Genetic, clinical, and neuroimaging characterization of Mitochondrial Disorders mimicking Multiple Sclerosis (MS).

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Abstract

Demystifying multiple sclerosis (MS) requires an understanding of their heritable disease mimics. To date, several single gene disorders have overlapping clinical and radiological features with primary progressive MS (PPMS) in adult populations. From this group, a growing amount of evidence suggests that metabolic and mitochondrial disorders (MDs) are common, thus requiring critical attention to such disorders to guide patient diagnosis, treatment, and management.

The first part of this thesis focused on identifying clinical and radiological features of mitochondrial PPMS mimics to define an updated list of mitochondrial PPMS mimickers by means of a scoping literature review. It was found that features of hearing loss, ptosis, and neuropathy, and more reports of gray matter involvement were key distinguishable features of MDs compared to PPMS. Over 499 unique variants were also identified with *POLG1, SLC25A15, DARS2,* and *SURF1* genes having the most variants reported for mitochondrial MS mimics at 110, 33, 32, and 23 variants, respectively.

The second part of this thesis focused on using whole exome sequencing (WES) and whole genome sequencing (WGS) using the American College for Medical Genetics (ACMG) guidelines to analyze candidate variants of 10 recruited adult subjects with white matter abnormalities atypical for PPMS. We found that 3 out of 10 subjects had disease-causing variants implicated in mitochondrial function (*MT-CO2, POLG1,* and *MT-ATP6*). We found 3 other subjects with highly probable candidate variants, awaiting functional studies and segregation analyses, while the 4 remaining subjects require routine WES and WGS reanalysis.

This thesis demonstrates the value of conducting a comprehensive scoping review to inform white matter specialists about the growing list of clinical phenotypes, radiological phenotypes and genetic features reported for mitochondrial disorders that mimic PPMS. It also showcases the importance of next-generation sequencing (NGS) in identifying and classifying candidate variants in clinical practices. We identified 2 novel variants in genes *MT-CO2* and *MT-ATP6* for subjects with a definite molecular diagnosis. We are certain the data generated from this thesis will help clinicians and scientists and inform future directions for rare adult leukodystrophies.

Resumé

Démystifier la sclérose en plaques (SEP) nécessite de comprendre ses mimétiques de maladies héréditaires. À ce jour, plusieurs troubles monogéniques présentent des caractéristiques cliniques et radiologiques chevauchantes avec la SEP progressive primaire (SEP-P) chez les patients adultes. Parmi ce groupe, évidence croissante suggère que les troubles métaboliques et mitochondriaux (TM) sont fréquents, ce qui nécessite une attention particulière à ces troubles pour guider le diagnostic, le traitement et la gestion des patients.

La première partie de cette thèse se focalise sur l'identification des caractéristiques cliniques et radiologiques des mimétiques mitochondriaux de la SEP-P afin de définir une liste mise à jour des imitateurs mitochondriaux de la SEP-P à travers une révision de la littérature de type « scoping ». Il a été constaté que des caractéristiques telles que la perte auditive, la ptose et la neuropathie, ainsi qu'un plus grand nombre de rapports concernant l'implication de la matière grise, étaient des éléments clés permettant de distinguer les TM de la SEP-P. Plus de 499 variantes uniques ont également été identifiées, les gènes *POLG1, SLC25A15, DARS2* et *SURF1* ayant respectivement le plus grand nombre de variantes rapportées pour les mimétiques de la SEP mitochondriale avec 110, 33, 32 et 23 variantes.

La deuxième partie de cette thèse se focalise sur l'utilisation du séquençage de tout l'exome et du séquençage de tout le génome en utilisant les directives du *American College for Medical Genetics* (ACMG) pour analyser les variantes candidates de 10 sujets adultes recrutés présentant des anomalies de la matière blanche atypiques pour la SEP-P. Nous avons trouvé que 3 des 10 sujets avaient des variantes causant des maladies impliquées dans la fonction mitochondriale (*MT-CO2, POLG1* et *MT-ATP6*). Nous avons également identifié 3 autres sujets avec des

variantes candidates hautement probables, en attente d'études fonctionnelles et d'analyses de ségrégation, tandis que les 4 sujets restants nécessitent une réanalyse routinière du séquençage de tout l'exome et du séquençage de tout le génome.

Cette thèse démontre la valeur de mener une revue systématique complète pour informer les spécialistes de la matière blanche sur la liste croissante des phénotypes cliniques, des phénotypes radiologiques et des caractéristiques génétiques rapportées pour les troubles mitochondriaux qui imitent la SEP-P. Elle souligne également l'importance du séquençage de nouvelle génération (NGS) dans l'identification et la classification des variantes candidates dans les pratiques cliniques. Nous avons identifié 2 variantes novatrices dans les gènes *MT-CO2* et *MT-ATP6* pour des sujets avec un diagnostic moléculaire définitif. Nous sommes certains que les données générées par cette thèse seront utiles aux cliniciens et aux scientifiques et informeront les orientations futures pour les leucodystrophies rares chez les adultes.

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I would like to take the opportunity to express my deep gratitude and appreciation to Dr. Roberta La Piana, an incredible role model/mentor, scientist, and supervisor. Her ability to motivate students, and teach with an open mind and heart, along with employing a critical mind when it comes to producing and contributing to science is beyond what I expected from my Master's experience. Thank you, Roberta, for your guidance and help during some unexpected moments throughout my degree and thank you for motivating me, along with the rest of the lab, to do our best and be independent thinkers. I couldn't have asked for a better supervisor – I really lucked out.

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Thank you to all the student volunteers in the lab, Rachel, Aziz, Lanyun & Aley. Your drive to help excel this field as young scientists and investigators helped me realize my own potential in this space. Thank you for helping me make my visions come true with my scoping review using your combined effort, unique skills and talents.

A special thank you goes out to Dr. Bernard Brais, Dr. Catherine Ashton, and Dr. Felipe Villa for their special care and dedication to the recruited subjects. Witnessing your care and expertise

towards these individuals and helping them was inspiring and reaffirmed my passions of wanting to be a clinician.

Thank you to all the labs and teams housed on the 6th floor of the Neuro. Having interesting conversations during the mornings and lunch time at the Neuro helped to get me into focus during hours of deep work. Observing your discipline of showing up consistently and doing your work was helpful for my own discipline and consistency. Special thank you to Vanessa for helping me coordinate my rare disease talk and your encouraging pep talk prior to presenting. Thank you to the security guards at the Neuro who let me stay past regular hospital hours and let me work in the lab late at night, during my peak focus hours.

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A special thank you to my 'Current Topics in Neuroscience' professor, Dr. Carl Ernst. Your passion for thinking about science multidimensionally reinforced how science can be an art just as much as it is a practice of objectiveness.

Thank you to my family, friends and IPN peers, your support means a lot.

Contribution of Authors

Aim 1. Identifying the research question

H.H. developed the research questions for the scoping review and Dr. Roberta La Piana, provided feedback on the final questions to be used.

Aim 1. Identifying relevant studies

H.H. developed the inclusion and exclusion criteria with Dr. Roberta La Piana.

Aim 1. Selecting studies

H.H. reviewed and selected studies to include in the scoping review using Covidence. Rachel Levin, volunteer student in the lab, assisted HH in the review and selection, under HH's and Dr. La Piana's supervision.

Aim 1. Charting the data

H.H. charted the data together using Microsoft Excel, with the assistance of Rachel Levin.

Aim 1. Collecting, summarizing, and reporting the results

H.H., collected relevant themes and information from the charted information of the scoping review with the help of Aziz Benbachir and Joyce Li (both students in La Piana's lab) and Dr. Roberta La Piana's guidance; Aziz Benbachir and Aleyeldin Hassan summarized the charted data using descriptive numerical summaries.

Aim 2. Participant recruitment

All participants were recruited by Dr. La Piana and her team. Specifically, H.H. and Dr. Roberta La Piana jointly recruited participants from cases 2, 5, 6, 7, 8, and 10; Dr. Roberta La Piana recruited participants from cases 1, 3 and 4; and Dr. Roberta La Piana and Ruwan Bedeir (MSc student in the lab) recruited the participant from case 9.

Aim 2. DNA extraction

H.H. performed all DNA extractions for all the cases described, except for case 3 and 9. Case 3's DNA extraction was done by Dr. Gabrielle Macaron's team at Centre de Recherche du CHUM (CRCHUM) and shared with us. Case 9's DNA extraction was performed by Ruwan Bedeir.

Aim 2. WES and WGS analysis of participant data

FASTQ formatted data obtained from WES and WGS analysis were post-processed in Dr. Tétreault's laboratory at the CRCHUM. H.H. performed all WES and WGS analyses as well as segregation analysis and further validation with guidance from Dr. Roberta La Piana, Justin Simo (PhD student in the lab), and Ruwan Bedeir.

List of Figures and Tables

Supplementary Table 5. List of included studies with their journal and year of publication from most recent to least recent.

