Integrating Genotype-Phenotype Correlations into Precision Medicine

Towards Individualized Patient Management, Preventive Strategies, and Advanced Diagnostics in Mitochondrial Diseases

Ph.D. Thesis

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Contents

The list of publications forming the basis of the thesis

First-author articles:

1. Expanding the Phenotypic Spectrum of SPG7 Rare Damaging Variants: Insights From a Hungarian Cohort. Jimoh, I. J., Balicza, P., Szlepak, T., Csaban, D., Gal, A., Geresi, A., ... & Molnar, M. J. (2025). *Clinical Genetics* Q2, IF:2,72
2. Wernicke–Korsakoff syndrome associated with mtDNA disease. Jimoh IJ, et al. (2020): Therapeutic advances in neurological disorders 13, 131756286420938972. Q1, IF:4,59
3. A mitochondrialis betegségek diagnosztikai és kezelési lehetőségei 2021-ben Jimoh, IJ ; Molnár, MJ (2021) Orvostovábbképző szemle

Other relevant co-author articles related to the scope of the Ph.D.:

4. Cornealis polymegathismus és retinalis pigmenthám-eltérések MELAS-szindrómában. István, L., Benyó, F., Csorba, A., Jimoh, I. J., Gál, A., Molnár, M. J., ... & Szabó, V. (2022). Szemészet, 159(2)
5. Broadening the phenotype of the TWNK gene associated Perrault syndrome. Fekete, B., Pentelényi, K., Rudas, G., Gál, A., Grosz, Z., Illés, A., ... & Molnar, M. J. (2019). *BMC Medical Genetics*, *20*, 1-8. Q3 IF:1,58

List of abbreviations

Abbreviation	Meaning	Abbreviation	Meaning
ACE	Addenbrooke's Cognitive Examination	LHON	Leber hereditary optic neuropathy
Acetyl-CoA	Acetyl coenzyme A	MELAS	Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes
ACMG	American College of Medical Genetics and Genomics	MLPA	Multiplex Ligation-dependent Probe Amplification
AD	Autosomal dominant	MMSE	Mini-Mental State Examination
AFG3L2	AFG3 Like Matrix AAA Peptidase Subunit 2	MNL	Motorneuron lesion
ALS	Amyotrophic lateral sclerosis	MRI	Magnetic resonance imaging
AR	Autosomal recessive	mtDel	mitochondrial DNA deletion
ATP	Adenosine triphosphate	mtDNA	Mitochondrial DNA
CIPO	Chronic intestinal pseudo-obstruction syndrome	MT-TL1	Mitochondrially encoded tRNA-Leu(UUA/G)
CIPO	Chronic intestinal pseudo-obstruction	NADH	Nicotinamide adenine dinucleotide, reduced form
COX	Cytochrome-C-oxidase	NGS	Next-generation sequencing
CPEO	Chronic progressive external ophtalomplegia	OXPHOS	Oxidative phosphorylation
DAMP	Danger-associated molecular patterns	PANSS	Positive and Negative Syndrome Scale
DaTscan	Dopaminergic Single Photon Emission Computed Tomography	PEO	Progressive ophtalmoplegia externa

DICOM	Digital Imaging and Communications in Medicine	PI3K-AKT	phosphatidylinositol-3-kinase –Akt
DNA	Deoxyribonucleic Acid	PM	Precision medicine
DRV	Damaging Rare Variant	PMD	Primary mitochondrial disease
EHR	Electronic health record	RDV	Rare Damaging Variant
EMA	European Medicines Agency	RNA	Ribonucleic acid
ERN	European Reference Network	ROS	Reactive Oxygen Species
ERN-NMD	European Reference Network Neuromuscular Disorders	SARA	Scale for the Assessment and Rating of Ataxia
ERN-RND	European Reference Network Rare Neurological Disorders	SDH	Succinate Dehydrogenase
$FADH_2$	Flavin adenine dinucleotide, reduced form	SE-GRI	Semmelweis University Institute of Genomic Medicine and Rare Disorders
FDA	U.S. Food and Drug Administration	SPG7	Spastic Paraplegia Gene 7
GWAS	Genome-wide association study	VUS	variant of uncertain significance
HP	Heteroplasmy	WES	Whole exome sequencing
HSP	Hereditary spastic paraplegia	WKS	Wernicke-Korsakoff syndrome
HSP7	Hereditary spastic paraplegia type 7		

1. Introduction

1.1 Paradigm shift in healthcare systems towards precision medicine

Over the past decades, modern medicine has achieved significant milestones in diagnostics, therapeutics, and disease prevention. Due to the relative decrease of communicable diseases and injuries, the focus has shifted to chronic diseases. However, despite technological and pharmacological advances, the overall life expectancy has shown only modest gains, and more critically, the healthspan[1] - the number of years lived in good health, free from chronic morbidity and functional limitations- did not improve significantly even in high-income countries[2] raising concerns about the long-term sustainability and effectiveness of current healthcare models.

Conventional medical practice continues to follow a generalized, population-based treatment approach, often referred to as the "one-size-fits-all" paradigm. This model, while historically having built a solid foundation for global and widely accessible healthcare systems, has notable limitations[3]. The conventional approach can fall short in addressing inter-individual variabilities, which can affect disease susceptibility, progression, and treatment response, leading to widespread issues such as inadequate therapeutic efficacy complicated by polypharmacy. Unoptimized use of resources leads to long waiting lists that are common in overstressed healthcare systems. These problems are further compounded by the demographic reality of an aging population, which is contributing to a sharp rise in chronic and multimorbid conditions globally[4–6]. Parallel to these trends and increasing demand, healthcare expenditures are increasing exponentially. Without reform, such expenditure trends threaten the economic viability of healthcare systems, even in high-income countries[7–9]. Thus, there is a critical need for a paradigm shift, one that emphasizes value-based, efficient, and individualized care strategies.

Personalized medicine and precision medicine (PM), represents a transformative approach to healthcare systems, representing advanced approaches that aim to tailor medical treatment to the individual characteristics of each patient. Although often used interchangeably, subtle distinctions exist between the two concepts. Personalized medicine integrates genetic, markers, whereas precision medicine represents a broader concept that encompasses not solely genomic data but also multiomic data integration and patient preferences[10]. Both approaches promise to increase quality of life, lead to more accurate diagnoses, targeted therapies, and preventive strategies tailored to individual patients[11]. As technological barriers are fading away and costs

of multiomics technologies - such as genomics, transcriptomics, and proteomics - continue to decline, it becomes increasingly feasible to integrate these tools into routine clinical practice. By implementing such approaches, medicine can become preventive, not just reactive[12,13] and can help predicting pharmaceutical trial outcomes[10,14–17].

Among the pillars of personalized medicine, genetics and genomics have a central role. Genetic insights provide a foundational understanding of disease pathogenesis, therapeutic targets, and inter-individual variability in drug response[18,19]. Many genetic disorders exhibit highly variable phenotypes, often involving multiple organ systems. Furthermore, certain genetic variants may exhibit pleiotropy or incomplete penetrance[20], or even variant specific inheritance pattern[21], posing challenges but also offering opportunities for nuanced risk stratification[22] and early intervention[23]. Although monogenic diseases are individually rare, genome-wide association studies (GWAS) and large-scale sequencing efforts have revealed that common genetic variants contribute cumulatively to the risk of prevalent diseases such as diabetes, cardiovascular, and neurodegenerative disorders, commonly considered as acquired or multifactorial disorders. These findings underscore the importance of integrating genetic data not only for diagnosing rare conditions but also for risk modeling in complex diseases and genetic traits[24] that showing increasingly convincing evidence of be feasible at large scale[25,26].

1.1.1. Patient pathways

Timely and accurate diagnosis represents a fundamental prerequisite for effective healthcare delivery[27]. Diagnostic delays or failure to identify patients not only exacerbate disease-related morbidity and mortality but also increase the overall socioeconomic burden[28]. The epidemiologic impact or even estimating the prevalence of rare disorders is challenging[29]. Inadequate characterization of disease impact may further lead to suboptimal resource allocation and misalignment of healthcare system priorities. These challenges are particularly critical in the era of rapid drug development, where the early identification of eligible patients is essential to ensure access to novel therapies and specialized care.

Mapping patient pathways and identifying unmet needs are key steps in prioritizing attention and optimizing resource distribution for specific disorders. The establishment of highly experienced, disease-focused clinical centers has been shown to enhance diagnostic yield and reduce underdiagnosis[30,31]. Beyond direct diagnostic interventions,

raising awareness and improving clinical suspicion can facilitate timely patient referral to expert centers. For inherited disorders with variable age of onset, patient identification poses additional challenges, particularly many patient get lost during the transition from pediatric to adult care[32]. Optimizing patient pathways can improve follow-up adherence and reduce patient attrition, which is pivotal for diseases with delayed or heterogeneous clinical presentations.

In the European context, rare disorders remain disproportionately affected by diagnostic delays[33]. European Reference Networks (ERNs) provide a pan-European framework for enhancing diagnostic capacity, standardizing care pathways, and facilitating cross-border expertise sharing. In Hungary the Institute of Genomic Medicine and Rare Disorders at Semmelweis University is a pioneer, being one of the first Hungarian centers to join the ERN initiative, participating in both ERN EURO-NMD and ERN-RND.

1.1.2. Patient registries and databases

Investigating the broad spectrum of genetic variation and advancing novel diagnostic and prognostic methodologies holds promise for enhanced patient stratification, personalized therapies, and more efficient healthcare resource allocation. The systematic collection of structured clinical data, especially through patient registries, facilitates the generation of real-world evidence that improves the understanding of disease courses, uncovers unmet clinical needs, and gives the possibility of optimizing patient pathways and health outcomes. Also, identifying clinical, digital, or molecular markers to facilitate diagnostics, enable patient stratification, and predict disease progression is a promise of such databases[34]. While developing comprehensive patient registries, clinical databases or biobanks demands significant technical, operational, and financial investment[35], even establishing minimal, clinician-friendly datasets[36] can yield a substantial impact, especially when future perspectives have been accounted for[37]. These simplified systems support sustainable, outcome-oriented healthcare by enabling the systematic capture of key patient-level data and improving long-term care management[38].

1.2 Mitochondrial disorders

Personalized medicine provides a comprehensive framework - supported by registries, biobanks, and large-scale biomedical databases - for integrating genomic information with

clinical data to advance disease characterization and treatment. This paradigm is particularly relevant to mitochondrial disorders, which stand out among heritable disorders as clear examples of the challenges and opportunities inherent in applying genomics to precision medicine. Their substantial genetic heterogeneity, together with the modifying influence of both nuclear and mitochondrial genetic backgrounds, contributes to the striking variability in clinical presentation[39–43]. As a result, mitochondrial disorders are exceptional examples where individualized patient stratification can be established, and understanding the physiological roles, molecular pathophysiology of the diseases, and cellular functions of mitochondria is indispensable for interpreting this complexity.

1.2.1 Physiological function of the mitochondria

Mitochondria are multifunctional organelles central to both cellular metabolism and signaling. Beyond their well-established role in energy production, they contribute to the synthesis of key biomolecules, including amino acids, fatty acids, cofactors derived from vitamins, and iron–sulfur clusters[40]. These organelles also participate in critical cellular processes such as signal transduction and the regulation of apoptosis, thereby influencing cell fate decisions. Despite their diverse functions, their most vital contribution lies in generating adenosine triphosphate (ATP), the primary energy currency of the cell[41,44]. This energy is produced through oxidative metabolism, wherein glucose, fatty acids, and amino acids are broken down to form acetyl-CoA. Acetyl-CoA enters the tricarboxylic acid cycle within the mitochondrial matrix, generating electron carriers NADH and $FADH_2$. These reduced cofactors supply electrons to the electron transport chain, a series of protein complexes located in the inner mitochondrial membrane, ultimately driving ATP synthesis via oxidative phosphorylation. The tricarboxylic acid cycle is essential for nutrient catabolism, reinforcing the mitochondria's role as a central integrator of cellular bioenergetics.

1.2.2 Prevalence of mitochondrial disorders

Primary mitochondrial diseases represent one of the most prevalent groups of inherited metabolic disorders, with a clinically confirmed estimated prevalence ranging from 1:4300 to 1:8,000, depending on geographical region and investigated disorders. However, the true prevalence is likely underestimated and difficult to estimate, as advancements in diagnostic technologies continue to reveal an increasing number of affected individuals. The clinical recognition of PMDs is often delayed due to their heterogeneous, multisystemic presentations and complex phenotypic variability[45–47].

1.2.3 Genetics of mitochondrial disorders

Historically, PMDs were predominantly attributed to maternally inherited mutations in mitochondrial DNA (mtDNA). MtDNA, a circular genome located within the mitochondrion, encodes a total of 37 genes: 13 polypeptides essential for oxidative phosphorylation, 22 transfer RNAs, and 2 ribosomal RNAs[48]. A hallmark of mtDNA is its maternal inheritance pattern, with only a few extremely rare exceptions reported in the literature[49]. In addition, each cell harbors numerous mitochondria, and each mitochondrion contains multiple copies of mtDNA, leading to a heterogeneous presence of mutant and wild-type genomes, a ratio known as heteroplasmy, which is tissue-specific[48]. Primarily postmitotic tissues tend to carry higher heteroplasmy levels. The phenotypic manifestation of mtDNA mutations is influenced by their relative abundance; when the proportion of pathogenic mtDNA exceeds a critical threshold - a phenomenon referred to as the threshold effect[50] - clinical symptoms are likely to emerge with variable severity and organ involvement. The intricate function of mitochondria is regulated not only by genes encoded within the mitochondrial genome but also by a large number of nuclear-encoded genes. With the advent of next-generation sequencing (NGS) and expanding insights into mitochondrial biology, it is clear that a substantial proportion of mitochondrial dysfunction arises from mutations in nuclear-encoded genes. In accordance with their essential physiological roles, these nuclear genes contribute to multiple mitochondrial processes, including the assembly of oxidative phosphorylation (OXPHOS) complexes, mitochondrial dynamics, protein import into the organelle, maintenance and quality control of mtDNA, as well as mitochondrial biogenesis. An increasing number of proteins encoded by nuclear genes have been implicated in one or more of these processes, leading to current estimates that approximately 1,136 nuclear genes are functionally associated with mitochondrial biology according to the Human MitoCarta3.0 database. However, a definitive pathogenic role has not been fully established for significant portion of these genes. Figure-1 summarizes the genetic heterogeneity of mitochondrial disorders[43,51,52].

