		* supplementation of other B vitamins and electrolytes should be considered	
Korsakoff syndrome		– alcohol discontinuation – social support/cognitive remediation – acetylcholinesterase inhibitors, memantine and methylphenidate	

Abbreviations: t.i.d: three times a day

[118]. A total of 16 patients have been reported, almost all presenting in childhood with acute encephalopathy [118-124]. Other than encephalopathy, these patients developed ataxia, dystonia, dysarthria, seizures and eye movement abnormalities. Initially, a normal psychomotor development is the norm, but after recurrent episodes of encephalopathy, psychomotor regression ensues. During the encephalopathy, elevated serum and CSF lactate, variable elevations in organic acids and low TPP levels are present [118]. Brain MRI is abnormal, with lesions developed in the cerebellum/dentate nuclei, striatum, thalamus, globus pallidus, brainstem and spinal cord [27, 123, 124].

Thiamine metabolism dysfunction syndrome 5 is a treatable disorder. When thiamine supplementation starts early, symptoms improve, normal neurodevelopment continues and MRI lesions reduce [118, 121, 123, 124]. Other treatment options like ketogenic diet showed modest or no efficacy [119, 125].

6. Treatment

Thiamine deficiency disorders, especially WE, are considered an emergency and thiamine administration should start upon diagnosis [126]. Patients with WE left untreated quickly progress to permanent neurological impairment, coma, and death [58, 127]. Undertreatment can also lead to the irreversible form of the encephalopathy, i.e. KS, in a large percentage of patients [58, 127].Treatment options for thiamine deficiency disorders are summarized in Table 3.

The ideal therapeutic regimen in patients with WE is not known. A Cochrane review by Day et al. identified only two studies regarding thiamine supplementation for WE, that fulfilled the inclusion criteria. The data from these two studies were insufficient to recommend an optimal therapeutic regimen [128]. Observational studies suggest that a dose of 100 to 200mg of intravenous thiamine per day is adequate for non-alcoholic patients with WE. In patients with alcoholic WE, thiamine supplementation should start with 500mg of intravenous thiamine three times a day [58]. If after 2-3 days no clinical response is observed, discontinuation is rational. If a clinical response is observed, continuation with a lower dose, 250mg three times a day, is recommended. Thiamine supplementation via the oral route should follow this iv regimen indefinitely. Concurrent electrolyte monitoring and supplementation is also important [129-131]. Especially magnesium, being a transketolase cofactor, potassium and other B-complex vitamins are important. Magnesium, being a transketolase cofactor, should given as 1 to 2 mL of a 50% solution of magnesium sulfate intramuscularly [38]. Other B-complex and water soluble vitamins should also be given at 5 to 10 times the recommended daily allowance for several weeks [38]. Following thiamine supplementation, recovery starts with the improvement of ocular symptoms within a few hours or days. This is followed by an improvement in gait, while delirium may take weeks to improve.Patients with KS have a less favorable prognosis, with most never recovering. Many of them need constant supervision and social support [86]. Treatment of alcoholic KS depends on abstinence [132]. Pharmacologic treatments are limited, so prevention and prompt treatment of WE before KS develops is imperative. Once KS develops, acetylcholinesterase inhibitors, memantine and methylphenidate can be tried. No controlled study is available, but anecdotal studies report efficacy [133-136]. Optimal treatment is mul-

tifaceted and incorporates medications, a balanced diet, cognitive remediation techniques and extensive psychosocial support [132].

Treatment of beriberi is also dependent on thiamine supplementation. In patients with mild polyneuropathy, 10 to 20mg/day for two weeks may be adequate to reverse the symptoms. If more severe, the neuropathy usually responds to doses of 20 to 30mg/day [39]. Treatment of wet beriberi depends first upon heart function optimization and second upon thiamine supplementation, with doses of 100mg/day recommended. Thiamine supplementation without addressing the heart failure can lead to low output states and worsening, due to reversal of vasodilation.

Inborn errors of metabolism respond to pharmacologic doses of thiamine. Their treatment has been addressed on the above passages.

In patients presenting in the ER with hypoglycemia, it is recommended that thiamine supplementation is given before or along with glucose to prevent WE. Although this is the classical teaching, this association between glucose administration and WE has not been rigorously studied, with most data coming from clinical observations and case reports [137]. Despite lack of high quality data, thiamine administration is advised before glucose in hypoglycemic patients [67]. Especially in alcoholic, malnourished and hyperemesis gravidarum patients, where thiamine deficiency is expected, 100mg of thiamine should be given concurrently. This should not delay glucose administration. Therapy with intravenous thiamine is considered safe, except for rare anaphylactic reactions [127, 138] which, however, can be prevented by proper vitamin dilutions.

Conclusions

Thiamine deficiency has a wide variety of manifestations, ranging from cardiovascular to neurologic, with often varied and non-specific presentation. Dietary thiamine deficiency was initially though exclusive to food-insecure settings, but is commonly recognized in populations of high income countries. A restrictive diet containing mainly processed food, eating disorders, alcoholism, bariatric surgery and chronic disease are common causes of thiamine deficiency. Mutations in genes implicated in thiamine metabolism also lead to symptoms of thiamine deficiency. Early identification of these disorders, as well as nutritional thiamine deficiency, is imperative. Prognosis depends on the timing of diagnosis and prompt thiamine supplementation must begin upon suspicion of thiamine deficiency. Treatment protocols differ between institutions, owning to lack of evidence-based guidelines.

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FABRY DISEASE: CLINICAL CHARACTERISTICS, DIAGNOSIS AND MANAGEMENT

Lina Palaiodimou, MD[1], Christina Zompola, MD[1], Maria-Ioanna Stefanou, MD[1], Konstantinos Melanis, MD[1], Aikaterini Theodorou, MD[1], Georgia Papagiannopoulou, MD[1], Sokratis Triantafyllou, MD[1], Michael Ioakeimidis, MD[1], Sotirios Giannopolos, MD[1], Panagiotis Kokotis, MD[2], Marianna Papadopoulou, MD[1], Alexandra Frogoudaki, MD[3], Agathi-Rosa Vrettou, MD[3], Dimitrios Petras, MD[4], Aris Anastasakis, MD[5], Georgios Tsivgoulis, MD[1, 6]

[1] 2nd Department of Neurology, National and Kapodistrian University of Athens, School of Medicine, "Attikon" Hospital, Athens, Greece
[2] 1st Department of Neurology, National and Kapodistrian University of Athens, School of Medicine, Eginition Hospital, Athens, Greece
[3] Second Department of Cardiology, National and Kapodistrian University of Athens, School of Medicine, "Attikon" Hospital, Athens, Greece
[4] Nephrology Department, Hippokration General Hospital, Athens, Greece
[5] Unit of Inherited and Rare Cardiovascular Diseases, Onassis Cardiac Surgery Center, Athens, Greece
[6] Department of Neurology, The University of Tennessee Health Science Center, Memphis, Tennessee, United States of America

Abstract

Fabry disease (FD) is an X-linked, lysosomal storage disorder characterized by the decreased activity of the lysosomal enzyme, alpha-galactosidase A (α-Gal A), related to mutations in the GLA gene (Xq21.3-q22). Deficient enzyme activity results in the accumulation of neutral glycosphingolipids and globotriaosylceramide (Gb3) in the plasma and cellular lysosomes of different tissues and organs throughout the body. Multi-system manifestations, such as progressive renal failure, cardiac disease, cerebrovascular disease, small-fiber and large-fiber peripheral neuropathy, and skin lesions, among other abnormalities complete the phenotype of FD. Diagnosis is based on the finding of reduced α-Gal A activity in leukocytes or plasma or the detection of Gb3 accumulation in plasma, urine or biopsy specimens, while it is confirmed by molecular genetic testing. Regarding the disease prognosis, end-stage renal disease and life-threatening cardiovascular or cerebrovascular complications limit the life-expectancy of untreated males and females compared to the general population. However, disease-specific treatments are currently available, including enzyme replacement therapy and small-molecule chaperone treatment. In adjunction to symptomatic management, these therapeutic options have the potential to modify the disease course and halt progression. Furthermore, other treatments such as substrate reduction therapies, mRNA-based therapies and gene therapies are being developed and tested in clinical trials. In this era when available FD-specific treatment options are actively expanding, increased clinical suspicion and prompt and accurate diagnosis of FD are critical for the early initiation of individualized treatment and change in the prognosis of FD patients.

Key words: Fabry disease, alpha-galactosidase A, globotriaosylceramide, enzyme replacement therapy, chaperone treatment

Introduction

Fabry disease (FD) is an X-linked metabolic disorder characterized by decreased activity of the lysosomal enzyme, alpha-galactosidase A (α-Gal A), related to mutations in the GLA gene (Xq21.3-q22) [1]. Deficient enzyme activity results in the accumulation of neutral glycosphingolipids and globotriaosylceramide (Gb3) in the plasma and cellular lysosomes of different tissues and organs throughout the body [2]. Hence, FD is categorized as a lysosomal storage disorder [3] with multi-system manifestations, such as progressive renal failure, cardiac disease, cerebrovascular disease, small-fiber and large-fiber peripheral neuropathy, and skin lesions, among other abnormalities [4].

FD is considered a rare disease (OMIM: # 301500) with an estimated annual incidence of 1 in 80,000 live births [5]. However, true prevalence might be underestimated due to later-onset subtypes of FD and under-diagnosed cases. Despite being highly variable among different countries, a prevalence of approximately 1 in 3,000 individuals has been suggested [2]. Diagnosis is based on the finding of reduced α-Gal A activity in leukocytes or plasma and is confirmed by molecular genetic testing [6]. Regarding the disease prognosis, end-stage renal disease and life-threatening cardiovascular or cerebrovascular complications limit the life-expectancy of untreated males and females with reductions of 20 and 10 years, respectively, versus the general population [2].

However, disease-specific treatments are currently available, including enzyme replacement therapy and small-molecule chaperone treatment. In adjunction to symptomatic management, these therapeutic options have the potential to modify the disease course and halt progression [7]. Furthermore, other treatments such as substrate reduction therapies, mRNA-based therapies and gene therapies are being developed and are currently being tested in randomized-controlled clinical trials [7].

In this narrative review, we aim to present the pathophysiology of FD, outline the main clinical characteristics that FD patients may exhibit and discuss the diagnostic and management considerations in FD.

1. *Pathophysiology*

Fabry disease is caused by the deficiency of α-Gal A, a lysosomal enzyme encoded by the GLA gene on the X-chromosome (region Xq22.1). Almost 1,000 mutations in the GLA gene have been described including missense and nonsense mutations [8]. The list of FD-associated mutations is continuously expanding, since novel GLA mutations or mutations that were previously considered non-pathogenic are being described and associated with FD diagnosis [9]. A potential limitation in the characterization of these mutations is the fact that many of them are private, being isolated in certain families only, further complicating the genotype-phenotype correlation [10].

Male patients carrying a FD-associated mutation in their single X-chromosome (hemizygous mutation) are expected to be impacted more severely and manifest FD-associated symptoms in a more characteristic manner, resulting in the "classic" presentation of symptoms. Female carriers may also be affected to a lesser extent, manifesting milder or atypical symptoms [11]. Inactivation of the wild-type GLA allele in heterozygous female patients is considered the most probable cause for disease manifestation [12, 13]. GLA-homozygous female patients have also been described in the literature, but are considered extremely rare [14, 15]. Therefore, female sex should not be considered as an exclusion criterion for disease diagnosis. Characteristically, the term "X-linked recessive" in the description of FD inheritance has been vigorously questioned and should better be replaced by the more general term "X-linked inheritance" to include the cases of carriers that manifest disease symptoms.

The normal function of α-Gal A is to participate in lipid metabolism by hydrolyzing and removing terminals α1, 3- and α1, 4-linked galactosyl residues from various glycoconjugates within lysosomes [16]. When α-Gal A is deficient, the primary substrate Gb3 cannot be digested and accumulates withing the lysosomes. Another deacylated form of Gb3 is the globotriaosylsphingosine (lyso-Gb3) which also contributes to disease pathogenesis, but, importantly, serves as a biomarker for FD diagnosis and treatment monitoring [17, 18]. Other glycoconjugates that accumulate –to a lesser extent compared to Gb3– in FD are digalactosylceramide and blood group B- and P1- glycosphingolipid antigens.

The association between Gb3 accumulation and disease manifestation seems to be dependent not only on lysosome function; other cellular organelles and mechanisms are implicated as well. Gb3 also accumulates within the plasma membrane of α-Gal A-deficient cells and potentially alters the function of various membrane proteins and channels [19, 20]. Mitochondrial and endoplasmic reticulum function is affected as well in FD, either directly through Gb2 accumulation or indirectly through impairment of the autophagic flux [21, 22]. Downstream results implicating fibrosis [23], inflammation [24] and increased oxidative stress [25], all contribute to the pathogenesis of the disease and its systemic manifestations.

2. *Clinical Characteristics*

FD has long been considered as an adult disease, predominately affecting male patients [2]. However, this notion could not be further from the truth. Indeed, significant morbidity in childhood has been associated with FD, even in the absence of major organ dysfunction [26]. Furthermore, both atypical symptoms and milder phenotypes exist, affecting female carriers. All organs may also be affected to varying extent, resulting in a phenotypic spectrum of disease manifestations. Characteristic symptoms of FD include neurological, cutaneous, renal, cardiac and cochleo-vestibular manifestations and are summarized in Table 1.

2a. Neurological Manifestations

Peripheral Nervous System

Neurological manifestations in FD can be observed even from an early stage of the disease, often during childhood. Those early FD neurological symptoms mostly implicate the peripheral nervous system, involving small nerve fibers of the peripheral somatic [27] and autonomic nerve systems [28].

The neurological symptoms most commonly experienced in childhood include neuropathic pain, gastrointestinal dysmotility, hypohidrosis and heat intolerance [29]. Neuropathic pain, which has been reported during childhood by 59% of males (median age 7 year) and 41% of females (median age 9 year) in a registry-based study [30], may manifest as acroparaesthesias or acute pain crises or a combination of both [31]. Symptoms, such as chronic neuropathic pain, acroparaesthesias or perspiration disorders may

Table 1. Organ-specific involvement and manifestations of Fabry Disease

Organ	Involvement %	Manifestations
Peripheral Nervous System	45%	Neuropathic pain Acroparaesthesias Acute pain crisis Thermal sensation deficits Perspiration disorders Gastrointestinal dysmotility Median nerve entrapment Cramp-Fasciculation Syndrome
Central Nervous System	34%	Ischemic Stroke Transient Ischemic Attack Transient Ischemic Attack Vascular Dementia Hemorrhagic Stroke
Kidney	45%	Microalbuminuria Proteinuria Progressive renal failure
Heart	68%	Left ventricular hypertrophy Right ventricular hypertrophy Congestive heart failure Arrhythmias Myocardial ischemia
Skin	34%	Angiokeratomas Lymphoedema Hoarse facial features
Eye	38%	Cornea verticillata Cataract
Ear	19%	Hearing loss Vestibular disturbances
Miscellaneous		Chronic cough Osteopenia Anemia Azoospermia Hypothyroidism

be under-reported by the patients. Therefore, during medical history investigation, patients should be specifically asked for those symptoms. Additionally, although these symptoms are not life-threatening, they clearly impact the health and quality of life of affected children [26, 32].

Peripheral neuropathy is a typical manifestation for adult FD patients, as well. Chronic, burning pain and superimposed attacks of severe pain, when temperature changes or other stressful situations occur (such as infectious illnesses or surgery), usually develop by the age of 20 years in 60-80% of patients with FD [33]. Thermal sensation deficits also occur and are more pronounced in the feet than in hands in a length-dependent manner [34]. Initially, the thermal sensation impairment involves cold perception, and only later warmth perception is diminished [35]. Accumulation of lipids in dorsal roots ganglion cells [36] and autonomic ganglia [37] may be accountable for these symptoms, via decompensation of Ad-and C-fiber function and absolute reduction in the intraepidermal nerve fiber density [38, 39]. Additionally, Gb3 accumulation within the endothelial cells of the vasa nervorum may precipitate thrombotic complications through laminal encroachment and occlusion and contribute to ischemic nerve damage [40]. Over time, the positive neuropathic symptoms seem to ameliorate, probably due to reduction of axonal sensory hyperexcitability and central sensitization, with disappearance of pain and progression to numbness and hypoalgesia.

Autonomic nerve dysfunction as part of the

small-fiber neuropathy may manifest with a variety of sympathetic and parasympathetic-associated symptoms. Gastrointestinal dysmotility including abdominal cramps, diarrhea and nausea is one of the most frequent and early general complaints among FD patients, affecting up to 52% of adult patients [41, 42]. Reduced tearing and saliva production may be also associated with autonomic disturbance in FD [28]. Impaired pupillary constriction with pilocarpine may also be apparent [28]. Perspiration disorders, including both hyperhidrosis and hypohidrosis, are characteristic for FD, even from the early stages of the disease, highlighting the involvement of autonomic nervous system [43]. Specifically in the case of hypohydrosis, pathogenesis lies not only to the autonomic neuropathy but partly to the sweat gland dysfunction due to Gb3 proliferation [44, 45]. Other autonomic-related symptoms include reduced increase in heart rate and blood pressure upon exercise and, less often, orthostatic hypotension [46]. However, cardiovascular autonomic involvement is considered rare in FD, while other end-organ manifestations may also significantly account for those cardiovascular decompensations [47].

Large nerve fiber dysfunction is much less common in FD [27, 48]. Motor function, position and vibration sensation are typically not affected until the later stages of the disease, through GL-3-induced cell dysfunction adding to the already negative influence of age per se in the large fiber integrity [27]. Other neurotoxic mechanisms, such as uremia due to FD-associated renal disease, may account as well for the development of large nerve fiber abnormalities.

On the other hand, median nerve is more commonly involved in FD, with a reported prevalence of carpal tunnel syndrome of approximately 25% for both male and female patients. Gb3 accumulation within the carpal tunnel structures is implicated in the pathogenesis of median nerve entrapment [49].

Cramp-fasciculation syndrome without apparent small-fiber neuropathy is another interesting and more atypical manifestation of peripheral nervous system involvement in FD, further expanding the spectrum of later-onset FD [50]. Although a clear pathologic association was not demonstrated in this reported case, the authors postulate that Gb3 deposits along the motor neuron or portions of the motor axons may account for this, otherwise unexplained, manifestation of lower motor neuron hyperexcitability [50].

Central Nervous System

The hallmark of central nervous system involvement in FD is cerebrovascular disease as a result of Gb3 accumulation in the vascular endothelium and smooth muscle cells of the small blood cerebral vessels [51, 52]. Other factors, such as the presence of a prothrombotic state, autonomic dysregulation and increased production of reactive oxygen species or even FD-associated cardiac arrhythmias, may also be implicated in the pathogenesis of cerebrovascular disease in affected patients. Cerebrovascular complications in FD may include transient ischemic attacks, ischemic strokes of multiple underlying mechanisms, intracerebral hemorrhage, subarachnoid hemorrhage, and vascular dementia [53, 54]. According to the Fabry registry, 6.9% of male patients and 4.3% of female patients were complicated with stroke, at a median age of 39 and 45.7 years, respectively [55]. Importantly, a significant proportion of patients may experience stroke as the first manifestation of FD disease, before experiencing any cardiac or renal events [55]. Therefore, screening for FD during etiopathogenetic investigation of stroke in young patients might be reasonable [56, 57]. In a cohort of 721 patients with cryptogenic stroke, 3.9% were diagnosed with FD [58]. In this cohort, proteinuria and infarction in the vertebrobasilar artery system were shown to be associated with FD diagnosis [58]. Dolichoectasia of the basilar artery, which is a characteristic finding of FD, may account for the high prevalence of strokes in the posterior circulation [59]. Screening gains even more clinical importance, when considering the high rates of stroke recurrence (76-86%) and the poor prognosis (mortality rate between 40-55%) of these patients [53].

Although clinically silent, white matter hyperintensities are the most common central nervous system manifestation of FD. This neuroimaging finding has been reported in 46% of FD patients, with a similar prevalence between male and female patients [60]. Cerebral white matter pathology has further clinical correlations as well, being associated with cognition and disease severity [61]. Even before apparent white matter hyperintensities in brain MRI are demonstrated, alterations of cerebral blood flow velocity are detected by transcranial doppler sonography in the posterior circulation of FD patients compared to controls [62]. Thus, cerebral hemodynamic changes detected by neurosonology may be used as a potential biomarker for the preclinical detection of neurovascular involvement in FD [62].

Another important neuroimaging finding of FD is the pulvinar sign, defined as T1-weighted symmetric hyperintensities in both lateral pulvinars. This sign is suggestive of dystrophic calcification, secondary to cerebral hyperperfusion and selective vulnerability of the pulvinar and adjacent thalamic nuclei [63]. Although previously considered pathognomonic, the prevalence of pulvinar sign is relevantly low, presenting in 3% of the FD patients among a retrospective cohort, and may also be found in other abnormal conditions, mostly when concomitant renal dysfunc-

tion is noted [64]. In fact, FD-associated pulvinar sign has been correlated with male sex and serious complications, such as hypertrophic cardiomyopathy and severe kidney involvement [65].

Apart from ischemic stroke, which is well recognized as a FD-associated complication, intracerebral hemorrhage has also been described in both classic and atypical FD patients [66]. Vessel wall remodeling and degeneration, in addition to arterial hypertension, have been associated with this dreadful manifestation. However, hemorrhagic strokes account for only 16.9 and 6.9 of all strokes in male and female FD patients, respectively [55]. Cerebral microbleeds may also be present in FD patients, with a prevalence ranging from 11% to 30% in different cohorts [67, 68]. These are predominately deep chronic microbleeds, significantly associated with white matter hyperintensities, underscoring the pathogenicity of small vessel vasculopathy for both manifestations.

Vessel fragility and possibility of rupture leading to intracerebral hemorrhage have been a matter of debate regarding the safety of recanalization therapies for acute ischemic stroke in FD patients. Despite scarcity of data, relevant published cases are in favor of intravenous thrombolysis in FD-associated stroke [69-71].

An uncommon manifestation of FD, resulting in cerebrovascular disease, is cervical artery dissection [72, 73]. Gb3 accumulation within the vessel walls renders the cervical arteries fragile and prone to dissection, even without a prominent traumatic history [74].

2b. *Renal Manifestations*

Renal involvement in FD is one of the most important causes of morbidity and mortality of the patients. The basis of the pathophysiology of renal manifestations in FD is the Gb3 depositions in the glomerular endothelium, mesangial and interstitial cells, podocytes, epithelium of the loop of Henle, as well as in the endothelial and smooth muscle cells of the renal arterioles [75, 76]. Initially, renal dysfunction may be masked by glomerular hyperfiltration, but with advancing age and Gb3 continuous accumulation, the existing nephrons cease to be able to adequately compensate for those affected, resulting in apparent kidney failure.

Microalbuminuria and proteinuria are the first signs of renal involvement in FD, typically manifesting during the second or third decade [77]. These have been shown to be present in 44% of male and 33% of female patients [78]. Furthermore, urinary protein excretion is considered to directly contribute to the progression of the Fabry nephropathy and is significantly associated with both systolic blood pressure [79] and renal disease progression in both sexes [80].