List of Abbreviations

ACMG – American college of medical genetics

ADEM – Acute disseminated encephalomyelitis

DSC - Diagnostic Scientific Committee

HHH – Hyperornithinemia-hyperammonemia-homocitrullinuria

IRDiRC - International Rare Diseases Research Consortium

LBSL – Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation

LHON – Leber hereditary optic neuropathy

MD – Mitochondrial disorder

MeSH – Medical subject headings

MRI – Magnetic resonance imaging

MS – Multiple sclerosis

mtDNA – Mitochondrial DNA

NARP – Neuropathy, ataxia, and retinitis pigmentosa

NGS – Next generation sequencing

NMO – Neuromyelitis optica

PRISMA-ScR – Preferred reporting items for systematic reviews and meta-analyses – scoping review

RRMS – Relapsing-remitting multiple sclerosis

SPMS – Secondary progressive multiple sclerosis

WES – Whole exome sequencing

WGS – Whole genome sequencing

VLCFA – Very long chain fatty acids

Introduction

Adult leukodystrophies often have insidious symptom onset, slowly progressive course, and non-specific imaging findings, easily leading to diagnostic challenges (Muthusamy et al. 2023). This is further compounded by the fact that adult leukodystrophies mimic or can be mimicked by other white matter disorders of acquired nature, like primary progressive multiple sclerosis (PPMS) (Weisfeld-Adams et al. 2015). The risk of misdiagnosis is particularly considerable for atypical clinical presentations of MS and, since the diagnosis of MS remains a diagnosis of exclusion, it is imperative to rule out genetic mimickers of PPMS that can account for 'atypical' PPMS phenotypes or for cases in which McDonald's criteria are incompletely fulfilled (Solomon et al. 2019). Among genetic MS mimickers, an increasing number is associated with mitochondrial disorders (MDs) (Weisfeld-Adams et al. 2015). However, many MS mimickers remain without a final molecular diagnosis -their disease-causing gene yet to be identified- with dramatic implications for patients and families. Due to the outdated available information on disease-causing genes and variants and the phenotype-genotype relationships of many MDs mimicking PPMS, I wanted to scope the literature for updated information, especially since the revolutionizing advent of next generation sequencing (NGS) techniques. I hypothesized that new forms of genetic – and specifically mitochondrial - MS mimickers can be identified by analyzing the NGS data of subjects with atypical PPMS after scoping available literature on mitochondrial MS mimickers.

My objectives and thesis were divided into 2 parts, 1) To define the updated list of mitochondrial MS mimickers, their disease-causing genes and variants, when available, and their phenotypes which represent the most considerable, yet often unrecognized proportion of genetic mimickers of MS, by means of a scoping review. Using standard Preferred Reporting Items for Systematic

reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) protocols (Tricco et al. 2018), I aim to gather all available literature describing the genetic, clinical, and radiological features of patients with MDs mimicking MS. 2) To identify the genes responsible for new forms of adult genetic MS mimickers using NGS techniques. Using NGS techniques – NGS panels, whole exome sequencing (WES), whole genome sequencing (WGS) - and bioinformatics strategies, I aim to identify candidate variants responsible for the disease phenotypes described in our cohort of recruited subjects, ultimately identifying either new forms or expanding the phenotypes of genetic MS mimickers.

Relevant literature

Genetically Determined Leukodystrophies

Leukodystrophies are hereditary white matter disorders that are phenotypically heterogeneous and genetically diverse affecting both children and adults (Patterson 2014). White matter is made up of myelinated axons, glial cells, and blood vessels (Van der Knaap and Bugiani 2017). Myelination is a process whereby glial cells, specifically oligodendrocytes, concentrically wrap their bodies around the axon unit of a neuron, creating a myelin sheath. These myelin sheaths provide high resistance and low capacitance electrical insulation for neuronal conduction, allowing information to flow much faster than in unmyelinated circuits (Williamson and Lyons 2018; Van der Knaap and Bugiani, 2017). Hereditary leukoencephalopathies are disorders primarily involving any of the white matter constituents: oligodendrocytes, astrocytes, microglia, axons and the glia-blood vessel interface (Van der Knaap and Bugiani 2017). Thus, according to the pathologic changes and pathogenetic mechanism, there are four classes of leukodystrophies: myelin disorders (hypomyelinating, when myelin production is inadequate from birth; demyelinating characterized by the normal development of myelin which is then progressively destroyed; and myelinolytic whereby there is myelin vacuolization); astrocytopathies; microgliopathies; leuko-axonopathies; and leuko-vasculopathies (Van der Knaap and Bugiani 2017).

In recent years, adult-onset leukodystrophies have been of increased interest to white matter disease specialists due to cohorts of patients presenting with signs and symptoms that are atypical for commonly diagnosed acquired white matter disorders, from vascular, inflammatory, or degenerative origins (Weisfeld-Adams 2015; Huang et al. 2022; Chen et al. 2023).

Leukodystrophies and Primary Progressive Multiple Sclerosis

MS is one of the most frequently diagnosed demyelinating diseases with an estimated 1.8 million people affected worldwide (Miller and Leary 2007; Antel et al. 2012; Weisfeld-Adams et al. 2015; WHO 2023). It is characterized by areas with focal demyelination of the central nervous system's white matter, with affected subjects having 1 of 3 forms of the MS disease course (Barcelos et al. 2019). The majority of patients with MS (more than 80%) start with a relapsing-remitting disease course, which in 50-60% of cases approximately evolves into a secondary progressive form. A minority of 15% of patients develop a primary progressive MS (PPMS) form (Antel et al. 2012; Coyle 2021). PPMS is characterized by a progressive disease course after the first symptoms surface, has a later onset at a mean age of 45 years, and an incidence affecting both males and females equally or with a slight male predominance, depending on the population studied (Kingwell et al. 2009; Miclea et al. 2018; Rommer et al. 2020; Coyle 2021; Luetic et al. 2022). For these features, the diagnosis of PPMS is specifically insidious since it can mimic other demyelinating diseases that are either acquired or genetic (Antel et al. 2012).

Since PPMS remains a diagnosis of exclusion, alternate diagnoses may go unseen and result in misdiagnoses for patients, with the consequent risk of being exposed to unnecessary immunomodulatory treatment (Weisfield-Adams et al. 2015) and delays in accessing genetic counselling services. The McDonald Criteria for MS were developed to provide specificity to the classification of MS using a patient's neurological history, physical examination and magnetic resonance imaging (MRI) patterns of the brain and spinal cord. This criterion was first established in 2001 and further developed and revised over the years with the latest update formulated in 2017 to accommodate a more simplified approach and amendments—an important one being the presence of oligoclonal bands in the cerebral spinal fluid (McDonald et al. 2001;

Polman et al. 2011; Mantero et al. 2018). While MS is a diagnosis of exclusion, applying the McDonald Criteria means looking for atypical signs to consider and exclude alternate diagnoses before a confirmed consensus is made on a diagnosis of MS for a patient (Polman et al. 2011). This also posits the capacity needed to identify and recognize alternate diagnoses (Rudick and Miller 2012). Over the years, the spectrum of disorders that overlap MS has grown with more expertise needed on MS mimickers. Overall, genetic leukoencephalopathies may share clinical and radiological characteristics with MS but with distinctly different courses and prognoses, thus it is important to invest efforts into investigating MS-like disease entities (Engell 1988; Solomon and Weinshenker 2013; Weisfeld-Adams et al. 2015)

Mitochondrial Disorders

Amongst the spectrum of MS-mimicking leukodystrophies that are the most difficult to diagnose, mitochondrial disorders (MDs) are notorious (Forny et al. 2021). Since every cell of the body with the exception of red blood cells has mitochondria, any nuclear or mitochondrial DNA defect may have multisystemic impacts (Forny et al. 2021; Shayota 2024). This leads to a vast range of heterogeneous phenotypic and neuroimaging characteristics in individuals. Given that the CNS has the highest energy demands supported by a ubiquitous supply of mitochondria, dysfunctional mitochondria make individuals especially vulnerable to neurologic deficits (Wallace 2010; Frazier et al. 2019). This becomes even more complicated as heteroplasmy is taken into consideration, a situation whereby mutant mitochondrial DNA (mtDNA) and wild type mtDNA coexist (Barcelos et al. 2019). Due to the nature of heteroplasmy, not all mitochondria will have an equal distribution of mutant mtDNA with wildtype mtDNA within tissues, suggesting that for disease to manifest, mutant mtDNA must pass a crucial threshold capable of impairing oxidative phosphorylation (Schaefer et al. 2004; Muzinin et al. 2010;

Pitceathly et al. 2014; Barcelos et al. 2019). Due to these features of heteroplasmy and the ubiquity of mitochondria throughout the body and CNS, clinical heterogeneity is particularly high in MDs, thus contributing to the diagnostic challenge of these forms (Forny et al. 2024). However, despite this, specialists in MDs use established guidelines, case report evidence, published recommendations, and theoretical concepts to identify MDs (Parikh et al. 2014).

Relationship between PPMS and Mitochondrial Disorders

The link between mitochondria and MS has been increasingly investigated in recent years (Barcelos et al. 2019; Chen et al. 2023). For instance, studies have shown how damaged mitochondria generate reactive oxygen species, creating a hostile environment for insufficient energy production and consumption, thus when further coupled with the disruptive neuro-inflammatory signalling that activates the innate immune response, as seen in MS, there is secondary damage to the mitochondria further impairing it and increasing its ROS generation (Barcelos et al. 2019; Chen et al. 2023). This vicious cycle prevents injured demyelinated axons from being remyelinated and instead begins the process of neuronal death, Wallerian degenerations, and subsequently, neurologic dysfunction (Barcelos et al. 2019). Given the vital role of proper mitochondrial function in axon remyelination and its contribution to the pathology of MS, it becomes clearer why MS shares characteristics with genetic mitochondrial disorders (Weisfeld-Adams et al. 2015).

Application of next-generation sequencing techniques and bioinformatics in leukodystrophy diagnostics

As NGS techniques are becoming more ubiquitous in clinical settings, diagnostic rates of genetic diseases have gone up significantly, as well as the discovery of novel pathogenic variants or genes (Perrier et al. 2023).

NGS panels are the most used NGS assay in clinical settings whereby a targeted panel sequences hundreds of genes. Genes to be incorporated into panels are predetermined, however, given the high volume of discovery in genetics research, the literature requires constant review to update panels and genes of interest (Qin 2019). In cases where an NGS panel is insufficient or provides negative or inconclusive results, a more in-depth review of patient genomic data is warranted utilizing WES or WGS. WES is an NGS assay that looks at nearly 95% of the coding region, constituting 1% of the human genome and representing the region where about 85% of disease-causing mutations are located (Rabbani et al. 2014; Qin 2019). For this reason, sequencing such regions has the potential to uncover variants responsible for monogenic rare diseases (Rabbani et al. 2014). This was notably exemplified in 2009 by Choi *et al.,* the first report of WES assisting with the diagnosis of chloride-losing diarrhea in a patient initially suspected of having Barter Syndrome. While WES is now a fast and cost-effective way of sequencing variants, structural variants or variants in the noncoding intronic regions of the genome are missed as these types of variants can be found through the sequencing of the entire genome (WGS). This type of data is more complex and is used to supplement NGS panels and WES data, as WGS requires time-consuming analyses for DNA structural alterations, deep intronic variants, or repeat expansions (Souche et al. 2022).