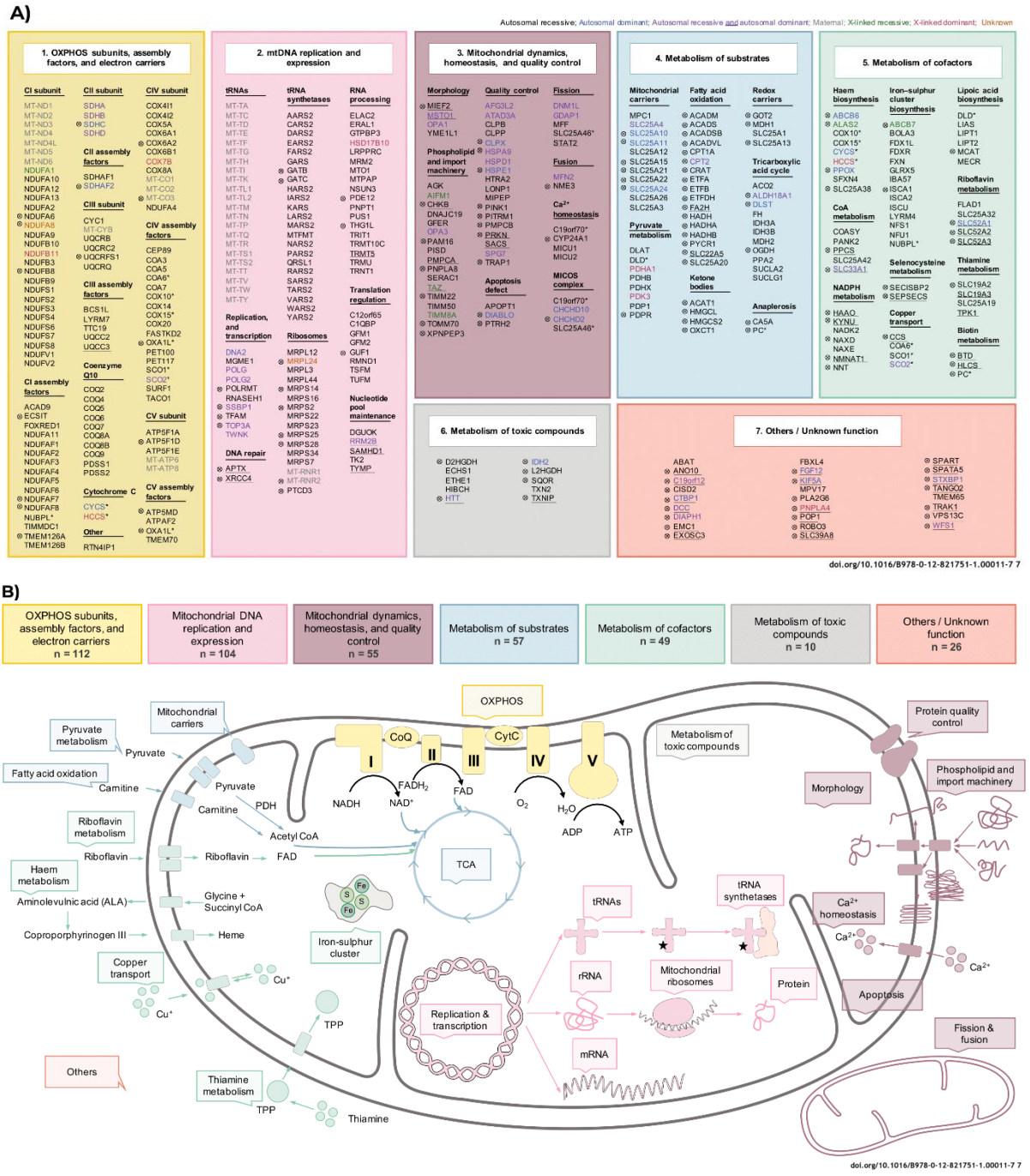


Figure 1 - Genes associated with the mitochondria

A) List of mitochondria-associated genes grouped by molecular functions. The function of the genes are indicated by the background color: yellow – genes associated with OXPHOS subunits, assembly factors and electron carriers; pink – genes associated with mtDNA replication and expression; brown – genes associated with mitochondrial dynamics, homeostasis, and quality control; light blue – genes associated with metabolism of substrates; teal – genes associated with metabolism of cofactors; gray – genes associated with metabolism of cofactors; orange – genes associated with unknown function. The color of the genes represents the mode of inheritance: black – autosomal recessive; light blue – autosomal dominant; purple – genes with both autosomal recessive and dominant inheritance; gray – mtDNA associated gene; orange – X-linked recessive; magenta – X-linked dominant; orange – unknown mode of inheritance. B) functional topology of the genes

1.2.4 Clinical representation and diagnostics of mitochondrial disorders

Mitochondrial disorders represent a heterogeneous group of diseases with high variability that depend on the physiological role of the causative gene. PMD-related dynamic imbalance influences mitochondrial morphology and function, culminating in increased oxidative stress, impaired calcium handling, disruption of ATP production, apoptosis and cellular death. Mitochondrial dysfunction predominantly affects tissues with high energy demands, such as the central nervous system, cardiac and skeletal muscle, and sensory organs. Within the central nervous system, this can lead to various manifestations such as encephalopathy, stroke-like episodes, paresis, ataxia, and sensory impairments manifesting as hearing or visual impairment. The above-mentioned metabolic vulnerability is particularly pronounced in dopamine-rich regions[53], including the basal ganglia, cortex, and limbic system. In these areas, dopamine metabolism, via monoamine oxidase or autoxidation, generates reactive oxygen species, which further damage mitochondrial integrity[54,55]. This oxidative stress impairs electron transport chain[24] function, reduces mitochondrial membrane potential, and disrupts neurotransmission, contributing to cognitive decline and, in some patients, neuropsychiatric manifestations such as psychosis. As a result, neurological and sensory symptoms dominate the clinical presentation of PMDs. Skeletal muscle involvement is also a frequent hallmark, manifesting in various degrees of myopathy. Beyond these core symptoms, mitochondrial dysfunction contributes to pathophysiological processes in other organ systems. In the heart, excessive ROS production promotes cardiac remodeling and fibrosis, predisposing patients to arrhythmias, heart failure, and cardiomyopathy. At the metabolic level, mitochondrial impairment disrupts key signaling pathways[56]. Mitochondrial dysfunction and insulin resistance can reinforce each other. Impaired OXPHOS and reduced ATP production weaken insulin-signaling pathways, including the PI3K-Akt pathway, which lowers glucose uptake and glycogen synthesis while disrupting fatty-acid metabolism and promoting lipid-intermediate accumulation, all of which worsen insulin resistance[57]. Additionally, damaged mitochondria can release danger-associated molecular patterns (DAMPs), which activate innate immune responses and amplify systemic inflammation, thereby exacerbating insulin resistance and other metabolic disturbances[58]. Taken together, these mechanisms highlight the systemic nature of mitochondrial dysfunction. Overall PMDs are classically defined as rare genetic disorders, their molecular underpinnings intersect with common, multifactorial diseases often considered acquired, such as

cardiovascular disease, diabetes, and neurodegeneration[59–63]. Thus, understanding the molecular basis of mitochondrial dysfunction offers critical insight into a wide spectrum of clinical phenotypes that extend beyond the traditional presentations of primary mitochondrial disorders. An overview of the phenotypic spectra associated with mitochondrial disorders is illustrated in Figure 2.

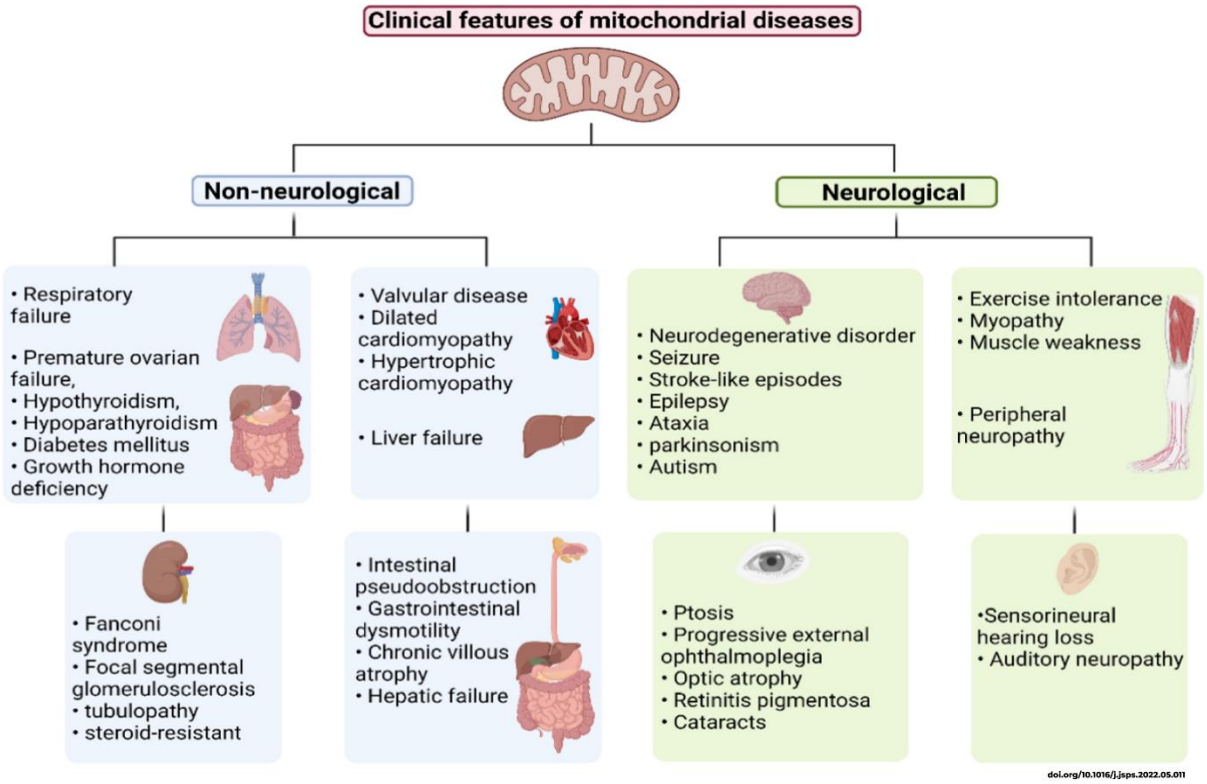


Figure 2 - Clinical manifestation of mitochondrial disorders

Mitochondrial disorders resulting from impaired cellular energy production give rise to a broad spectrum of multisystemic symptoms. Based on their prevalence among affected individuals, these manifestations can be pragmatically categorized into neurological and non-neurological groups. Non-neurological symptoms encompass dysfunctions of the respiratory, gastrointestinal, renal, and cardiovascular systems. In contrast, neurological symptoms involve central nervous system manifestations such as neurodegenerative disorders, seizures, stroke-like episodes, ataxia, and parkinsonism, as well as neuropsychiatric conditions including autism spectrum disorder. Additionally, muscle involvement and sensory organ dysfunctions are frequently observed.

Altogether, mitochondrial disorders represent a clinically and genetically heterogeneous group of diseases, with the most severe manifestations historically classified into distinct syndromes. Despite these well-established clinical frameworks, a substantial proportion of patients cannot be reliably categorized within traditional syndromes. Interestingly, individuals with different genetic mutations may present with remarkably similar clinical features, while, conversely, individuals within the same family, even though having identical pathogenic variants, can exhibit striking phenotypic variability, highlighting the complexity of genotype–phenotype correlations in mitochondrial disease. These features make establishing the diagnosis and

differential diagnosis challenging, especially in adult-onset cases[64][*Figure 3*] leading to complicated patient routes, diagnostic delays, and underdiagnosis of mitochondrial disorders.

The clinical expression of mitochondrial dysfunction is further influenced by factors such as heteroplasmy levels and nuclear-encoded gene variants with incomplete penetrance. In many cases, individuals may remain asymptomatic unless (often environmental) stressors trigger disease onset. Accordingly, both the severity and onset of symptoms vary considerably among patients, reflecting the multifactorial nature of mitochondrial disease expression involving genetic and environmental factors.