Fibrosis, sclerosis and tubular atrophy typically follow in the third to fifth decades of life, leading to rapid progression of FD-associated nephropathy and end-stage renal failure [81]. Even when chronic hemodialysis is undertaken, mortality in FD-associated nephropathy has been shown to be higher compared to other standard nephropathies, with a 5-year survival rate of 41% in FD patients compared to 68% in other pathologies [82, 83]. Furthermore, the severity of chronic kidney disease is also closely related to cardiovascular disease progression, including left ventricle hypertrophy, arrhythmias and sudden death [84]. Characteristically, according to the findings of the Fabry registry, 57% of the patients who died due to cardiovascular causes had previously received renal replacement therapy [85].

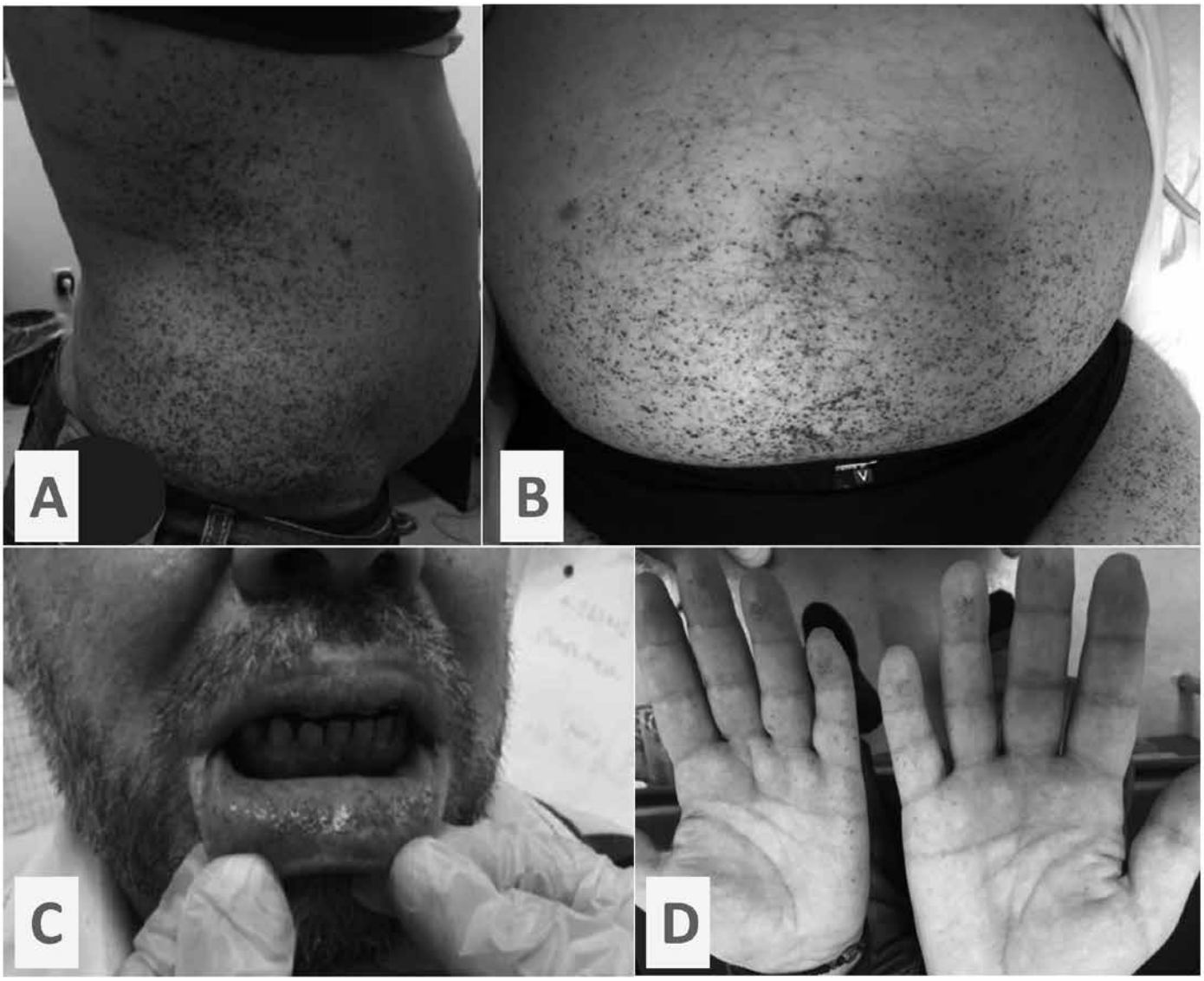
2c. *Cardiac Manifestations*

Cardiac involvement in FD may manifest with structural changes, such as left ventricular hypertrophy, arrhythmia and electrocardiographic abnormalities, and less often with coronary artery disease [86-88]. Cardiovascular system is involved in approximately 40-60% of FD patients [81].

Diastolic dysfunction and concentric left ventricular hypertrophy without left ventricular outflow tract obstruction are the most characteristic signs of cardiac involvement in FD [89]. Systolic function seems to be preserved until the later stages of disease. Septum thickness is another important finding to recognize in FD-related cardiomyopathy, since the posterior wall is more commonly affected by the fibrosis [90]. Congestive heart failure may occur at the end stage of FD [91, 92]. Right ventricular structural changes were previously considered less common. However, right ventricular hypertrophy is found in almost 40% of FD patients, equally affecting both genders [93].

Structural changes and remodeling, together with autonomic disturbance, may lead to prominent electrocardiographic abnormalities in FD patients, including a short PR interval, ST segment depression, prolonged QRS and QT intervals, atrioventricular blocks, bundle branch blocks, intermittent supraventricular tachycardia and other arrhythmias [94]. Excessive Gb3 may also accumulate in the endothelium of coronary arteries, leading to abnormal coronary microvascular function [95]. As a result, reduced exercise capacity, angina, myocardial ischemia and infarction may present as FD-associated complications [95, 96]. Arrhythmias and myocardial infarction may be the cause of sudden death in a significant proportion of patients [97]. Therefore, prompt diagnosis and management are important. Aortic root dilatation has also been demonstrated in 24% of affected male patients and was found to be associated with basilar dolichoectasia [2].

Figure 1. Patients with diagnosed Fabry disease presenting angiokeratomas in typical locations, such as the abdomen and thighs (Panel A & B), the inner part of the lips (Panel C) and the palms (Panel D). Angiokeratoma corporis diffusum is characteristic for Fabry disease and presents as small, red-to-purple, raised skin lesions, which are superficial angiomas due to excessive deposition of Gb3 in the vascular endothelial cells of the skin



Atypical variants of FD restricted to cardiac involvement, hence the "cardiac variant", have also been reported in the literature [98, 99]. In patients with this variant, left ventricular hypertrophy and other cardiac manifestations present during middle age, without any other signs of FD [100]. Relatively high residual α-Gal A activity, as well as certain mutations have been shown to be associated with this cardiac phenotype [101].

2d. *Dermatological Manifestations*

A characteristic skin lesion that manifests early in the course of FD is angiokeratoma corporis diffusum [102]. Angiokeratoma presents as small, red-to-purple, raised skin lesions, localized in the buttocks, groin, thighs, abdomen (i.e., the "swimsuit area") and mucosal areas as well, such as the inner part of the lips or the genitals (Figure 1) [103]. These lesions are actually superficial angiomas due to excessive deposition of Gb3 in the vascular endothelial cells of the skin. The superficial dilated capillaries are also accompanied by epidermal proliferation. Angiokeratomas are benign and are found in 83% of males and 80% of females with FD [104]. With progressive age, they tend to increase in number and size. Nevertheless, angiokeratomas are not pathognomonic for FD, since they may appear in other diseases as well, such as hereditary haemorrhagic telangiectasia or Fordyce's angiokeratoma [105].

Another cutaneous manifestation is lymphoedema, which was firstly described in the original description of FD by Anderson [106]. Although heart and renal impairment may partly account for this manifestation, actual Gb3 deposition within the cells of lymphatic vessels actually occurs, leading to the lymphatic microangiopathy [107]. Dysfunction of the lymphatic circulation subsequently leads to lymphoedema [108].

Facial dysmorphia may also be present in FD, as in other lysosomal storage disorders. Although not striking, hoarse facial characteristics, with prominent supraorbital ridges, frontal bossing, broad nasal base and thickening of the lips, can be found in males and to a lesser extend in female FD patients [109, 110]. The exact pathophysiology of facial dysmorphisms remains yet unclear; however, Gb3 accumulation within the growing facial bones and connective tissues may probably be a putative link between FD and abnormal facial appearance [109].

Other dermatological manifestations are acroparaesthesia and perspiration disorder have been previously described under FD-associated peripheral nerve involvement.

2e. *Ophthalmological Manifestations*

Cornea verticillata, revealed by slit-lamp examination, is one of the most common signs of FD disease, affecting more than 70% of the patients, including both males and females [111, 112]. Cornea verticillate actually refers to vortex opacities located in the superficial corneal layers and characteristically does not affect the visual acuity of the patient. Thus, every patient with suspected FD should be evaluated ophthalmologically for the presence of ocular manifestations, even when no relevant symptoms are reported by the patients.

Another ocular manifestation is the so-called "Fabry cataract", which is defined as a posterior subcapsular cataract consisting of posterior lens opacities with a radiating appearance [113]. Fabry cataract is a more specific finding for FD compared to cornea verticillata (which can also be found in other disorders, such as chronic amiodarone administration) and can affect males with a higher prevalence compared to female FD patients [112].

Retinal and choroidal vessels are affected as well in FD due to Gb3 accumulation within the endothelial cells and may present increased tortuosity, giving a corkscrew appearance during fundoscopic examination or fluorescein angiography [114, 115].

2f. *Otological Manifestations*

Hearing loss, tinnitus and vertigo have also been reported as otological manifestations of FD [116, 117]. Both progressive and acute hearing loss may complicate FD patients, implicating both neurologic and vascular damage as the pathogenetic link [116]. Vestibular disturbances may also be present in a significant proportion of patients [118].

2g. *Other Manifestations*

FD, being a multi-system disorder, also presents respiratory manifestations, including exercise-induced dyspnea, chronic cough and airway obstruction [119, 120]. Furthermore, skeleton involvement may also be present in 50% of the cases, with either osteopenia or osteoporosis [121, 122]. Finally, rare cases have been reported with FD-associated anemia [123], azoospermia [124], priapism [125] and hypothyroidism [126].

3. Diagnosis

Significant diagnostic delays have been reported in FD, with an approximate interval of 14- and 16-years for male and female patients, respectively, between onset of symptoms and disease confirmation [78]. However, delaying diagnosis might have important clinical consequences. Irreversible damage of organs might occur, leading to a reduced response to available treatments [29]. Additionally, there is a higher risk for FD-related clinical events, such as cardiovascular or renal events, underscoring the need for early diagnosis and treatment initiation, before the onset of severe organ damage [127]. Prompt FD diagnosis requires increased awareness and clinical suspicion regarding the phenotypic spectrum of the disease. In case of suspected FD, appropriate biochemical and genetic confirmation is warranted [128]. A summary of the laboratory findings in FD, according to the phenotype, is presented in Table 2. If positive, biochemical tests are highly indicative of disease diagnosis, especially in male patients; however, diagnosis of FD is not possible without genetic confirmation for both males and females [129].

3a. *Biochemical analysis*

The activity of α-Gal A can be measured in plasma or in leukocytes and the demonstration of its reduction is the mainstay of initial diagnosis in male FD patients [130]. However, in the case of the female patients, this result can be quite inconclusive, since false negative results may be quite common [131]. Genotypic analysis is more informative regarding the female patients [132]. Several pitfalls in the measurement of α-Gal A activity in plasma may occur, mostly regarding the buffering and incubation of the specimens [133]. Therefore, measurement in the leukocytes is considered more accurate compared to plasma [134]. Examination of samples derived from dried blood spots is also quite applicable and accu-

Table 2. Summary of findings in Fabry disease

	Classical Males	Females	Non-classical Patients
Residual Enzyme Activity	Absent	Low to normal	Reduced, but not absent
Mutation	Severe (null, missense)	Carrier	Mild (missense)
Phenotype	Severe	Variable	Milder, variable
Typical symptoms, including angiokeratomas, cornea verticillata, acroparaesthesia	Present	Variable	Usually absent

rate, allowing for storage and delivery to reference laboratories worldwide [135].

Detection of Gb3 accumulation may also assist in the diagnosis of FD. This can be performed by the measurement of the deacylated form of Gb3 in the plasma or urine, called the lyso-Gb3, which has been proven an important biomarker for FD diagnosis and monitoring [136]. Liquid chromatography tandem mass spectrometry is considered the most reliable method for lyso-Gb3 measurement in the plasma [137]. Increased lyso-Gb3 values are indeed very suggestive for FD [138]. An additional important aspect is that lyso-Gb3 increase may also identify the clinically relevant GLA mutations among those with unknown significance [139]. One limitation is that normal Gb3 values cannot exclude FD, especially with regard to non-classical phenotypes or female patients. However, lyso-Gb3 measurement appears to be more useful in FD diagnosis compared to α-Gal A activity [140].

3b. *Genetic analysis*

More than 900 mutations have been described in the GLA gene. Not all of them, however, are considered pathogenetically associated with FD. Direct molecular analysis of the GLA gene is applicable using dried blood spots. High-performance liquid chromatography and Multiplex Ligation-dependent Probe Amplification are useful methods to identify point mutations and deletions in the GLA gene [15, 141]. When a pathogenetic variant is identified, FD diagnosis can be confirmed.

Novel mutations and mutations of unknown significance present as the challenge in the molecular diagnosis of FD. For example, the mutation D313Y, which was previously considered as non-pathogenic variant, has been recently associated with FD classical phenotype and disease diagnosis [9, 142]. Despite the fact that genetic testing is considered mandatory for FD confirmation, its results should be evaluated critically and in adjunction with the clinical signs of the disease and the other biomarkers as well [129].

3c. *Histopathological analysis*

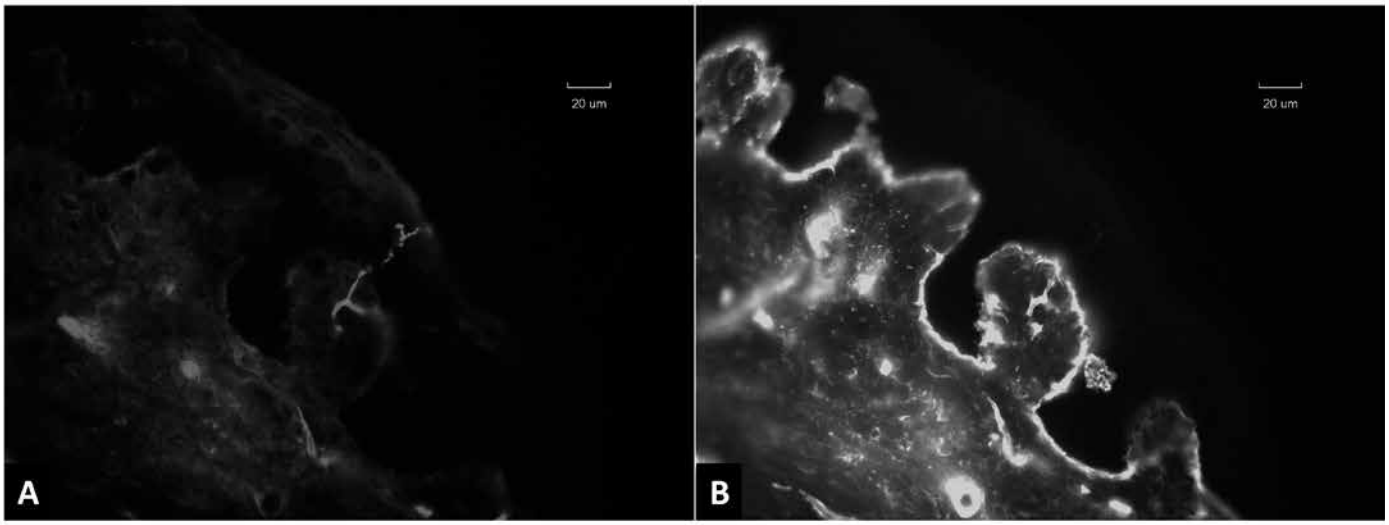
Although quite invasive, histopathological analysis of kidney or myocardial biopsies under electron microscopy may provide useful information, especially in ambiguous cases or in cases presenting novel GLA mutations or mutations of unknown significance [143]. Glomerulosclerosis and tubulointerstitial fibrosis are non-specific findings in kidney biopsies of FD patients, while Gb3 deposits in podocytes, glomerular endothelial cells, mesangial cells, tubular epithelial cells and vascular endothelial cells can also be evaluated and are more characteristic of the disease [144]. Intracellular osmiophilic, lamellar inclusions, called the "zebra bodies" are very indicative of FD, but drug-induced renal phospholipidosis may also mimic this ultrastructural appearance [145]. Lysosomal storage and "zebra bodies" may also be visualized in the cardiomyocytes during electron microscopy assay [146]. Furthermore, skin biopsies may also be used, revealing Gb3 deposits which are specific in FD patients with classical GLA mutations [147]. Importantly, the utility of skin biopsies also includes the evaluation of peripheral small fiber innervation and the involvement of peripheral nervous system as part of FD manifestations [148].

3d. *Other diagnostic tests*

During the initial diagnosis of an FD patient and throughout the entire follow-up, a variety of ancillary diagnostic tests should be performed to evaluate the involvement of each target organ.

With regards to peripheral nerve involvement, electrophysiological studies should be performed, including sympathetic skin response, thermal quantitative sensory testing, sensory and motor nerve conduction studies and electromyography [149]. Skin biopsy may also be evaluated for estimating intradermal nerve fiber density (Figure 2) [38]. Central nervous system involvement may be assessed by brain MRI, assessing for strokes, leukoencephalopathy, cerebral microbleeds or pulvinar sign [150]. Transcranial doppler ultrasonography may also early evaluate cerebral

Figure 2. Double immunofluorescence staining of a 3 mm skin specimen, collected 10 cm above lateral malleolus, showing reduced number of intraepidermal nerve fibers penetrating the basal membrane of the epidermis; magnification 40X; secondary staining with Cy3 (Panel A) and Daylight 488 (Panel B)



hemodynamic changes indicative of microvascular disruption [62].

Renal manifestations may be assessed by serum biochemical assays, evaluating plasma urea, creatinine, and age-corrected eGFR [151]. Initial and more subtle involvement may be determined by microalbuminuria and proteinuria [80]. Imaging techniques, such as renal ultrasound or MRI, may also be performed [152].

Electrocardiography and transthoracic echocardiography are mandatory when assessing an FD patient [86]. Transthoracic echocardiography reveals a rather symmetric pattern of myocardial hypertrophy, compared to the more common pattern of asymmetric hypertrophy of hypertrophic cardiomyopathy. Quite often, the patient may also need long-term rhythm monitoring for possible detection of rhythm abnormalities [153]. The mainstay of cardiac involvement evaluation is cardiac MRI that is able to detect cardiac hypertrophy and fibrosis [154]. Newer cardiac MR sequences, most notably native T1 mapping, can aid the differential diagnosis in unexplained myocardial hypertrophy that can be observed in other forms of cardiomyopathy, such as cardiac amyloidosis, hypertrophic cardiomyopathy or hypertensive heart disease [155]. As lipids shorten T1 time, intracellular cardiomyocyte accumulation of glycosphingolipids leads to characteristic shortening of native T1 mapping, in contrast to areas with fibrosis where it is prolongated. However, these scar areas are well identified on late gadolinium enhancement (LGE) images. Therefore, cardiac MR with native T1 mapping exhibits an advantage in Fabry disease, as it can help diagnose myocardial involvement in earlier disease stages without the need of contrast agent administration. Notably, iron overload, in patients with thalassemia or hereditary hemochromatosis, also shortens native T1 mapping times, however the clinical context differs between the two disease entities.

Specialized dermatologist, ophthalmologist and otolaryngologist, as part of the multidisciplinary team, should also examine a FD patient at initial diagnosis and during follow-up.

4. Management

4a. *Supportive Management*

The so far available FD-specific treatments do not cure the disease, but rather halt its progression. Thus, supportive management continues to be the mainstay of treatment. An organized and multidisciplinary approach is desirable to provide the FD patient a holistic treatment.

Adequate pain management and avoidance of a pain crisis can be achieved by the avoidance of trigger factors, such as temperature changes or physical stress. Other conservative measures of treatment include rest, holding icepacks, or administration of acetaminophen during febrile periods, However, in the cases of chronic neuropathic pain and acroparaesthesias, symptoms are more persistent and require pharmacological treatment. Carbamazepine, phenytoin and gabapentin have a proven efficacy in managing the pain in FD [156]. Serotonin-norepinephrine reuptake inhibitors and duloxetine may also be used in the management of chronic pain in FD. On the other hand, non-steroidal anti-inflammatory drugs and narcotic analgesics should generally be avoided or maybe used for the acute relief during a pain crisis [157].

Secondary stroke prevention therapy is recommended in the cases of FD-associated stroke, including antiplatelets, statins, and adequate control of other risk factors, such as arterial hypertension, diabetes mellitus or hyperhomocysteinemia [158].

Renal disease can be managed by administrating angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs), which may limit proteinuria and also control hypertension [159-161]. Dialysis and kidney transplant should be considered in the cases of end-stage renal disease [162-164].

Symptomatic anti-anginal and antihypertensive therapy is recommended in FD cardiac involvement, including calcium antagonists, ACEis's and ARBs. B-blockers should be avoided to avoid sinus bradycardia and atrioventricular block [165]. When clinically significant arrhythmias occur, cardiac pacing and cardioverter defibrillator implantation may be necessary. In end-stage heart failure, heart transplant may be a viable option [166].

4b. *Disease specific therapies*

Apart from supportive care, there has been a wide range of developments in treatment of lysosomal storage disorders in general, and in FD specifically. Different steps along the metabolic pathway have been targeted as therapeutic options, including enzyme replacement therapy and chaperone therapy, while substrate reduction therapy and gene therapy are under development (Table 3).

Enzyme Replacement Therapy

Agalsidase alfa at a dose of 0.2 mg/kg biweekly and agalsidase beta at a dose of 1.0 mg/kg biweekly are authorized FD-specific treatments, replacing the deficient α-Gal A enzyme in FD patients [167, 168]. Safety and efficacy of both drugs have been proven in randomized controlled clinical trials [169-172], as well as in investigator-sponsored studies independent from the industry [173-176].

The efficacies of agalsidase alfa and agalsidase beta are comparable [177, 178]. However, both treatments should be administered early in the course of FD, since the window of opportunity exists before irreversible damage is evident [179]. Both male and female patients should initiate treatment upon first evidence of early FD-associated clinical signs [179]. Significant reduction of neuropathic pain has been recorded in patients treated with both regimens [180]. Additionally, left ventricular mass was found to be reduced in treated patients [181]. Progression of renal disease may also be delayed during treatment [182, 183]. FD-associated major events, including renal, cardiac, cerebrovascular complications and death, were found to be lower upon treatment [184]. In addition, a recent meta-analysis comprising 7 cohort studies and 2 randomized controlled clinical trials involving 7513 participants; 1471 on ERT vs. 6042 on native treatment) showed that the stroke recurrence ratio in the ERT treatment group was 8.2% (95%CI: 3.8-12.6) and in the native-treatment group was 16.0% (95%CI: 10.2-21.7) [185]. Effect differences favored ERT treatment group over native treatment group ($p = 0.03$) [185]. However, in both treatments, no obvious effect on brain lesions was noted, possibly due to the fact that these large molecules cannot cross the blood-brain barrier [186]. Treatment effect can be sufficiently monitored through lyso-Gb3 measurement in the plasma, further underscoring the utility of this biomarker in FD [18, 187].