The analysis of WES and WGS data needs to incorporate essential information such as minor allele frequency (< 0.001), conservation, predicted pathogenicity, disease associations, both established or predicted biological functions and familial segregation when filtering for candidate variants (Fernandez-Marmiesse et al. 2018). These types of data are generated as annotations from NGS panels performed in clinically accredited laboratories, while for WES or WGS data, the analysis should be performed by accessing various databases such as ClinVar,

GnomAD, OMIM and GeneCards (Landrum et al. 2014; Hamosh et al. 2021; Gudmundsson et al. 2022). In silico prediction tools like PolyPhen-2, SIFT, Dynamut2, CADD, and Franklin provide generated predictions of pathogenicity based on various database results and algorithm calculations which provide significant insight on the possibility of pathogenicity of a variant (Ng et al. 2003; Adzhubei et al. 2010; Rentzsch et al. 2019; Rodrigues et al. 2020; Steinhaus et al. 2021; Rodrigues et al. 2022).

Using these types of prediction tools and databases, researchers use the additional guidelines to classify variants known as the American College of Medical Genetics and Genomics (ACMG) guidelines (Miller et al. 2023). To accommodate the standardization of high throughput sequencing and variant interpretation, ACMG released guidelines to follow which are recommendations that describe the process of classifying variants into 5 categories: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign (Richard et al. 2015). Variants grouped into these categories consider the population data, computational data, functional data, and segregation data of specific variants (Richard et al. 2015). These guidelines are used routinely in understanding the pathogenicity of variants, however, are limited in scope in identifying the continuum of effects caused by variants (Masson et al. 2022).

Methods

Aim 1. Scoping Review "Genetic, clinical and neuroimaging characterization of mitochondrial disorders mimicking MS."

A scoping review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines described by Tricco et al. (2018) and its methodological framework was done according to Arksey and O'Malley (2005) which identifies 6 stages to a scoping review: identifying the research questions; identifying relevant studies; study selection; charting the data; collating, summarizing, and reporting the results; and the optional stage to consult stakeholders.

Identifying the research questions

This review was designed from the following questions:

1. What clinical, genetic, and radiological data exist for mitochondrial disorders that mimic primary progressive MS?
2. Which article types were published over time on these types of mitochondrial disorders?

Identifying relevant studies

Identifying relevant studies occurred in a stepwise process initiated by a comprehensive literature search which was performed in MEDLINE (Ovid and PubMed), Scopus, and Web of Science. The search terms and medical subject headings terms (MeSH) were derived from 4 concepts guided by the questions defined for this review: Mitochondrial Disorders, Acquired Demyelinating Disorders, Clinical Evidence, and Neuroradiological Evidence (Supplementary Table 1). The search was filtered to include English language literature from 1990 onwards on humans of all ages. The rationale for including studies from the year 1990 onwards was

determined due to MRI being more routine in clinical settings after 1990 (Kevelam et al. 2016). Preliminary searches began on February 13th, 2023 and an updated search for more recent and relevant articles was conducted on April 24th, 2023.

Selecting studies

Studies were imported to the workflow platform Covidence in .csv format. Covidence is a web-based collaboration software platform that streamlines the production of systematic and other literature reviews (Babineau 2014). Once uploaded, duplicate studies were removed, and studies were then screened in a 2-step process. First, studies were screened based on titles and abstracts, and then included studies were further filtered by conducting full-text reviews. Two reviewers (H.H. and Rachel Levin, R.L., an undergraduate student in La Piana's lab) voted blindly during both screening processes to minimize bias. During conflicting votes on a study that was either to be included or excluded, a final blind conflict-resolving vote was given to the last reviewer, upon which a tiebreaking vote was made to either include or exclude the study in the data extraction phase (Supplementary Table 5).

Exclusion criteria were unanimously discussed and assessed post-hoc, consistent with scoping review processes (Arksey and O'Malley 2005) (Supplementary Table 2). Primarily, methodological, animal, and cell studies were excluded due to our study selection and research questions focusing on the clinical, genetic and radiological data collected on humans. Furthermore, literature discussing co-morbidities not relevant to mitochondrial disorders mimicking MS were excluded.

Charting the data

Data from the data extraction phase were organized and inputted in Microsoft Excel (2023) by HH and RL. The number of papers was divided into 4 sections and reviewers alternated between sections to extract. After each extraction, the other reviewer looked through the entries to assess and verify the consistency of the entries. This process was repeated for all sections and the entire data extraction process. If available, the charted information included: disease(s) (e.g., which mitochondrial disorder mimicking MS was discussed or diagnosed), genetic information, types of biochemical and/or laboratory tests performed, clinical features, rare clinical features (e.g., rare features reported by the author that broadened the phenotype of the disease), magnetic resonance imaging features (e.g., location and type of modality used), main findings of the paper, significant findings relevant to the review studies, recommendations of the article, and conclusions of the paper. For a full list of the parameters used to chart data, see Supplementary Table 3.

Collecting, summarizing and reporting the results

According to Arksey and O'Malley, and Tricco et al., the charted information was based on qualitative thematic analyses and descriptive numerical summaries. Emerging themes were summarized and reported.

Aim 2. NGS analyses of 10 participants

Participant Recruitment

This project is part of a bigger study on undiagnosed white matter diseases of probable genetic origin, which received approval from the McGill University Health Centre Research Institute Research Ethics Board (2021-7463). Adult patients with 'atypical MS' were referred by MS clinicians to the Rare White Matter Disorders clinic at the Montreal Neurological Institute. Subjects underwent a comprehensive neurological and clinical examination by a neurologist and neurogenetics fellows before being recruited to this study. 'Atypical MS' was defined as when one or more revised McDonald diagnostic criteria (Polman et al. 2011; Thompson et al. 2018) are lacking AND/OR when either radiological (symmetric and/or diffuse involvement) or clinical (insidious onset, long-standing symptoms) characteristics are not specific for MS AND/OR when there are multiple affected family members. For this project, we included all the participants with 'atypical MS' recruited from September 2022 to September 2023. Upon recruitment, subjects underwent blood collection for genomic DNA extraction.

DNA Extraction

All subjects underwent a next-generation sequencing panel performed in recognized clinical laboratories (MNG laboratory, NGS panel for genetic leukoencephalopathy, including 220 genes + mitochondrial DNA). Additionally, upon negative or inconclusive results from the NGS panel, participants' DNA was extracted from whole blood samples using PureGene QIAGEN kit (Cat no. 158489) protocols. NanoDrop Lite (Thermo Scientific manufacturer no. 840281500) was used to detect purity and quantity of DNA before being shipped to Genome Quebec, Sainte-Justine Hospital for WES and WGS.

WES and WGS analysis of Participant Data

WGS and WES were performed at Genome Quebec of Sainte Justine Hospital, Montreal, using the Illumina NovaSeq PE150 (1200M reads) with an aimed coverage of 100X for WES and 30X for WGS. Data obtained in FASTQ format were then post-processed in Dr. Tétreault's laboratory at the Centre de Recherche du CHUM using a pipeline designed for rare mendelian disorders. FASTQ reads were aligned to a reference genome (Hg38) using BWA. Variant annotation was done using ANNOVAR.

Exome data from either WES or WGS was analyzed by HH on Microsoft Excel 2020. Priority was given to nonsynonymous variants (missense, nonsense, small indels, and splice site intronic variants) with a minor allele frequency (MAF) of less than 5% or 0.005 in 1000 genomes. A filtering strategy was used to generate candidate variants by looking at inheritance patterns (based on information collected upon recruitment), pathogenicity prediction scores (Polyphen-2, SIFT, CADD, MutationTaster2021), looking for known variants associated with leukodystrophies and mitochondrial disorders, using literature and databases to supplement variant investigations, and looking at the conservation of variants (Table 1). Candidate variants for subjects with available family members (with priority for parents) were considered for segregation analysis. I prioritized variants in genes known to be associated with genetic leukoencephalopathies, specifically mitochondrially related, and, secondly, in pathways involved in glial cell physiology and inflammation. Participants with no strong variants were re-analyzed using updated databases after 3 months using the same filtering strategy, based on previous evidence that an additional 15% of undiagnosed cases can be solved by repeated NGS analysis (Gibson et al. 2018). Submissions to ClinVar for the same variant suspected in our participants were further investigated and authors of these submissions were contacted, if necessary.

Table 1. In-silico prediction tools, databases, and tissue expression program used for variant analyses and phenotype-gene relationship analyses.