1.2.5 Mitochondrial disorders in precision medicine

This extensive genetic and phenotypic variability presents significant challenges for epidemiological studies and complicates efforts to accurately assess the societal and healthcare impact of PMDs. Among mitochondrial disorders, Barth syndrome is currently the only condition for which a disease-modifying therapy has received approval from the FDA or the EMA[65]. Although many clinical aspects of Barth syndrome, such as cardiomyopathy and skeletal myopathy, are also observed in other mitochondrial disorders, no FDA- or EMA-approved therapies currently exist for other mitochondrial conditions. Agents such as elamipretide and other investigational compounds are still under evaluation for effectiveness[66] and standardized guidelines for drug safety and therapeutic interventions in mitochondrial diseases remain under active development[67] Among substances available outside of clinical trials, nutritional supplements are the most widely used. These include antioxidants, cofactors (such as vitamins B2, B3, C, E, folic acid, and alpha-lipoic acid) and electron acceptors (such as coenzyme Q10 and carnitine) as well as L-arginine. Nevertheless, there is growing interest from the pharmaceutical industry in targeting mitochondrial diseases, and numerous clinical trials are currently ongoing[68,69].

Given these, the creation and characterization of trial-ready patient cohorts and patient registries are becoming increasingly important. Altogether, investigating and evaluating the clinical relevance of PMDs offers a unique model for exploring broader principles in drug development and personalized medicine. They exemplify the need for integrated, data-driven approaches that combine genomic, biomarker, and clinical information to guide management strategies for patients with complex, multimorbid conditions.

1.3 Mitochondrial model disorders in the scope of the study

Among the wide spectrum of mitochondrial syndromes, we selected SPG7- and MT-TL1-associated disorders for detailed investigation, as their estimated global prevalence, suggests they may represent some of the most common[70,71] manifestations of PMDs.

1.3.1 mt-TL1-associated Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes (MELAS)

MELAS syndrome is an mtDNA-associated PMD mitochondrial disorder that exemplifies the complexity and multisystemic impact of mitochondrial dysfunction. It is characterized by a broad spectrum of neurological, muscular, and systemic manifestations resulting from mutations in mtDNA, which disrupt cellular energy metabolism[39,72]. The disease follows a maternal inheritance pattern. However, phenotypic expression is highly variable and influenced by factors such as heteroplasmy levels, which are further complicated by tissue-specific heterogeneity of heteroplasmy and nuclear-mitochondrial interactions.

The most common genetic cause of MELAS is the m.3243A>G point mutation in the MT-TL1 gene, that accounts for approximately 80% of clinically diagnosed MELAS cases. Other associated pathogenic variants include m.3271T>C (7–10% of cases), as well as less frequent variants such as m.3252A>G (also in MT-TL1), m.13513G>A in MT-ND5, m.10158T>C and m.10191T>C in MT-ND3, and m.1644G>A in MT-TV. These variants predominantly affect mitochondrial tRNA genes or subunits of Complex I of the respiratory chain, ultimately impairing mitochondrial protein synthesis and disrupting OXPHOS and associated symptoms. The clinical presentation typically includes recurrent stroke-like episodes, progressive encephalopathy, seizures, lactic acidosis, myopathy, and various systemic complications such as sensorineural hearing loss, short stature, diabetes mellitus, and cardiomyopathy.

Epidemiological estimates on MELAS prevalence are highly variable, reflecting both diagnostic challenges and population-specific genetic backgrounds. Reported prevalence rates range from as low as 0.18 per 100,000 individuals in Japan to as high as 236 per 100,000 in certain Australian cohorts, depending on whether clinical or genetic diagnostic criteria are used. In most Western populations, the prevalence is estimated between 10 and 20 per 100,000, with the highest numbers reported when screening for carriers of the m.3243A>G mutation irrespective of clinical presentation[73].

1.3.2. SPG7-associated hereditary spastic paraplegia type 7 (HSP7)

SPG7 encodes paraplegin, a mitochondrial metalloprotease of the AAA family, localized to the inner mitochondrial membrane. It plays a critical role in mitochondrial protein quality control by removing misfolded or damaged proteins[74]. SPG7 is essential for the assembly and stability of complexes I and IV of the oxidative phosphorylation (OXPHOS) system and influences mitochondrial morphology and fusion. Additionally, SPG7 participates in Ca^{2+}- and ROS-induced permeability transition pore opening. Beyond its role in protein homeostasis, SPG7 contributes to the functional integrity of the OXPHOS system as part of the mitochondrial permeability transition pore[75,76].

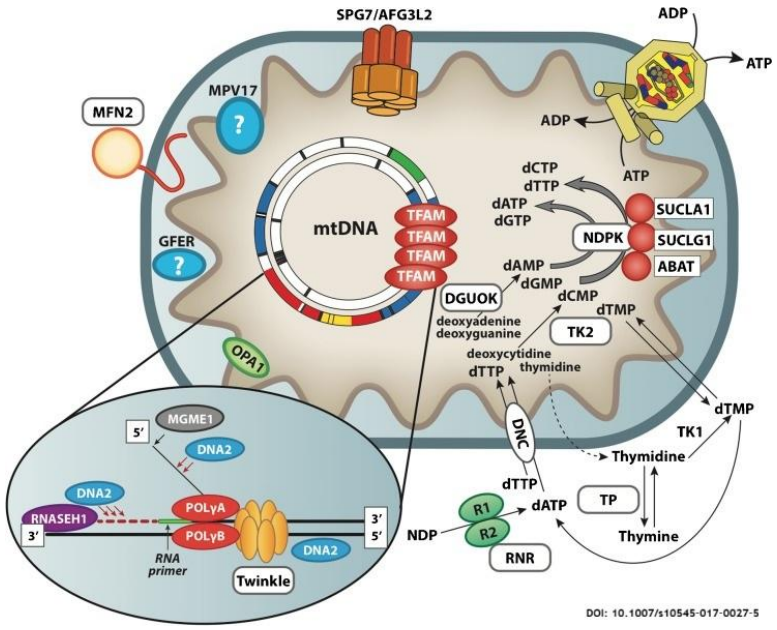


Figure 3 – The physiological role of SPG7 in the mitochondria

The figure demonstrates SPG7 as a participant in pathways involved in mtDNA maintenance.

Pathogenic variants in the *SPG7* gene have been associated with HSP7, a neurodegenerative disorder traditionally classified within the spectrum of pure hereditary spastic paraplegias (HSPs), ie. *SPG7*-related disease was primarily characterized by lower limb spasticity and weakness, often accompanied by impaired vibratory sensation and urinary dysfunction[70,77–79]. However, subsequent reports have substantially expanded the phenotypic spectrum to include cerebellar ataxia, optic neuropathy, and other neurological features, thereby reclassifying a subset of patients as complex HSP cases[80–84]. Spasticity, widely considered the hallmark feature of SPG7-related disorders, may be absent in a substantial proportion of affected individuals[85,86].

Not considering SPG7 screening in non-spastic patients contributes to diagnostic uncertainty and underrecognition of these conditions.

Despite its relatively low estimated prevalence - ranging from 1.55% to 12% among all HSP cases and 0.22–0.72 per 100,000 in the general Caucasian population - the true burden of *SPG7*-related disease is likely underestimated due to variable expressivity and the broad clinical spectrum. This underdiagnosis is further complicated by the gene's central role in mitochondrial function, implicating it in overlapping other phenotypes often not considered for SPG7 screening. While *SPG7* variants are predominantly inherited in an autosomal recessive (AR) manner, emerging evidence supports possible dominant-negative effects in specific alleles, as well as the contribution of structural variants (e.g., deletions or duplications), non-loss-of-function variants as well as and digenic interactions, particularly involving *AFG3L2*[87–90].

Given these complexities, a comprehensive understanding of the clinical and molecular spectrum associated with both MT-TL1 and SPG7 mutations remains incomplete. These two genes can serve as models for the genetic and phenotypic heterogeneity that defines mitochondrial disorders, bridging the gap between monogenic and non-Mendelian disease mechanisms[91]. Elucidating the intricate relationships among genotype, phenotype, and disease progression in these contexts is critical not only for improving diagnostics and prognostic assessment, but also for informing the development of precision therapies. As the field of mitochondrial medicine advances, integrating genetic insights with clinical phenotyping will be key to unlocking novel, patient-specific therapeutic strategies and identifying homogenous patient sub-groups.

2. Objectives

Mitochondrial disorders are associated with considerable clinical and genetic heterogeneity, with significant variability in both prevalence and phenotypic expression. The primary objective of our research was to investigate the prevalence and clinical variability of selected mitochondrial disorders. To create patient cohorts, two model diseases were selected based on their relative frequency among mitochondrial disorders and the anticipated availability to identify a sufficient number of patients in Hungary to support meaningful analysis, with a solid foundation of well-characterized patient registries.

1. Evaluate the genetic epidemiology of SPG7 associated disorders in Hungary
2. Observe the phenotypic spectra of SPG7 and mt-TL1 associated conditions
3. Establish genotype-phenotype correlations (including zygosity and heteroplasmy levels) affecting penetrance, age of onset, phenotypic variability
4. Assessing the therapeutic effectiveness of cariprazine based on its molecular effects in a pilot patient with MELAS syndrome
5. Identify underrepresented patient groups to improve diagnostics and patient pathways
6. Building an electronic health record (EHR)-based framework for creating trial-ready patient cohorts, registries and biobanks to support genotype–phenotype correlation studies, as well as the discovery of clinical, digital, and molecular biomarkers

3. Materials and Methods

3.1 Ethics statement

Ethical approval was provided by the Ethical Committee of the Medical Research Council (TUKEB 44599–2/2013/EKU 535/2013, SE RKEB 241/2024.) and all participants provided written informed consent.

3.2. Patient EHR data collection and data structure

Availability of detailed clinical records for retrospective and/or prospective evaluation was also an inclusion criterion. Data in the SPG7 cohort were collected in a retrospective manner. Patients in the Mt-TL1 m.3243A>G cohort were selected based on the presence of pathogenic or likely pathogenic variants in the *MT-TL1* mitochondrial gene. A total of 27 patients (mean age 40,83 ±13.98 years), including 9 males, 18 females) from 17 unrelated families were enrolled in the study.

To ensure long-term sustainability and robust data capture for rare disorders, all data collection was conducted under informed consent and with ethical approval. Patients were pseudonymized and enrolled in ERN databases and the GENOMIT rare disease registry to provide data for international datasets and increase statistical robustness of future studies. Data were collected at multiple levels to capture both population-wide and disease-specific information. (1)Population-level dataset: Demographic and basic clinical data were recorded, including age, residential address, diagnosis, and vital status (alive/deceased). (2)Disease-specific registry data: Structured data were extracted from EHRs and included age at disease onset, genomic data, and detailed clinical phenotypes. (3) Unstructured clinical data: Complementary un-structured data were collected, including MRI DICOM files, raw electrophysiological recordings, and family pedigrees in our data repositories.

3.2.1. SPG7 cohort

Since SPG7 is one of the more commonly implicated mitochondrial genes in disease, our study aimed to identify individuals carrying rare, damaging SPG7 variants (SPG7 DRV). Patient enrollment was guided by clinical presentation and aimed to capture a broad phenotype spectrum. Four main groups were included:

(1) **Classical HSP phenotype**: Patients presenting with spastic paraparesis of presumed genetic origin, consistent with the typical *SPG7*-associated hereditary spastic paraplegia (HSP7) phenotype.

(2) **Cerebellar ataxia group**: Patients with clinical features suggestive of inherited cerebellar ataxia, representing an extended, non-spastic phenotype associated with *SPG7* variants.

(3) **Motor neuron lesion (MNL) group**: Patients exhibiting MN signs without significant amyotrophy. In this group, electrophysiological (including EMG) findings, brisk deep tendon reflexes, pyramidal signs, and El Escorial[92] and Gold Coast[93] criteria, as well as the absence of amyotrophy were assessed, supporting motor neuron disorder.

(4) **Mitochondrial phenotype group (mt-cohort)**: Previously not investigated in the literature, but based on the molecular function of the SPG7 protein product paraplegin. This group comprised individuals with clinical signs suggestive of mitochondrial central nervous system involvement, including progressive external ophthalmoplegia (PEO), optic atrophy, myopathologic findings indicative of mitochondrial dysfunction, or abnormal results on lactate stress testing, suggestive of impaired aerobic metabolism.

The presence of acquired neurological diseases served as an exclusion criterion. For patients with suspected mitochondrial disease, multisystemic involvement was not an exclusion criterion. Spasticity was present in 267 patients (mean age: 53.16 ±15.11 years); 182 had ataxia as the presenting symptom (mean age: 54.67 ±14.44 years); 116 were referred due to suspected lower motoneuron disease or electromyography-identified motoneuron lesions without amyotrophy (mean age: 55.74 ±15.13 years); and in 113 cases, mitochondrial disease was suspected (mean age: 54.95 ±14.58 years). Some patients exhibited more than one symptom or phenotypic feature at the time of examination, leading to their inclusion in multiple subcohorts. Consequently, 214 patients were enrolled in more than one subcohort. Altogether, 437 probands (mean age: 53.22 ±15.24 years) were selected for SPG7 RDV screening, including 197 females and 240 males.

3.2.2. Mt-TL1 m.3243A>G cohort

In the mt-TL1 cohort, patients were selected based on a genetic diagnosis made at the Institute of Genomic Medicine and Rare Disorders, Semmelweis University (SE-GRI). To ensure statistical power and comparability, only patients with the m.3243A>G variant were included. The MT-TL1 cohort comprising data from 27 patients (9 males and 18 females) representing 17 distinct families. The mean age at the most recent follow-up was 41 ± 13.15 years. Alongside comprehensive phenotyping and clinical assessments, longitudinal follow-up data were available for 18 patients, with a mean observation period of 5.99 ± 4.41 years, including retrospective information. From this cohort, one pilot patient was selected to evaluate the potential therapeutic effect of Cariprazine in mitochondrial disease.