Safety issues during enzyme replacement therapy mostly concern moderate infusion-related reactions, such as fever, flushing and rigors, which were present in 57% and 59% of the patients during the administration of agalsidase alfa and beta, respectively [169, 170]. In general, these reactions can be managed conservatively and may also be prevented by premedication with an antihistamine, paracetamol and/or dexamethasone, and by reducing the infusion rate [188].

Another significant concern is the formation of specific neutralizing antidrug antibodies against the enzymes in about 40% of all treated patients, which may attenuate the treatment efficacy [189, 190]. Plasma lyso-Gb3 have been found to be higher in patients with neutralizing antibodies [191], while increased left ventricular mass and progression of renal disease have been associated with the inhibition of enzyme replacement therapy [190]. The formation of the antibodies is typically observed within 3-6 months after initiating treatment [192]. The administered dose seems to be the most important trigger factor for neutralizing antibodies; this association also might explain the increased risk of antibody formation in patients receiving agalsidase beta compared to aglasidase alfa [193]. However, head-to-head comparisons have not been performed yet and future studies are warranted.

Chaperone Therapy

Chaperone therapy with migalastat has recently become available for FD patients aged ≥16 years with amenable mutations. In general, chemical chaperones are small molecules that assist the folding, maturation, binding or trafficking of the deficient enzymes and have been used in several inherited metabolic disorders, including lysosome storage disorders [194]. In the case of FD, migalastat is able to stabilize the endogenous a-Gal A enzyme of the patients, facilitates its trafficking from the endoplasmic reticulum to the lysosomes, and increases the enzymatic function [195-197].

Table 3. Current and future treatment options in FD

Therapies	
Available Therapies	
Enzyme Replacement Therapy	Agalsidase alfa
	Agalsidase beta
Chaperone Therapy	Migalastat
Developing Therapies	
Enzyme Replacement Therapy	Pegunigalsidase alpha
Substrate Reduction Therapy	Lucerastat
	Venglustat
mRNA-based Therapy	Exogenous mRNA via lipid nanoparticles
Gene Therapy	Hematopoietic stem cells (ex-vivo)
	Recombinant adeno-associated virus vectors (in-vivo)

Migalastat is an oral treatment that is administered every other day at a dose of 123 mg. Despite the fact that patients with amenable mutations account for 37% to 60% of all the FD-patients in different cohorts [198, 199], its treatment efficacy is well established in this subset. Real-world data show that, indeed, α-Gal A activity is significantly increased, while lyso-Gb3 levels are stabilized, and FD-specific manifestations and symptoms remain stable in patients under treatment [200, 201]. Additionally, a significant reduction in left ventricular mass has been noted in patients receiving this treatment [200, 201]. The efficacy regarding FD-associated neurological complications remains to be elucidated; however, it should be noted that migalastat, as a small molecule, is able to cross the blood-brain barrier.

In a phase III, randomized-controlled clinical trial, it has been shown that migalastat had a comparable efficacy to enzyme replacement therapy, underscoring that migalastat may be used as a viable alternative treatment option [202]. Furthermore, switching from enzyme replacement therapy to migalastat can be safely performed without requiring any special procedure, and may be considered in patients with amenable mutations, according to individualized criteria and patients' preferences [203, 204].

Developing Disease Specific Therapies

Other forms of exogenous enzyme replacement therapies are being currently investigated, with the most promising being a pegylated form of α-Gal A, named pegunigalsidase alpha. Pegunigalsidase alpha was found to have a much longer half-life compared to the available enzyme replacement therapies, with preliminary clinical data suggesting efficacy in reducing peritubular capillary lyso-Gb3 as measured in kidney biopsies, while the drug was well tolerated with no significant adverse events [205].

Gene therapy, either ex vivo by transplanting hematopoietic stem cells that express α-Gal A, or in vivo by infusing recombinant adeno-associated virus vectors are also under development [206]. Another approach concerns the administration of mRNA which directly codes wild-type α-Gal A, with preclinical data providing promising results in the tested FD-models [207]. Whether the produced α-Gal A enzyme will trigger the formation of neutralizing antibodies in these cases, remains to be determined [206].

Another therapeutic target in the metabolic pathway associated with FD is the substrate reduction therapy. Two molecules, lucerastat and venglustat, are currently under investigation in clinical trials. Lucerastat acts as a glucosylceramide synthase inhibitor, limiting the production of ceramide and, as a result, the accumulation of Gb3 [208]. Promising results regarding the efficacy in reducing plasma glycosphingolipids have emerged in one small trial evaluating treatment with lucerastat combined with available enzyme replacement therapies versus enzyme replacement therapies alone [209]. Venglustat has a similar action to lucerastat and has been shown to reduce skin capillary endothelial cell Gb3, plasma Gb3, plasma lyso- Gb3, and urine Gb3 in treatment-naïve FD-patients included in a phase II clinical trial [210]. Apart from the obvious metabolic controls, the patients were clinically stabilized, with no evidence for development of cardiovascular disease, stabilization of proteinuria and eGFR and overall lack of significant clinical progression [210]. Further data

from ongoing clinical trials are warranted; however, it seems that the treatment arsenal against FD is largely expanding and a variety of therapeutic options may be soon available for FD patients following a more individualized approach.

Conclusions

FD is a progressive, multi-systemic disorder affecting both male and female patients, even from an early age. The potentially disabling manifestations, emanating from the nervous system, and the renal and cardiac implication, and the increased mortality warrant prompt and accurate diagnosis, followed by an appropriate treatment. Diagnosis can be achieved by metabolic and molecular testing in patients with high clinical suspicion, and supportive treatment should be administered according to FD manifestations. Since the early 2000's, FD-specific treatment became available for the patients and provided sufficient metabolic and clinical control. Apart from the enzyme replacement therapies, more recent drugs such as the already-approved migalastat and the under-investigation substrate reduction therapy and gene therapy expand the treatment options for FD patients, providing the opportunity for personalized medicine in FD.

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POMPE DISEASE: CLINICAL CHARACTERISTICS, DIAGNOSIS, AND MANAGEMENT

Maria Moschou[1], Maria Gavriilaki[1*], Konstantinos Notas[1, 2], Marianthi Arnaoutoglou[2], Martha Spilioti[1], Vasilios K. Kimiskidis[1]*

[1] 1st Department of Neurology, AHEPA University Hospital, School of Medicine,Aristotle University of Thessaloniki, Thessaloniki, Greece
[2] Laboratory of Clinical Neurophysiology, AHEPA University Hospital, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece
** Maria Moschou and Maria Gavriilaki contributed equally to this review*

Abstract

Pompe disease is a genetic neuromuscular disorder caused by a deficiency of the acid α-glucosidase enzyme leading to lysosomal glycogen accumulation. Disease phenotypes range from the severe infantile-onset Pompe disease to the slowly progressive late-onset. Until 2006, the management of Pompe disease has been limited to supportive and palliative care. Since 2006, enzyme replacement therapy (ERT) has been available. The requirement of weekly life-long intravenous infusions along with the inability of the enzyme to reach skeletal muscle led to the development of several alternative forms of ERT and the initiation of gene therapy trials in Pompe disease. With the advent of new therapeutic options, a timely diagnosis of Pompe disease is of critical importance, as the prompt therapy has a significant clinical impact on disease course. This article aims to review current literature on Pompe disease and present the latest insights about clinical characteristics, diagnosis, novel therapies, and the impact of COVID-19 pandemic on the management of this treatable neuromuscular disorder.

Key words: Glycogen Storage Disease Type II; Pompe Disease; GAA protein, human; Therapeutics; Review

1. Introduction

Pompe disease (PD) (glycogen storage disease type II, OMIM ID: 232300) is a genetic neuromuscular disorder caused by deficiency of the enzyme acid α-glucosidase (GAA) also known as acid maltase. PD is inherited in an autosomal recessive patternand leads to lysosomal glycogen accumulation [1].

Dr. Joannes Cassianus Pompe, a Dutch pathologist, first described in 1932 an autopsy case of an infant with excessive accumulation of glycogen in her heart muscle, resulting in fatal heart hypertrophy. Dr. Pompe named the disease "cardiomegalia glycogenica" and described the presence of vacuolar storage of glycogen in many organs and tissues, including the myocardium [2]. It was not until 1963 when Dr Henri-Gery Hers recognized that a deficiency in the activity of α-Glucosidase (GAA) was the cause of PD [3] and a few years later, Dr. Andrew G Engel described the same enzyme deficiency in adults presenting with similar clinical manifestations as PD [4].

GAA is a lysosomal enzyme catalyzing alpha 1, 4 and alpha 1, 6 linkages of lysosomal glycogen and hydrolyzing it to glycose [5]. PD is caused by homozygous or compound heterozygous mutations in the GAA gene on chromosome 17q21-23, leading to unstable mRNA producing deficient or null product α-Glucosidase [6]. GAA deficiency causes progressive glycogen lysosomal accumulation, which eventually causes lysosomal rupture and release of the hydrolytic products in myocytes of cardiac, respiratory, skeletal, and smooth muscles [7]. This results in impaired contractile ability and subsequently tissue destruction [8, 9]. Moreover, the accumulation of these substrates in the lysosomes activates several pathogenic mechanisms, including oxidative stress, autophagy, mitochondrial dysfunction, calcium homeostasis and disruption of the mTOR (mammalian target of rapamycin) signaling pathway, contributing altogether to tissue damage seen in PD [10].

The clinical spectrum is variable and ranges from the severe infantile-onset Pompe (IOPD) disease to the slowly progressive late-onset PD (LOPD) [11]. Classic IOPD is characterized by a progressive hypertrophic cardiomegaly and generalized muscle weakness with hypotonia [1]. Without treatment, IOPD progresses to cardiorespiratory failure and death within the first years of life. Patients with LOPD present with a progressive lower limb girdle muscle weakness followed by respiratory symptoms, usually without cardiomyopathy [12]. The phenotypes of the disease are related to the residual enzyme activity. Thus, in IOPD GAA activity is less than 1% of normal, while in LOPD the GAA-activity varies between 1 to 30%. This broad phenotypic spectrum makes the diagnosis challenging.

Table 1. Common clinical features of infantile-onset and late-onse Pompe disease

	Clinical features	Frequency
IOPD	Cardiomegaly/cardiac failure	92%-100%
	Hepatomegaly	90%
	Hypotonia	88%
	Delay or failure in motor development	63-96%
	Respiratory distress	78%
	Hearing loss	75%
	Macroglossia	62%
	Feeding difficulties	53% to 57%
LOPD	Proximal muscle weakness	95%
	Respiratory insufficiency	na
	Exercise intolerance	na
	Gastroesophageal reflux, constipation, diarrhea, vomiting, nausea, and bowel incontinence	na
	Cerebral vasculopathy	67%
	Arterial dolichoectasia of the vertebrobasilar system	52%
	Scoliosis	33%
	Cerebral aneurysms	14%
	Polyneuropathy	na
	Chewing and swallowing difficulties	na

na: not applicable
IOPD: infantile-onset Pompe disease
LOPD: late-onset Pompe disease

PD has an estimated incidence of 1 in 40,000 for all phenotypes in the Caucasian population, while IOPD has a reported frequency of 1 in 138,000 live births [13, 14]. Recently, a novel method based on various registries for GAA variants estimated that PD's incidence was 1 in 23, 232 [15].

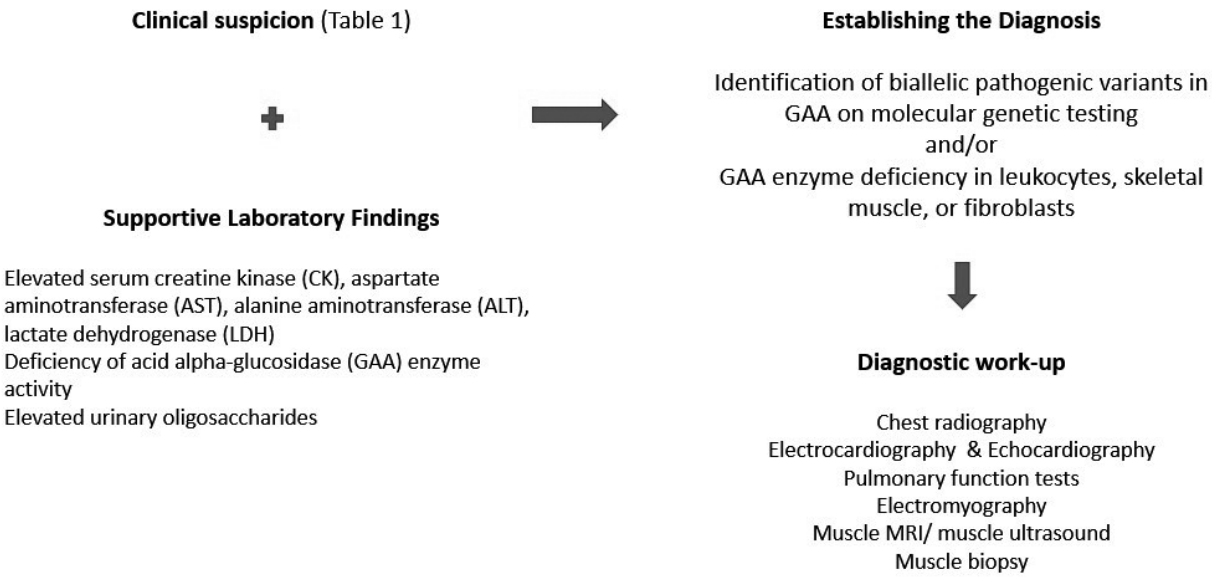
2. Clinical characteristics

PD is classified into infantile-onset (IOPD) and late-onset (LOPD) based on the age at symptom onset and the residual GAA activity. The American College of Medical Genetics (ACMG) Work Group on Management of Pompe Disease in 2006 differentiated the infantile form into "classic infantile" and "non-classic infantile". Classic infantile phenotype includes patients presenting with cardiomyopathy during the first year of life, while non-classic infantile form corresponds to patients with symptom onset at less than 12 months of age, but with mild or no cardiomyopathy and slower disease progression. On the other hand, late-onset form includes childhood or juvenile variant with symptom onset after the 12 months of age and adult-onset with onset of symptoms from adolescence to late adulthood. Typically, these forms do not develop severe cardiomyopathy [16].

Patients with IOPD have a mean age of symptoms onset at two months [17] and their most common presenting symptom is hypotonia [18]{van den Hout, 2003 #42264;Kishnani, 2006 #42270;Marsden, 2005 #42267}. Progressive hypertrophic cardiomyopathy is the key feature of classic IOPD and the median age of death without treatment is six months [1]. Typical signs for infantile onset form include cardiomegaly causing cardiac failure (92%), hypotonia (88%), progressive muscle weakness leading to delay or failure in motor development (63 to 96%), hepatomegaly (90%), macroglossia (62%), poor feeding with subsequent failure to thrive (53% to 57%), respiratory distress (78%), and hearing loss (75%) [1, 17, 19].

Furthermore, brain white-matter abnormalities have been described in adult patients with IOPD that survived after the introduction of enzyme replacement therapy (ERT). IOPD-related white matter lesions

Figure 1. A proposed workflow for the diagnosis of Pompe Disease



seem to follow a characteristic symmetrical pattern, with slow progression over time, affecting the neuropsychological development of these patients in a varying degree [20].

Based on recent data from the Belgian Pompe registry, LOPD patients have a median age of symptoms onset at 28.9 years (range 7 months-68 years) but the diagnosis was reached with a mean delay of 12.9 years [12]. The most common mutation in LOPD patients is c.-32-13 T > G [12, 21] and in contrast to IOPD, patients with this mutation rarely present severe cardiac dysfunction [22].

LOPD patients typically present with proximal muscle weakness, affecting initially the lower limbs and the paraspinal muscles. Progression of the disease is characterized by diaphragm involvement and respiratory distress, and respiratory failure is the most common cause of death in LOPD patients [23]. In a small number of patients (13%), respiratory symptoms may be the presenting manifestation of the disease, while non-invasive ventilation (NIV) is needed in 37% of the patients fifteen years after symptom onset [12]. Macroglossia and hepatomegaly are rarely found.

Nowadays, LOPD is considered as a multi-system disease, affecting in a varying degree not only the musculoskeletal and respiratory system but also the vascular, gastrointestinal, nervous and genitourinary system [24]. According to recent studies, gastrointestinal symptoms may be observed before the diagnosis in 42% of the LOPD patients. The most common manifestation is gastroesophageal reflux, followed by constipation, diarrhea, vomiting, nausea and bowel incontinence [25].

Moreover, involvement of the central nervous system can be found in a significant number of LOPD patients, including the presence of cerebral vasculopathy (67%), arterial dolichoectasia of the vertebrobasilar system (52%), and cerebral aneurysms (14%) [22, 26]. Scoliosis presents in 33% of LOPD patients [27], while polyneuropathy (mainly motor neuronopathy and small fiber neuropathy) has been described as an atypical clinical manifestation [28, 29]. Swallowing disturbances and dysphagia may be also present in LOPD patients, due to bulbar and facial muscles weakness, while ptosis and strabismus may also be rarely observed [30].

The vast clinical spectrum observed in PD, and specifically in LOPD, can be explained by the presence of numerous GAA genotypes caused by the significant number of GAA variants. The last update of the PD GAA variant database described 648 disease-associated variants [24]. Furthermore, the same GAA genotypes may correspond to different phenotypes in LOPD, implicating the impact of secondary modifying genetic factors [8, 20, 31]. On the contrary, a strong genotype-phenotype correlation in IOPD is usually observed [32].

3. Diagnosis

A proposed diagnostic workflow is presented at Figure 1. IOPD should be suspected in any infant with generalized hypotonia and hypertrophic cardiomegaly [33]. Moreover, a history of recurrent respiratory infections or/and liver enlargement is also suggestive of IOPD, although it is not unique to this myopathy. Additional laboratory parameters that should alert physicians towards this diagnosis are the increased levels of creatine kinase (CK), serum glutamic-oxaloacetic transaminase (SGOT), serum

Table 2. Main phenotypic appearance of specific acid α-glucosidase (GAA)

GAA pathogenic variant	Population[1]	GAA enzyme activity	Phenotype
p.Glu176ArgfsTer45 (c.525delT)	Dutch	negligible	severe - predicts IOPD
p.Gly828_Asn882del (c.2482_2646del)	Dutch	negligible	severe - predicts IOPD
c.336-13T>G	na	greatly diminished	LOPD - not associated with IOPD
p.Asp645Glu (c.1935C>A)	Taiwan and China	na	founder effect - IOPD
p.Arg854Ter (c.2560C>T)	African	na	founder effect - IOPD

na: not applicable
IOPD: infantile-onset Pompe disease
LOPD: late-onset Pompe disease

glutamic-pyruvic transaminase (SGPT) and lactate dehydrogenase (LDH). The diagnostic work-up should also include a chest x-ray, an electrocardiogram and a cardiac ultrasound scan, where an enlargement of the cardiac silhouette, a biventricular hypertrophy with short PR intervals and giant QRS complexes and left ventricular noncompaction cardiomyopathy could be observed respectively. The study of glucose tetrasaccharide Glc (4) levels in the urine of infantile-onset patients is a primary diagnostic and therapeutic monitoring biomarker, as it reflects disease progression and correlates with ERT efficacy [34, 35].

Adults with LOPD typically present with progressive muscle weakness, involving proximal lower limbs and paraspinal muscles. The electromyogram (EMG) study demonstrates myopathic discharges, along with numerous myotonic discharges and fibrillations, especially in the paraspinal muscles [36], and although it is not specific, it can strengthen clinical suspicion. Respiratory muscle involvement in adults may occur early in the disease course and it is revealed by the substantially reduced forced vital capacity (FVC) [37]. Additionally, the majority of LOPD patients has elevated CK levels. However, in 5% of these patients CK levels are within the normal range [33]. Screening for the deficiency of acid alpha-glucosidase enzyme (GAA) activity in adults with asymptomatic hyperCKemia has facilitated the early diagnosis of LOPD [38-43].

A confirmed diagnosis of PD, regardless of its form (IOPD or LOPD), warrants confirmatory laboratory testing [44]. The diagnosis is established by the identification of either GAA enzyme deficiency in leukocytes, skeletal muscle, or fibroblasts and/or findings of biallelic pathogenic mutations of the GAA gene on genetic testing [45].

In everyday clinical practice, the preferred method appears to be the measurement of GAA activity. Traditionally, GAA enzyme analysis was performed in skin fibroblasts or muscle biopsy samples. Nowadays, novel methods such as dried blood spot (DBS)-based GAA activity assays are being adopted as a rapid, minimally invasive and reliable first tier test for screening [42, 45]. Of note, a positive DBS test must be subsequently confirmed by a secondary test such as GAA activity assays in tissue samples (whole blood sample, skin, or muscle biopsy) and/or DNA analysis.

With the advent and subsequently decreasing cost of gene sequencing, whole-exome sequencing is being increasingly used in the diagnostic work-up of PD [46]. Gene sequencing offers a high diagnostic yield in patients with suggestive symptoms of PD in whom other differential diagnoses such as muscular dystrophies could not be excluded [47, 48]. In 83 to 93 per cent of patients with impaired or absent GAA enzyme activity GAA sequence analysis may yield two pathogenic variants [49, 50]. Of note, the use of sequence analysis may lead to the detection of benign variants or variants of uncertain significance. If only one or no pathogenic variant is identified, the next step could be gene-targeted deletion/duplication analysis considering that this method may detect deletions or duplications missed by sequence analysis of DNA coding regions [51, 52]. DNA analysis provides genotype-phenotype information as presented at Table 2, is essential for genetic counseling, and facilitates a prenatal diagnosis if the pathogenic mutation is known in the family.

Despite the advances in the field and the increased scientific knowledge about the disease, the confirmation of the diagnosis may still be delayed. According to the worldwide Pompe Registry, the time from symptom onset to initiation of therapy appears to be increased in the year 2008 compared with previous years [53]. Considering that ERT, available since 2006, has been associated with a better disease course in children and adults, it is of paramount importance to diminish diagnostic delay [54, 55].

During the last decade, many countries are implementing newborn screening programs for GAA deficiency, based on several studies in which an early diagnosis followed by an early initiation of therapy was associated with a favorable impact on survival, motor function outcomes and patients' quality of life [56, 57].

4. Management

Until 2006, the management of PD was restricted to supportive and palliative care. During that year, ERT with recombinant human GAA (alglucosidase alfa) administered by intravenous infusions was introduced and substantially changed the natural course of the disease.

The first approved drug therapy (alglucosidase alfa, Myozyme®, Genzyme Corporation) has been available for individuals affected by IOPD disease [58, 59], and another recombinant alglucosidase alfa manufactured at a larger scale (alglucosidase alfa, Lumizyme®, Genzyme Corporation) was subsequently approved for LOPD patients without cardiomyopathy [54]. Alglucosidase alfa is delivered every two weeks as an intravenous infusion at a recommended dosage of 20 mg/kg body weight. In 2014, approval was expanded to patients of all ages irrespective of cardiac involvement, based on the ADVANCE trial, a phase IV, open label study in which patients older than one year of age were included [60].