Type of Tools	Tool/database	Purpose	Score(s) and output(s)	Score(s) and output(s) interpretations
In-silico Prediction tools	PolyPhen-2	In-silico prediction tools looking at the damaging impact of an amino acid substitution on human protein's structure and function (Adzhubei et al. 2010)	Score range: 0 to 1	0 is closer to benign and 1 is closer to pathogenicity
	SIFT	Prediction tool looking at the functional consequences of a protein from mutations that cause amino acid changes (Ng et al. 2003)	Score range: 0 to 1	0 is deleterious and 1 is tolerated
	Dynamut2	Prediction looking at the effects of missense variations on protein stability and dynamics to elucidate the effects on disease manifestations and uses Gibbs free energy calculations (Rodrigues et al. 2020)	Score range: $\Delta\Delta G >$ 0.0 kcal/mol or $\Delta\Delta G <$ 0.0 kcal/mol	$\Delta\Delta G >$ 0.0 kcal/mol is stabilizing and $\Delta\Delta G <$ 0.0 kcal/mol is destabilizing
	CADD	Prediction tool that uses surrounding sequence context, gene model annotations, evolutionary constraint, epigenetic measurements, and functional predictions to rank variants (Rentzsch et al. 2019)	Score range: 1 to 99	Less than 15 is considered benign; 15 to 20 is considered 'neutral' (uncertain significance) and 20 + is considered pathogenic
	MutationTaster	Predict pathogenicity of DNA variant effects by integrating variant frequencies in populations, known mutations, various gene lists and panels, evolutionary conservation and pathway interactions to interpret variants (Steinhaus et al. 2021)	Scores: 'Deleterious' or 'Benign'	N/A
	Franklin	Predicts pathogenicity of variants using the ACMG variant classification system through AI-based interpretation engine that uses collected data from databases, population statistics, and literature and text mining evidence (Rodrigues et al. 2022)	Scores: *ACMG classification system	N/A
Databases	GnomAD	Variant and gene interpretation by using population frequency data (Gudmundsson et al. 2022)	N/A	Minor allele frequencies; variant classification
	OMIM	Describes the clinical features and gene and variant evidence of over 7500 Mendelian conditions, many of which are rare (Hamosh et al. 2021)	N/A	Phenotype-gene relationships; History of condition; Inheritance pattern

	ClinVar	Archive of reports of phenotype and variants relationships and variant information (Landrum et al. 2014)	N/A	Phenotype-gene relationships; location of variation on human assemblies; variant interpretations
	Genecards	Gene information mined and integrated from various data sources	N/A	Phenotype-gene relationships; domains; expression; function of gene; genomics; localization; orthologs
Tissue Expression	GTEx	Used to study the relationships between gene variations, expression and other molecular phenotypes in different tissues (Lonsdale et al. 2013)	N/A	Bulk tissue and single tissue gene expressions of gene(s)

Results

Aim 1: Results from Scoping review "Genetic, clinical and neuroimaging characterization of mitochondrial disorders mimicking MS."

We screened a total of 755 abstracts and titles before 316 full-text studies were included to be screened. From this full-text screen, 214 studies were included whereby through post-hoc screening, 181 studies were finally included for review (Figure 1).

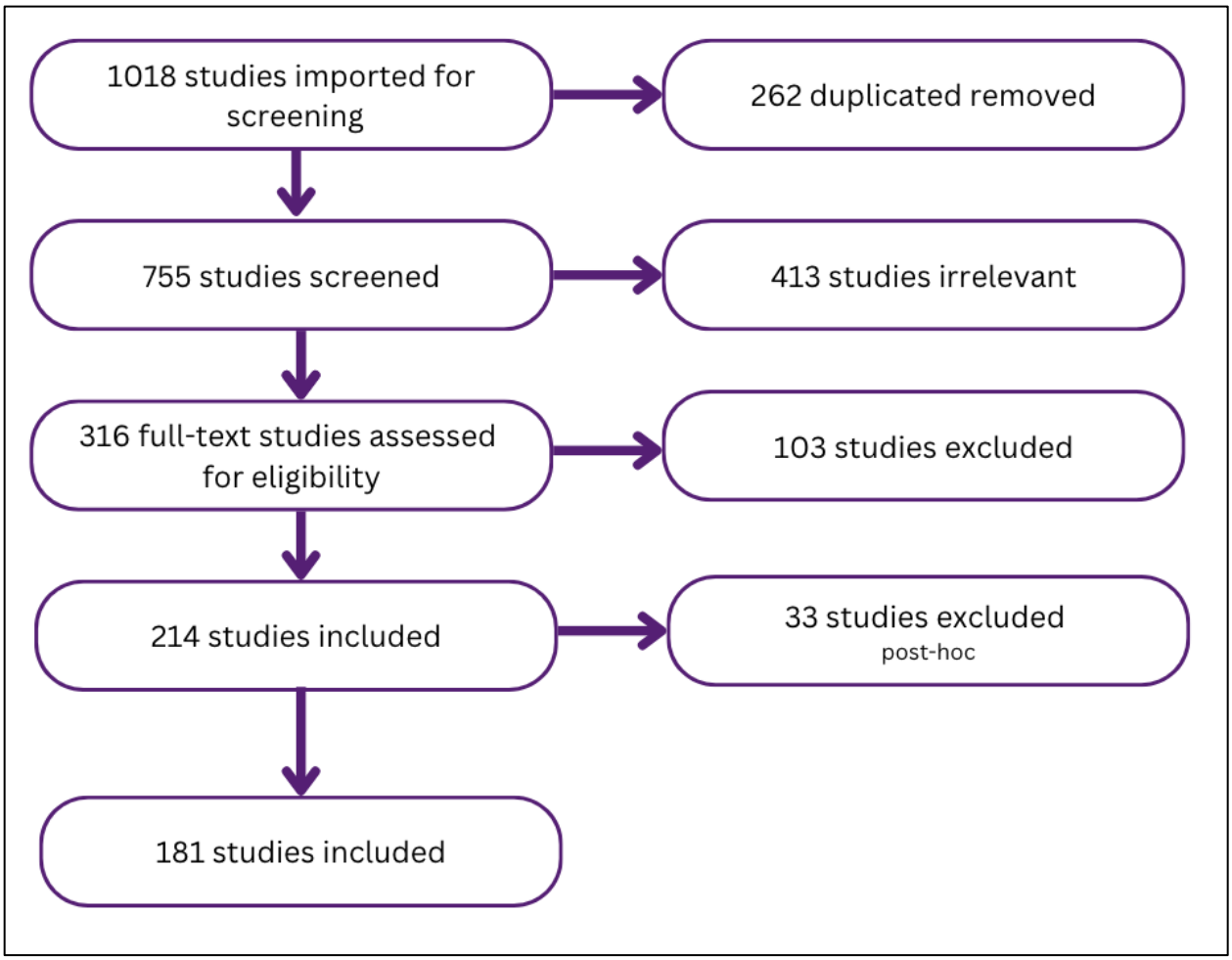

Figure 1. Scoping review screening results. Covidence was used to screen all literature by 2 reviewers. 214 articles were included for full data extraction upon which 30 were excluded. The final number of articles included was 181.

1.1 Genetics: Genes and variants associated with mitochondrial MS mimickers.

We found 307 unique genes implicated in mitochondrial functions, 499 unique variants in genes implicated in mitochondrial functions, and 287 genes reported as associated with conditions mimicking MS but without details on their variants. Of these genes, *POLG1* variants were reported the most, with 110 unique variants. The second most reported gene was *SLC25A15,* with 33 unique variants, and *DARS2* was the third most reported gene with a total of 32 unique variants (Figure 2). There were 76 disorders associated with the variants and genes found in the data as mitochondrial MS mimickers (see Supplementary Table 4). Out of all the disorders mentioned in the review, the heterogeneous category 'other mitochondrial-related disorders' which includes disorders involving the mitochondrial respiratory chain complexes and those reported as 'mitochondrial disorders' with no specific designation, were the most frequent, associated with 86 different genes, followed by Leigh syndrome (associated with 52 genes), and Mitochondrial Encephalopathy Lactic Acidosis and Stroke-like episodes (MELAS) (associated with 18 genes).

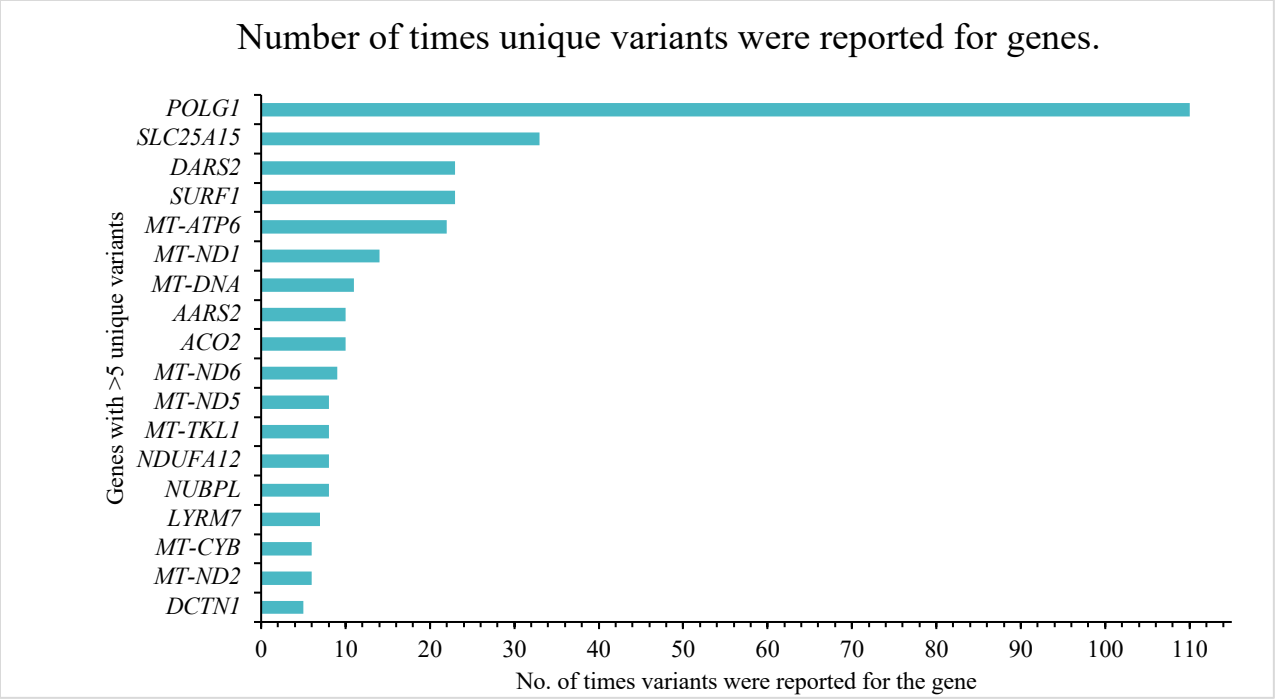

Figure 2. Number of times unique variants were reported for genes. Genes with unique variants greater than 5 are included in this graph. Gene *POLG1* had the most reported variants at 110 unique variants, *SLC25A15* had 23 variants reported, *DARS* and *SURF1* genes had 23 reported variants.