3.2.3. Clinical assessments

All participants were examined by a board-certified neurologist or clinical geneticist. As part of the deep phenotyping, individuals carrying SPG7 variants underwent standardized assessments including the Ashworth Spasticity Scale, manual muscle testing (MRC scale), and the Scale for the Assessment and Rating of Ataxia (SARA). Besides physical examination and deep phenotyping, electrophysiological (ENG/EMG), MRI, and DatScan were performed on selected cases.

3.3. Applied genetic diagnostic methods

3.3.1 PCR-Based Methods (Long-PCR, PCR-RFLP)

DNA was extracted from blood and muscle tissue using the QIAamp DNA Blood/Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol.

Long-PCR was used to detect single and multiple mtDNA deletions in a 20 µl reaction containing 20 pmol primers, 0.2 µl Phusion DNA Polymerase, 4 µl GC Reaction Buffer (Finnzymes, Vantaa, Finland), 0.4 µl dNTP mix, and 12.4 µl RNase-free water (qPCR grade, AMBION). PCR was performed with primers Fw: 5'-TAAAAATCTTTGAAATAGGGC-3', Rev: 5'-CGGATACAGTTCACTTTAGCT-3', using the following cycling conditions: initial denaturation at 98°C for 30 sec; 30 cycles of 98°C for 10 sec, 63°C for 10 sec, 72°C for 3 or 8 min; final extension at 72°C for 7 min. Products were run on 2% agarose gel, stained with ethidium bromide, and analyzed using QuantityOne Software (Bio-Rad, UK).

The pathogenic mitochondrial MT-TL1 variant, m.3243A>G (associated with MELAS) was screened by PCR-RFLP (GeneAmp PCR System 9700, Applied Biosystems) in a 20 µl reaction with 20 pmol primers, 10 µl ImmoMix (Bioline, USA), and 7 µl RNase-free water. The MELAS mutation region was amplified using Fw: 5'-GCAATTCCCGGACGTCTA-3' and Rev: 5'-GCGAACAGATTTTCGTTCAT-3' primers (300 nmol/L) with the following PCR program: 94°C for 5 min; 35 cycles of 94°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec; and a final extension at 72°C for 7 min.

MLPA testing was performed for patients with monoallelic SPG7 DRVs (Probemix P213-B3) to screen for disease-causing large SPG7 deletions.

3.3.2 Sanger sequencing

Primer sequences for segregation analysis of SPG7 relatives and targeted sequencing of AFG3L2 (RefSeq: NM_006796.3) exons (3, 7, 11, and 14–16) with a potential digenic effect[88,89,91] were performed on patients with monoallelic SPG7 DRV ($n = 25$) in monoallelic patients.

Primer list for AFG3L2 gene

Exon	Forward primer	Reverse primer
Exon4	TGCAAGTATAGCCCTTGGGAG	CCGGATCTCTTGAGCAGCAA
Exon7	TCACCACCATTATTAAAGCCAGT	GCCAAACTGATAAAGGCCTGT
Exon11	TGCAGTGCTCATATGTTCCTGA	ACCCTGGAGTTAAAGATGCCA
Exon14	CTGCGCCTGGCCTTGATTAG	CTCTCGCCTGCTTTTGGACA
Exon15	GTGCGGCTGTTGTGCTTATT	GCCTAAAACAGTGAAGCACAACT
Exon16	CCTTTGTGCACTTTAGCCGG	CCGGGACAGTGTCAACTTCT

PCR amplification was performed using 35 cycles: initial denaturation at 94°C for 4 min, followed by 94°C for 30 sec, annealing (Tm) for 30 sec, 72°C for 1 min, and a final extension at 72°C for 4 min. Amplified products were purified using SureClean®, followed by sequencing with the BigDye Terminator Kit. Final purification was done with the NucleoSeq® kit (BIOLINE). Sequencing was performed on an ABI PRISM 3100 Genetic Analyzer using capillary gel electrophoresis. Data were analyzed with the 3100 Data Collection Software, and sequence chromatograms were evaluated using SequenceScanner v1.0. Sequences were aligned to the human genome reference using NCBI BLAST, and variants were identified via the Ensembl database.

3.3.3 NGS panel and WES investigations

For patients with monoallelic heterozygous variants, personalized extended NGS panel or WES was performed to search for possible intergenomic interactions or RDVs in alternative HSP disease-causing genes. In patients with Parkinsonian phenotype Parkinson's disease-related gene panel investigation was performed.

The full coding sequence, exon–intron boundaries, and flanking regions of the *SPG7* gene were analyzed using either Sanger sequencing (ABI Prism 3500 DNA Sequencer, Applied Biosystems, USA) or targeted next-generation sequencing (NGS) panels for ataxia, HSP, and ALS-associated genes. Genomic DNA was fragmented and libraries were prepared using Agilent SureSelectQXT Human All Exon v5 and SureSelectQXT Target Enrichment kits (Agilent Technologies, USA). Custom probe sets were used to capture coding and flanking regions of *SPG4*, *SPG7*, and *SPG11*. Sequencing was performed on Illumina platforms using the HiSeq2500 with HiSeq SBS Kit v4 or MiSeq with Reagent Kit v2 (300 cycles), following the manufacturer's protocols.

3.3.4. Bioinformatic analysis and variant classification

Variant identification from NGS data was conducted using GATK HaplotypeCaller (v3.3-0), in accordance with the GATK Best Practices. Variants were annotated using SnpEff and the ClinVar database. Filtering of exome data was performed with Franklin Genoox and VariantAnalyzer, developed at the Budapest University of Technology and Economics. Novel variants were classified based on ACMG guidelines, and segregation analysis was conducted when possible. Key interpretation criteria are summarized in Table 1; additional ACMG scoring elements were also considered during evaluation[94–97].

3.4. Myopathologic examinations

For investigations to identify signs of mitochondrial dysfunction NADH, Modified SDH and Cytochrome-C-Oxidase (COX) staining were applied, besides Hematoxylin & Eosin staining.

3.5. Statsitical analyses

Statistical analysis was performed using Prism GraphPad V7.0b and SigmaPlot (2015) software. One-way analysis of variance and Spearmen correlation was used for multiple

group comparisons, and independent samples *t* tests and χ^2 tests were used to compare two groups.

4. Results

4.1 Results from the SPG7 screening cohort

Results presented in the SPG7 screening cohort chapter are based on, and partly cited from, previously published work[81]. These findings will contribute to the original and unpublished content of the discussion chapter in this thesis.

4.1.1. Epidemiology and sub-cohort specific diagnostic rate of SPG7

At disease onset, the most frequent symptoms were ataxia, paraparesis, dysarthria, or spasticity. SPG7 RDV was detected in 13.5% (50/437) of the cohort, excluding probands' relatives. RDV prevalence varied across four partly overlapping cohorts, with the highest diagnostic yield seen in the ataxia group. Among patients carrying biallelic RDVs, 48% initially presented with paraparesis, 48% with ataxia, and 12% with visual impairment. For those with monoallelic RDVs, presenting symptoms included spasticity (36%), paraparesis (21.4%), ataxia (35.7%), visual complaints (3.6%), and fasciculations or mitochondrial-related symptoms such as myalgia, fatigue, exercise intolerance, or PEO (14.2%). Figure 4 details RDV prevalence in each subcohort. Damaging biallelic RDVs were most common in the ataxic and spastic groups, while monoallelic DRVs were more frequent in the mitochondrial cohort. In other cases with monoallelic or biallelic variants, early corticospinal tract involvement was detected later, progressing to spasticity or ataxia. The SE-GRI is the country center laboratory in the diagnostics of SPG7, thus based on our observations, the minimum prevalence of SPG7-associated conditions can be estimated to be approximately 0.46 per 100,000 in Hungary between 2018.11.22. and 2023.06.05.

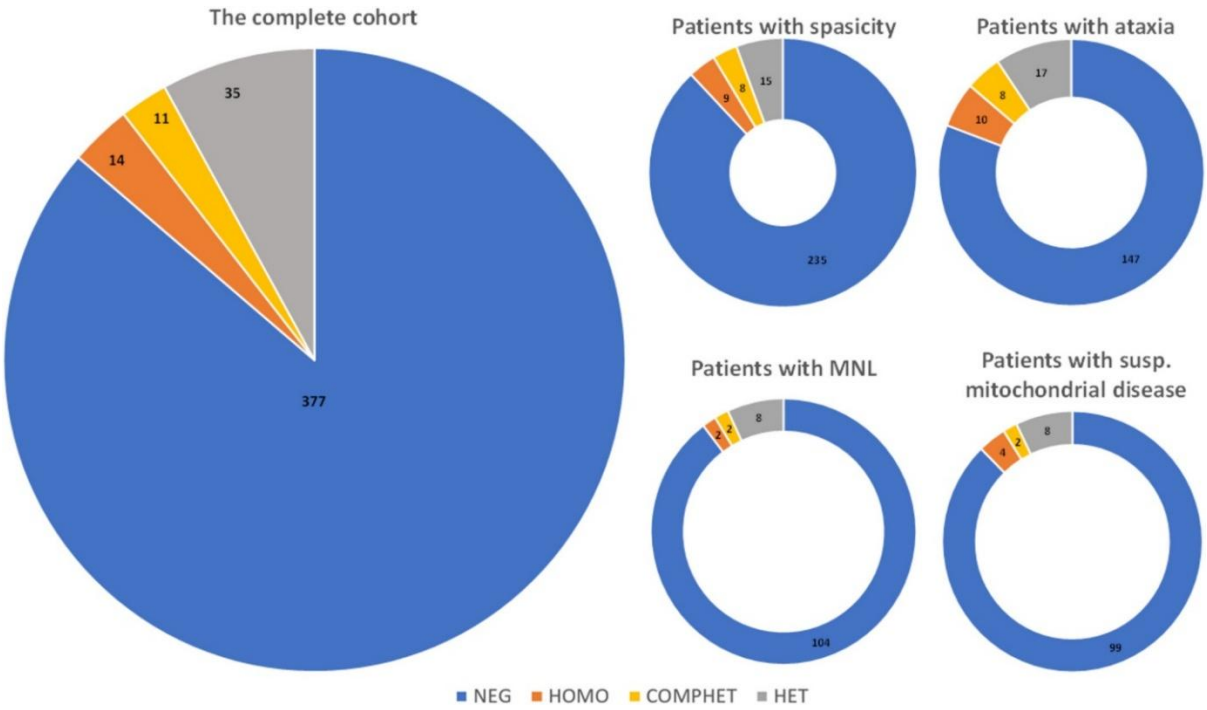


Figure 2 – Prevalence of SPG7 RDVs in different subcohorts

Prevalence of rare damaging variants in patients with spasticity, ataxia, motoneuron lesion (MNL), patients with suspected mitochondrial disorders. The thickness of the circle corresponds to the size of the cohorts. COMPHET, patients with compound heterozygous variants; HET, patients with heterozygous variant; HOMO, patients with homozygous SPG7 RDV; NEG, patients without SPG7 RDV.

4.1.2. Identified disease-associated SPG7 variants

In the SPG7 gene, 16 rare damaging variants (RDVs) were identified among 58 symptomatic individuals. These included 41 missense, 35 nonsense, 3 frameshift, and 4 splice-site alleles including patients both with bi- and monoallelic variants. MLPA analysis revealed no deletions or duplications in SPG7. Based on ACMG criteria, 10 variants were classified as pathogenic or likely pathogenic. In addition, several variants of uncertain significance (VUS) were detected; five of these were considered RDVs due to in silico predictions and supporting ACMG evidence, and were included despite not meeting full pathogenicity criteria.

The most common RDVs were p.Leu78Ter (n=23) and p.Ala510Val (n=21). p.Leu78Ter appeared as heterozygous (n=9), compound heterozygous (n=5), or homozygous (n=9); p.Ala510Val occurred in homozygous (n=5), compound heterozygous (n=7), and heterozygous (n=9) forms. Other RDVs were rarer and sometimes observed in asymptomatic carriers. The variant p.Arg486Gln, a known ALS risk factor, was found

heterozygously in five cases. The identified DRVs were identified in 23 probands from families with biallelic SPG7 mutations, 22 symptomatic monoallelic carriers, and five individuals with the ALS risk allele. In total, 58 symptomatic individuals carried at least one DRV or ALS-associated variant. Among them, 14 were homozygous, 11 compound heterozygous, 28 heterozygous, and five carried the risk allele. Variant distributions by zygosity and clinical status are detailed in Table 1 and are visualized in Figure 5. Family testing revealed two additional symptomatic homozygotes, six symptomatic monoallelic carriers, and five asymptomatic relatives with monoallelic DRVs. Notably, digenic cases involving SPG7 and AFG3L2 variants have been reported but, none of our patients harbored secondary AFG3L2 variants.