If left untreated, classic IOPD patients will present an unremitting deterioration, leading to death from cardiac insufficiency during the first two years of life [17]. Infants with non-classic IOPD suffer from less severe cardiomyopathy and may have a prolonged survival based on natural history studies [61]. In untreated patients with LOPD, the estimated survival rate 30 years after the initial diagnosis was 40% [62]. It has become evident that patients with an infantile severe form of the disease benefit the most from ERT, considering that the natural history of the disease and the rate of treatment response varies among individuals with LOPD [54, 63-65].

Apart from ERT, the management of a PD patient of any age should include a multidisciplinary care team consisting of a neurologist or a pediatrician, a geneticist, a physical medicine and rehabilitation physician, a cardiologist, a pulmonologist, an orthopedic, and a nutritionist. Additionally, an international consensus regarding the required outcome measures regularly performed on clinical follow-up and its timeline should be established.

Efficacy of ERT

Several case series and small cohort studies confirmed the short-term and long-term benefits of ERT administration in survival, cardiorespiratory function, and motor development of IOPD patients [59, 66-69]. Prompt initiation of ERT in less severely affected individuals through newborn screening resulted in significant improvement in cardiac, motor and pulmonary function, and survival of these infants [70]. Another prospective study in ten IOPD treated patients diagnosed through newborn screening demonstrated that all patients achieved independent ambulation and none of them required mechanical ventilation after a median treatment duration of 63 months [71]. However, a progressive pelvic girdle muscle weakness was observed in patients older than 2 years old, revealing some limitations of ERT.

The efficacy of ERT in juvenile-onset (2 to 18 years) LOPD patients was recently examined by a systematic review [72]. Based on low quality of evidence, administration of ERT may improve short-term muscle activity and pulmonary function, but no evidence exists about the effect of ERT on survival of these patients. ERT is more effective when administered in younger juvenile-onset patients with a milder disease at baseline assessment.

Between 2006 and 2010, the therapeutic management of LOPD consisted of off-label administration of intravenous ERT with GAA, based on early case-series and case reports [73-76]. In 2010, the first randomized placebo-controlled trial of alglucosidase alfa in 90 LOPD patients revealed a favorable outcome on the 6-minute walk test and stabilization of FVC over 18-months of treatment, and subsequently led to drug approval [54]. Since then, recommendations for managing LOPD patients have been published for many countries [77-81]. In LOPD patients, a duration of ERT up to five years has been proved to improve or stabilize muscle strength, motor and pulmonary function, and survival [64, 82-86]. Several systematic reviews and meta-analyses have been conducted, reporting a significant beneficial effect of ERT in the walking distance achieved by LOPD patients [64, 87]. Recently, a large real-world data study examined the long-term efficacy of alglucosidase alfa in the LOPD population and reported that the initial favourable outcome was followed by a secondary deterioration in multiple outcome measures, highlighting the need for novel therapeutic options [88]. In that context, the European Pompe Consortium (EPOC) developed a specific guidance on starting and stopping ERT in adult patients, taking into consideration the increased costs of a life-long ERT administration [44]. Furthermore, EPOC proposed a minimal set of outcome measures consisting of skeletal muscle strength tests [manual muscle testing (MRC), six minute walk test, timed tests], pulmonary function tests, the fatigue severity scale and patient reported outcomes for monitoring ERT efficacy in PD patients [89]. All patients with PD should perform yearly check-ups at specialized centres by a multidisciplinary neuromuscular team.

Adverse effects of ERT

The most commonly encountered adverse effects (AE) of ERT infusions are severe hypersensitivity and/or infusion reactions [59, 67, 68]. Slowing of the infusion rates and implementation of anaphylaxis pro-

Table 3. Summary of ongoing clinical trials investigating the management of Pompe disease

Interventions	Title	AgesEligible	ClinicalTrialPhase/ Status	Trial Number
Enzymereplacementtherapy				
Alglucosidasealfa	Study to Evaluate Efficacy and Safety in Chinese Patients With Late Onset Pompe Disease With Alglucosidase Alfa Treatment (APOLLO-LOPD)	≥ 3 yearsold	IV/Recruiting	NCT04676373
	Higher Dose of Alglucosidase Alpha for Pompe Disease	upto 60 Years	Observational/ Notyetrecruiting	NCT05017402
	Growth and Development Study of Alglucosidase Alfa	upto 24 Months	IV/Active, notrecruiting	NCT00486889
	A Prospective Study to Observe & Describe Clinical Outcomes of Alglucosidase Alfa Treatment in Patients Upto 6 Months of Age With Infantile-onset Pompe Disease (IOPD)	upto 6 Months	Observational/ Recruiting	NCT04848779
	In Utero Enzyme Replacement Therapy for Lysosomal Storage Diseases (IUERT)	Maternal pregnant women of age 18-50, carrying a male or female fetus at 18 0/7 weeks to 34 6/7 weeks	I/Recruiting	NCT04532047
	PompeLactationSub-Registry	Child, Adult, Older Adult	IV/Recruiting	NCT00566878
Avalglucosidasealfa	A Study to Assess Safety and Efficacy of Avalglucosidase Alfa Administered Every Other Week in Pediatric Patients With Infantile-onset Pompe Disease Previously Treated With Alglucosidase Alfa (Mini-COMET)	6 monthsto 17 yearsold	II/Active, notrecruiting	NCT03019406
	Clinical Study for IOPD Participants Less Than or Equal to 6 Months of Age to Evaluate Efficacy and Safety of Enzyme Replacement Therapy (ERT) With Avalglucosidase Alfa (Baby-COMET)	upto 6 Months	III/Recruiting	NCT04910776
	Avalglucosidase Alfa Extension Study (NEO-EXT)	Child, Adult, Older Adult	II-III/ Active, not recruiting	NCT02032524
AT-GAA [Cipaglucosidase Alfa (ATB200)/ Miglustat (AT2221)]	PROPEL Study - A Study Comparing ATB200/AT2221 With Alglucosidase/ Placebo in Adult Subjects With LOPD	≥ 18 yearsold	III/Completed - No results Posted	NCT03729362
	ZIP Study - A Study of the Safety, Pharmacokinetics, Efficacy, Pharmacodynamics, and Immunogenicity of ATB200/AT2221 in Pediatric Subjects Aged 0 to < 18 Years WithPompe Disease	0-18 yearsold	III/Recruiting	NCT03911505

Table 3. Continuity

Interventions	Title	AgesEligible	ClinicalTrialPhase/ Status	Trial Number
AT-GAA [Cipaglucosidase Alfa (ATB200)/ Miglustat (AT2221)]	Rossella: A Study to Evaluate the Safety, PK, Efficacy, PD and Immunogenicity of Cipaglucosidase Alfa/ Miglustat in IOPD Subjects Aged 0 to <18	0-18 yearsold	III/Recruiting	NCT04808505
	A Study to Assess the Long-term Safety and Efficacy of ATB200/ AT2221 in Adult Subjects With LOPD	≥ 18 yearsold	III/Active, notrecruiting	NCT04138277
Genetherapy				
Recombinant Adeno-Associated Virus Acid Alpha-Glucosidase (rAAV9-DES-hGAA)	Re-administration of Intramuscular AAV9 in Patients With Late-Onset Pompe Disease (AAV9-GAA_IM)	18 to 50 yearsold	I/Active, notrecruiting	NCT02240407
AAV2/8-LSPhGAA	A Phase 1 Study of the Safety of AAV2/8-LSPhGAA in Late-onset Pompe Disease	≥ 18 yearsold	I-II/Recruiting	NCT03533673
SPK-3006	A Gene Transfer Study for Late-Onset Pompe Disease (RESOLUTE)	≥ 18 yearsold	I-II/Recruiting	NCT04093349
AT845	Gene Transfer Study in Patients With Late Onset Pompe Disease (FORTIS)	≥ 18 yearsold	I-II/Recruiting	NCT04174105
Other				
Filgrastim/ Genetically modified autologous bone marrow cell product	Clinical Specimen Collection From-Pompe Disease Patients	3 to 30 yearsold	Observationalstudy/ Recruiting	NCT04476550
NeuRxDiaphragm-pacer (DPS)	Response to Diaphragmatic Pacing in Subjects With Pompe Disease	2 to 65 years old	Observationalstudy/ Recruiting	NCT02354651
Clenbuterol	Phase II Clinical Trial of Clenbuterol in Adult Patients WithPompe Disease	≥ 18 yearsold	II/Notyetrecruiting	NCT04094948

tocols have been applied to avoid these AE. In IOPD, treatment outcome has been negatively affected by cross-reactive immunologic material (CRIM) status. High titers of antibodies against the exogenous GAA have been identified in CRIM-negative IOPD patients, which can lead to clinical deterioration and decreased survival [90]. Individuals with LOPD experience less severe infusion-related AE [91], and AE leading to death have been scarcely reported [92, 93].

Novel therapies in the pipeline

As discussed above, there is an unmet need for a more efficient and cost-effective therapeutic approach for the long-term management of Pompe disease. A summary of ongoing clinical trials investigating novel therapeutic drugs is presented at Table 3.

The requirement for weekly life-long intravenous infusions, along with the inability of the available products to cross the blood-brain barrier, and the fading of ERT efficiency observed over time, led to the development of several gene therapy trials in PD. The first successful phase I/II trial of adeno-associated virus (AAV)-mediated alpha-glucosidase gene therapy in five ventilator-dependent children previously treated with ERT marked a milestone in the management of PD [94]. Gene therapy trials in the pipeline should focus on the safety profile and the long-term therapeutic effect on pulmonary and motor function.

Another drawback of the currently used ERT is the inability of the enzyme to reach skeletal muscle, due to the limited number of mannose-6-phosphate (M6P) groups on alglucosidase alfa and the decreased expression of the cation-independent mannose 6-phosphate receptor (CI-M6PR) on the surface of

muscle cells [95, 96]. Hence, alternative forms of ERT with an increased affinity for this receptor are being developed. Avalglucosidase alfa (Nexviazyme®, Sanofi Genzyme) is a second generation recombinant human GAA enzyme which recently received an approval by the U.S. Food and Drug Administration (FDA) for the treatment of LOPD patients older than one year of age [97]. In a phase III trial, treatment-naive patients with LOPD who received avalglucosidase alfa demonstrated a greater improvement in pulmonary and motor function outcomes compared with alglucosidase alfa treated individuals [98]. The European Medicines Agency's (EMA) final recommendation is expected.

In another effort to enhance the efficiency of ERT, a combination of alglucosidase alfa with clenbuterol was studied in a phase I/II trial, demonstrating a positive safety profile along with motor function improvement [99]. Additionally, AT-GAA (Amicus Therapeutics, USA) is a novel ERT combining recombinant human GAA with a pharmacological chaperone that is evaluated in PD patients of any age.

Finally, aerobic exercise along with a low carbohydrate - high protein diet have been studied as adjunctive therapies to ERT [100, 101], while another ongoing trial examines diaphragmatic pacing as a rehabilitative tool to minimize mechanical ventilation requirements based on a previous case series presenting positive results [102].

Impact of COVID-19 pandemic on Pompe disease management

As of September 7 2021, the COVID-19 pandemic has affected more than 220 million individuals with over 4.5 million deaths worldwide [103]. Despite the abundant scientific data rapidly published about the clinical syndrome caused by COVID-19 and the rapid development of effective vaccines, humanity is still fighting to control virus spread almost two years after COVID-19 outbreak [104]. COVID-19 associated restrictions and the recurrent so-called lockdowns have negatively impacted economic and healthcare systems worldwide.

Patients with chronic neuromuscular diseases such as PD had restricted access to the hospitals due to the increased risk of contamination during the first year of the pandemic. According to the French Rare Health Care for Neuromuscular Diseases Network (FILNEMUS) guidelines, in-hospital ERT infusions had to be postponed over a period of 1 to 3 months and home infusions were suggested [105]. Indeed, the transition to home-therapy seemed to be the most effective access to ERT during pandemic and was implemented in many countries around the world [106, 107]. However, in a recent German study, interruption of ERT in LOPD patients for a mean time of 49.42 days (SD ± 12.54) was associated with a significant deterioration in pulmonary and motor function tests and other objective outcome measures [108]. Hence, in order to provide PD patients with the best medical care and access to the hospitals, physicians should encourage all patients who are not taking immunosuppressive agents to receive COVID-19 vaccines, as suggested by American Association of Neuromuscular & Electrodiagnostic Medicine (AANEM) [109].

5. Conclusions

Even though PD was a neuromuscular disorder associated with a very poor prognosis, the approval of ERT in 2006 has substantially changed the natural course of the disease, and mainly the severe form of IOPD. Nevertheless, an unmet need for a more efficient therapeutic approach for the long-term management of these patients remains until today. With the advent of alternative forms of ERT and gene therapy applied in PD, the timely diagnosis is still of a critical importance, as the earliest initiation of the therapy has a significant clinical impact on disease course.

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X-LINKED ADRENOLEUKODYSTROPHY: CLINICAL CHARACTERISTICS, DIAGNOSIS AND MANAGEMENT

Chrysoula Koniari, Georgios Koutsis

Neurogenetics Unit, 1st Department of Neurology, National and Kapodistrian University of Athens, Eginitio Hospital, Athens, Greece

Abstract

Adrenoleukodystrophy (X-ALD) is a rare X-linked peroxisomal disease, which usually presents in affected males with one of three phenotypes: adult-onset adrenomyeloneuropathy (AMN, 45%), childhood-onset cerebral demyelination (CALD, 35%), or primary adrenal insufficiency (PAI, 10%). Occasionally, X-ALD patients may suffer from less specific symptoms resembling those of other neurological conditions. Variability in presentation and age of onset can make the diagnosis challenging. AMN should be considered in patients presenting with spastic paraparesis. Female carriers manifest a late-onset mild form of myelopathy and/or neuropathy. Inclusion of testing for X-ALD in newborn screening programs is expected to expand our understanding of the disorder's natural history. Newborn screening should enable early detection of affected individuals and allow timely therapeutic interventions. Such interventions include corticosteroid replacement therapy for PAI, allogeneic hematopoietic stem cell transplantation for CALD, and approved gene therapy (elivaldogene autotemcel) for suitable candidates with childhood CALD. Currently, no effective treatment exists for the neurological manifestations of AMN, the commonest presentation of X-ALD. Hopefully, in the near future, novel gene therapy approaches, similar to those recently approved for other rare neurogenetic diseases may also be developed for X-ALD.

Key words: X-linked adrenoleukodystrophy; adrenomyeloneuropathy; primary adrenal insufficiency; HSCT; elivaldogene autotemcel

Introduction

X-linked adrenoleukodystrophy (X-ALD, OMIM #300100) is a lipid-storage disease, which primarily affects the nervous system, adrenal cortex, as well as testicular function [1-4]. X-ALD represents the most common peroxisomal disorder, with a recently estimated birth incidence of approximately 1:18,000 [3]. As shown in Box1, clinical manifestations of the disorder vary, with patients presenting with either of the following three basic clinical phenotypes; cerebral X-ALD (CALD), adrenomyeloneuropathy (AMN), and primary adrenal insufficiency (PAI) [4].

X-ALD is due to mutations in the *ABCD1* gene, located on chromosome Xq28, coding for ALDP, a

Box 1. X-ALD Clinical Phenotypes

Males
• Cerebral form (CALD) ○ Childhood ○ Adolescent ○ Adult • Adrenomyeloneuropathy (AMN) o With cerebral involvement o Without cerebral involvement • Primary adrenal insufficiency (PAI), "Addison's only" phenotype • Asymptomatic/Presymptomatic
Females
• AMN-like phenotype • Asymptomatic/Presymptomatic

peroxisomal ATP-binding cassette protein [5-7]. As of June 11, 2021, 3, 247 variants of *ABCD1* have been reported, of which 913 are non-recurrent and 247 are variants of unknown significance (https://adrenoleukodystrophy.info/mutations-biochemistry/mutation-statistics). No correlation between genotype and disease phenotype has been found, even within the same family [8].

ALDP serves as a transmembrane channel which enables the transportation of very long-chain fatty acids ≥ C22:0 (VLCFA) into the peroxisome, for β-oxidation [9]. The defective function of ALDP prevents degradation of VLCFA, resulting in its accumulation in tissues and plasma, which is regarded as X-ALD's biochemical hallmark [10]. VLCFA levels are increased in the plasma of all male carriers and in approximately 80-85% of female carriers [11, 12].

In 2006, Hubbard et al. found raised levels of C26:0 lysophosphatidyl choline, measured via LC-MS/MS assay, in postnatal venous dried blood spots from X-ALD males [13]. This finding has allowed the emergence of a newborn screening diagnostic test for X-ALD with several countries incorporating it in their newborn screening programs [14]. Therapeutic management has also evolved, with corticosteroid replacement therapy and hematopoietic stem cell transplant (HSCT) remaining the mainstay of treatment, but with new therapeutic options, such as gene therapy for eligible candidates suffering from CALD, also emerging [15, 16]. As X-ALD patients have no neurological deficits at birth, early diagnosis through newborn screening has opened a "window of opportunity" for the use of these therapies.

In this review, we intend to enlighten the reader about the disorder's basic pathophysiology and clinical characteristics, as well as provide an update on diagnosis and management of X-ALD.

2. X-ALD Basic Pathophysiology

VLCFA mainly accumulate in the nervous system, adrenal cortex, and testicular Leydig cells, a process already taking place in utero [17-20]. Unidentified molecular events prompt the transition from the metabolic phase, i.e. VLCFA accumulation, to neuroinflammation and demyelination in the brain in CALD, or to axonal degeneration in the spinal cord in AMN [21].

In 2010, Singh and Pujol, in an attempt to describe the mechanisms underlying CALD, proposed the "three-hit hypothesis"; excess of VLCFA and lower plasmalogen levels (antioxidant phospholipids) result in oxidative stress (first hit) that successively, with the contribution of environmental, genetic or epigenetic factors, triggers a neuroinflammatory response (second hit), which further disrupts the peroxisomal function (third hit), leading to a progressive inflammatory demyelinating disease [22-24]. Similarly, regarding the myeloneuropathy's pathophysiology, oxidative stress along with defective mitochondrial function result in the disruption of ATP-dependent axonal transport, inducing a distal non-inflammatory dying-back axonopathy [25].

In the adrenal glands, VLCFA are preferentially found in the zona fasciculata and zona reticularis, thus glucocorticoid and androgen deficiencies are more common in X-ALD [19, 26, 27]. The suggested mechanisms that elicit the aforementioned deficiencies are summarized as follows: a) VLCFA accumulation exhibiting a direct cytotoxic effect to cells, followed by apoptosis due to oxidative stress [23], b) inadequate free cholesterol availability for steroid hormones' formation owing to the accumulation of cholesterol esters with VLCFA [28], c) incorporation of VLCFA into cell membranes, interfering with adrenocorticotropic hormone's (ACTH) ability to attach to its receptor [29]. A similar pathology is thought to lead to abnormal hormonogenesis in testes' Leydig cells, with low testosterone levels leading to testicular dysfunction [30].

3. X-ALD Clinical Characteristics

Various different X-ALD clinical phenotypes have been described, each of them characterized by specific clinical manifestations (see Table 1). However, considering the disorder's progressive nature and the fact that some phenotypes evolve into others, one could speak of a clinical spectrum of disease (see figure 1). Interestingly, the X-ALD phenotype shows no correlation, either to VLCFA plasma levels or to the *ABCD1* pathogenic variant involved, even in the same family [31-33]. Moreover, in contrast to previous understanding, it is now widely accepted that X-ALD not only affects males, but, female heterozygotes as well, who eventually develop primarily AMN symptoms [34].

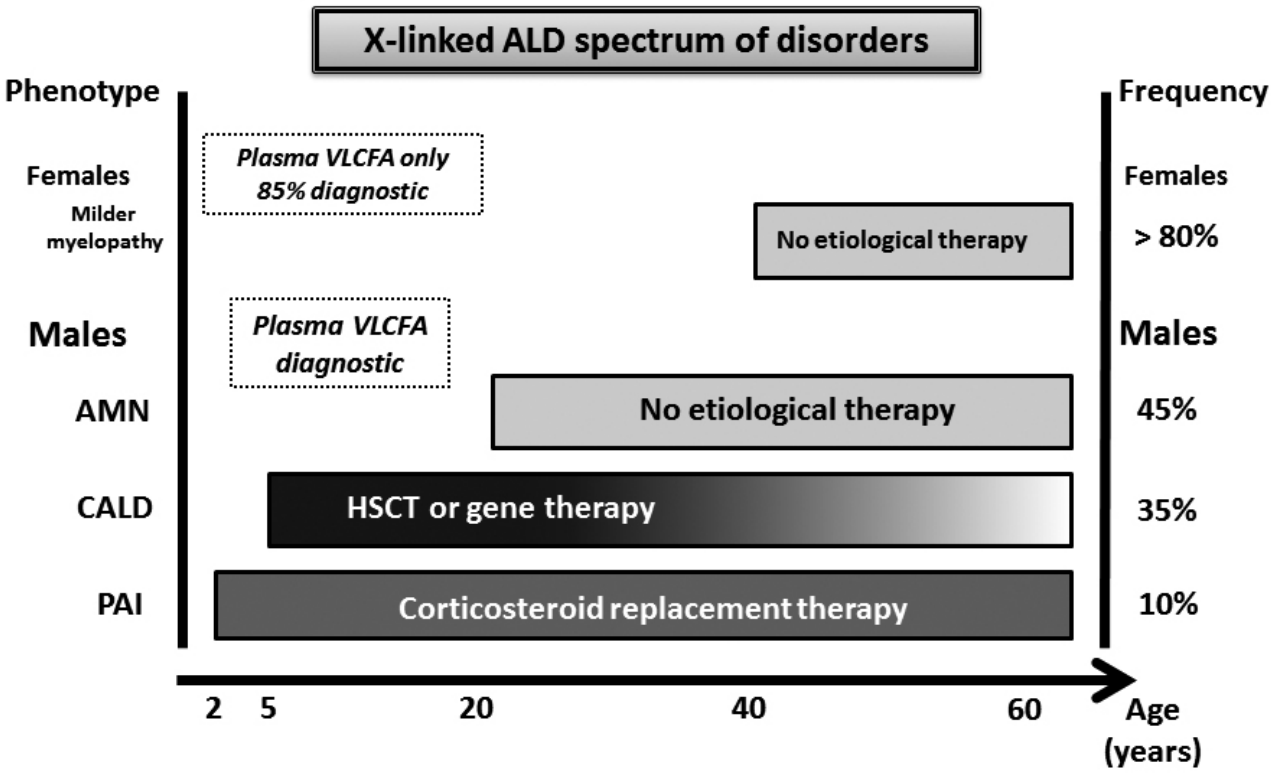
3a. Presentations Most Commonly Seen in Affected Males

The cerebral form of X-ALD can occur at any age (childhood, adolescence, early adulthood), but mostly between four and eight years of age, with the childhood cerebral form occurring in ~35% of affected individuals [35, 36]. Affected schoolboys exhibit cognitive deficits (behavioral and/or learning), which may resemble those of attention deficit hyperactivity disorder. As the demyelinating process progresses, more serious symptoms arise, such as hemiparesis or spastic tetraparesis, apraxia, astereognosia, difficulty in understanding speech despite intact hearing, lack of spatial orientation, visual disturbances, cerebellar ataxia, and seizures [36]. By the time the neurologic disturbances appear, most patients have

Table 1. Clinical manifestations of X-ALD's most common phenotypes and their lifetime prevalence

Phenotypes	Clinical manifestations	Lifetime prevalence
Males		
Cerebral form	cognitive deficits resembling attention-deficit hyperactivity disorder, signs of dementia, visual disturbances, aphasia, apraxia, astereognosia, auditory agnosia, lack of spatial orientation, hemiparesis or spastic tetraparesis, cerebellar ataxia, seizures	~60%
AMN	spastic paraparesis, sensory ataxia, sphincter disturbances, sexual dysfunction	~100%
Adrenocortical insufficiency	increased skin pigmentation, orthostatic hypotension, fatigue, anorexia, weight loss or poor growth, gastrointestinal symptoms	~80%
Females		
AMN-like phenotype	sensory ataxia, sphincter disturbances, sensory symptoms, gait spasticity, neuropathic pain	>80%

Figure 1 legend. Diagram summarizing main diagnostic, phenotypic and therapeutic issues in X-linked adrenoleukodystrophy. Percentages, presented separately for males and females, represent phenotypes at presentation; ALD: adrenoleukodystrophy; AMN: adrenomyeloneuropathy; CALD: cerebral ALD; PAI: primary adrenal insufficiency; HSCT: Hematopoietic stem cell transplantation; gene therapy refers to elivaldogene autotemcel



also developed adrenocortical insufficiency. Eventually, progressive functional decline leads to total disability followed by death, two to four years after symptom onset.