1.2 Clinical features

Nine clinical phenotype categories emerged from summarizing the clinical features of mitochondrial disorders mimicking MS: Cerebellar involvement; Visual deficit; Oculomotor dysfunction; Pyramidal and extrapyramidal signs; Sensory signs (other than visual); Cortical signs (seizures and myoclonus); Cognitive and psychiatric symptoms; Peripheral nervous system (PNS) involvement; and Metabolic and extraneurological involvement. From these categories, 1021 unique terms used to identify symptoms were quantified and sorted using content analyses methods for all studies whereby 'Ataxia', 'Seizures', and 'Vision loss' was mentioned 72, 48, and 40 times, respectively (Figure 3). The 3 major categories that emerged from grouped terms

were ‘Pyramidal and extrapyramidal involvement’ ‘Cerebellar involvement’, and ‘Metabolic and extraneurological’, with 17.5%, 15.7%, and 14.7% of the terms in each category, respectively.

Content analysis of terms used to describe clinical features throughout collected literature quantified for scoping review.

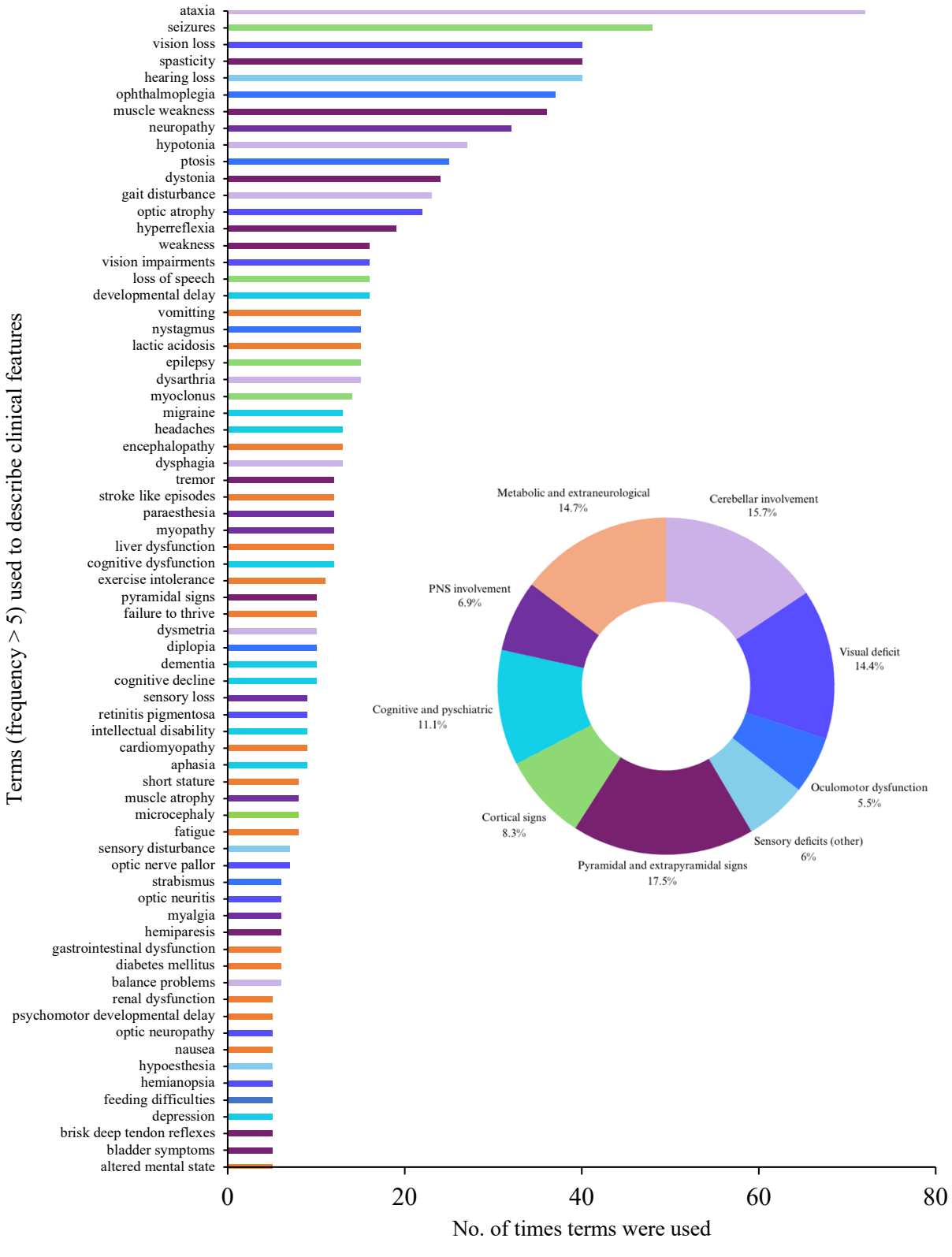

Figure 3. Content analysis of clinical features reported in the literature for Mitochondrial MS mimickers. Unique symptoms quantified (number of times mentioned) for all studies included in the scoping review (bar graph) 'Ataxia', 'Seizures', and 'Vision loss' were mentioned 72, 48, and 40 times respectively out of 1021 terms. Categories for symptoms (donut chart) are color-coded in the bar graph. 17.5%, 15.7%, and 14.7% of the terms fall under symptom categories of 'Pyramidal and extrapyramidal involvement' 'Cerebellar involvement', and 'Metabolic and extraneurological', respectively.

1.3 MRI features

Eleven MRI categories emerged from summarizing the MRI features of mitochondrial disorders mimicking MS: involvement of Basal ganglia, Brainstem, Cerebellum, Cerebrum, Cortex, Limbic system, Spinal cord, Thalamus, white matter Tracts, Ventricles, and 2 or more structures combined. From these categories, 186 unique terms used to identify symptoms were quantified and sorted using content analyses methods for the first all studies describing MRI features whereby the top 3 most used terms were 'Periventricular', 'Cerebellum', 'Basal ganglia' mentioned 44, 35, and 34 times, respectively (Figure 4).

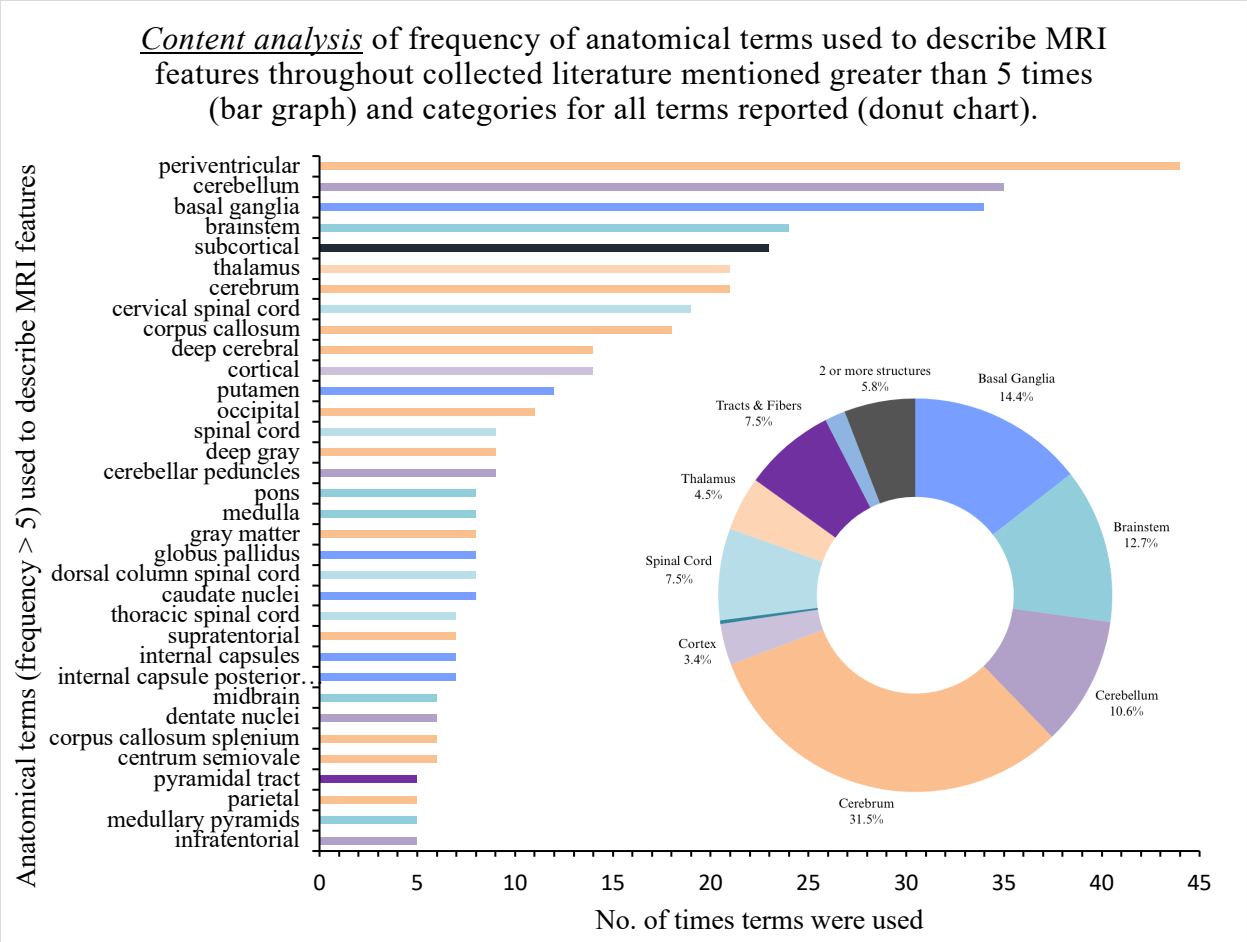

Figure 4. Content analysis of MRI features reported in the literature for Mitochondrial MS mimickers. Unique MRI features quantified (number of times mentioned) for all studies included in the scoping review (bar graph) with a frequency of being reported > 5 times. 'Periventricular', 'Cerebellum', and 'Basal ganglia' were mentioned 44, 35, and 34 times respectively out of 182 terms. Categories for MRI features (donut chart) are color-coded in the bar graph. 31.5%, 14.4%, and 12.7% of the terms fall under symptom categories of 'Cerebrum', 'Basal ganglia', and 'Brainstem', respectively.