Variant	Exon	Variant Type	CADD	GERP	ACMG	GnomAD 2.1 all AF	All affected cases / Families	Biallelic / Monoalleic cases	Bi / monoallelic cases with spasticity	Bi / monoallelic cases with ataxia	Bi / monoallelic cases with LMNL	Bi / monoallelic cases with mitochondrial dysfunction	Asymptomatic carriers
p.Leu78Ter	2	Nonsense	33	5.32	PATH	0.00039	23 / 18	14 / 9	11 / 7	14 / 9	1 / 0	2 / 2	1
p.Lys340Glu	8	Missense	25.9	5.85	VUS_LP	0	1 / 1	0 / 1	0 / 0	0 / 1	0 / 0	0 / 1	1
p.Gly344Asp	8	Missense	25.7	5.85	LP	0.00001	2 / 1	1 / 1	1 / 1	1 / 1	0 / 0	0 / 0	0
p.Gly352AlafsTer87	8	Frameshift	NA	5.85	LP	0	1 / 1	1 / 0	1 / 0	1 / 0	0 / 0	0 / 0	0
p.Gly352Ser	8	Missense	29.4	5.85	LP	0.00001	1 / 1	1 / 0	0 / 0	1 / 0	0 / 0	0 / 0	0
p.Val379Met	8	Missense	27.9	5.85	VUS_LP	0.00003	1 / 1	0 / 1	0 / 1	0 / 1	0 / 0	0 / 0	0
p.Arg398Ter	9	Nonsense	41	5.63	PATH	0.00002	2 / 2	2 / 0	1 / 0	2 / 0	1 / 0	2 / 0	0
p.Tyr406Cys	9	Missense	29.5	5.63	LP	0.00001	1 / 1	0 / 1	0 / 1	0 / 1	0 / 0	0 / 0	0
p.Arg486Gln	11	Missense	22.6	5.42	RISK FACTOR	0.004722	5 / 5	0 / 5	0 / 2	0 / 4	0 / 1	0 / 0	0
p.Gln507Ter	11	Nonsense	49	5.42	PATH	0	1 / 1	1 / 0	0 / 0	1 / 0	0 / 0	0 / 0	0
p.Ala510Val	11	Missense	27.8	5.42	PATH	0.00289	21 / 19	12 / 9	9 / 5	12 / 7	1 / 2	2 / 2	3
c.1552+1G>T	11	Splice	34	5.42	PATH	0.00002	4 / 4	2 / 2	1 / 1	2 / 1	1 / 1	0 / 0	0
p.Val540Met	12	Missense	21.6	5.47	VUS	0.00002016	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0
p.Ser645Thr	14	Missense	14.82	5.93	VUS	0.00076	2 / 2	0 / 2	0 / 1	0 / 2	0 / 0	0 / 1	0
p.Asn739fs	17	Frameshift	NA	5.59	PATH	0.00001	2 / 2	2 / 0	1 / 0	2 / 0	1 / 0	2 / 0	0
p.Tyr740Cys	17	Missense	25.7	5.59	VUS	0.00004599	1 / 1	0 / 1	0 / 0	0 / 1	0 / 0	0 / 0	0

Table 1 The - Identified rare damaging variants in the cohorts

Abbreviations: Variant type STOP-nonsense; Miss – missense, FRAME – Frameshift, SPLICE – splice site variants, ACMG classifications: PATH – pathogenic; LP – likely pathogenic – VUS – variant with unknown significance. AF allel frequency; AC – Allele count, Homo# - number of homozygous patients; CompHet# - compound heterozygous patients to the variant, Het# number of symptomatic carriers

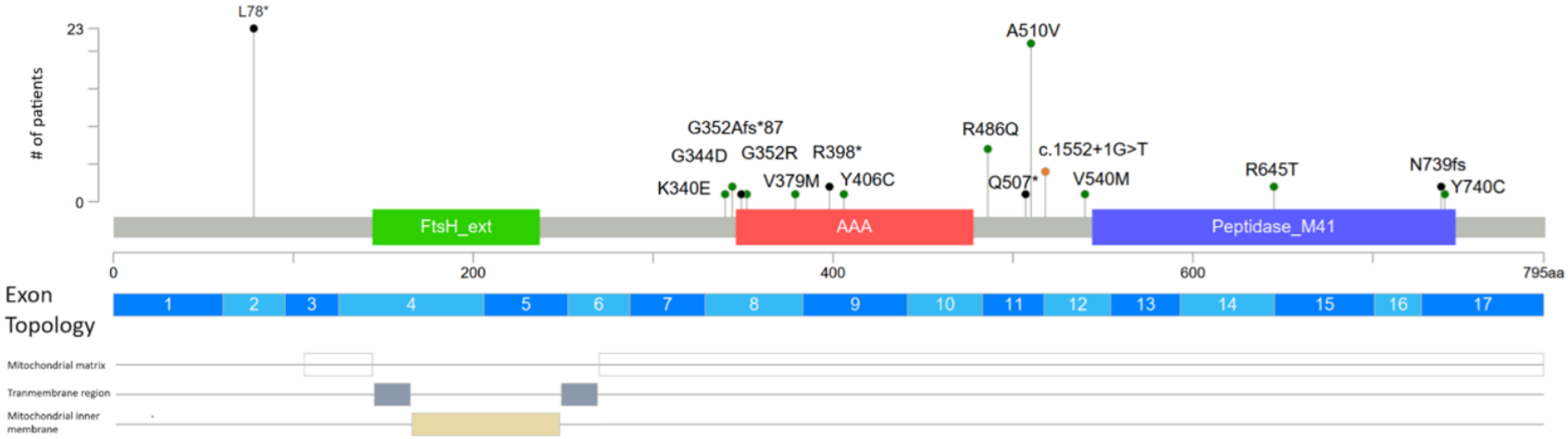


Figure 3 Lolliplot of identified SPG7 rare damaging variants

Lollipop height represents allele count, and color denotes variant type: green for missense, black for frameshift/nonsense, and orange for splice site variants. Variants are mapped across three layers: (1) Functional domains of the SPG7-encoded paraplegin protein - FtsH, AAA ATPase, and Peptidase M41; (2) Genomic/exonic positions - dark and light blue bars indicate exon or genomic locations; (3) Mitochondrial localization - bottom layer shows all variants, except the L78 truncating variant, are situated within the mitochondrial matrix. All variants share the same zygosity type

4.1.3 Clinical phenotype of patients with identified SPG7 RDV

The MRI and imaging findings in the patients were consistent with previous studies in terms of cerebellar volume and T2 signals. The prevalence of cerebellar atrophy was 36.84% (19/52) in the biallelic cases. In addition to cerebellar atrophy, the following findings were identified in our patients' brain MRIs: dentate nuclei hyperintensity, nonspecific focal, and confluent T2 white matter hyperintensity. Additionally, we identified the “ears of the lynx” sign in one of our patients, which has not previously been associated with SPG7-related disorders. These findings were not specific to a particular variant, variant type, or zygosity. Some patients with atypical parkinsonism underwent DaTscan, and none of them showed impaired dopaminergic function.

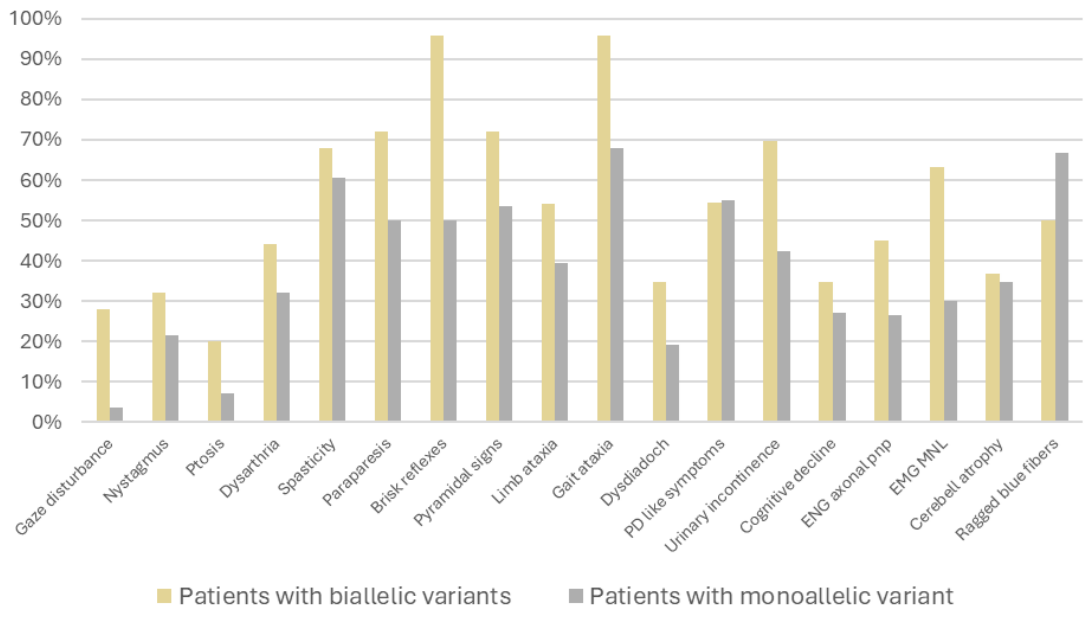
4.1.4 Genotype–phenotype correlations

The average age at onset in affected individuals was as follows: 41.12 (±9.97) years for patients with biallelic variants (39 ± 9.73 years for homozygous, 44.3 ± 9.95 years for compound heterozygous), 45.86 (±13.37) years for monoallelic heterozygous patients, and 51.6 (±19.65) years for patients with the risk allele. Asymptomatic relatives with monoallelic RDVs had a relatively young age (mean age 30.75 ± 2.9 years). The type and location of the SPG7 variant did not significantly affect the age of onset or the symptoms. However, homozygous and compound heterozygous patients appeared to differ in terms of clinical phenotype, particularly in the severity of spasticity, muscle strength, and SARA scores. Monoallelic patients exhibited similar symptoms to those with biallelic variants, but they tended to have a milder phenotype [Figure 6]. Although multiple investigations have been conducted, we did not find a significant correlation between the mutation type, age of onset, or symptom severity.

In the mitochondrial screening cohort, 77 patients underwent muscle biopsy, including seven carrying SPG7 RDVs. Mitochondrial dysfunction with ragged-blue fibers was observed in the following cases: P4 and P11C (homozygous p.Leu78Ter), P32 and P46 (heterozygous p.Ala510Val), P38 (heterozygous p.Ser645Thr), P48 (heterozygous p.Val540Met), and P49

(heterozygous c.1552 + 1G>T). Muscle histology supported (but was not able to fully confirm) the pathogenicity of the p.Ser645Thr variant, which remains a VUS, although it showed a feature typical of SPG7. mtDNA deletion screening was performed on 52 blood and 6 muscle samples, revealing multiple deletions in 15.38% of blood samples (8/52) and 66% of muscle samples (4/6). Integrating histology and mtDNA data, mitochondrial dysfunction was identified in 33.3% of heterozygous and 16.0% of biallelic patients. Statistical tests showed no significant association between mtDNA deletions and age of onset (t test p=0.1213; ANOVA p=0.1575).

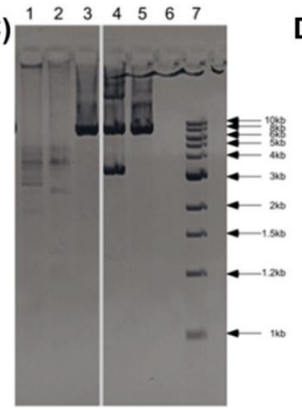
A)



B)

	Risk allele carriers	Heterozygous RDV	Biallelic RDVP	Comphet RDVP	Homozygous RDVP	2 truncating RDVP	1 truncating 1 missense RDVP	2 missense RDVP	1 truncating RDVP	1 missense RDVP
Sex (F/M)	2/3	15/13	5/20	2/9	3/11	4/8	0/6	1/6	5/6	10/7
AOO	51.60	45.86	41.12	43.55	39.21	38.75	39.67	46.43	43.91	47.12
Vision	0.00	0.07	0.20	0.09	0.29	0.25	0.17	0.14	0.00	0.12
Ptosis	0.00	0.07	0.20	0.09	0.29	0.25	0.33	0.00	0.09	0.06
Gaze	0.00	0.04	0.54	0.60	0.50	0.50	0.50	0.67	0.00	0.06
Dysarthria	0.40	0.14	0.79	1.20	0.50	0.25	1.33	1.33	0.09	0.18
Paraparesis	4.20	4.29	3.68	3.91	3.50	3.42	4.17	3.71	4.23	4.32
Dysphagia	1.00	0.76	1.22	1.05	1.36	0.75	2.25	1.14	0.45	0.97
Gait (SARA)	2.25	1.40	3.11	3.61	2.79	3.54	2.00	3.40	0.60	1.93
Standing (SARA)	1.50	1.14	2.11	3.11	1.46	2.21	1.33	2.80	1.10	1.17
Sitting (SARA)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Finger chase (SARA)	1.00	0.23	0.83	0.78	0.88	0.82	0.90	0.80	0.27	0.18
Nose finger test (SARA)	0.67	0.25	0.36	0.39	0.33	0.23	0.40	0.60	0.18	0.31
Fast altern. (SARA)	0.67	0.42	0.69	0.61	0.75	0.82	0.90	0.20	0.30	0.50
Heel-Shin (SARA)	0.88	0.52	0.83	0.94	0.75	0.71	0.92	1.00	0.36	0.64
SARA score	7.13	4.16	7.24	9.45	5.50	6.08	8.33	8.29	3.27	4.74
Spasticity	1.40	1.13	2.60	2.73	2.50	2.58	2.50	2.71	0.86	1.29
Brisk Reflex	0.40	0.46	0.96	0.91	1.00	1.00	1.00	0.86	0.09	0.71
Pyramidal sign	0.40	0.54	0.68	0.64	0.71	0.75	0.50	0.71	0.36	0.65
Cognitive decline	0.40	0.21	0.32	0.27	0.36	0.33	0.50	0.14	0.27	0.18
Depression	0.40	0.36	0.17	0.10	0.21	0.17	0.33	0.00	0.40	0.33
Continence	0.00	0.42	0.63	0.55	0.69	0.82	0.33	0.57	0.45	0.40
ENG apnp	0.00	0.33	0.25	0.13	0.33	0.20	0.40	0.20	0.75	0.13
EMG mnl	0.33	0.42	0.40	0.50	0.33	0.50	0.00	0.60	0.25	0.50
MRI T2	0.00	0.50	0.48	0.50	0.45	0.88	0.00	0.43	0.22	0.69
Cerebellar atrophy	0.33	0.23	0.33	0.30	0.36	0.25	0.50	0.29	0.22	0.23
Mito involvment	0.00	0.32	0.24	0.09	0.36	0.25	0.33	0.14	0.18	0.41