A proportion of affected males suffering from CALD may develop the so-called "chronic or arrested cerebral X-ALD". In such cases, a spontaneous arrest of the demyelinating process is observed, followed by several years of disease stability. Nevertheless, a small number of those patients may, at some point, shift to the progressive form of CALD, reflecting the recurrence of the cerebral demyelinating process [37, 38].

The proportion of affected individuals presenting with symptoms suggestive of adrenomyeloneuropathy is estimated as high as ~40-45% [39]. AMN typically affects men between their second and fourth

decade of life, who develop progressive symptoms such as spastic paraparesis, sensory ataxia, sphincter disturbances, and sexual dysfunction [37]. As made apparent by its clinical manifestations, AMN should be considered in patients with spastic paraparesis of undetermined cause [40, 41]. Adrenal insufficiency is usually already evident as of AMN diagnosis' establishment. Despite initial absence, about 40%-45% of patients suffering from AMN eventually develop some degree of brain involvement, which in some cases becomes severely progressive and may even lead to death [42].

Approximately 10% of male carriers exhibit, at presentation, signs of primary adrenal insufficiency, i.e. increased skin pigmentation, orthostatic hypotension, fatigue, anorexia, weight loss or poor growth, and gastrointestinal symptoms, resulting in a diagnosis of Addison's disease [37]. PAI, if left untreated, may lead to adrenal crisis, a potentially life-threatening situation. Therefore, testing for X-ALD seems reasonable in all male probands showing signs of Addison's disease [43]. Although most affected male individuals will exhibit signs of adrenal dysfunction at some phase of the disease, adrenal function is usually normal in female carriers [44].

Another 5%-10% of affected males present with one the following constellation of symptoms: a) signs of localized brain disease including hemiparesis, aphasia, and visual field defects, and symptoms due to elevated intracranial pressure, such as headache, b) progressive behavioral disturbance, dementia and paresis in an adult, c) ataxia in a child or adult, d) only evidence of sphincter and sexual dysfunction in male carriers [45]. Lastly, a small proportion of male carriers may remain asymptomatic/ presymptomatic. This marked phenotypic heterogeneity has been attributed to the existence of possible disease modifiers, such as environmental triggers but also genetic factors [46]. Numerous reports from studies that aim at identifying modifier genes in X-ALD have been published, an extensive discussion of which is beyond this review's intentions [47].

3b. Presentations Most Commonly Seen in Female Carriers

For a long time, X-ALD was regarded as a disorder that only affects males. However, nowadays, studies have shown that despite adrenocortical insufficiency and CALD affecting less than 1% of female carriers, more than 80% show signs of neurological dysfunction by the age of 60 years [48]. As expected, the disease is less severe and progresses at a slower rate in female carriers. Symptom onset predominantly occurs between the 4th and 5th decade of life, resembling an AMN-like phenotype. Affected women typically present with symptoms suggestive of myelopathy and/or peripheral neuropathy such as sensory ataxia, fecal incontinence, bladder dysfunction, sensory symptoms and gait spasticity [49]. They often suffer from neuropathic pain, a symptom that is not generally present in males with AMN [50]. Women very rarely develop the cerebral form of the disease, this occurring only in the context of two possibilities a) woman carrier of two mutated *ABCD1* alleles, or b) complete inactivation of the normal X-chromosome in all tissue cells [51].

4. X-ALD: Establishing the Diagnosis

The implementation of X-ALD testing in newborn screening programs has undoubtedly led to a new era regarding X-ALD diagnosis in several countries [52]. In such countries, as expected, the burden has now been placed on monitoring of diagnosed infants and extensive discussions are underway, regarding monitoring protocols. Families that give birth to infants with a positive newborn screen are referred to specialized centers for confirmatory testing and genetic advice. The infant is respectively closely followed by pediatric neurologists and endocrinologists [14].

In countries where newborn screening is not available, diagnosis remains a matter of clinical suspicion. When X-ALD is suspected in a male, measurement of VCLFA in blood is diagnostic, with high specificity and sensitivity as all males with X-ALD have elevated VLCFA levels. However, about 15% of female carriers might have normal plasma VLCFA levels, rendering mutation analysis as the diagnostic test of choice in females [35, 53]. In males suffering from primary adrenal insufficiency, the clinician should consider the possibility of X-ALD and test for plasma VLCFA levels [54]. Similarly, boys and young adults with suggestive neurological symptoms, with or without typical lesions on brain MRI, should be considered for X-ALD. Furthermore, both male and female patients with symptoms consistent with chronic myelopathy should be tested for X-ALD, after ruling out more common causes, such as multiple sclerosis, vitamin deficiencies, compressive lesions, radiation, infections, or primary lateral sclerosis, and before or at the same time as embarking on genetic testing for hereditary spastic paraparesis [55]. In males, the coexistence of progressive myelopathy and early baldness may facilitate establishing the diagnosis, as most affected males will already have some signs of adrenal insufficiency by the time they develop myelopathy.

5. Monitoring of X-ALD patients

Once the diagnosis is established in a patient, genetic testing seems reasonable in the entire family. If the affected individual is a male infant/child, the American Pediatric Endocrine Society suggests laboratory screening of cortisol and ACTH levels (every

Box 2. X-ALD diagnosis

Suspect X-ALD
• In males with symptoms and signs compatible with **adrenal insufficiency**. • In males with neurological symptoms and signs compatible with **cerebral** X-ALD. • In patients with **progressive myelopathy** after exclusion of most frequent causes.
If X-ALD is suspected
• Obtain plasma **VLCFA levels**. • Proceed to ***ABCD1* gene mutation analysis**.
Ancillary paraclinical evaluation
• Brain and/or spinal cord MRI. • Laboratory testing for ACTH, cortisol, electrolytes (potassium, sodium), glucose.

Box 3. Sample collection for plasma VLCFA levels determination

According to the Greek Institute of Child Health, sample collection should be carried out as follows:
Time of blood collection: In the morning, fasting blood sample.
Type of sample: Take 5 cc of blood in a heparinized syringe (Heparin sodium) and transfuse in an empty tube.
Transport conditions for samples: Preferably immediate after blood sample collection. If not possible, place samples in cold storage at +2°C to +8°C until shipment via cooled transport (4 °C).
Special cautions: Inform the laboratory in case of prior blood transfusion. In case of hemolysis, obtain a new sample as hemolysis might increase VLCFA levels. Other factors affecting VLCFA levels include: ketogenic diet, liver disease, and dyslipidemia.

3-4 months in the first 2 years and every 4-6 months after 2 years of age) [56]. Regarding MRI surveillance, the following protocol has been recently proposed: a) Obtain an MRI between 12 and 18 months old; b) Obtain a second MRI 1 year after baseline; c) Between 3 and 12 years old, obtain a contrast-enhanced MRI every 6 months; d) After 12 years, obtain an annual MRI. For the time being, no guidelines exist for neuroimaging in adults [57]. T2 hyperintensities can be observed in the involved areas, i.e. corpus callosum, visual pathway, supratentorial white matter and major projection fibers. The presence of gadolinium enhancing lesions reflects the disruption of the blood brain barrier and marks the transition to the demyelinating stage of the disease [38].

The Loes MRI Severity Score is a grading system used to assess the severity of MRI lesions and may range from 0, meaning no disease activity, to 34, which is indicative of the most severe disease. This score is of importance as therapeutic interventions are indicated for subjects with a score between 0.5 and 9 [58]. If the affected individual is female, routine monitoring is not recommended, neither for adrenal insufficiency nor for cerebral ALD. Spinal cord atrophy may be observed amongst individuals suffering from AMN [37]. A recent study showed that patients' spinal cord cross-sectional area correlates with the severity of myelopathy, suggesting that it may serve as a monitoring tool for AMN patients [59].

6. Therapeutic management of X-ALD patients

As expected, a multidisciplinary approach is recommended for the therapeutic management of X-ALD patients. For patients suffering from adrenomyeloneuropathy, no established etiological therapeutic options exist so far. Physical therapy along with treatment of urologic complications and counseling might be of value [60, 61]. If adrenal insufficiency is diagnosed in an affected male, corticosteroid replacement therapy is vital [56, 62]. X-ALD newborn screening in some countries, along with the improvement of imaging modalities, have allowed for more timely intervention, as hematopoietic stem cell transplantation (HSCT) is only indicated for early cerebral disease states. Substantial research is currently underway, using various approaches, in order to identify new effective therapeutic options, offering promising prospects for X-ALD patients [63].

6a. Allogeneic Hematopoietic stem cell transplantation

Allogeneic Hematopoietic stem cell transplantation (HSCT) is a treatment, suitable for boys and adolescents in early stages of CALD. HSCT is thought to halt neurologic progression when performed in these stages, though the underlying mechanism remains unclear [64, 65]. In contrast to neurologic progression, HSCT has no impact on the progression of ad-

Box 4. Monitoring of X-ALD patients

Upon establishment of X-ALD diagnosis, the following management protocol is proposed:
- **Adrenal insufficiency** surveillance
  - Every 3-4 months, if age ≤ 2 years.
  - Every 4-6 months if age > 2 years.

In case of **abnormal** findings, refer to an **endocrinologis**t for **corticosteroid replacement** therapy.

- **MRI** surveillance
  - Obtain an MRI between 12 and 18 months old.
  - Obtain a second MRI 1 year after baseline.
  - Between 3 and 12 years old, obtain a contrast-enhanced MRI every 6 months.
  - After 12 years, obtain an annual MRI.

In case of **abnormal** findings, refer to an **HSCT/gene therapy** specialized center.

- **Genetic testing** and **counselling** seem reasonable in possibly affected family members.

renal insufficiency. Transplantation, when performed in appropriate candidates, not only offers a survival advantage, but also prevents the development of major functional disability. In order to be considered for HSCT, patients must have few lesions on brain MRI and remain in a good clinical condition, as determined by the ALD-specific Neurologic Function Scale (NFS) and the Loes MRI severity score [58]. HSCT is ineffective in patients with advanced disease and cannot reverse neurologic impairment already present at the time of the procedure. In addition, disease stabilization occurs 3-24 months after HSCT, leading to a possible accumulation of disability in the meantime [15, 66]. As expected, allogeneic HSCT may be associated with acute mortality and late complications, such as failure of donor cell engraftment and graft-versus-host disease [15]. Finally, recent reports have drawn attention to a potential beneficial effect of HSCT even in adult patients with CALD [67, 68].

6b. Gene therapy

For the purpose of overcoming allogeneic HSCT's limitations, i.e. finding of a suitable donor and possibility of developing graft versus host disease, transplantation of autologous, genetically-modified hematopoietic stem/progenitor cells (HSPCs) was proposed [69]. A normal copy of the responsible gene is delivered via gene transfer to the HSPCs, which are then infused into the patient. Before the infusion, busulfan, a myeloablative agent with the ability to facilitate the engraftment of the transplanted HSPCs in the hematopoietic and the central nervous system, is administered to the patient [70].

In July 2021, elivaldogene autotemcel (SKYSONA™, eli-cel; Lenti-D™ gene therapy) received approval for the treatment of early cerebral X-ALD in patients below the age of 18, for whom an HLA-matched sibling-hematopoietic stem cell (HSC) donor is not available. The approval study protocol involved 30 boys aged 4 to 14 years with early CALD. According to the study's results, after two years, 90% of the treated boys showed no signs of major functional disability and approximately 96% of the boys experienced a stable Gross Neurological Function Measure score (a score measuring the developing child's ability to achieve expected motor milestones) after two years. Furthermore, there was evidence of continuing benefit for up to 8 years [71]. Developing gene therapy techniques, such as antisense oligonucleotides and small interfering RNAs, may also have a place in the future treatment of X-ALD [72].

6c. Lipid modulation

Lorenzo's oil is a mixture comprising a 4:1 mix of oleic and erucic acids,that, in conjunction with a low-fat diet, normalizes plasma VLCFA levels. Despite several studies arguing against its efficacy in preventing CALD progression once it already exists, Moser et al., reported that it could prevent the development of CALD in presymptomatic subjects [73]. There have also been some reports of benefit of Lorenzo's oil in males suffering from AMN, but came from studies with questionable methodology [74].

In a recently published paper, Moser et al. reported the VLFCA-lowering effect of the antihypertensive irbesartan in cultured skin fibroblasts from an X-ALD patient, implying a potential beneficial effect in X-ALD that should be further validated [75].

Lastly, it has been recently shown *in vivo*, that metabolic rerouting of saturated to monounsaturated VLCFAs by upregulating the enzyme Stearoyl-CoA Desaturase-1 (SCD1 induction) may decrease lipid toxicity, a strategy that may prove of benefit in X-ALD [69].

6d. Antioxidant therapy

In a small open-label trial, 13 patients suffering from AMN were administered a high dose of α-tocopherol, N-acetylcysteine, and α-lipoic acid in combination. Normalization of biomarkers suggestive of oxidative stress and inflammation was observed, as well as a beneficial effect on the 6-min walk test, justifying larger future placebo-controlled trials [76].

Leriglitazone is a newly developed full PPARγ agonist which can cross the blood brain barrier. It has been proved to decrease oxidative stress, increase adenosine 5'-triphosphate concentrations, as well as exert a neuroprotective effect in animal models of AMN. The study's findings also suggest a potential beneficial role for cerebral X-ALD, as it was shown to prevent the progression to disrupted blood-brain barrier [77].

7. Conclusion

Increasing understanding of X-ALD pathophysiology has resulted in the emergence of new promising treatment options. Newborn screening for X-ALD enables the identification of patients at high risk for life-threatening adrenal insufficiency and cerebral ALD early in the disease course, allowing early corticosteroid replacement therapy and timely HSCT or gene therapy for suitable candidates. Hopefully, gene therapy approaches similar to those recently approved for other neurologic monogenic diseases, such as spinal muscular atrophy and Duchenne muscular dystrophy, may be developed for X-ALD in the near future.

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GAUCHER DISEASE: A LYSOSOMAL STORAGE DISORDER WITH MANY TYPES

Chrysoula Marogianni, Efthimios Dardiotis, Georgia Xiromerisiou

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

Abstract

Gaucher disease is a chronic multisystem disease with a genetic background. It is inherited in an autosomal recessive way and belongs to the lysosomal cumulative diseases. The symptomatology is due to the decreased activity of the lysosomal enzyme glucocerebrosidase and the accumulation of glucosylceramide in macrophages. The disease presents three types, with the predominant type being Gaucher Disease type 1 (GD1), which does not show neurological involvement and the types GD2, GD3 that present with various, usually severe neurological symptoms. There are two main therapeutic approaches for the disease, the treatment of enzyme replacement and the one that involves the reduction of the therapeutic substrate.

INTRODUCTION

Gaucher disease is the most common sphingolipidosis. Sphingolipidoses are part of lysosomal cumulative diseases and relate to the dysfunction in the process of catabolism of certain critical metabolites that are either in the cell membrane or are regulators of signaling pathways [1].

Gaucher disease is a rare, autosomal recessive genetic disorder, first described in 1882 by Philippe Gaucher. The French dermatologist, while he was still a medical student, recognized in a 32-year-old woman a clinical picture of marked splenomegaly without leukemia, and despite attributing the clinical picture to some type of cancer, he published his findings in his doctoral thesis. Many years later the biochemical nature of the disease, which took his name, was fully understood [2].

Gaucher disease (GD) is caused by mutations in the gene for Acid beta-glucocerebrosidase or beta-glucosidase (GBA1), which is located on chromosome 1 (1q21). These mutations cause a severe decrease in the activity of the lysosomal enzyme glucocerebrosidase (GCase), which normally hydrolyzes glucosylceramide (GlcCer) to ceramide and glucose [3]. Up to date, more than 400 mutations in the GBA1 gene have been described. Mutations in the GCase activator, saposin C, and more specifically in the PSAP gene, cause a similar clinical picture, but are much rarer [3, 4].

EPIDEMIOLOGY

GD is a disease found in all nationalities and races, although it has an extremely high prevalence among Ashkenazi Jews. Specifically, among the latter, it has been detected a frequency of 1: 850, while the carriers are 1:17 [5]. In the general population, the incidence of the disease is approximately 1/40,000 to 1/60,000 births [6].

PATHOPHYSIOLOGY

Mutations in the GBA1 gene that cause a decrease in glucocerebrosidase activity lead to the accumulation of glucosylceramide in macrophages, transforming them into Gaucher cells [7]. Gaucher cells are found mainly in the bone marrow, spleen, and liver. The pathophysiological mechanism leading to the neurological complications of the disease has not yet been fully elucidated [8]. An autophagic dysfunction is hypothesized, as well as the amplification of another metabolic pathway resulting in the accumulation of glucosylsphingosine which causes neuronal dysfunction and death.

CLINICAL PICTURE

Gaucher disease is characterized by hepatosplenomegaly, cytopenia, severe bone damage and in some cases, serious neurological complications. There are three types of the disease (table 1, Figure 1).

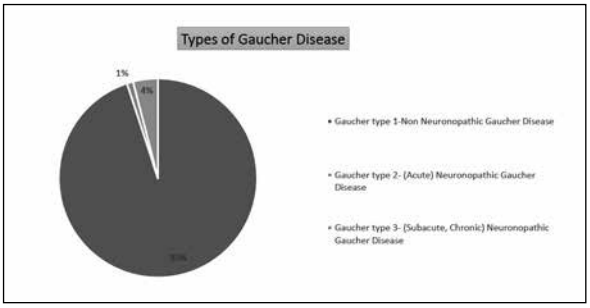
Type 1

Type I (GD1) is the most common (prevalence 90-95% in Europe and North America). It has a varied clinical picture, and its spectrum extends from asymp-

Table 1. Symptomatology of Gaucher disease types

Gaucher type 1	Gaucher type 2	Gaucher type 3
Splenomegaly	Cerebellar symptoms	Horizontal saccadic movement disorder
Hepatomegaly	Opisthotonus -trunk rigidity	Ocular muscle apraxia
Bone disease, bone pain crises	Eye movement dysfunction	Myoclonus
Anemia, cytopenia	Epileptic seizures	Epileptic seizures
Fatigue	Medullar dysfunction	Extrapyramidal symptoms
Abdominal pain	Apnea crises	Dementia

Figure 1. Diagram showing the prevalence of Gaucher Disease subtypes



tomatic patients throughout their lives to forms with early onset in childhood. The mean age of diagnosis is between 10-20 years [9].

The symptoms of the disease can vary and affect different organs, but without neurological complications. Half of the patients report intense fatigue, which affects their daily life [10]. One of the most common symptoms is osteopenia or even osteoporosis, which occurs at a much younger age than normal [11]. Pathological fractures often occur as well (mainly of the long bones and vertebrae) [12]. In addition to the reduction of bone density, that is mainly responsible for them, fractures can often be the result of focal osteolytic lesions. In cases involving mainly the lower jaw, cystic lesions locally can even lead to serious dental abnormalities [13, 14]. With the use of magnetic resonance imaging, both bone marrow infiltration and bone infarction, lytic and osteonecrotic lesions can be assessed [15]. On X-ray, lesions can be found around metaphysis/diaphysis of the femur. The deformity consists of lack of modeling of this specific area of the bone with abnormal cortical thinning and lack of the concave di-metaphyseal curve, resulting in an Erlenmeyer flask-like appearance. These lesions occur mainly in childhood [16].

Gaucher cells are also found in other organs such as the lungs, mainly in patients homozygous for the 1448G (L444P) mutation [17]. Patients with GD1 and pulmonary involvement may have a picture of interstitial lung disease, which could potentially lead to pulmonary fibrosis or pulmonary hypertension. Pulmonary hypertension is more common in patients who have had a splenectomy, especially women.

Rarely, proteinuria and hematuria are found after the infiltration of renal glomeruli by Gaucher cells [18]. Complications from the cardiovascular system, the eyes or the skin are rare in GD1 [19]. More specifically, in some cases, there is a yellow-brown pigmentation of the skin in the anterior region of the tibia as well as on the cheeks.

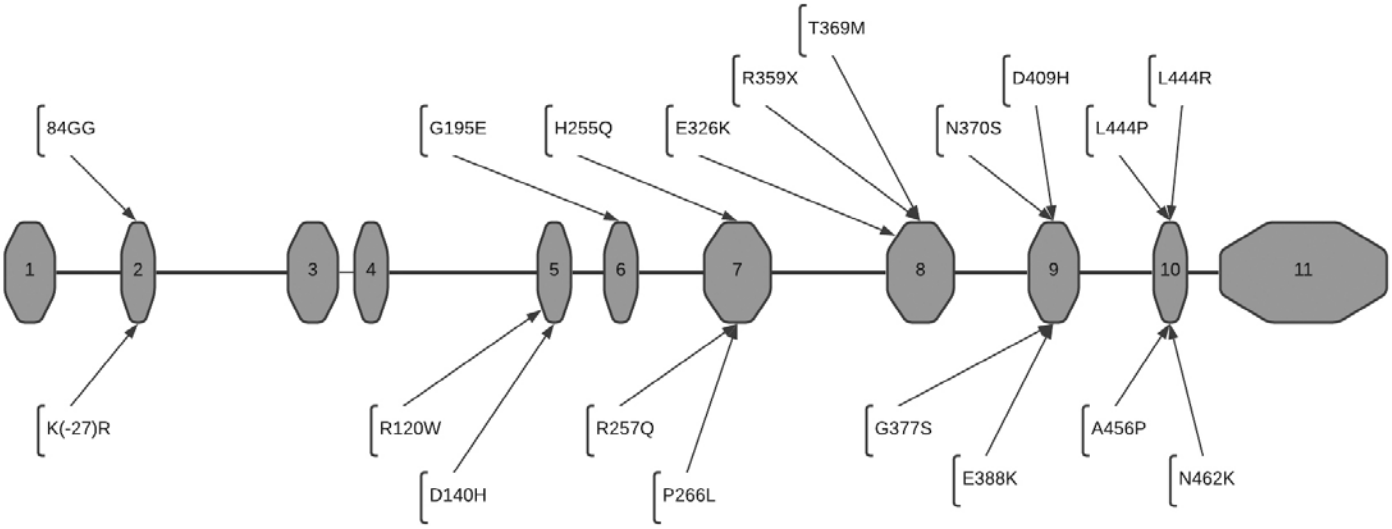
Interestingly, there is an association between GD1 disease and an increased risk of developing Parkinson's disease. Therefore, mutations in GBA are observed in 2-30% of patients with Parkinson's disease [20]. In fact, Parkinson's disease in patients with GD1 as well as in heterozygous carriers occurs at a younger age compared to the general population and often occurs with dysautonomia and dementia [21, 22].