Aim 2: Results of NGS analyses in 10 Participants

We recruited a total of 10 subjects fulfilling the criteria of 'atypical MS'. The mean age for the participants was 50.6 years old (age range: 34 - 75 years old), with the sex distribution of 6 females and 4 males. After the analysis of their genetic data, we identified a definite molecular diagnosis in 3 (30%) and highly probable candidate variants in 3 (30%). The analysis remained

inconclusive in 4 (40%) and will require further genetic as well as functional investigations (Figure 5). Of the 6 participants with a definite diagnosis and those awaiting functional studies, 3 were determined via NGS panel results, while 3 via WES analyses. Seven out of 10 participants underwent WES or WGS following negative NGS panel results.

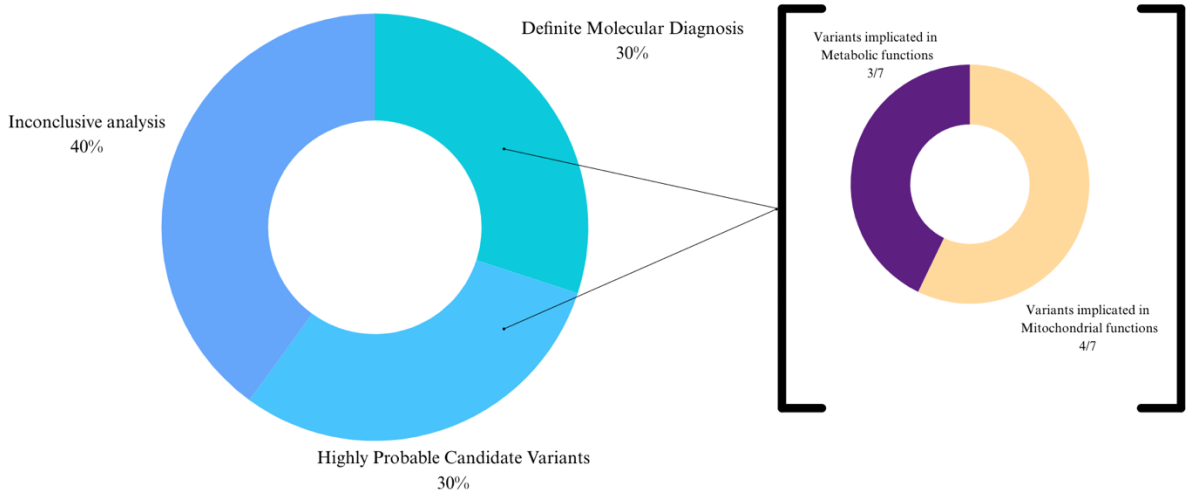

Figure 5. NGS analyses and variant category results of 10 participants and 15 candidate/confirmed variants. 40% of participants have ongoing analyses due to inconclusive results, while 30% were identified as having a 'Definite Molecular Diagnosis' and 'Highly Probable Candidate Variants'. The latter 2 NGS analysis categories had 4/7 variants that were implicated in mitochondrial functions and 3/7 that were implicated in metabolic functions.

The variants identified for the 'Definite Molecular Diagnosis' category were all variants implicated in mitochondrial functions (100%), while for 'Highly Probable Candidate Variants' and 'Definite Molecular Diagnosis' categories, 57.1% of the variants were implicated in mitochondrial functions.

2.1 Definite Molecular Diagnosis

Case 1. *MT-CO2*

This participant (75-year-old, female) with familial origins from Montmagny and Huron, Canada presented with symptoms at age 16 when she was suspected of having MS. The clinical picture, though, include elements suggestive of a mitochondrial disorder as, at the most recent examination, this participant has short stature, slight weight and present with ataxic and spastic features. The radiological features show confluent white matter hypersignals in the periventricular and deep white matter with the posterior limbs of the internal and external capsule involved (Figure 6A). Grey matter structures such as the basal ganglia and thalamus are affected and the splenium, cerebellum, and cerebral hemispheres show volume loss (Figure 6A). We performed a NGS panel for the known forms of genetic leukoencephalopathies (including mtDNA sequencing) and documented a *MT-CO2* gene variant (m.8203 C>G, p.Phe206Leu). The allele frequency of the variant is 0.00% and it showed 14% heteroplasmy, a PolyPhen2 prediction score of probably damaging, a SIFT score of deleterious low, and a Franklin score of 'variant of uncertain significance (VUS) warm'. The *MT-CO2* gene codes for subunit 2 of complex IV in the mitochondria, to which defects result in metabolic distress whereby affected individuals show a range of neuromuscular and non-neuromuscular symptoms (Koenig 2008). In a case report published by Courtois *et al*. (2024), a patient with a variant (m.8091G>A) in *MT-CO2* gene has a radiological picture that highly resembles that of our patient. Additionally, this participant has glaucoma for which the pathophysiology can be tied to glaucomatous injury seen in reduced complex IV activity from this participant's variant. For this evidence, this variant was considered disease-causing.

Case 2. *POLG1*

This participant (66-year-old male) with familial origins from Chambly and Richelieu, Canada presented to the clinic with a mild clinical picture of frequent headaches accompanied by aura. The radiological picture showed bilateral white matter abnormalities located in the periventricular and deep white matter, particularly in the posterior regions, sparing the brainstem, basal ganglia and posterior fossa (Figure 6B). We performed an NGS panel for the known forms of genetic leukoencephalopathies (including mtDNA sequencing) and found compound heterozygous variants c.752C>T (p.Thre251Ile) and c.1760C>T (p.Pro587Leu) in *POLG1* gene, with minor allele frequencies of 0.00155 and 0.00157, respectively. Both variants were predicted to be pathogenic on Franklin, supported by numerous reports of affected individuals with the same variants in cis variant phase and some reports exhibiting mild clinical features like that of our participant (Scuderi et al. 2015). *POLG1* codes for DNA polymerase gamma. *POLG1* variants are commonly associated with progressive external ophthalmoplegia (Scuderi et al. 2015) - which is absent in our patient - with or without ataxia and neuropathy. Multifocal white matter changes as those observed in our participant have been previously reported in association with POLG1-related MS mimicker (Echaniz-Laguna et al. 2010). Given the association of migraine, especially with aura, and *POLG1* variants (Burow et al. 2021), and specifically with these two variants in literature, we conclude that these variants are disease-causing for the multifocal white matter changes and mild clinical symptoms seen in this participant. The muscular biopsy performed recently on the participant's bicep muscle did not document any specific pathological abnormalities, in keeping with the mild clinical phenotype observed and the absence of extra-neurological symptoms; the analysis of the respiratory chain complexes is ongoing.

Case 3. *MT-ATP6*

This participant (53-year-old, female) had her first symptoms at the age of 35 when she presented dystonic movements followed by progressive cognitive deterioration, hemiparesis, apraxia, ataxia, spasticity, and hemiparetic gait. The picture and evolution were interpreted as suggestive of PPMS. The MRI displayed confluent and multifocal bilateral lesions, predominantly in the supratentorial regions with small intralesional cavitations (Figure 6C). A NGS panel for the known forms of genetic leukoencephalopathies (including mtDNA sequencing) was performed and documented a variant in the *MT-ATP6* gene (m.9104T>C, p.Phe193Ser). Heteroplasmy levels were not available; in silico prediction tools such as PolyPhen-2, SIFT, and Franklin describe this variant as possibly damaging, tolerated, and VUS warm, respectively. The frequency of the variant was absent in thousand genomes and GnomAD, however, was found to be 0.01% in a multi-ethnic population of 30,000. *MT-ATP6* gene codes for subunit 6 of complex V, the bioenergetic pump found within the inner membrane of the mitochondria. While the m.9104T>C variant is not previously reported in cases or literature, this region is associated with decreased coupling of proton flow with adenosine triphosphate (ATP) production. Diseases like Leigh syndrome, Neuropathy ataxia retinosa pigmentosa (NARP), and atypical Leber hereditary optic neuropathy (LHON)-like disease are associated with pathogenic variants in this gene. Other variants downstream and upstream of this variant are reported to have decreased baseline respiratory complexes activities, decreased ATP hydrolysis and/or synthesis, and increased sensitivity to glucose deprivation (Ganetzky et al. 2019). This participant is currently considered to have a dual diagnosis of MS and mitochondrial disorder caused by a pathogenic *MT-ATP6* variant.

2.2 Highly Probable Candidate Variants

Case 4. *ABCD1*

Participant 4 (49-year-old, male) with family from Greece had clinical features including a slow progression of ataxia, spasticity, and cognitive decline. His family history was negative for neurological disease. His radiological features showed cerebellar atrophy, bilateral involvement of the cerebellar white matter, supratentorial bilateral multifocal white matter involvement predominant in the posterior regions, and atrophy of the splenium of the corpus callosum (Figure 6D). The patient was initially diagnosed with PPMS, although atypical. NGS panel for the known forms of genetic leukoencephalopathies (including mtDNA sequencing) was negative. Through WES analyses I found 4 variants in *ABCD1* gene, c.1699C>T (p.Gln567Ter), c.1700A>G (p.Gln567Arg), c.1744G>A (p.Val582Ile), and 1748T>A (Val583Glu) - the last 3 variants reported to be benign on Clinvar. The *ABCD1* c.1699C>T is a stop gain variant reported by in silico prediction tools to be a loss of function variant, it has also been reported to be pathogenic by 2 sources on ClinVar and of uncertain significance by 2 sources on ClinVar. This variant has a minor allele frequency of 0.0004, a CADD prediction score of 36, and a Franklin prediction reported as *pathogenic*. *ABCD1* gene encodes for a half transporter located in the peroxisome with pathogenic variants associated with a build-up of very long chain fatty acids (VLCFA) in tissues. This metabolic dysfunction causes X-linked Adrenoleukodystrophy, predominantly affecting males. Due to the progressive clinical picture and MRI findings compatible with adult-onset X-linked adrenoleukodystrophy, we classified this candidate variant as “Highly probable”. Unfortunately, neither the patient nor his family members are available for VLCFA testing and segregation analysis which would confirm the diagnostic hypothesis.