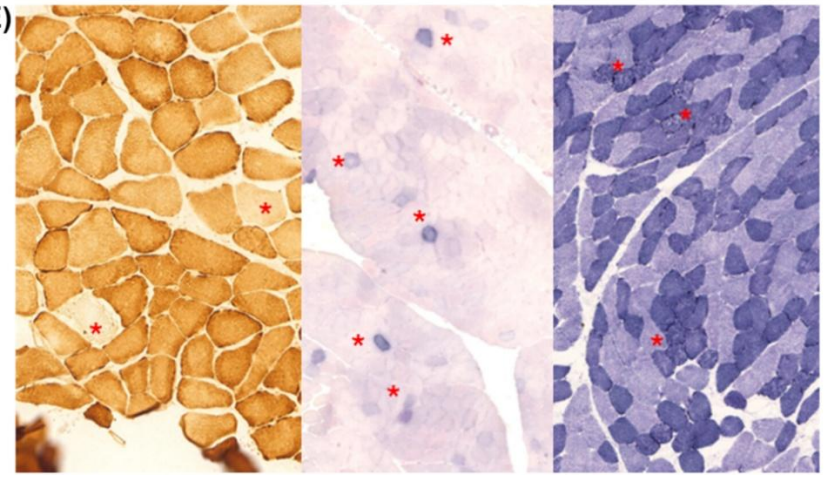
C)



D)

	Prevalence of mtDel in bDNA	Ratio*	Prevalence of mtDel in mDNA	Ratio**	Overall prevalence of mtDysf	Ratio***
Monoallelic RDV patients	16.67%	4/24	80%	4/5	33.33%	9/27
Biallelic RDV patients	16.00%	4/25	NCI	0/0	24.00%	6/25
Compound heterozygous RDV patients	9.09%	1/11	NCI	0/0	9.09%	1/11
Homozygous RDV patients	21.43%	3/14	NCI	0/0	35.71%	5/14
Patients with 2 truncating RDV	16.67%	2/12	NCI	0/0	25.00%	3/12
Patients with 2 missense RDV	16.67%	1/6	NCI	0/0	33.33%	2/6
Patients with 1 truncating and 1 missense RDV	14.29%	1/7	NCI	0/0	14.29%	1/7
Patients with 1 truncating RDV	18.18%	2/11	NCI	0/0	18.18%	2/11
Patients with 1 missense RDV	15.38%	2/13	80%	4/5	43.75%	7/16

E)



F)

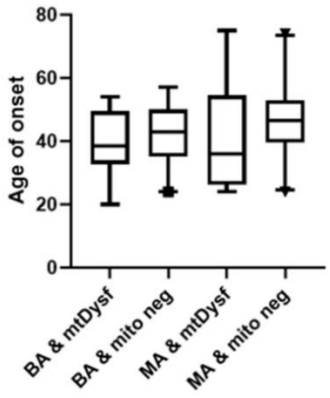


Figure 4 - Genotype–phenotype observations in patients with SPG7 RDV

A) The figure compares the frequency of SPG7-related symptoms between patients with biallelic and monoallelic non-risk factor rare damaging variants (RDVs).
B) The figure shows the distribution of key clinical features across SPG7 genotype groups. Columns represent genotype categories, while rows indicate specific clinical signs. On the left, patients are grouped by zygosity (heterozygous, biallelic, and compound heterozygous). On the right, patients are grouped by RDV type: two truncating RDVs, one truncating + one missense, two missense, one truncating, or one missense variant.
SARA subscore means are labeled as "(SARA)." Heatmap colors are scaled within each row to emphasize the genotype group most associated with each feature. AOO, age of onset; DD, disease duration; EMG mnl, motor neuron lesions detected by electromyography; ENG apnp, axonal polyneuropathy identified via electroneurography; Fast altern., fast alternating movements; Mito involvement, mitochondrial dysfunction evidenced by muscle histology or mtDNA multiplex deletions. MRI T2, T2 hyperintense foci observed on MRI.
C) *Representative long-range PCR results. Lanes 1–2: patient with multiple mtDNA deletions; lane 3: patient without mtDNA deletions; lane 4: control with a single mtDNA deletion; lane 5: wild-type mtDNA control; lane 6: no-template control; lane 7: molecular-weight marker.*
D) *Summary of deletion analysis results. bDNA and mDNA: DNA extracted from blood and skeletal muscle, respectively. Ratio*: proportion of bDNA samples positive for multiple mtDNA deletions relative to all bDNA cases. Ratio**: proportion of mDNA samples positive for multiple mtDNA deletions relative to all mDNA cases. NCI: no investigation performed in cases meeting the criteria. mtDysf: patients with mitochondrial dysfunction confirmed by muscle histology or mtDNA deletion analysis. Ratio***: proportion of mtDysf-positive cases among all investigated for mtDysf.*
E) *Representative muscle histology. From left to right: COX-negative fibers (patient P38), ragged-blue fibers (patient P46), and subsarcolemmal granular aggregates on NADH staining (patient P11C).*
F) *Age-of-onset distribution in patient subgroups. BA: biallelic RDV carriers; MA: monoallelic RDV carriers; mito neg: patients without histologic or deletion-analysis–confirmed mitochondrial dysfunction.*

4.2 Results from the MT-TL1 m.3243A>G cohort

4.2.1. Characterics of the MT-TL1 m.3243A>G patients

The mean age of the cohort at the last follow up was 41 ± 13.15 years. In addition to detailed phenotyping and clinical assessment, longitudinal follow-up data from 18 patients were analyzed over a mean period of 5.99 ± 4.41 years, including retrospective data. From this cohort, a single pilot patient was selected to assess the potential beneficial effect of Cariprazine in mitochondrial disease.

4.2.2. Effect of HP on age of onset

During the initial and follow-up clinical evaluations, all patient presented MELAS-associated symptoms. In 5 cases, childhood onset was observed. Based on our observations, HP showed significant effect on the onset. Spearman correlation: r -0,6126; 95% KI: -0.8123 to -0.2843; two-tailed P: <.001

4.2.3. Clinical phenotype of MT-TL1 m.3243A>G patients

Given the multisystemic nature of MT-TL1 m.3243A>G-related MELAS, individuals in our cohort exhibited a broad and heterogeneous spectrum of clinical manifestations, reflecting the systemic impact of mitochondrial dysfunction[Figure 7]. The most prevalent clinical features were sensorineural hearing loss, present in 57.69% of patients, and diabetes mellitus, diagnosed in 42.31%. Stroke-like episodes, a hallmark of MELAS, occurred in 30.77%, while migraine was reported by 15.38% of individuals, often preceding or accompanying neurological decline. Cardiac involvement was identified in 38.46% of the cohort, with subtypes including hypertrophic cardiomyopathy (19.23%), dilated cardiomyopathy (7.69%), and cardiac arrhythmias (7.69%). Myopathic features such as muscle weakness, exercise intolerance, and elevated serum creatine kinase were observed in 30.77%, consistent with primary mitochondrial myopathy. In addition to neuromuscular symptoms, gastrointestinal and metabolic comorbidities were also common. Notably, chronic intestinal pseudo-obstruction (CIPO), commonly presented as food intolerance or gastrointestinal complaints by patients, was diagnosed in 19.23% of individuals. Radiological evaluation revealed nonspecific T2-weighted hyperintensities on brain MRI as the most frequent, but nonspecific imaging finding, detected in 80% of patients. These

lesions, often located in subcortical and cortical regions, were not restricted to vascular territories and are characteristic of MELAS-related encephalopathy. Psychiatric assessment was conducted in 15 patients, highlighting a significant neuropsychiatric burden. Among them, anxiety disorders were present in 40.00% (6/15), while cognitive impairment was noted in 35.71% (5/14). Depression affected 33.33% (5/15), and childhood neurodevelopmental disorders were identified in 13.33% (2/15). Additionally, schizophrenia spectrum disorders were diagnosed in 13.33% (2/15) of patients in our cohort.

4.2.4. The effect of HP on comorbidities

Although HP levels measured in blood may not fully reflect mutation loads in clinically relevant other tissues such as muscle or brain, peripheral blood remains the most accessible and routinely used source for mitochondrial DNA (mtDNA) analysis. To explore potential genotype–phenotype correlations in patients with the m.3243A>G mutation, individuals were stratified into subgroups based on HP percentages measured from blood-derived DNA[Figure 8].

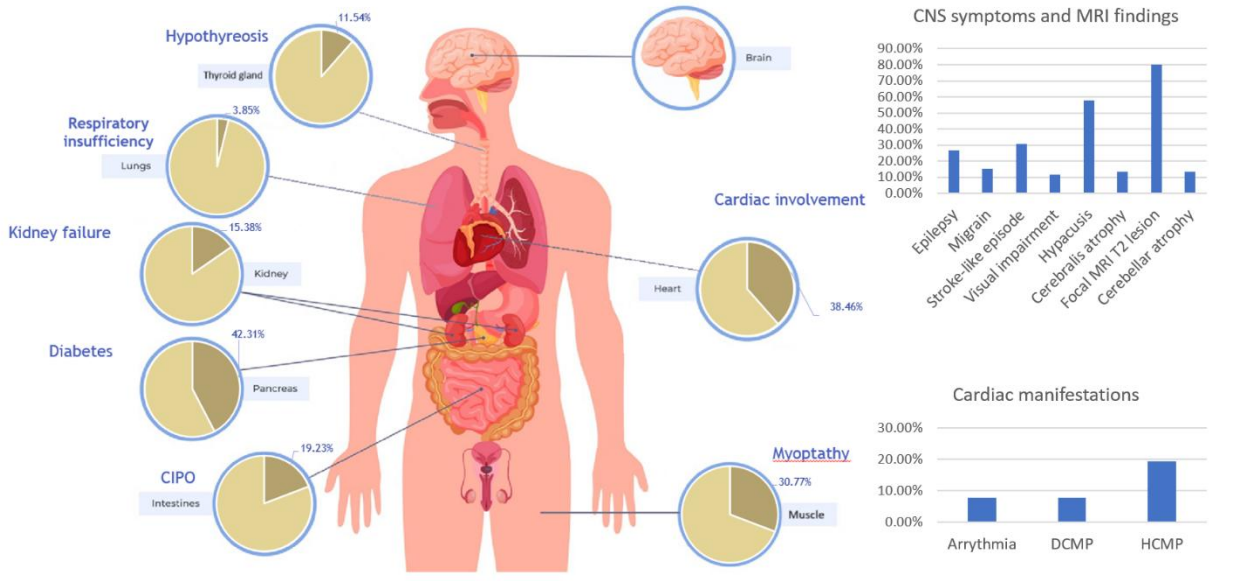


Figure 7 - Clinical phenotype of patients with m.3243A>G variant

Legend: CNS – central nervous system; DCMP – dilatative cardiomyopathy, HCMP – hypertrophic cardiomyopathy; CIPO – chronic intestinal pseudo-obstruction syndrome

In the low heteroplasmy group (≥10% to <25%), the most frequently observed clinical manifestations were sensorineural hearing loss and diabetes mellitus, typically with adult-onset presentation. Notably, no patients in this subgroup exhibited pediatric-onset disease, and there was an overall absence of severe neurological or multisystemic involvement.

The intermediate heteroplasmy group (≥25% to <30%) demonstrated a broader clinical phenotype. In addition to hearing loss and diabetes mellitus, patients often exhibited muscle weakness and exercise intolerance, cardiac involvement (including arrhythmias or cardiomyopathy), and stroke-like episodes. Although disease onset remained predominantly in adulthood, symptoms tended to emerge earlier than in the lower HP subgroup, suggesting a trend toward increasing clinical complexity with higher HP.

Patients in the moderate-to-high HP range (≥30% to <35%) presented with significantly more severe and early-onset phenotypes. Common features included epilepsy, stroke-like episodes, and early cardiac manifestations, sometimes occurring in childhood or adolescence. While some adult-onset presentations resembled those observed in lower HP groups, the frequency and severity of symptoms were notably increased in this category, indicating a clear shift toward more aggressive disease expression.

In the high HP group (≥35%), patients exhibited the full clinical spectrum of MELAS, often with childhood onset of symptoms. Multisystem involvement was frequent, including neurological, endocrine, cardiac, gastrointestinal, and psychiatric features. The early appearance and multiplicity of symptoms in this group highlight the strong association between higher HP levels and both earlier disease onset and greater clinical severity. These findings support a dose-dependent relationship between blood-based HP levels and phenotypic expression, with increasing HP correlating with earlier onset, broader systemic involvement, and more severe disease progression.