Finally, it has been reported an increased risk of certain malignancies, especially multiple myeloma in patients with GD1.

Type 2

Gaucher type 2 disease is very rare (affects <5% of cases in most countries) and is characterized by very serious neurological complications that begin in neonatal life, while a fetal subtype is described (extremely rare, <1% of cases and the most severe form) [23]. The neurological burden begins in infants 3-6 months of age, who most commonly initially show hepatosplenomegaly. In 60% of cases splenomegaly is associated with thrombocytopenia. The clinical triad that enhances the diagnosis of the disease consists of cervical and trunk stiffness, bulbar signs and oculomotor dysfunction. Pyramidal and extra-pyramidal signs are found in the neurological examination of newborns. Affected infants often suffer from episodes of apnea, which are associated with a gradually increasing duration of laryngeal spasm

Figure 2.



[24, 25]. Seizures, usually of the type of refractory myoclonic epilepsy and slowing of neonatal psychomotor development are common in GD2. Bone complications in type 2 are not described. There may also be involvement of the lung, most commonly with lesions resulting from repeated aspirations as well as pulmonary infiltration by Gaucher cells. Death usually results from prolonged apnea or mass aspiration and occurs before the first 3 years of patients' lives [26]. The fetal form of the disease is characterized by hydrops, hepatosplenomegaly, ichthyosis, arthrogryposis, facial deformity, and fatal thrombocytopenia. These embryos usually end up in the womb or shortly after birth. Nevertheless, the diagnosis of the disease is considered very important in order to offer the suitable genetic guidance and advice to the parents [26].

Type 3

Gaucher type 3 disease (affects 5% of cases, but there are studies describing that it can reach up to 33% of them), is a combination of type 1 and 2, as in GD3 coexist the visceral complications of type 1 and neurological complications [27]. It is often referred to as adolescent or subacute neurological GD, as it most commonly occurs before the age of 20 years. Type 3 neurological complications may be mild, such as slowed horizontal saccadic eye movement, but the disease may present with severe neurological complications such as progressive myoclonic epilepsy (affecting 16% of patients with type GD3) or cerebellar ataxia and spasticity [28-30]. Systemic complications on the other hand are usually mild (hepatosplenomegaly, bone disorders, cytopenia). Patients with the mutation c.1342G > C (D409H) show progressive calcification of the aorta and heart valves, as well as corneal and hydrocephalus lesions [31]. The patients with the rare deficiency of saposin C present with a clinical picture corresponding to GD3, having all the main neurological complications of the disease [32].

DIAGNOSIS

The diagnosis of the disease is made by measuring the activity of the enzyme glucocerebrosidase. The measurement is made in circulating leukocytes or monocytes or in fibroblast culture [33, 34]. Most commonly, the enzyme activity is at 10-15% of normal value. We can detect the decrease in the enzyme activity by checking the blood monocytes with flow cytometry, although this method remains to be certified by more centers [35].

If the activity of the enzyme glucocerebrosidase is normal, but there is a high clinical suspicion or biomarkers that point to the possibility of GD (especially when chitotriosidase is very high), then the PSAP gene should be tested for mutation in sapocin C [36].

Bone marrow biopsy is not a necessary test to confirm the diagnosis of GD. In fact, as Gaucher-type cells can be seen in certain hematological diseases such as lymphoma, chronic lymphocytic leukemia, and others, it is advisable to avoid the test, as it may be misguiding [37].

The genotyping of the GBA1 gene encoding GCase will confirm the diagnosis and identify the responsible mutation. More than 400 mutations of the disease have been described in the GBA1 gene, the most common of which are the following: c.1226A > G (N370S), c.1448T > C (L444P), c.84dup, c.115 + 1G > A (IVS2 + 1G > A) and R120W/RecNciI (39.40) (Figure 2).

Biomarkers of the disease

Chitotriosidase is an enzyme produced by activated macrophages, and in the case of GD by Gaucher cells.

Its levels in patients with GD who do not receive treatment are particularly high and therefore can act as a biomarker of the disease, as well as an indicator of the response to treatment [36]. The limitation of the usefulness of the measurement of chitotriosidase is its presence in other lysosomal cumulative diseases (such as Niemann-Pick, although in this case it is at lower levels), in granulomatous diseases, such as sarcoidosis and other diseases (b thalassemia, Alzheimer's disease, multiple sclerosis) [38]. Also, there is a mutation in chitotriosidases' gene that lead to a deficiency in the activity of the enzyme in the general population. This fact makes it difficult to use the enzyme measurement as a biomarker for the disease and especially as a biomarker between patients with GD, measuring the severity of their disorder or their response to treatment. Finally, there are different techniques for measuring the enzyme levels, which may also worsen the comparison of the results between different reference centers [39].

Another biomarker of GD is the chemokine CCL18/PARC, which like chitotriosidase is secreted by Gaucher cells. In the plasma of patients with GD this chemokine is found 20 to 50 times higher than normal [36].

The transmembrane protein gpNMB (glycoprotein nonmetastatic melanoma protein B) has also been found to be increased 50-fold in the plasma of patients with GD1 [40]. A recent study has confirmed this association, while another study describes the presence of elevated levels of this protein in the CNS of patients with GD3 [41].

Glucosylsphingosine is a biomarker under investigation, which appears to be more specific for the disease than chitotriosidase and CCL18. While further studies are needed to support the wider use of glucosylsphingosine, it has recently been found to make a significant contribution to monitoring patients' response to treatment [42, 43].

Finally, ferritin, although not a specific indicator of the disease, seems to provide useful information at different stages of the disease. It is elevated in most patients with GD, with serum iron levels and transferrin saturation being normal [44, 45]. Iron stores are mainly found in the liver and bone marrow, so ferritinemia also functions as a predictor of the onset of bone complications of the disease. Splenectomized patients with GD also have high ferritin.

TREATMENT

There are two types of treatment for Gaucher disease. The treatment is not suitable for all types of the disease, nor for all its stages. It is important to diagnose the disease early, before permanent and irreversible complications of the disease, such as osteoarthritis, vertebral fractures, osteonecrosis as well as massive fibrous splenomegaly, hepatic or pulmonary fibrosis [46, 47].

Enzyme replacement therapy

The principle of enzyme replacement concerns the supply to the cells of the glucocerebrosidase that they lack. The drugs on the market are analogs of recombinant DNA produced by the human enzyme β-glucocerebrosidase (imiglucerase, velaglucerase) but also by plant-derived glucocerebrosidase (taliglucerase) [48-50]. The administration of the above treatments is intravenous, while the dosage and frequency of administration are determined based on recommendations from the International Working Group on Gaucher Disease (ICGG), as well as the guidelines and the treatment goals. A typical starting dose for children and adults with severe symptoms is 60 U/Kg body weight every 2 weeks, with the dose reduced by half when a therapeutic effect is achieved [51]. Lower doses may reduce the cost of treatment and are recommended for use in patients with a stable clinical picture of GD1. The evaluation of the response to treatment is done by controlling a blood analysis report of the patients, the bone density and their quality of life in general through various scales (pain, etc.) [52].

All enzyme replacement therapies can be given to patients suffering from GD1, who are symptomatic or have laboratory/biological abnormalities, and only imiglucerase has been officially approved for GD3 [53, 54]. Up to date, no cure has been found for GD2, that can reverse, stabilize or delay the course of the disease. This is partly due to the very rapid progression of the disease and the serious neurological complications. Enzyme replacement therapy is usually well tolerated, with 2-14% of patients developing antibodies to the enzyme after a while. Allergic reactions are rare [55].

Substrate reduction therapy

Substrate reduction therapies aim to reduce the excess glucosylceramide, substantially reducing its production. Miglustat, which is an inhibitor of glucoceramide synthase, works by reducing its production in Gaucher cells [56]. It is prescribed in mild to moderate GD1, when it has failed or for some reason no enzyme replacement therapy can be given [57]. The most important benefit of the drug is the control of the increase in the size of the liver and spleen, while it also reduces the levels of chitotriosidase. Its effectiveness in hematological and bone complications of the disease seems to be limited [58]. It is an oral medicine, and the recommended dose is 100 mg, three times a day. It should not be given during pregnancy and patients should take contraceptive measures when they are on medication [59].

Eliglustat is a newer drug, which is a potent and specific inhibitor of glucosylceramide synthase and acts as a substrate reduction therapy for patients with GD1 [60]. It is classified as a first-line treatment, and while it appeared to be similar to Miglustat in most comparisons, eliglustat appeared to provide higher protection to patients with respect to GD1's bone complications [61]. Special care should be taken before initiating the drug, as it has serious side effects when administered to patients with rapid or intermediate metabolism of CYP2D6. In every case and before the first dose, patients should be tested by CYP2D6 genotyping to determine metabolic status [62]. Eliglustat is contraindicated in patients with severe heart disease or in patients receiving class IA and III antiarrhythmics drugs.

Substrate reduction therapies do not appear to help in cases of GD2, GD3, specifically in the neurological complications of the disease, although Miglustat appears to cross the blood-brain barrier.

Treatment with small accompanying molecules -chaperones

Chaperones are small molecules that bind to proteins in the endoplasmic reticulum, helping target proteins to form properly and thus stabilizing their structure. This is a treatment that helps to enhance the activity of the enzyme, in this case glucocerebrosidase, as mutations in the GCase gene often causean abnormal folding of the protein, resulting in its early degradation [63]. Ambroxol has been studied in high doses in combination with enzyme replacement therapy with good results [64]. Isophagomine is still being studied with encouraging in vitro results [65].

Gene therapy

The first results from the use of gene therapy in Gaucher disease were not very encouraging. Human glucocerebrosidase cDNA was successfully transferred to mouse and human hematopoietic stem cells and progenitors, with satisfactory expression of glucosidase in transplanted mice, but with a low rate of expression in vivo, and therefore no clinical benefit reported [66]. The above study was performed in patients with GD3.

Symptomatic treatments

Following the widespread use of enzyme rehabilitation therapies, routine splenectomy in patients with GD1 has been abandoned. It is now recommended in rare cases of non-response to enzyme therapy or in cases of splenic rupture [67].

For bone pain attacks, short-term bed rest and strong analgesic treatment are recommended. Orthopedic surgical evaluation is imperative in cases of pathological fractures and osteonecrosis. Regarding the use of bisphosphonates, they do not seem to improve bone density, considering that the pathophysiological mechanism has not been clarified yet. However, they are mainly given to postmenopausal women, more commonly in the case of severe osteoporosis [68]. Patients with GD should always be tested for possible coagulation disorders before any invasive procedure.

DISEASE MONITORING

Gaucher disease is a chronic, multisystemic disease. Patients with GD need both clinical monitoring (by a physician, hematologist, neurologist in the case of GD2, 3), as well as regular laboratory and imaging tests.

Enzyme replacement therapy can improve hematological disorders, reduce biomarker levels, control hepatosplenomegaly and long-term bone density disorder. Regular monitoring is also recommended for asymptomatic patients in diagnosis.

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HYPERHOMOCYSTEINEMIA

Nikolaos P. Margos[1], John Ellul[2]

[1] School of Medicine, University of Patras, University Campus, Rio, Greece
[2] Department of Neurology, University Hospital of Patras, Patra, Greece

INTRODUCTION

Homocysteine is a non-proteinogenic amino acid and differs from cysteine by an additional methylene bridge. It is synthesized from methionine and can be recycled into methionine or converted into cysteine in the presence of certain B vitamins (Figure 1).

Homocystinuria, a rare autosomal disorder characterized by steep elevation of homocysteine in plasma and urine, is always accompanied by systemic clinical manifestations. On the contrary, hyperhomocysteinemia is characterized by a less markedly elevated level of homocysteine (above 15 μmol/L) in serum measurements [1]. Hyperhomocysteinemia is more common than homocystinuria and affects 5-7% of the general population [2].

High levels of homocysteine are associated with increased cerebrovascular, cardiovascular, and thromboembolic diseases, and appear to be an independent marker of atheromatic disease [3, 4]. There is clear evidence that lowering homocysteine levels is beneficial in both slowing the acceleration of brain atrophy [5] and in decreasing cardiovascular risk in patients with homocystinuria [6]. Nonetheless, the evaluation and treatment of hyperhomocysteinemia still remains controversial, as studies have shown that homocysteine-lowering therapies do not significantly affect the prevention of stroke and/or coronary heart disease [7, 8].

ETIOLOGY

Genetic factors: The most common form of genetic hyperhomocysteinemia results from a mutated methylene tetrahydrofolate reductase (MTHFR) that has reduced enzymatic activity, thus leading to the accumulation of homocysteine in serum, especially in mutated MTHFR C677T homozygotes [4, 9]. A marked ethnic and geographical variation of the mutated enzyme has been observed [10], with 8% to 20% of the affected individuals being homozygous in North America, Europe, and Australia [11]. Additionally, *MTHFR* A1298C polymorphism has been associated with an increased risk for schizophrenia [12].

Vitamin deficiencies: Vitamin B12, folate and vitamin B6 deficiencies can all lead to increased blood levels of homocysteine since they are used as cofactors in the enzymatic pathways of homocysteine metabolism [13, 14]. Decreased B12 absorption and intake may play an important role in elevating serum homocysteine levels in older adults, while low intake of folate as a cause of hyperhomocysteinemia is relatively common in the general population.

Other causes include: chronic kidney disease (due to impaired renal excretion), drugs (i.e. fibrates, nicotinic acid, metformin, methotrexate) though with uncertain clinical significance, smoking and hypothyroidism [3, 15, 16].

CLINICAL PRESENTATION

The clinical presentation of hyperhomocysteinemia depends on the underlying etiology, and in most cases moderately increased homocysteine serum levels produce no apparent symptoms. However, markedly elevated levels are associated with atherogenic and prothrombotic properties. Intimal thickening, elastic lamina disruption, smooth muscle hypertrophy, marked platelet accumulation and the formation of platelet-enriched thrombi can all be signs of vascular injury induced by homocysteine [17].

Most importantly, homocysteine induced vascular injury can cause cardiovascular and cerebrovascular disease, peripheral arterial disease, and heart failure [4, 7, 18, 19]. Studies have shown that a 5μmol/L increase in homocysteine levels is associated with a 20% higher risk for coronary heart disease (CHD) [20]. Ischemic stroke has also been associated with hyperhomocysteinemia [21, 22]. However, lowering homocysteine levels does not seem to decrease cardiovascular events in contrast to the well-known effectiveness of traditional vascular risk factors management [23, 24].

Moreover, the association of hyperhomocysteinemia with venous thromboembolism (VTE) remains to this day controversial. Even though some studies conclude that hyperhomocysteinemia is a risk factor for VTE [25, 26], additional research suggests that this may be due to confounding factors [27, 28]. An association with neurodegenerative diseases such as Alzheimer disease, cognitive decline – dementia and Parkinson's disease, has also been reported [29]. Furthermore, a clear relationship between hyperhomocysteinemia and obstetric complications has not been established. Some studies have reported

Figure 1. Homocysteine metabolic pathway [46]

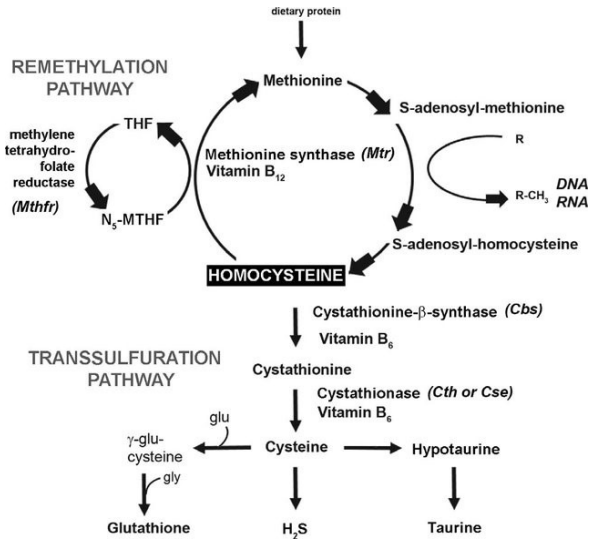
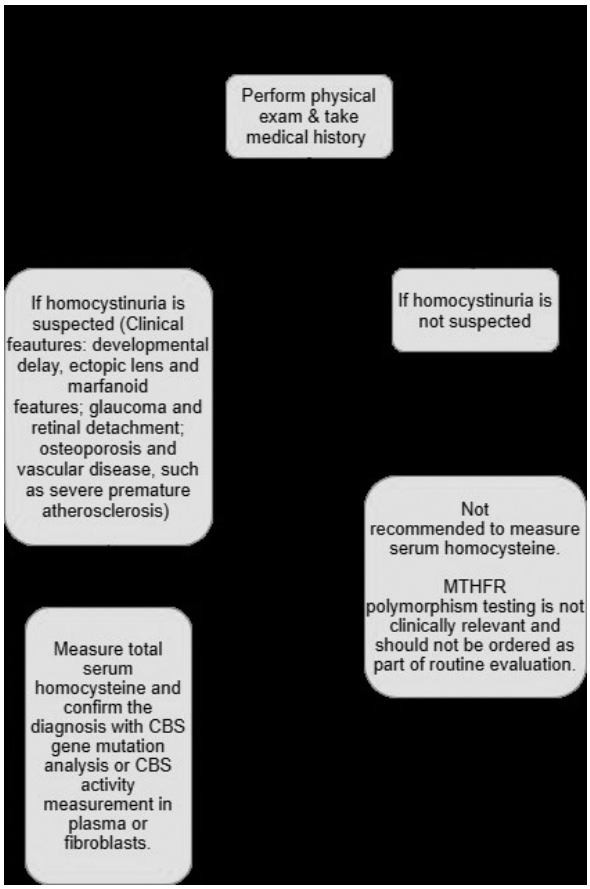


Figure 2. Evaluation of hyperhomocysteinemia



preeclampsia, abruptio placentae, fetal growth restriction and neural tube defects as possible side effects [30, 31]. However, a more recent meta-analysis showed no connection between MTHFR mutations and obstetric complications [32].

Other disease associations such as hip fractures, schizophrenia and osteoporosis [33, 34] have been reported, but are not always clearly linked with hyperhomocysteinemia [35]. Hyperhomocysteinemia and possible disease associations are shown in Table 1.

EVALUATION

Evaluation (Figure 2) should initially include a complete physical examination and thorough medical history. Although there is no recommended screening for hyperhomocysteinemia, physicians should always check homocysteine levels if homocystinuria (cystathionine β-synthase (CBS) deficiency) is suspected. The four most prevalent CBS mutations include p.Ile278Thr, p.Thr191Met, p.Gly307Ser, and p.Trp323Ter [36]. Clinical features of this disease include developmental delay, ectopic lens and marfanoid features. Children and young adults often present with glaucoma and retinal detachment, alongside osteoporosis and vascular disease, such as severe premature atherosclerosis [37]. Homocystinuria due to CBS deficiency is a rare, but potentially lethal disease, thus prompt diagnosis leads to better prognostic outcomes. Diagnosis should be made by measurement of total serum homocysteine (tHcy).

Table 1. Hyperhomocysteinemia & possible disease associations

Hyperhomocysteinemia & possible disease associations
Vascular disease (cardiovascular, cerebrovascular)
Venous Thromboembolism
Alzheimer disease
Cognitive decline - dementia
Parkinson disease
Obstetric complications (i.e. preeclampsia, abruptio placentae, fetal growth restriction)
Hip fractures, osteoporosis
Schizophrenia

Elevated tHcy accompanied by borderline or high plasma methionine concentrations makes the diagnosis very likely. CBS deficiency should be confirmed by mutation analysis of the biallelic pathogenic CBS variant and/or measurement of CBS activity in plasma or fibroblasts [38].

In patients who lack homocystinuria clinical features and present with cardiovascular disease, stroke or venous thromboembolism, it is not recommended to measure tHcy. MTHFR polymorphism testing is not clinically relevant and, should not be ordered as a part of a routine evaluation [39].

Total homocysteine must be measured in patient plasma/serum. The samples must be collected after the patient has fasted overnight and analyzed as soon as possible to avoid falsely decreased or elevated homocysteine levels. Refrigeration of the samples inhibits homocysteine accumulation for at least 4 hours [40].

For the record, normal homocysteine levels range between 5-15 μmol/L.

Kang et al. classified hyperhomocysteinemia in 1992 [1]:

- Moderate: 15-30 μmol/L.
- Intermediate: 30-100 μmol/L.
- Severe: >100 μmol/L.

TREATMENT

A large meta-analysis that included 71,422 participants showed that homocysteine lowering therapy (HLT) such as folate, vitamin B12, or vitamin B6 does not reduce the risk for myocardial infarction [41].

Interventions to reduce plasma homocysteine level in post-stroke patients did not have any effect in mitigating the severity of ischemic stroke outcome [23]. In addition, recurrent VTE is not prevented in patients with hyperhomocysteinemia that are treated with HLT [42]. Similarly, no clear evidence that HLT is useful in neurodegenerative diseases exists.

Nevertheless, advocates of treating hyperhomocysteinemia with B vitamins and folate still exist. For instance, a small study reported that HLT can improve cognitive function in patients with hyperhomocysteinemia and schizophrenia. A possible mechanism linking hyperhomocysteinemia to schizophrenia depends on the interaction of homocysteine with NMDA receptors. It also initiates oxidative stress, causes mitochondrial dysfunction and cell apoptosis. [34] Additionally, a review paper suggests HLT initiation when serum levels exceed 15 μmol/L [43].

Finally, the only medical condition where treating hyperhomocysteinemia would have an effect in patient prognosis and symptom management is homocystinuria. HLT in this patient group does indeed lower cardiovascular risk [44, 45].

CONCLUSION

In conclusion, hyperhomocysteinemia still remains a controversial occurrence, which is often considered a secondary manifestation and possibly entails several cardiovascular and metabolic implications. Still, more research is needed for clear correlations with diseases to be made, and for more clinically relevant therapeutic options to be developed.

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HEREDITARY TRANSTHYRETIN AMYLOIDOSIS: CLINICAL CHARACTERISTICS, DIAGNOSIS AND MANAGEMENT

Efthimios Dardiotis[1], Theodoros Kyriakides[2]

[1] Laboratory of Neurogenetics, Department of Neurology, University Hospital of Larissa, Faculty of Medicine, School of Health Sciences, Larissa, Greece.
[2] University of Nicosia Medical School, Nicosia, Cyprus-corresponding author

Abstract

Hereditary transthyretin amyloidosis (hATTR) is an adult onset, lethal, autosomal dominant, multisystemic disease due to the deposition of mutated transthyretin (TTR) in various tissues, mainly the peripheral nerves and the heart. Circulating mutated TTR tetramers are unstable and dissociate into misfolded monomers which then polymerize into amyloid. Although there are more than 120 mutations in the TTR gene, the p.Val30Met mutation is by far the commonest and typically presents with a length dependent sensory and autonomic axonal neuropathy or a mixed phenotype that also includes cardiomyopathy. Due to its multisystemic phenomenology patients may present to non-neurologists and diagnosis can be delayed. Treatment is now available and includes TTR stabilizers as well as gene silencing therapies. Timely diagnosis is of paramount importance for a better prognosis.