Case 5. *ZFYVE26* and *GALC*

Participant 5 is a French Canadian 34-year-old female with a family history of autism, Alzheimer's disease and consanguinity in maternal grandparents. Clinical features showed a slow disease progression starting at the age of 5 when she experienced difficulties with ballet movements and was described as a 'toe walker'. By age 15, she was unable to keep up with sports due to balance issues which progressed to 'increased difficulties' with her left side by age 16. In 2014, at age 25, upon being diagnosed with MS, she was started on Tysabri for her left leg symptoms but had a progression of symptoms involving her right side and upper limbs resulting in the inability to walk unsupported. The participant was noted to have depression and anxiety related to the disease. MRI patterns revealed considerable brainstem involvement, multifocal periventricular signal abnormalities in the anterior portion of both cerebral hemispheres, and, upon secondary review, the "ear-of-the-lynx' sign along the minor forceps of the corpus callosum (Figure 6E). A NGS panel for the known forms of genetic leukoencephalopathies (including mtDNA sequencing) resulted negative. Through WGS analyses, 2 candidate genes were identified: *ZFYVE26* and *GALC.* This participant has 2 heterozygous *ZFYVE26* gene variants, c.3722G>C, p.Arg1241Gln, and a splicing-extension variant, c.6987-3C>T, with allele frequencies of 4.39%, and 0.354% in non-founder populations, respectively, according to GnomAD. Available prediction scores for the *ZFYVE26* c.3722G>C were low for pathogenicity and predicted to be benign with a CADD score of 3.46, and a Franklin classification of benign strong. Available prediction scores for *ZFYVE26* c.6987-3C>T were low for pathogenicity and predicted to be benign with SIFT, PolyPhen-2, CADD, and Franklin scores/classifications as 0.91, 0.579, 0.7, and benign strong, respectively. *ZFYVE26* is a gene associated with SPG15, an autosomal recessive hereditary spastic paraplegia, that becomes apparent during childhood and

adolescence primarily affecting the lower limbs and with “ear-of-the-lynx” MRI features (Ebrahimi-Fakhari et al. 2021). Despite the relatively high frequency and low predicted pathogenicity of the *ZFYVE26* variants, the clinical and radiological picture could be compatible with SPG15. Therefore, we will proceed with a segregation analysis of the available family members.

This same participant also requires further analysis to identify a possible intronic variant to supplement the finding of a heterozygous *GALC* gene variant that was found in a splicing region (c.1339-2A>C) with an allele frequency of 0.0% in GnomAD. This variant has an in-silico prediction score of 23.2 in CADD, and a Franklin score of pathogenicity strong due to its low frequency and predicted loss of function. *GALC* is associated with Krabbe disease, an autosomal recessive lipid storage disorder, which can present during adolescence with progressive spasticity and MRI features of posterior periventricular involvement (Baba and Weerakkody 2023). This participant passed away during the analysis, however, the parents of the participant have recently consented to be recruited to undergo segregation analysis for the identified variants.

Case 6. *MFF*

Participant 6, a 48-year-old French Canadian female with family origins from Gaspesie, Quebec experienced her symptoms in 2010 with paraesthesia of the right side of her face and upper limb followed by regression of symptoms and a migraine. A few months later, she developed blurry vision in the right eye that lasted a few days. Symptoms then showed a slow progression over time with on-and-off evolution of paraesthesias, pain (mainly localized to joints), tremors, vertigo, and impaired balance. She also described a slow deterioration of motor abilities and strength. MRI features showed symmetric, multifocal, predominantly posterior white matter involvement. The abnormalities were mainly located in the posterior periventricular white

matter, the parietal subcortical regions and the posterior limb of the internal capsules (Figure 6F). This participant has a strong candidate heterozygous variant in *MFF* gene (c.37_38del, p.Arg13fs) with an allele frequency of 0.00%, and a prediction score of likely pathogenic by Franklin. *MFF* gene encodes for MFF protein which has a primary role in controlling mitochondrial fission, of which overexpression or loss of function can lead to various neurodegenerative phenotypes and syndromes (Otera et al. 2010; Chen et al. 2014). Biallelic pathogenic *MFF* gene variants have been previously associated with early onset encephalopathy due to mitochondrial and peroxisomal fission 2 (EMPF2) which manifests with Leigh syndrome-like MRI changes involving the basal ganglia (Koch et al. 2016). The clinical and MRI features of Participant 6 are not suggestive of Leigh-like syndrome. However, de novo heterozygous variants in the DNM1L gene, another key player in mitochondrial and peroxisomal fission that strongly interacts with MFF, were described in an adult subject with slowly progressive complex neurological syndrome including optic atrophy, sensory neuropathy, ataxia, and normal brain MRI (Lhuissier et al., 2022). Further investigation of this variant with segregation analysis and, if needed, functional studies are thus warranted.

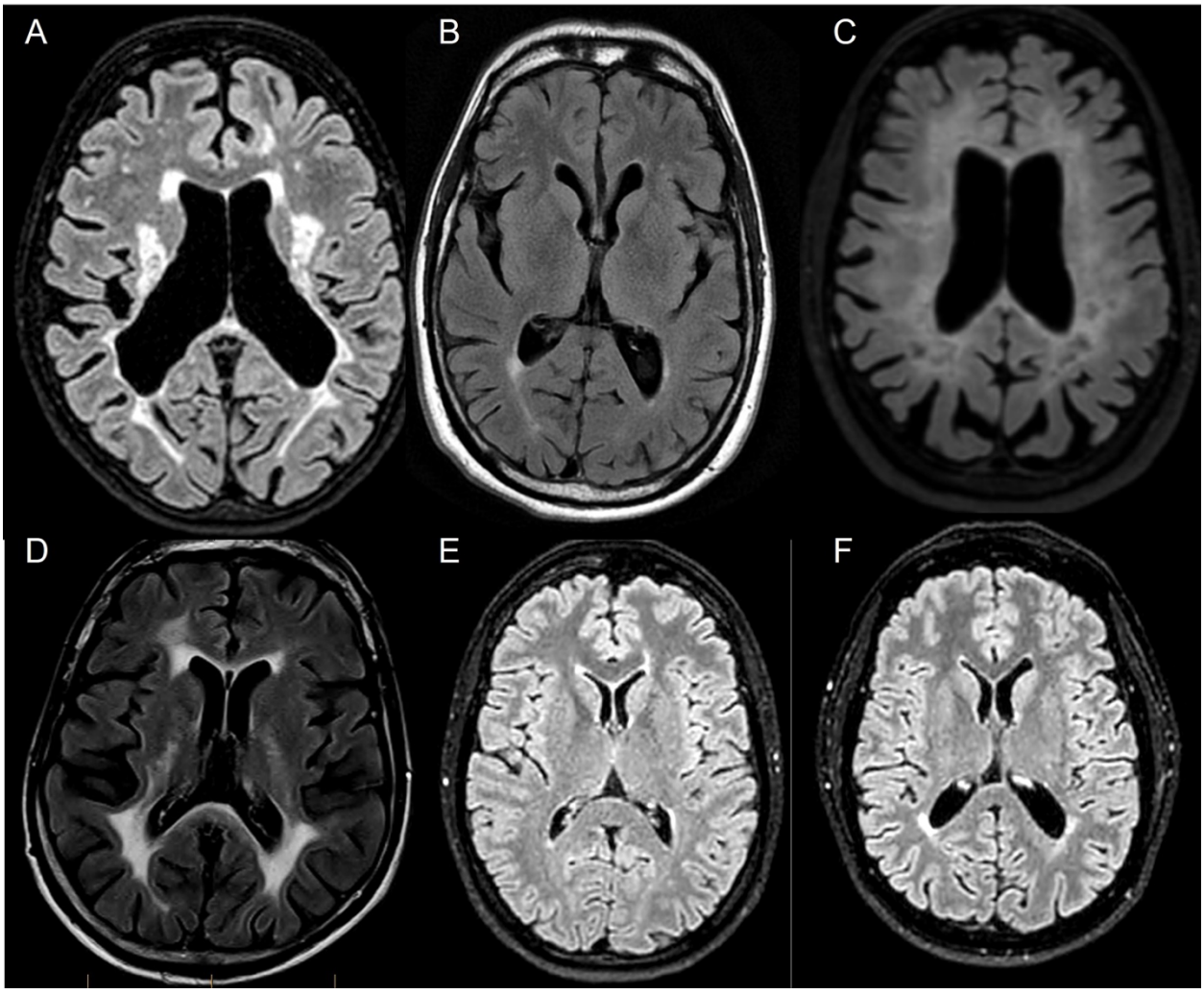

Figure 6. Selected axial T2-weighted MR images of the 6 participants with confirmed or suspected disease-causing variants. A) image of participant 1 showing severe posterior periventricular white matter loss, frontal juxtacortical lesion and involvement of the basal ganglia. B) image of participant 2 showing posterior periventricular white matter abnormalities. C) image of participant 3 showing diffuse confluent white matter involvement in which focal areas of hypointense signal can be recognized. D) image of participant 4 with severe periventricular involvement, predominant posteriorly, involvement of the splenium and internal capsules. E) image of participant 5 with anterior periventricular involvement, appearing as “ear-of-the-lynx” on the left side. F) image of participant 6 showing focal areas of involvement in the posterior periventricular white matter.

Table 2. Summary of 10 recruited participants including their case status, gene(s) of interest, clinical and radiological features, and next steps. The next steps are abbreviated with the following definitions: IF = participant or referring physician informed of disease-causing variant for next steps, SA = segregation analysis, IV = intronic variant analysis, RA = re-analysis, FS = functional studies.