HP group: ≥10% to <25% (n=7)	<18yrs	19-24yrs	25-34yrs	35-44yrs	>45yrs
Cardiac manifest.	0.0%	0.0%	0.0%	14.3%	28.6%
CIPO	0.0%	0.0%	0.0%	14.3%	14.3%
CKD	0.0%	14.3%	14.3%	14.3%	14.3%
DM	0.0%	0.0%	0.0%	42.9%	71.4%
Hypothyreosis	0.0%	0.0%	14.3%	14.3%	14.3%
Myoptathy	0.0%	0.0%	0.0%	0.0%	0.0%
Respir. insuff.	0.0%	0.0%	0.0%	0.0%	0.0%
Epilepsy	0.0%	0.0%	14.3%	28.6%	28.6%
Migrain	0.0%	0.0%	0.0%	14.3%	14.3%
Stroke like ep.	0.0%	0.0%	0.0%	14.3%	14.3%
Visual imp.	0.0%	0.0%	0.0%	0.0%	0.0%
Hearing imp.	0.0%	0.0%	14.3%	71.4%	71.4%

HP group:≥25% to ≤30% (n=5)	<18yrs	19-24yrs	25-34yrs	35-44yrs	>45yrs
Cardiac manifest.	0.0%	20.0%	20.0%	40.0%	40.0%
CIPO	0.0%	0.0%	0.0%	0.0%	20.0%
CKD	0.0%	0.0%	0.0%	20.0%	20.0%
DM	20.0%	20.0%	20.0%	60.0%	60.0%
Hypothyreosis	0.0%	0.0%	0.0%	0.0%	0.0%
Myoptathy	0.0%	0.0%	20.0%	40.0%	40.0%
Respir. insuff.	0.0%	0.0%	0.0%	0.0%	0.0%
Epilepsy	0.0%	0.0%	0.0%	20.0%	20.0%
Migrain	0.0%	0.0%	0.0%	0.0%	0.0%
Stroke like ep.	0.0%	0.0%	0.0%	20.0%	40.0%
Visual imp.	0.0%	0.0%	0.0%	0.0%	0.0%
Hearing imp.	0.0%	0.0%	20.0%	20.0%	40.0%

HP group ≥30% to <35% (n=5)	<18yrs	19-24yrs	25-34yrs	35-44yrs	>45yrs
Cardiac manifest.	20.0%	20.0%	20.0%	20.0%	20.0%
CIPO	0.0%	0.0%	0.0%	0.0%	0.0%
CKD	0.0%	0.0%	0.0%	0.0%	0.0%
DM	0.0%	0.0%	0.0%	0.0%	0.0%
Hypothyreosis	0.0%	0.0%	20.0%	20.0%	20.0%
Myoptathy	0.0%	20.0%	20.0%	40.0%	60.0%
Respir. insuff.	0.0%	0.0%	0.0%	0.0%	0.0%
Epilepsy	20.0%	20.0%	20.0%	40.0%	40.0%
Migrain	0.0%	0.0%	20.0%	40.0%	40.0%
Stroke like ep.	20.0%	20.0%	20.0%	40.0%	40.0%
Visual imp.	0.0%	0.0%	0.0%	0.0%	20.0%
Hearing imp.	0.0%	20.0%	40.0%	60.0%	80.0%

HP group ≥35% (n=9)	<18yrs	19-24yrs	25-34yrs	35-44yrs	>45yrs
Cardiac manifest.	22.2%	22.2%	44.4%	44.4%	55.6%
CIPO	11.1%	33.3%	33.3%	33.3%	33.3%
CKD	11.1%	22.2%	22.2%	22.2%	22.2%
DM	0.0%	0.0%	11.1%	33.3%	33.3%
Hypothyreosis	11.1%	11.1%	11.1%	11.1%	11.1%
Myoptathy	22.2%	22.2%	33.3%	33.3%	33.3%
Respir. insuff.	11.1%	11.1%	11.1%	11.1%	11.1%
Epilepsy	11.1%	11.1%	11.1%	22.2%	22.2%
Migrain	0.0%	0.0%	11.1%	11.1%	11.1%
Stroke like ep.	11.1%	11.1%	11.1%	33.3%	33.3%
Visual imp.	11.1%	22.2%	22.2%	22.2%	22.2%
Hearing imp.	11.1%	22.2%	44.4%	44.4%	44.4%

Figure 5 - Comorbidities by age in HP subgroups

Legend: Cardiac manifest – cardiologic findings including arrhythmia, dilatative and hypertrophic cardiomyopathy; CIPO – chronic intestinal pseudo-obstruction syndrome; CKD – chronic kidney disease; DM – diabetes mellitus, Respir. Insuff. – respiratory insufficiency; Stroke like ep. – stroke-like episode; Visual imp. – visual impairment, Hearing imp. – hearing impairment; yrs-years of age; n – number of patients in the HP group

4.2.5. Effect of clinical symptoms on diagnostic time

The mean diagnostic delay across the entire cohort was 6.96 ± 6.81 years. To identify clinical domains where earlier recognition may be improved, diagnostic latency was analyzed based on initial symptom type. Epilepsy was associated with the shortest time to diagnosis (3.0 ± 4.0 years), reaching statistical significance ($p = 0.04$), suggesting it may prompt earlier referral and diagnostic workup. In contrast, hypothyreosis (12.33 ± 10.02 years, $p = 0.15$), cardiomyopathy (8.56 ± 8.38 years), and CIPO (7.8 ± 8.2 years) were linked to longer diagnostic delays, although these did not reach statistical significance. Symptoms such as

diabetes mellitus (7.6 ± 7.3 years), myopathy (7.1 ± 7.6 years), hypacusis (7.87 ± 8.21 years), stroke-like episodes (6.0 ± 6.8 years), and migraine (5.75 ± 3.6 years) were associated with intermediate diagnostic intervals. These findings underscore the variable timelines across disciplines and highlight the need for increased awareness of mitochondrial disease presentations beyond neurology, particularly in endocrinology, cardiology, and gastroenterology.

4.3. Experience with Cariprazine in a pilot m.3243A>G patient

A 27-year-old female was referred to our clinic with suspected Wernicke-Korsakoff syndrome (WKS), characterized by the classical triad of nystagmus, ataxia, and memory impairment with limited response to parenteral thiamine supplementation.

Since her late teens, the patient experienced dysmenorrhea with diarrhea and generalized anxiety and began using cannabis at age 17. At 21, she developed several week-long episodes of vomiting and diarrhea without an infectious cause. Symptoms briefly improved on a gluten-free diet, but gluten-sensitive enteropathy was excluded. She was later diagnosed with gastroesophageal reflux disease and hepatic focal nodular hyperplasia.

By age 26, she presented with ataxia, memory impairment, fatigue, and depression. High-dose parenteral vitamin B1 (200 mg/day) improved ataxia, but cognitive deficits and mood fluctuations persisted. Brain MRI revealed mild bilateral thalamic lesions, supporting a diagnosis of WKS. These symptoms emerged following significant weight loss due to recurrent episodes of severe vomiting, in the absence of alcohol consumption.

A 27-year-old female was referred to our clinic. The patient had received partial thiamine supplementation prior to presentation, with limited clinical response, which suggested other non-classical confounding mechanisms, including possible genetic contributors. Family history was suggestive of maternal inheritance: the proband's mother had diabetes mellitus, gastrointestinal pseudo-obstruction, and short stature, while the proband's sister exhibited exercise intolerance, anxiety, and short stature. Neurological evaluation included brain magnetic resonance imaging (MRI), which revealed bilateral mild thalamic lesions and

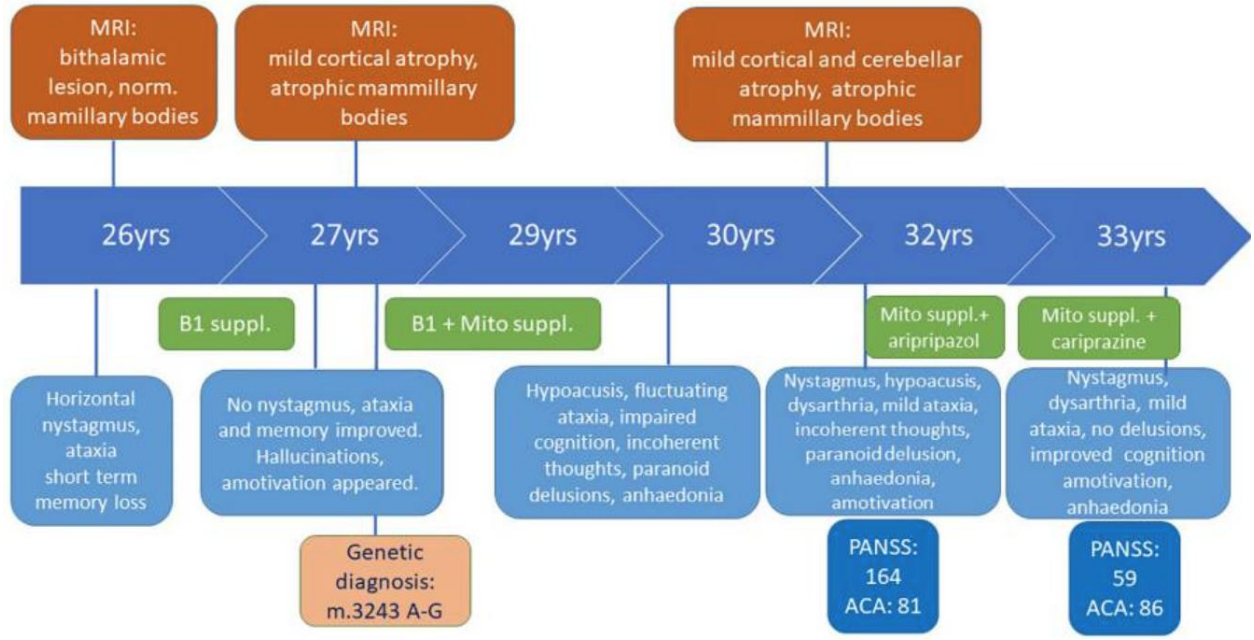
atrophy of the mammillary bodies, consistent with a diagnosis of WKS. Molecular genetic testing was performed following DNA extraction from peripheral blood samples. Sequencing identified the pathogenic m.3243A>G mutation in the MT-TL1 gene in all three symptomatic family members. Heteroplasmy levels were 48% in the proband, 38% in the mother, and 28% in the sister.

The proband was treated with high-dose intravenous thiamine, and mitochondrial-targeted therapy was initiated (CoQ10, L-arginine, vitamins B1, B2, B3, C, D3, and E) alongside dietary modifications, leading to stabilization of weight and partially improved cognitive functions. During the follow-up of the proband, many MELAS-related symptoms were observed. The proband's mother also experienced transient confusion, hyponatremia, and reversible cognitive dysfunction. At age 29, the patient developed hypoacusis. A year later, cognitive decline, disorganized and paranoid thinking, and emotional flattening were observed. At age 32, neurological examination revealed horizontal nystagmus, mild dysarthria, lower limb paresis, and hyperreflexia. Gait was slightly unstable during blind walking; the heel–shin test was normal, while the finger–nose test was inaccurate bilaterally. EMG showed mixed-type muscle changes, and a lactate stress test indicated impaired aerobic metabolism. Although neurological changes were significant, the clinical picture was dominated by a psychotic syndrome characterized mainly by negative symptoms, along with cognitive decline. Eventually, initiation of antipsychotic treatment became necessary. Prior to antipsychotic treatment, the patient exhibited prominent psychiatric symptoms, including incoherent thought processes, auditory hallucinations, blunted affect, apathy, lack of motivation, marked psychomotor retardation, and poor self-care. The Positive and Negative Syndrome Scale (PANSS) total score was 164, indicating severe psychopathology. Cognitive assessment using the Addenbrooke's Cognitive Examination (ACE) yielded a total score of 81/100, with subdomain scores as follows: verbal fluency 9/14, orientation 9/10, concentration 8/8, memory 22/35, language 28/28, and visuospatial abilities 5/5. The Mini-Mental State Examination (MMSE) score was 29/30. Initial treatment with aripiprazole (7.5 mg twice daily) was ineffective over a 1-month period. Consequently, due to the persistence of negative symptoms and cognitive deterioration, therapy was switched to

cariprazine (3 mg/day), considering the underlying mitochondrial disorder and the pharmacodynamic profile of cariprazine, which led to significant clinical improvement without adverse effects. After three months of cariprazine treatment, the patient resumed employment, re-engaged in social activities, and showed progressive recovery in memory and learning. At six months, the ACE score improved to 86/100, and the PANSS score decreased to 59, reflecting marked reduction in both positive and negative symptoms. During the investigation period, no extrapyramidal signs, elevation of resting lactate levels, nor other significant side effects were observed. The patient initiated personal relationships as well as started a part-time job. The timeline of clinical events is presented in Figure 9.

Figure 9 – Clinical timeline of the CPZ treated patient

The neurological, psychiatric status, the brain MRI findings, the major neuropsychological status and the administered medication are illustrated chronologically. Abbreviations: ACA, Addenbrooke Cognitive Assessment; mito, mitochondrial; MRI, magnetic resonance imaging; PANSS, Positive and Negative Syndrome Scale; suppl, supplements



Discussion

Mitochondrial disorders are associated with considerable clinical and genetic heterogeneity, with significant variability in both prevalence and phenotypic including the selected two model disorders.