Introduction

Hereditary transthyretin amyloidosis (hATTR) is an adult onset, autosomal dominant, multisystemic disease due to the deposition of misfolded mutated transthyretin (TTR) in various tissue beds [1]. Peripheral nerves and the heart are most commonly affected but clinical phenotype is variable. The TTR gene consists of four exons and is located on chromosome 18 [2]. TTR circulates as a homotetramer in plasma and each monomer consists of a 127-amino acid peptide with a predominant β-pleated sheet secondary structure. TTR is primarily synthesized by the liver (>90%), retinal pigment epithelium and the choroid plexus. In the circulation, the primary functions of TTR are to be a carrier of vitamin A, in association with the retinol binding protein, and a carrier of about 15% of thyroxine [3]. The name trans-thy-retin is derived from its function in plasma. However, an increasing number of physiological roles for TTR are being recognized both in the peripheral and central nervous systems [4].

To date, there are more than 120 TTR mutations associated with hATTR with the most common being the p.Val30Met mutation which causes predominantly a neuropathic or mixed phenotype, the latter occurring particularly in late onset patients, defined as disease onset after the age of 50 years [5]. The clinical phenotype of hATTR depends on the predominant tissue bed affected which in turn is predominantly determined, via poorly understood mechanisms, by the specific mutation. Penetrance of specific mutations may vary in different populations and is probably modulated by genetic modifiers [6].

Epidemiology

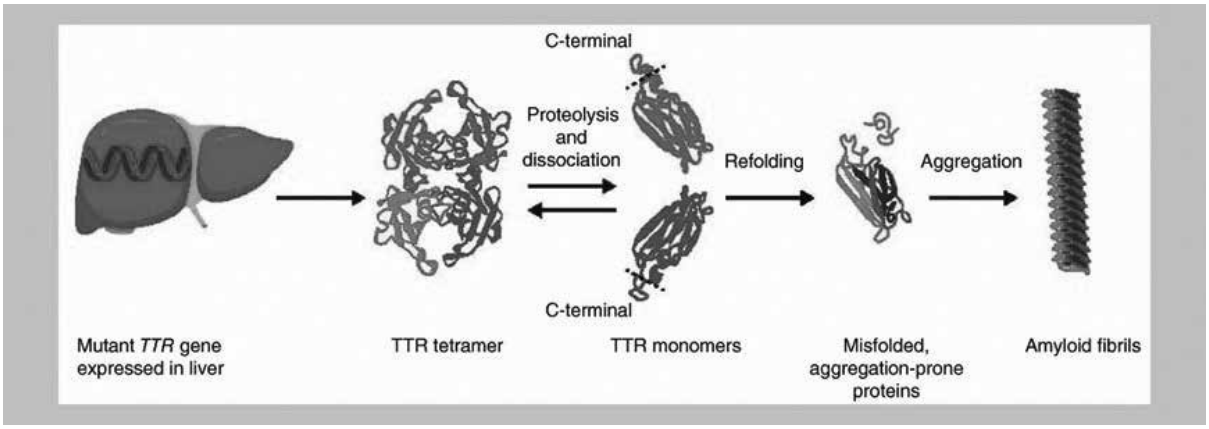
Hereditary transthyretin amyloidosis is found worldwide with major endemic foci in Northern Portugal, Sweden and Japan [7]. More recently smaller endemic regions have been identified in Cyprus and Majorca while in Greece there are several families in Crete [6, 8-10]. In most of Europe, mutations in the ATTR gene are heterogeneous while in smaller endemic foci such as Cyprus the only pathogenic mutation found so far is p.Val30Met.

Pathogenesis

ATTR circulates in plasma as homotetramer, produced and released by the liver. The tetramer naturally dissociates at a certain rate into monomers which, due to their intrinsic secondary structure, tend to misfold and polymerise into prefibrillar and fibrillar structures as amyloid (figure 1) [11]. The phenomenon of dissociation and misfolding of TTR occurs even in the absence of any mutation, as is seen in late onset ATTRw (wild type amyloidosis) cardiomyopathy [12].

In the presence of a pathogenic mutation the rate of homotetramer dissociation is increased and the misfolded monomers penetrate various tissue beds where amyloidogenesis takes place.

Figure 1. Mutated TTR increases destabilization of the homotetramer resulting in increased levels of monomers which misfold and aggregate via various intermediates into amyloid in various tissues beds (From "Patisiran, an RNAi therapeutic for the treatment of hereditary transthyretin-mediated amyloidosis" with kind permission of Future Medicine Ltd).



The mechanisms whereby specific mutations in ATTR give rise to various phenotypes is poorly understood but likely involves tissue bed protein homeostasis, mutated TTR peptide folding kinetics and chaperone protein metabolism both local and remote [13, 14]. The phenomena of variable penetrance of the p.Val30Met mutation in various populations, giving rise to the same neuropathic phenotype, as well as the phenomenon of anticipation (which is not based on a repeat nucleotide mechanism) suggests that genetic background as well as epigenetic factors may play a role. In the Cypriot and Portuguese populations complement C1Q polymorphisms impact the age of onset of the p.Val30Met mutation [6, 15, 16].There is also animal data that complement participates in amyloidogenesis and modulates disease expression [17].

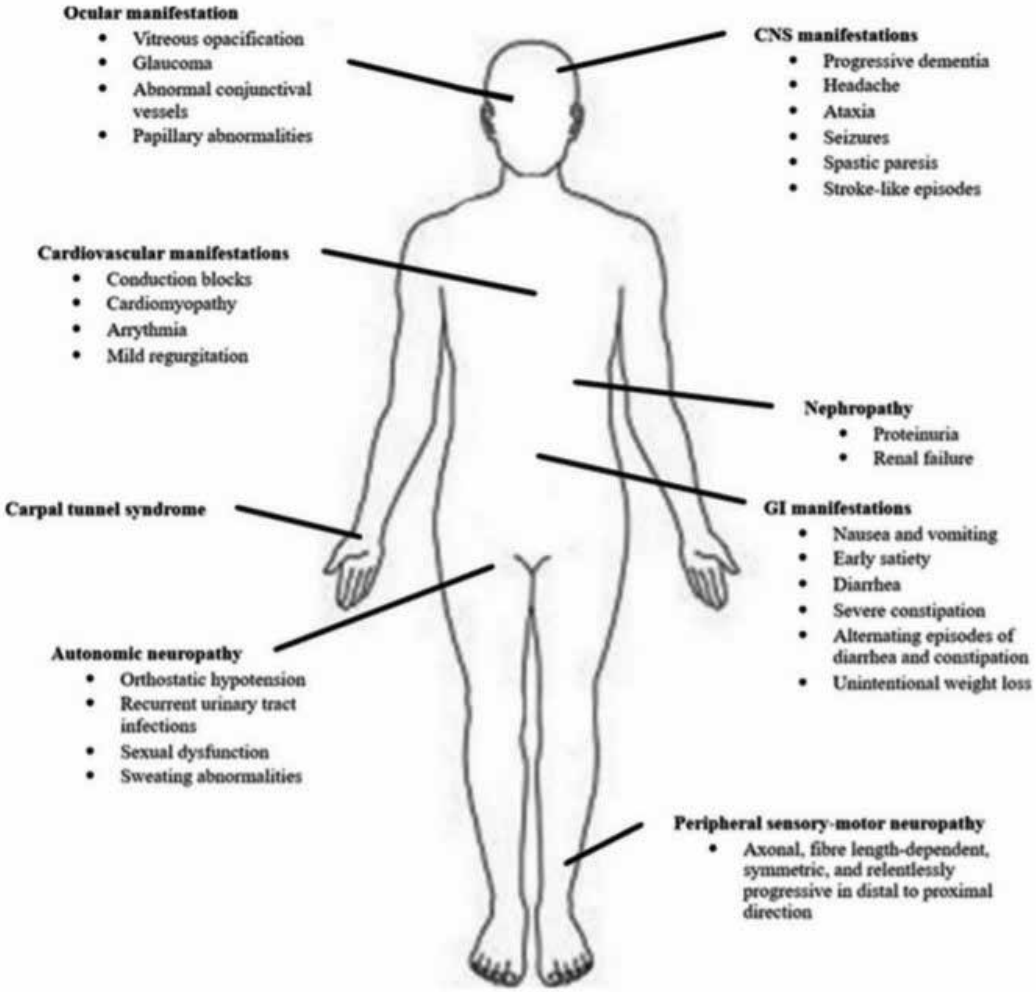
Clinical phenotypes

Hereditary transthyretin amyloidosis is a multisystemic disease with the phenotype largely dependent on the mutation and various organs can be simultaneously or sequentially involved (figure 2) [18]. There are more than 120 TTR mutations but the most common mutation worldwide is the p.Val30Met mutation. This mutation gives rise to a predominantly neuropathic phenotype and patients present with symptoms of a length dependent small fibre and autonomic neuropathy which, with the passage of time, involves large sensory and motor nerve fibres as well. Traditionally this was called Familial Amyloid Neuropathy (FAP) and was originally described in Portugal by Andrade in 1952 [19]. Early onset FAP presents before the age of 50 years and has the typical phenotype as described below. Late onset FAP has mixed features that also includes a cardiomyopathy and presents after the age of 50 years [1]. Late onset FAP is more likely to be sporadic while early onset FAP is usually familial especially in endemic areas.

Age of onset in hATTR neuropathy is variable and in familial cases anticipation is common. In both p.Val30Met and non-p.Val30Met mutations, late onset patients tend to have a more aggressive course. The typical phenotype of early onset p.Val30Met neuropathy is that of a length dependent sensorimotor and autonomic axonopathy presenting with sharp shooting pains in the feet, nausea and early satiety, change in bowel habit, diarrhoea and/or constipation, weight loss, bladder involvement, impotence, and changes in sweating, such lack of sweating in the feet and hands [1]. On examination there is dissociated sensory loss in the feet and postural hypotension is not uncommon. Sometimes, in the first twelve months of symptom onset, clinical examination may not be unequivocally abnormal, in which case quantitative sensory testing and/or Sudoscan testing may be carried out for small diameter and autonomic fibre assessment [20]. In the author's experience inserting the tuning fork in a glass of iced water and comparing perception of coldness on the dorsum of foot, mid shin and knee is quite a reliable and practical way to pick up small fibre dysfunction. It is important not simply to ask if the coldness can be felt but also how quickly it is appreciated at each level in the leg. Furthermore, temperature appreciation tends to be more affected than pain sensation with touch and joint position involved later by which time muscle wasting and weakness sets in (figures 3a and 3b).

It is crucial to think of hATTR neuropathy to diagnose it. In this respect a careful family history is paramount and should also include enquiry for less typical symptoms such as carpal tunnel syndrome,

Figure 2. Multisystem involvement in hATTR. The TTR mutation as well a disease duration determines the clinical phenotype (From "Diagnosis and Treatment of Hereditary Transthyretin Amyloidosis (hATTR) Polyneuropathy: Current Perspectives on Improving Patient Care" with kind permission of Dovepress)



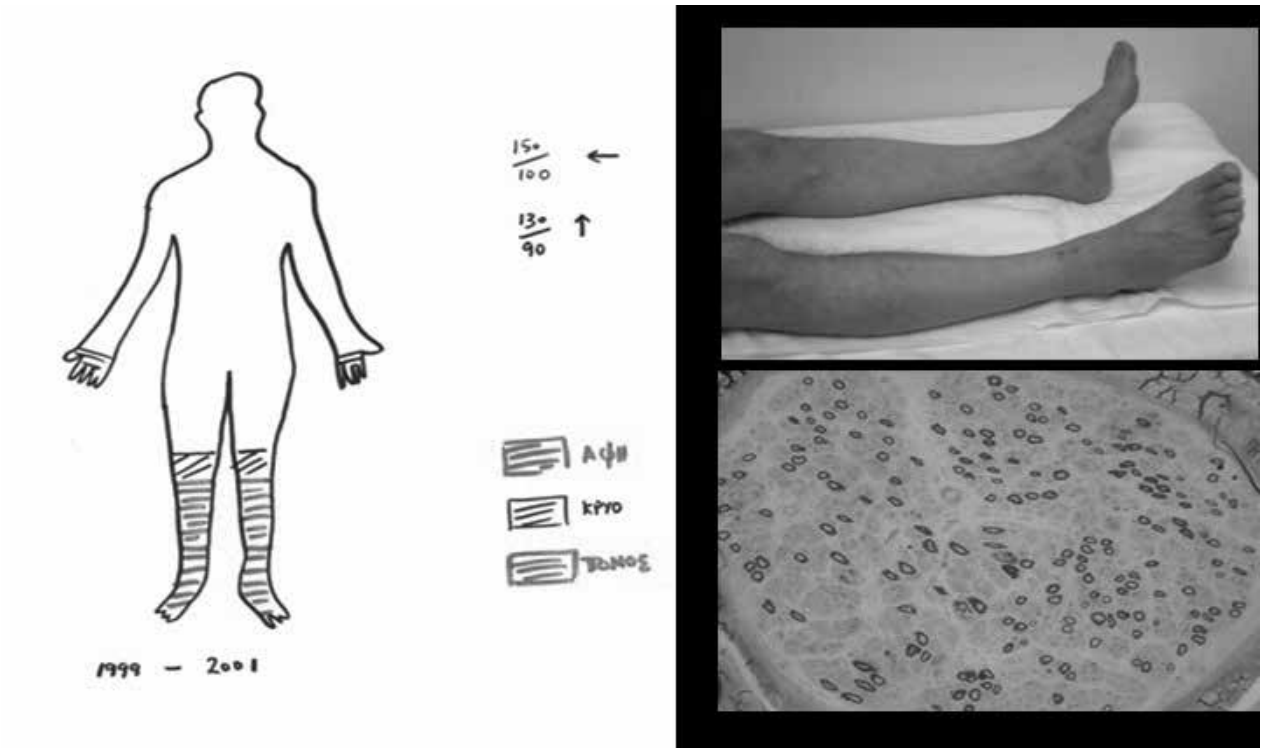
lumbar canal stenosis, biceps tendon rupture and cardiomyopathy in the family.

One of the authors has personal experience of patients misdiagnosed as Crohn's disease, irritable bowel syndrome, Charcot foot, sick-sinus syndrome needing a pacemaker and recurrent urinary tract infections due to a neurogenic bladder. All of these patients, in retrospect, had a family history of hATTR neuropathy but perhaps more importantly they all had dissociated sensory loss which was not recognized at the time of their first presentation. In fact, it is not uncommon that patients with hATTR neuropathy take several years to be diagnosed especially sporadic cases in non-endemic areas.

As a result of liver transplantation, and disease survival in p.Val30Met neuropathy has increased, further manifestations of this mutation have become apparent such an eye disease and central nervous system (CNS) complications [6, 21-24]. In the Cypriot cohort of liver transplanted patients, the prevalence of ocular, cardiac and central nervous complications are 60%, 20% and 16% respectively [6].

The ocular and CNS manifestations are due to the production of mutated TTR by the retinal epithelium and ciliary body in the eye and choroid plexus and ependymal cells in the brain respectively. Ocular complications include scalloped pupils, kerato-conjunctivitis sicca, vitreous opacities and glaucoma [22]. CNS complications occur after a mean of 16 years following liver transplantation and all patients also have ocular involvement, mainly glaucoma [21]. The most commonly reported transient focal neurological episode (TFNE) in the Cypriot cohort was expressive dysphasia, followed by dysarthria, facial distortion, and unilateral limb numbness. The TFNEs tend to be stereotyped in phenomenology but not in duration in any individual patient. In some patients different hemispheres are involved. Rarely, more than one TFNE could occur in the same patient on the same day after a period of recovery. The tempo of the onset to peak

Figure 3A. Patient diagnosed three years after onset. Dissociated sensory loss with temperature (red) > pain (blue) > touch (green) loss and no significant muscle wasting. Sural nerve biopsy shows severe loss of small myelinated fibres and to a lesser degree large myelinated fibres. Years correspond to time from symptom onset to diagnosis



for TFNEs varies over several minutes and are alike conventional transient ischemic attacks [21]. In the Cypriot cohort of patients with CNS complications there was 30% mortality due to cerebral haemorrhage (figure 4).

It is worth mentioning that there are certain mutations in hATTR amyloidosis that preferentially cause oculoleptomingeal amyloidosis early in their presentation such as the Ala25Thr and Tyr69His mutations [25, 26].

As has been mentioned genotype mainly determines phenotype but age at onset, duration of disease, genetic background and epigenetic factors probably modulate phenotype (table 1) [18]. Some variants, such as p.Val122Ile, p.Ile68Leu, p.Thr60Ala and p.Leu111Met, cause a predominant cardiomyopathy presentation known as Familial Amyloid Cardiomyopathy (FAC).

Cardiac amyloidosis is not prominent in early onset p.Val30Met mutation although cardiac conduction block can occur and patients are usually inserted a pacemaker prior to liver transplantation.

FAC is an infiltrative restrictive cardiomyopathy characterized by heart failure with preserved ejection fraction, a speckled appearance on ECHO and apical sparing [12]. Numerically ATTRw cardiomyopathy is more important and is increasingly recognized as it is now treatable [27]. It should be suspected in men, aged >70 years with a cardiomyopathy and a history of carpal tunnel syndrome, lumbar spinal stenosis and biceps tendon tear.

Lastly, renal involvement is not an early feature of the p.Val30Met mutation but can occur later on in the course of the disease due to amyloid deposition and in, liver transplanted patients, due to drugs [28].

Diagnosis

In endemic regions, where family history is often present, and the family members are themselves aware of the common symptoms, diagnosis is straight forward and confirmed with clinical examination and DNA testing. In endemic regions, there are amyloid clinics where confirmed carriers are followed up and diagnosis is often made in less than a year from symptom onset. The situation is more difficult in non-endemic areas where there may be more late onset cases with uninformative family history and less typical symptoms which may delay diagnosis for years. In the case of the p.Val30Met mutation late onset cases may exhibit less autonomic involvement and more likely to exhibit cardiomyopathy or less typical features such as carpal tunnel symptoms, lumbar canal stenosis and biceps tendon rupture. It is useful to keep in mind the multisystemic involvement of

Figure 3B. Patient diagnosed eight years after onset. Dissociated sensory loss, extending almost throughout the body, severe muscle wasting in the limbs, catheterised due a neurogenic bladder. Systolic pressure was none recordable on standing up. Sural nerve biopsy (semithin section) shows severely depleted of nerve fibres of all sizes and replaced by amorphous material consisting of amyloid. Years correspond to time from symptom onset to diagnosis

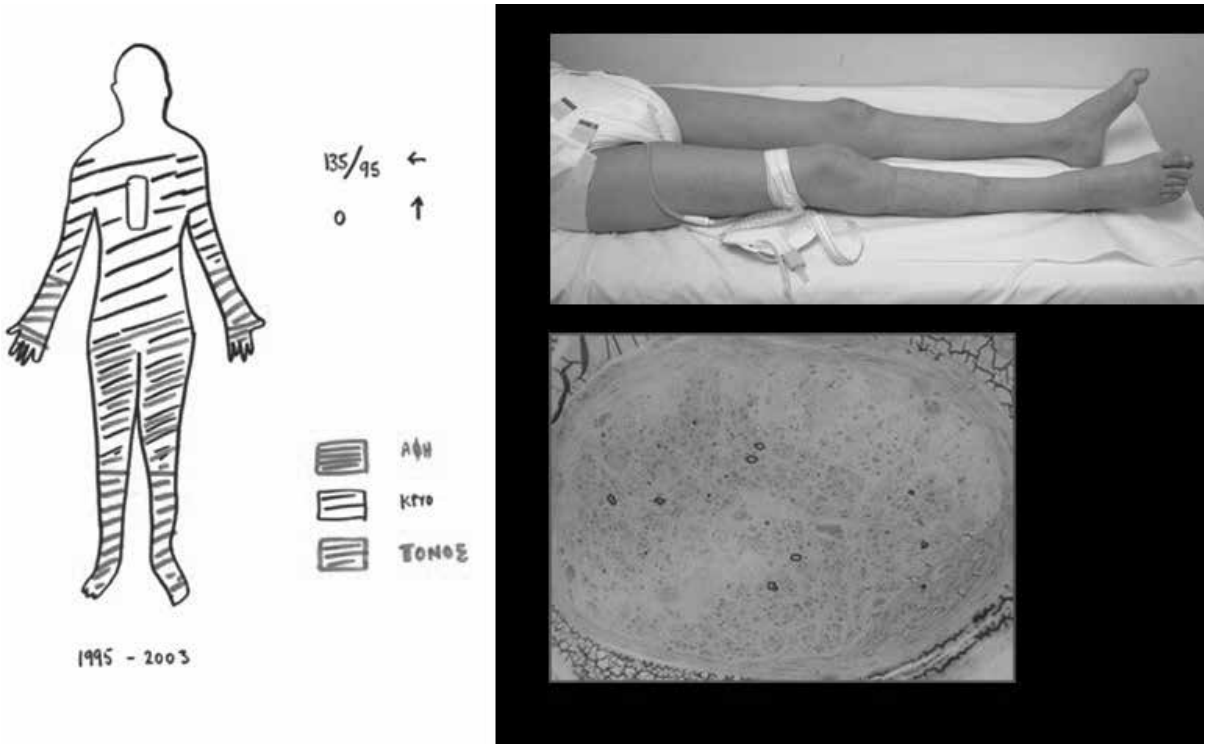
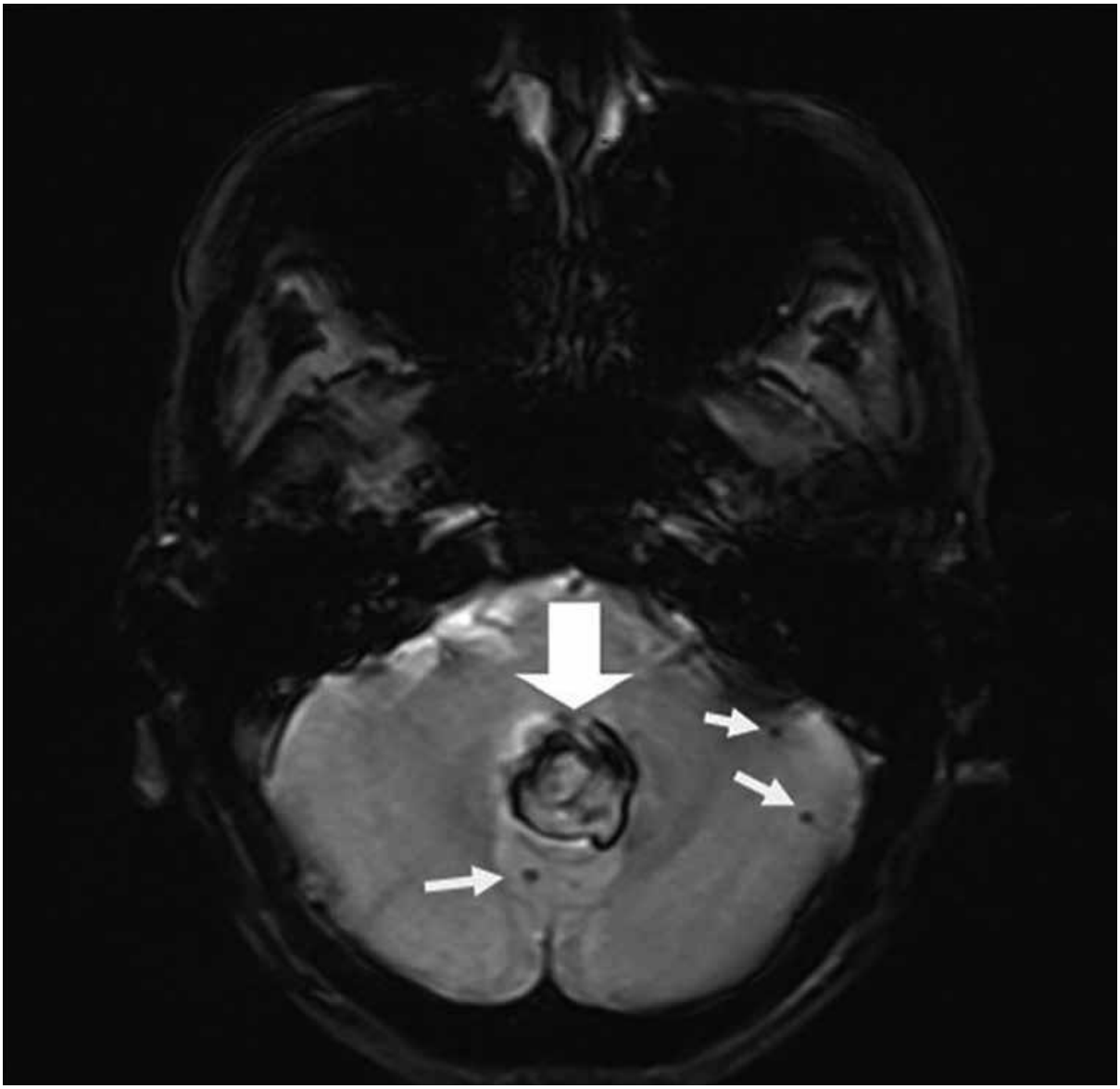


Table 1. Genotype is the main determinant of phenotype (From Diagnosis and Treatment of Hereditary Transthyretin Amyloidosis (hATTR) Polyneuropathy: Current Perspectives on Improving Patient Care with kind permission of Dovepress)

Mutation	Epidemiology	Peripheral Neuropathy	Autonomic Neuropathy	Cardomyopathy	Ocular Involvement	Gastrointestinal Involvement	Renal Involvement
Val30Met (early onset)	Portugal, Brazil	++	+++	±	+	++	+
Val30Met (late onset)	Japan, Sweden, USA, Italy, France	+++	+	++	+	+	±
Val122Ile	USA	±	±	+++	±	±	±
Thr60Ala	UK, USA	+	+	+++	±	±	±
Glu89Gln	Italy, Bulgaria	++	++	++	±	+	±
Ser50Arg	Japan, France, Italy, Usa	+++	+++	±	±	+	±
Phe64Leu	USA, Italy	++	++	++	±	+	±
Ile68Leu	Germany, Italy	±	±	+++	±	±	±
Ser77Tyr	USA, France, Israel	++	++	++	±	+	±
Ile107Val	USA, France, Brazil	++	++	++	±	±	±
Asp38Ala	Japan	++	++	++	±	±	±

Notes: The number of "+" provides an indication of the likelihood of presence of symptoms, with "±" indicating an unknown likelihood as the symptom is present in some patients and not in others.