5.1. Genetic epidemiology of certain primary mitochondrial disorders in Hungary

Our SPG7 cohort constitutes one of the largest reported cohorts worldwide, providing a unique opportunity to evaluate the prevalence of SPG7-associated disorders within Hungary, as our center represents the sole national facility for genetic testing of this condition. The cumulative prevalence of SPG7-associated conditions in Hungary was determined to be 5.1 per 1 million individuals (0.05 per 10,000), aligning closely with prevalence estimates reported internationally. While SPG7-related disorders are rare, SPG7 remains among the most frequently altered nuclear mitochondrial genes. In contrast, establishing the prevalence of MELAS is particularly challenging due to its marked phenotypic heterogeneity. Based on predicted prevalence in European cohort estimates, approximately 900 to 1900 cases of MELAS would be expected in Hungary, however, the number of genetically confirmed cases identified to date falls markedly below this estimate, highlighting potential underdiagnosis.

5.2. Landscape of SPG7-related disorders

We observed the genetic landscape of SPG7-related disorders. We investigated the genetic landscape of Hungarian SPG7 variants during our investigations, in our cohort, in contrast to other published cohorts[98,99], the most common disease-causing variant was the p.Leu78Ter variant, which might be a region-specific observation, highlighting the necessity of population-specific cohort to evaluate country-specific impact of genetic conditions[100], especially in the light of the semi-dominant effect of the variant.

5.3. Genotype-phenotype correlations

5.3.1 Genotype-phenotype correlations in the SPG7 cohort

Regarding genotype-phenotype correlations, our study was the first to deep-phenotype monoallelic patients as well as the prevalence of SPG7 DRVs in a wide variety of patient cohorts, including a

previously not investigated subcohort of patients with suspected mitochondrial disorder. Paraplegin is expressed in a wide variety of cells in the central nervous system which may contribute to the colorful phenotypic spectra, but disease manifestations can be explained by the molecular mechanisms behind disease development as well as the physiological function of the SPG7 gene.

Corticospinal tract impairment, leading to spastic paraparesis, is a common consequence of SPG7 RDVs and the hallmark symptom of HSP7[101]. While patient phenotypes can evolve over time, spasticity was absent in 8% of individuals with biallelic RDVs and 40% with monoallelic RDVs during follow-up period of our investigations. The pathomechanism underlying corticospinal tract damage resembles upper motor neuron lesions, similar to those in ALS. In our cohort, 3.4% of patients with suspected motor neuron disease (without amyotrophy) had biallelic SPG7 RDVs, and 4.3% had monoallelic RDVs. EMG identified motor neuron lesions in 40% of monoallelic and 55% of biallelic RDV cases. Although the p.Arg486Gln variant is classified as benign under ACMG guidelines, it has been suggested as an autosomal dominant risk[102,103] or modifying factor for ALS[104]. Among five patients carrying this variant in heterozygous form, two were referred to us with suspected ALS. These patients exhibited diverse phenotypes, often with late onset, including ALS-like features and SPG7-related symptoms such as spastic ataxia. However, the role of this variant as a risk factor remains uncertain and requires further study. Less severe SPG7 variants, alongside pathogenic ones, may contribute to ALS or motor neuron syndromes. Indeed, SPG7 mutations have been implicated in 7% of slowly progressive upper motor neuron syndromes[105]. Diagnosing HSP versus ALS is particularly challenging when patients with paraparesis undergo EMG without visible amyotrophy, a feature typically absent in early ALS[106]. Findings such as denervation or neurogenic lesions can mimic early ALS, emphasizing the need for SPG7 genetic screening in these cases.

Paraplegin's role in energy production in Purkinje cells and cerebellar nuclei links SPG7 RDVs to their degeneration. This dysfunction manifests in imaging and clinical signs, including cerebellar ataxia. In our cohort, cerebellar atrophy was observed in 33% of biallelic and 26.3% of monoallelic RDV patients. A recent study also reported increased T2-weighted MR hyperintensity in cerebellar dentate nuclei among SPG7 patients[107]. In our cohort, 45.5% of patients with biallelic RDVs and 57.9% of patients with monoallelic RDVs had T2 hyperintense white matter foci on T2-weighted

MR images, making it a frequent but nonspecific feature. Brain atrophy and cognitive impairment have also been reported in patients with SPG7 RDVs [84,108] several years after the onset of the disease, which aligns with our observations.

Interestingly, five of our patients exhibited Parkinsonian-like phenotypic features. In a limited cohort, it has been proposed that paraplegin is expressed in the globus pallidus within the basal ganglia[109,110]. Patients presenting Parkinsonian-like phenotypes did not show a response to dopamine agonists. All patients tested negative for the most common monogenic Parkinsonian syndromes. Additionally, in patients who underwent DaTscan (n = 2), no dopaminergic dysfunction was detected. Thus, Parkinsonian-like features may represent a rare and interesting manifestation of SPG7 RDVs.

5.3.2. Genotype-phenotype correlations in the MT-TL1 m.3243A>G MELAS cohort

MELAS syndrome is traditionally considered a predominantly neurological disorder, yet its multisystemic nature is frequently underrecognized. While classical manifestations such as epilepsy, stroke-like episodes, migraine, and myopathy are well described, a substantial proportion of our cohort presented without neurologic involvement. In our study, 75% of patients did not develop epilepsy, stroke-like episodes, or migraine, and 57.1% showed no evidence of myopathy. This clinical heterogeneity likely reflects the variable energy demands of affected tissues and the tissue-specific threshold effect of mitochondrial heteroplasmy.

Among patients with low HP, hearing impairment was one of the most frequent non-neurologic manifestations. This can be attributed to the high energy requirements of cochlear structures, including the stria vascularis, inner hair cells, and spiral ganglion neurons, which heavily rely on mitochondrial oxidative phosphorylation. Impaired ATP production and oxidative stress-induced apoptosis in these metabolically demanding cells contribute to the development of sensorineural hearing loss in mitochondrial disorders[111]. Cardiac involvement represented another prominent non-neurologic feature, predominantly in patients with moderate to high HP. The myocardium's high energy demand renders it particularly susceptible to mitochondrial dysfunction, leading to degeneration of cardiac fibers, microangiopathy, and interstitial fibrosis. Histopathologic patterns resemble those of

idiopathic hypertrophic cardiomyopathy, suggesting shared pathophysiologic mechanisms of mitochondrial cardiomyopathy[112,113] complicated by vascular remodellation[114]. Interestingly, diabetes mellitus emerged as a common comorbidity in the low to moderate heteroplasmy group, and in two patients it was the only manifestation aside from hearing loss. In 21.4% of cases, diabetes developed in the absence of any neurologic or myopathic symptoms. This is explained by the critical dependence of pancreatic β-cells on mitochondrial ATP production for glucose-stimulated insulin secretion. Even low-level HP may be sufficient to disrupt the ATP/ADP ratio signaling in β-cells, leading to impaired insulin release and age-dependent onset of diabetes or glucose intolerance[114–116]. In the pilot patient selected for cariprazine treatment, the observed effectiveness may be attributable to the drug's potential to mitigate mitochondrial dysfunction via its high dopamine D3 receptor affinity. Activation of the D3 receptor has been associated with reduced mitochondrial ROS production, preservation of mitochondrial membrane potential, and enhanced cellular apoptosis resistance [117,118], a mechanism that can be prominently impaired in dopaminergic neurons of mitochondrial patients.

5.4. Effect of genotype on inheritance and penetrance

5.4.1. Inheritance of SPG7-related disorders

Although SPG7 is generally considered an AR disorder, we propose that certain SPG7 RDVs in monoallelic form may be associated with symptoms. Information on patients with heterozygous SPG7 variants is limited. According to literature, SPG7-associated disease may originate from digenic variants. Among the potential interacting genes, AFG3L2 emerges as the most convincing candidate, which has been implicated in several phenotypes. Consequently, the presence of digenic factors exists, but based on literature and our observations, it is unable to fully explain the widespread occurrence of monoallelic presentations.

The abundance of heterozygous patients in our subcohorts, may be related to the molecular function of paraplegin, which is involved in mitochondrial quality control, ribosome assembly, and OXPHOS biogenesis[119]. The decreased activity of protein impairs respiratory complex I and increases sensitivity to reactive oxygen species [86,102]. Some other nuclear-encoded mitochondrial genes (e.g., POLG1, TWNK[120]) have variant-specific inheritance patterns and clinical spectra.

Thus, the physiological function may explain the proposed AD effects of SPG7[80,83,121,122]. Based on segregation and phenotypic data, the presence of mitochondrial dysfunction, and MRI features in patients having monoallelic SPG7 RDVs, the p.Ala510Val, and p.Leu78Ter variants showed the most reliable dominant or semi-dominant effects, in our cohort. While our findings do not establish definitive evidence of dominant inheritance, they further support the notion that monoallelic variants in SPG7 may independently contribute to pathogenicity. Based on our observations, truncating variants may be particularly likely to cause disease in a monoallelic form due to their more severe impact on protein function. We observed 11 patients with monoallelic truncating variants, with the p. Leu78Ter variant being the most common. However, the missense p.Ala510Val variant was also frequently observed in monoallelic form, potentially due to its specific location. Additionally, environmental factors such as medications, infections, or alcohol-related mitochondrial dysfunction might influence the onset of the disease and the penetrance of certain variants. The influence of environmental factors has been noted in mitochondrial disorders. Patients with monoallelic RDVs generally exhibited a relatively milder phenotype, similar to the pattern seen in POLG-associated disorders.

5.4.2. Penetrance of MT-TL1 m.3243A>G variants

Our observations indicate that even HP levels can contribute to the development of MELAS-related manifestations, underscoring the molecular and clinical complexity of mtDNA-associated disorders. By stratifying disease prevalence across heteroplasmy-defined patient groups and analyzing outcomes in an age-dependent manner, we were able to refine risk predictions for individual patients. This approach is clinically relevant, as family screening following the diagnosis of a proband frequently identifies pathogenic variants in relatives who are either asymptomatic or previously misdiagnosed.

These findings emphasize that the classical threshold effect model should be applied with caution, as HP levels can vary substantially between tissues, leading to organ-specific vulnerability and heterogeneous clinical expression. Furthermore, 17.9% of our cohort presented with childhood-onset disease, consistent with the impact of HP on age of onset, as lower tissue-specific thresholds in metabolically demanding organs can trigger early manifestations.

5.5. Room for optimization in patient pathways

Rare damaging variants in SPG7 are disease-causing genetic alterations, even in cases where classical spasticity is not a prominent feature. SPG7 variants are associated with a spectrum of mitochondrial disorder phenotypes, including progressive external ophthalmoplegia, and myopathy. Genetic testing for SPG7 should be considered in the differential diagnosis of lower motor neuron lesions, especially when amyotrophy is absent. Further investigations are needed to elucidate the penetrance and expressivity of such variants. Our findings align with previous studies on SPG7-related disorders, further emphasizing the link between SPG7 RDVs and mitochondrial dysfunction. Mitochondrial dysfunction has been identified in patients who were not initially suspected of having mitochondrial disease. As clinical trials for mitochondrial disorders advance, demonstrating evidence of mitochondrial dysfunction is likely to become a critical component of clinical practice in the future.

MELAS syndrome is often considered to be a mainly neurologic condition, and multisystemic involvement may be overlooked. Cardiologic and diabetology examinations should be applied even for asymptomatic patients, because on certain occasions patients can be seen asymptomatic until major healthcare events. These cases highlight the importance of clear patient pathways. Especially important fields based on our observations are cardiology and endocrinology or diabetology to increase identification of MELAS patients.

5. Conclusion

The thesis newly establishes national-level epidemiological benchmarks for mitochondrial disorders in Hungary.

   a. It presents one of the world's largest SPG7-associated spastic paraplegia cohorts, enabling the first national prevalence estimate (5.1 per 1 million individuals).
   b. The compiled MELAS (m.3243A>G) cohort represents one of the largest in Central and Eastern Europe, highlighting underdiagnosis and limited accessibility of genetic testing in the region.

2. As the bases of the thesis a previously not existent patient registries have been established, that demonstrates that the integration of structured patient registry data and unstructured data-lake resources can generate reliable real-world evidence for mitochondrial disorders. This methodology contributed to the Europe-wide data collection in the ERN and GENOMIT databases, strengthening cross-border harmonization of diagnostic and research standards.
3. The thesis newly identifies clinically actionable genotype–phenotype correlations that redefine the target populations for genetic screening.
   a. It proposes SPG7 testing in patients with suspected motor neuron disease without amyotrophy and m.3243A>G screening in individuals with isolated hearing loss, cardiomyopathy, or diabetes mellitus.
   b. Strengthens and widens possibly SPG7 variants with dominant effect, by evaluating patients with monoallelic SPG7 DRVs, who previously lacked a systematic review.
   c. These findings refine diagnostic algorithms and improve the clinical detection of mitochondrial disease.

4. The thesis newly demonstrates that function-based diagnostic approaches, including myopathology and mitochondrial DNA deletion analysis, can serve as cost-effective preliminary screening tools for SPG7. It further reveals that deep phenotyping and genetic evaluation may challenge existing inheritance models in SPG7 and revise pathogenic thresholds in m.3243A>G-related MELAS.

5. The thesis provides the first Hungarian pilot example of genotype-guided therapy in a patient with an m.3243A>G variant, representing an early translational step toward personalized medicine in mitochondrial disorders that mostly lack EMA- or FDA-approved therapies.

6. The thesis contributes to the improvement of genetic counseling and multidisciplinary follow-up by defining variant-specific prognostic recommendations, particularly emphasizing cardiology and diabetology care for m.3243A>G carriers and comparable management strategies for SPG7 patients.

7. Beyond its scientific results, the thesis enhances national awareness and professional capacity in mitochondrial medicine through dissemination activities, including publication in national medical journal, professional lectures, and contributions to registry development and international collaborations.

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