Figure 4. Gradient Echo (GRE) sequence MRI, demonstrating microhemorhages (small arrows) and the major hemorrhage (big arrow) in the cerebellum (from The frequency of central nervous system complications in the Cypriot cohort of ATTRV30M neuropathy transplanted patients with kind permission of Springer nature)



hATTR amyloidosis particularly in patients presenting with peripheral neuropathies of unknown origin and be aware that diabetes mellitus can be a distractor.

In familial cases the presence of the typical symptoms and signs and confirmation of the endemic TTR gene mutation are often adequate for diagnosis. Ideally, confirmation of amyloid deposits, preferably in symptomatic tissue, with the demonstration of the typical apple green birefringence under polarized light with the Congo red stain is desirable. Biopsies can be obtained from abdominal fat, rectal mucosa, labial salivary glands, skin, nerve or myocardium [29]. The sensitivity of biopsy is variable (up to 80%) and depends on local expertise but one should bear in mind that amyloid deposition is often patchy. If biopsy is positive for amyloid then the precursor protein should be typed by immunohistochemistry or mass spectroscopy. If biopsy is negative and suspicion is high then then TTR sequencing should be carried out.

For peripheral neuropathy nerve conduction studies (NCS) may be performed but the reader should keep in mind that conventional NCS assess myelinated nerve fibers larger than eight microns. Small fibre neuropathy can be diagnosed with skin biopsy while the Sudoscan is increasingly used to assess sudomotor fibres. The latter is very easy to perform and can be done periodically in patients with symptoms suggestive of small fibre sensory and autonomic neuropathy [20].

For patients with possible infiltrative cardiomyopathy, cardiac ECHO with strain imaging looking for the speckled pattern as well as magnetic resonance imaging are useful [12]. The use of scintigraphy with bone markers such ^{99m}Tc-2,3-dicarboxypropane-1,1-diphosphonate (DPD), ^{99m}Tchydroxymethylene diphosphonate (HMDP) or ^{99m}Tcpyrophosphate (PYP) is becoming more popular due to its non-invasiveness and good specificity [30]. In the p.Val30Met mutation conduction abnormalities is an early sign and Holter monitoring has an important role. Similarly in monitoring for incipient cardiac failure, N-terminal prohormone (NT-proBNP) is very useful in the context of heart failure with preserved ejection fraction [12].

Treatment

Treatment in hATTR amyloidosis aims at eliminating the source of mutated TTR and stabilizing TTR in the circulation so that there is no further dissociation and deposition of misfolded TTR peptides in the various organs. Secondly since hATTR amyloidosis is a multisystemic disease symptomatic treatment needs to be provided by a multidisciplinary team. The multidisciplinary team will need to include among others; a gastroenterologist and dietitian, a cardiologist, an ophthalmologist, a renal physician and a neurologist.

Liver transplantation was first performed for hATTR amyloidosis in 1990 since liver is the main site of TTR production (accounts for 99% of circulating TTR) [31]. Although the transplanted liver produces normal TTR, this may continue to be deposited in previously seeded tissues such as the heart. Nevertheless life survival at 20 years after transplantation is 55% compared to 10 years life survival after disease onset due to the p.Val30Met mutation [32]. Late onset p.Val30Met mutation and non- p.Val30Met mutation do worse than early onset p.Val30Met mutation. Liver transplantation however has recently been abandoned following the introduction of TTR stabilizers and gene silencing therapies.

Oral TTR stabilizers, include Tafamidis and the non-steroidal anti-inflammatory drug Diflunisal, both of which dock into one of the carrier sites of the TTR tetramer, stabilize it and reduce the dissociation rate of the homotetramer. Less dissociation of the tetramer results in less misfolded monomers and reduced amyloid formation in the various organs. Phase 3 trials that have included both p.Val30Met and non-p.Val30Met mutations have shown reduction in neuropathy progression in most but not all patients [33, 34]. Advanced neuropathy and/or old age were bad prognostic factors for a good response.

Tafamidis, both at 20mg and 80mg, have been shown to reduce mortality and cardiovascular-related admissions in patients with hATTR and ATTRw cardiomyopathy with the latter dose been the most effective [35, 36].

Recently two gene silencing therapies have been approved for downregulating, by more than 80%, TTR production in the liver. The first is Patisiran which consists of a small interfering RNA (RNAi), encapsulated in lipid nanoparticles, given intravenously every three weeks and taken up by liver hepatocytes. Patisiran mediates the degradation of TTR mRNA in the cytoplasm. The APOLLO trial was a phase 3 study double blind trial of Patisiran, lasting 18 months that included patients with p.Val30Met mutations of both early and late onset as well as non p.Val30Met mutations. The Patisiran treated patients had a significant improvement in their mNIS+7 score (modified Neuropathy Impairment Score), a score that combines both clinical and neurophysiological neuropathy related measurements, compared to worsening in the placebo treated patients [37]. A number of quality of life measures also improved in the treated but worsened in the untreated group. A 12-month follow up study to APOLLO has confirmed both safety and effectiveness of Patisiran [38]. A substudy of APOLLO, examining the effect of Patisiran on cardiomyopathy showed a halt or even a reversal in the progression of cardiomyopathy [39].

Inotersen, the second approved gene silencing therapy, is an antisense oligonucleotide administered subcutaneously three times a week in the first week and then once weekly. The mechanism of action is to destroy the TTR RNA transcript in the nucleus through a RNase H1 mechanism[40]. Again, the treatment group fared better than the control group on the mNIS+7 as well as quality of life assessments. There were two serious adverse events in the treatment group which were glomerulonephritis and thrombocytopenia which occurred in 3% of patients each.

Patients on three weekly Patisiran are subjected to regular intravenous steroids while those on weekly subcutaneous Inotersen are exposed to potentially serious side-effects. Thus gene silencing using these two molecules lifelong is not without risks. Recently, an alternative to mRNA targeting-based gene silencing has been tested in a phase 1 study using the clustered regularly interspaced short palindromic repeats and associated Cas9 endonuclease (CRISPR-Cas9) system to achieve in vivo knock out of the transthyretin gene. Serum TTR was reduced by 87% by day 28 with the higher dose used. Admittedly only six patients with ATTR amyloid neuropathy were included and follow up data were very short but experiments on non-human primates show sustained TTR reduction for up to 360 days [41].

Conclusion

ATTR amyloidosis is an excellent example of the tri-

umph of translational medicine in neurology, whereby progress in molecular medicine actually translates into saving lives. ATTR neuropathies, a worldwide autosomal dominant multisystemic disease, with a life span of ten years, is now treatable by a variety of approaches involving gene silencing either via RNA editing or direct DNA silencing of the TTR gene. Wildtype ATTR cardiomyopathy, a much commoner condition, is similarly treatable with TTR stabilizers and also probably, with the gene silencers although further studies are needed. There are however, several unmet needs that escape effective treatment in ATTR amyloidosis and these include the eye and CNS complications of ATTR amyloidosis. These manifestations are becoming an increasing cause of morbidity and mortality since the above therapies do not impact on these privileged tissue sites. Perhaps other modes of delivery may be needed but there is certainly scope for optimism.

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NIEMANN-PICK TYPE C: CLINICAL CHARACTERISTICS, DIAGNOSIS, AND MANAGEMENT. A MINI-REVIEW

Vasileios Siokas[1], Athina-Maria Aloizou[1], Georgia Xiromerisiou[1], Georgios M. Hadjigeorgiou[1, 2], Efthimios Dardiotis[1]

[1] Department of Neurology, Laboratory of Neurogenetics, University Hospital of Larissa, Faculty of Medicine, School of health Sciences, University of Thessaly, Larissa, Greece.
[2] Department of Neurology, Medical School, University of Cyprus, Nicosia, Cyprus

Abstract

Niemann–Pick type C (NPC), is a lysosomal storage disorder and belongs to a group of diseases characterized by defective cholesterol trafficking. It is inherited with the autosomal pattern of inheritance. Early NPC onset is mainly characterized by visceral manifestations, while late NPC onset is characterized by neurological manifestations. The definite diagnosis of NPC is confirmed by the presence of 2 alleles with a known disease-causing mutations in the NPC1 or NPC2 genes. Miglustat is a compound that has been approved for NPC treatment in many countries, while other treatments are also under investigation. Here, we present a brief synopsis regarding the epidemiology, clinical characteristics, biomarkers, genetics, differential diagnosis and management of NPC.

Key words: Niemann-Pick type C, NPC, miglustat, neurogenetics

1. Epidemiology, Genetics, and Pathophysiology

Niemann-Pick type C (NPC), is a lysosomal storage disorder, such as Tay-Sachs and Gaucher's disease [1]. It belongs to a group of diseases characterized by defective cholesterol trafficking [2]. NPC is a genetic disorder, inherited with the autosomal pattern of inheritance [3], while its incidence is estimated to span from 0.61 to 1:100 000 births [2]. Moreover, it accounts for 1 to 2 percent of autosomal recessive cerebellar ataxias (ARCAs) [4].

Mutations in the NPC1 (Chromosome 18: 23,506,184-23,586,506, http://www.ensembl.org/Homo_sapiens/Gene/Summary?g=ENSG00000141458;r=18:23506184-23586506) and the NPC2 (Chromosome 14: 74,476,192-74,494,177, https://www.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000119655;r=14:74476192-74494177) genes [5-7], have been reported to cause an impaired trafficking of lipids and their consequent accumulation within cells [5-7]. Mutations in NPC1 genes are the commonest accounting for more than 9 out to 10 NPC cases, with more than 30 reported genetic alterations [8].

From pathophysiological aspect, a defective function of NPC1 or NPC2 does not allow the excretion of the cholesterol from lysosomes, leading to the accumulation of toxic cholesterol within them, and therefore leads to an injury of cells and organs, such as the brain, spleen, liver and lungs [9, 10].

2. Clinical features

Phenotypically, NPC is a slowly progressive heterogeneous disorder, with the core manifestations related to the age of onset [11]. More precisely, early onset (perinatal and infancy) is presented mainly with predominantly visceral manifestations, while the NPC with late onset is characterized by neurological manifestations [12]. An earlier onset of the neurological manifestations has been associated with poor prognosis (faster progression, increased morbidity and mortality) [13].

An overview of phenotypic appearance in patients with NPC classified by the age of onset, is presented in Table 1 [12].

3. Diagnostic Assessment

History, neurological examination, laboratory, and genetic testing contribute to the diagnosis of NPC [14]. Physicians should suspect NPC, when visceral and psychiatric/neurological manifestations are pres-

BOX 1

Early NPC onset is mainly characterized by visceral manifestations. Late NPC onset is characterized by neurological manifestations.

Table 1. Commonest phenotypic features of patients with NPC classified by the age of onset.

	Age at NPC onset				
Clinical sign/symptom	**Pre-/peri-natal (<2nd month)**	**Early infantile (<2nd year)**	**Late infantile (2nd - 6th year)**	**Juvenile (6th - 15th year)**	**Adolesence/Adulthood (>15th year)**
Systemic manifestations					
Hepatomegaly	+	+	+	+	
Jaundice	+	+	+		
Hydrops	+				
Foetal ascites	+				
Liver failure	+				
Splenomegaly	+	+	+	+	+
Pulmonary disorder	+				
Thrombocytopenia	+				
Neurological/Psychiatric features					
Hypotonia	+	+			
Motor development delay		+	+		
Speech delay		+	+		
VSGP		+	+	+	+
Spasticity		+	+		
Ataxia			+	+	+
Dystonia			+	+	+
Dysphagia		+	+	+	+
Dysarthia				+	+
Dysmetria				+	
Dyskenisia				+	+
Tremor					+
Clumsiness			+		+
Seizures			+	+	
Cataplexy			+	+	+
Hearing loss			+		
Falls			+	+	
Behavioral				+	
Cognitive decline/ Dementia					+
Psychosis					+
Depression					+

NPC, Niemann-Pick type C; VSGP, vertical supranuclear gaze palsy.

BOX 2

The definite diagnosis of NPC is confirmed by the presence of 2 alleles with a known disease-causing mutations in the NPC1 or the NPC2 genes.

BOX 3

While the Filipin test is no longer considered as the 'gold standard' for the diagnosis of NPC, it is useful in cases suspected for NPC where only one pathogenic mutation in NPC1 and NPC2 genes, is detected.

ent. However, we should bear in mind that atypical also forms exists [15-18], while specific tools are available for estimating disease severity and identifying patients in need of further investigation [12, 14, 19].

3.1. Genetic testing

Following the suspicion of NPC, patients should undergo biochemical and genetic testing. The diagnosis of NPC is confirmed by the mutation analysis of NPC1 and NPC2 genes, in patients with biomarker profile and clinical features suggestive for NPC [12, 14]. The presence of 2 alleles with a known disease-causing mutations in either the NPC1 or the NPC2 genes confirms the diagnosis of NPC [12].

Initial genetic screening for mutations in the NPC1 and NPC2 genes could be performed with Sanger sequencing, which applies polymerase chain reaction (PCR) targeting the 30 coding exons, and intron-exon boundaries, or next-generation sequencing (NGS). Full genomic DNA sequencing with NGS should be spared for patients with clinical and/or biochemical profile compatible with NPC but in which routine sequencing has not identified two known pathogenic mutations, as this methods could identify variants that cannot be interpreted easily [20], and could possibly require mRNA and/or protein studies in specialized laboratories [14].

3.2. Routine laboratory testing.

Results from routine laboratory routine tests (e.g. peripheral blood analyses, levels of lipids in plasma, levels of bilirubin levels, liver function tests, and renal test) are usually unremarkable [12]. However, increased glutamic-pyruvic transaminase (SGPT), and chitotriosidase levels, low high-density lipoprotein (HDL) cholesterol, and low blood platelet count levels can be observed [12, 21-23].

3.3. Biomarkers

Three types of biochemical markers, namely plasma oxysterols (cholesterol oxidation products), plasma lysosphingolipids and urine bale acids, can be used to increase the sensitivity and specificity of the diagnosis of NPC [12, 24, 25]. More precisely, the oxysterols cholestane-3β, 5α, 6β-triol (C-triol) and 7-ketocholesterol (7-KC), the plasma lysosphingolipids [lyso-sphingomyelin (lyso-SM) and lysosphingomyelin-509 (lyso-SM 509], and finally, the specific urine bale acides [3β-sulfooxy-7β-N-acetylglucosaminyl-5-cholen-24-oic acid (SNAG-Δ5ίCA) (and itsglycine- and taurine- amides)] appear to be elevated in NPC [12, 24, 25]. However, there are few limitations regarding their applications, such as the fact that they can also be found elevated in other metabolic disorders or in heterozygotes of NPC genes mutations, that some of them can only be measured in specific research institutions at the moment, and there are difficulties in shortage [12]. This, ultimately highlights the genetic screening for mutations in NPC genes as the optimal option for the proof NPC diagnosis.

3.4. The Filipin test

While the Filipin test (a method for detection of accumulated unesterified cholesterol within the lysosomes suggesting impaired intracellular cholesterol transport and homeostasis) was considered as the 'gold standard' for the diagnosis of NPC, it is no longer considered a first line test [12,14, 26, 27].

However, it can be still be useful in the diagnostic process, especially in cases where the genetic analysis and/or biomarkers yielding inconclusive results [12]. The flipping test, is especially useful in cases suspected for NPC and only one pathogenic mutation in NPC1 and NPC2 genes, is detected [12].

3.5. Imaging

Brain imaging of NPC patients usually reveals abnormalities only in late disease stages [28]. However, atrophy (corpus callosum, mild cerebral, cerebellar vermis), demyelination, hypometabolism (in the frontal cortex, in the prefrontal cortex and thalamus), hypermetabolism (in the cerebellum, pons, and the lenticular nucleus of the basal ganglia), have been reported in studies using Magnetic Resonance Imaging (MRI), Magnetic Resonance Spectroscopy (MRS)

Table 2. Differential diagnosis of NPC based the age of disease onset.

Age at NPC onset		
Neonatal/Infatile	**Childhood**	**Adolesence/Adulthood**
Biliary atresia	Hydrocephalus	Alzheimer's Disease
TORCH infections	Brain tumor	FTD/ALS spectrum disorders
Histiocytosis	ADD	PSP
Lymphoma	subacute necrotizing encephalomyelopathy	Pick Disease
Leukemia	HIV encephalopathy	Lysosomal storage disease
Niemann-Pick disease type A	Depression	HIV dementia
Niemann-Pick disease type B	Periodic Paralyses (Hypo-, Hyper-kalemic)	Syphilis dementia
Gaucher disease	Maple syrup urine disease	
Alpha-1-antitrypsin deficiency	Wilson Disease	
Tyrosinemia type I	Neuronal ceroid-lipofuscinosis	
	Tay-Sachs disease	
	Maple syrup urine disease	
	Glutaric acidemia type 1	

NPC, Niemann-Pick type C; TORCH, Toxoplasma gondii, other agents, rubella, cytomegalovirus (CMV), and herpes simplex virus (HSV); FTD, Frontotemporal Dementia; ALS, amyotrophic lateral sclerosis; PSP, Progressive supranuclear palsy; ADD, attention-deficit disorder

and Positron Emission Tomography (PET) imaging techniques [29-32].

4. Differential Diagnosis

Considering the differences in the clinical presentations of the NPC according to the age of onset, the differential diagnosis can include various sets of diseases [33]. The differential diagnosis of NPC based on the age of disease onset is presented in Table 2 (https://www.ncbi.nlm.nih.gov/books/NBK1296/) [34].

5. Follow-up

After the diagnosis of NPC, functional assessments should be performed on regular basis in order to provide an appropriate control and management of clinical features and function. Consequently, physical examination, neuropsychiatric evaluation, calculation of the NPC clinical severity score, hearing examination, swallowing assessment, ophthalmological examination, and developmental or cognitive assessment are usually performed at diagnosis/baseline and then every 6 -12 months [12, 35-38].

6. Management

A causative treatment for NPC has not been developed yet; treatment efforts aim to alleviate symptoms and delay disease progress. Patients are also often encouraged to seek multidisciplinary guidance at large academic centers that can provide better solutions for the multifaceted health issues that arise [12, 14]. Besides the usual symptomatic treatment and frequent medical checks, substrate reduction therapy is also applied in NPC; of note, cholesterol-lowering agents have been long known to be inefficient in NPC [39]. N-butyldeoxynojirimycin (miglustat) inhibits the synthesis of glycosphingolipids and gained approval for the treatment of neurological manifestations in NPC in 2009. It has been shown to stabilize clinical progress and inhibit neurodegeneration [40]. Its commonest side-effects come from the gastrointestinal tract and seem to subside after the first 6 months of treatment [14]. The available guidelines for NPC [12] recommend miglustat -albeit not in the ultimate degree- for patients with neurological manifestations, but not for those with advanced neurological deficits and dementia, or for a concomitant disease that could lead to death within a year. Moving on, acetyl-DL-leucine has been also reported as effective for ataxic symptoms in NPC [41] and will continue to be examined in clinical trials [42]. In this regard, several ongoing clinical trials (clinicaltrials.gov) will assess other therapeutic options in NPC, namely acetyl-DL-leucine (NCT05163288, NCT03759639), arimoclomol (NCT02612129, NCT04316637), human acid sphingomyelinase (NCT01722526), intravenous and intrathecal hydroxypropyl betacyclodextrin (VTS-

270 or adrabetadex) (NCT04958642, NCT02939547, NCT04860960, NCT03471143, NCT03887533, NCT03893071, NCT02912793, NCT02534844, NCT01747135, NCT03879655, NCT03643562), vorinostat (NCT02124083), lithium carbonate (NCT03201627), intrathecal umbilical-cord-blood-derived oligodendrocyte-like cells (NCT02254863), hemopoetic stem cell infusion (NCT01372228) and transplantation of placental-derived human stem-cells (NCT01586455).

7. Conclusions

Niemann-Pick Type C disease is a rare autosomal recessive disease that based on its age of onset can exhibit various symptoms. An early disease onset is associated with visceral manifestations, while a later onset mostly presents neurological manifestations. The diagnosis is primarily set with genetic testing, aided by biochemical marker testing. Treatment-wise, a multi-disciplinary approach is necessary for tackling the heterogeneous symptoms that arise in the course of the disease. One drug, miglustat, is currently approved for NPC patients with neurological manifestations, while many more options are currently under examination.

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