Case Status	Case no.	Patient (age, sex)	Gene of Interest	Clinical Phenotype	Radiological Picture	Next Steps
Definite Molecular Diagnosis	1	75 y/o, Female	*MT-CO2*	Short stature, slight weight, ataxia, spasticity	Confluent WM hypersignals in periventricular and deep white matter with the posterior limbs of the internal and external capsules involved	IF
	2	66 y/o, Male	*POLG1*	Mild clinical picture with frequent headaches and auras	WM abnormalities in both hemispheres sparing the brainstem, basal ganglia and posterior fossa	IF
	3	53 y/o, Female	*MT-ATP6*	First symptoms of dystonic movements with progressive symptoms of cognitive deterioration, hemiparesis, apraxia, ataxia, spasticity, and hemiparetic gait	Symmetric confluent and multifocal bilateral lesions predominantly in the supratentorial regions with small intralesional cavitations	IF
Highly Probable Candidate Variants	4	49 y/o, Male	*ABCD1*	Slow progression of ataxia, spasticity, and cognitive decline	Cerebellar strophy, atrophy of corpus callosum(splenium)	IF, FS
	5	34 y/o, Female	*ZFYVE26, GALC*	Gradual weakness and balance difficulties, neurological symptoms worsen during infections, progressive spastic paraparesis with childhood onset	WM abnormalities localized mainly to the anterior periventricular regions, ears of the lynx sign	SA, IV
	6	48 y/o, Female	*MFF*	Dysmetria, ataxic gait, and on/off evolution of paresthesia, pain, tremor, vertigo and localized pain in joints, issues with memory, urinary frequency	Several T2 hyperintense lesions with 3 located in the periventricular white matter, symmetric and predominantly posterior	FS

Ongoing analysis	7	44 y/o, Male	*PEX5**	Paranoid schizophrenia since age 18, mild head trauma at 13, progressive spastic and ataxic motor deterioration, slowed speech, slow and broad-based gait	Bilateral periventricular multifocal abnormalities, cervical cord involvement, diffuse involvement	RA
	8	53 y/o, Female	*FAT2, ADCK3, PCNA, RNF216*	Severe neurological disability with progressive asymmetric weakness and incoordination (ataxia) and cerebellar features	Multifocal lesions, bilateral diffuse symmetric WM involvement, cerebellar atrophy	RA
	9	N/A, Male	*MIEF2, MT-RNR1*	Traumatic lesion of ulnar and median nerves following car accident, rapid cognitive decline, monoliteral optic neuritis that developed contralaterally	Multifocal confluent bilateral WM abnormalities, spinal cord lesion at the level of C2	RA
	10	34 y/o, Female	*NEK1*	Episodes of hypoglycemia, episodic numbness and paraesthesias, asthma, episodic difficulties with speaking or writing the wrong word, polydipsia and urinary frequency	Diffuse symmetric, involvement of some structures of the posterior limb of the internal capsule and temporal poles, Dawson's fingers	RA

*PEX5 was initially suspected to be a strong candidate gene, until multiples of this same variant was detected in other WES files, suggesting false positive readings.

Discussions

The scoping review results on the genetic, clinical, and radiological features of mitochondrial PPMS mimickers show interesting findings amongst those that were expected.

My thesis generated an up-to-date list of genes implicated in mitochondrial disorders with 307 unique genes and 499 unique variants (see Figure 2). It is known that panels offered across different laboratories vary from each other in genetic testing (Hall et al. 2014; Reid and Pal 2020). These discrepancies can result in variable interpretation and varying clinical results, thus, by the recommendations of the ACMG, there needs to be a 6-month yearly review for reanalyzing diagnostic panels (Bean et al. 2020; Reid and Pal 2020). The ACMG recommends that these reviews include the expertise of relevant clinicians and researchers, literature surveys, and the curated results of databases. Thus, the findings from this scoping review's literature survey, enable clinicians and researchers to find results that reflect the most recent literature and findings in the rare disease sphere, as well, facilitates discussions between clinical diagnostic laboratories and treating physicians.

Of the 499 unique variants, *POLG1*, *SLC25A15*, *DARS2*, and *SURF1*, were reported on the most with 110, 33, 32, and 23 variants, respectively. These findings suggest that these genes have been studied in depth due to the number of variants described in the collected and reviewed literature. Consequently, these genes and their respective variants should be reasonably considered in WES and WGS screenings of those with atypical PPMS phenotypes suspected of having an MD.

Considering the intimate relationship between mitochondrial dysfunction and neuroinflammatory pathways, it is unsurprising that mitochondrial genes like *POLG1*, *SLC25A15*, *DARS2*, and *SURF1* gene variants have been implicated in mimicking PPMS phenotypes. POLG-related

disorders in many cases present as PPMS due to key features of optic neuritis, varying brain white matter hyperintensities, and unmatched cerebral spinal fluid oligoclonal bands (Harding et al. 1992; Enchaniz-Laguna et al. 2010). However, there are also instances of patients with *POLG* features indistinguishable from PPMS, developing more mitochondrial disorder-like features (ie., ptosis, myopathy, ataxia, dysphagia, hearing loss, and cognitive impairment) as their disease states progressed. This suggests that these individuals had mitochondrial disorders mimicking PPMS in the early stages (Enchaniz-Laguna et al. 2010; Yu-Wai-Man et al. 2011; Barcelos et al. 2019). Moreover, the finding that 110 of variants were related to *POLG1* shows that the literature is robust enough to aid clinicians and researchers in the diagnostic process and the future development for patients with *POLG* related PPMS mimics. For these reasons, along with our findings of 110 unique *POLG1* variants, it is important to screen for the *POLG1* gene during genetic workups on atypical PPMS cases. We identified pathogenic *POLG1* variants in one of the 10 subjects included in our study, thus further supporting the high frequency of POLG-related MS mimickers. *SLC25A15* gene variants were reported 2nd most with 33 unique variants. These variants were included in one paper rather than being aggregated from multiple papers like the other genes. This particular paper published by Martinelli et al. in 2015 was included in our study due to most of the clinical features in the abstract matching that of mitochondrial PPMS mimickers. In fact, *SLC25A15* is a mitochondrial gene that does not result in a perfect PPMS mimic however, it can be regarded as a gene to investigate during the genetic workup of atypical presentations of MS spectrum disorders like neuromyelitis optica (NMO) and acute disseminated encephalomyelitis (ADEM). *SLC25A15* pathogenic variants are associated with Hyperornthinemia-hyperammonemia-homocitrullinuria (HHH) syndrome which has a variable presentation in adulthood with some key features including lethargy, disorientation, episodic

confusion, unexplained seizures, intellectual disabilities, recurrent vomiting, cerebellar and pyramidal tract signs, ataxia, muscle weakness, and spasticity (Camacho and Rioseco-Camacho 2020). Due to HHH syndrome's acute nature along with other features such as vomiting and confusion, which are regarded as rare and atypical features of PPMS (Popescu et al. 2011; Levinthal et al. 2013), HHH syndrome should be considered in the differential for other acquired inflammatory demyelinating disorders like NMO and ADEM.

DARS2 gene had the 3rd highest number of unique variants reported. Pathogenic variants in *DARS2* manifest as a rare autosomal recessive neurological disorder known as leukoencephalopathy with brainstem and spinal cord involvement and elevated white matter lactate (LBSL). LBSL has some overlapping radiological and clinical features with PPMS and thus is considered an MS mimicker, especially due to its cervical spine involvement that is uncommon in other mitochondrial diseases that mimic MS (Isohanni et al. 2010; Weisfeld-Adams et al. 2015; Engelen et al. 2021). Due to their overlapping features, a study in Finland looked at whether common *DARS2* mutations contributed to the risk of developing MS in 321 patients with MS (Isohanni et al. 2010). The study concluded that although common *DARS2* mutations did not correlate to increased MS risks, DARS2-related disorder is nonetheless important for neuroradiologists to recognize due to the high *DARS2* variant carrier frequency in European-derived populations and overlapping features with MS. Our study further validates this recommendation, especially when excluding PPMS as a diagnosis in patients. The *SURF1* gene is one of many nuclear genes with pathogenic variants associated with Leigh Syndrome (LS), which is a known MS mimicker (Weisfeld-Adams et al. 2015; Jabeen et al. 2016). LS was first characterized in 1996 by Rahman et al., and in 1998 Zhu et al. described the role of *SURF1* in

LS. Given that LS and MS have many clinical and radiological overlaps, *SURF1* gene variants should be considered in the differential diagnosis when considering atypical MS cases.

The scoping review results on the clinical features of MDs that mimic PPMS showed overlapping features with PPMS alongside some key differences. For instance, out of the top 10 features that were reported for MDs that mimic PPMS, it was shown that terms commonly used to describe MDs like 'ataxia', 'seizures', 'spasticity', 'ophthalmoplegia', and 'muscle weakness' (see Figure 3) are also commonly used to describe MS features (Fernàndez et al. 2020). These findings further support that MDs can mimic PPMS based on clinical features that are present in patients. Despite this, the results also indicate that 1 out of the top 10 features ('hearing loss') describing MDs are more specific for MDs and exceedingly rare for PPMS, while 4 out of the top 10 ('neuropathy', 'hypotonia', 'ptosis' and 'vision loss') are not used to describe PPMS features at all (Mirmosayyeb et al. 2022).

The content analysis of clinical terms (Figure 3) showed that 3 out of the top 4 categories are common in PPMS. This is the case for 'pyramidal and extrapyramidal signs', 'cerebellar involvement', and 'visual deficit' accounted for 17.5%, 15.7%, and 14.4% of terms used by authors, respectively, to describe MD features. However, the category of 'metabolic and extraneurological involvement' accounting for 14.7% of the terms used to describe MD, is unusual for PPMS. This is consistent with what is reported in the literature as MDs have both metabolic and multisystemic involvement.

Overall, the findings on content analysis by clinical terms showed that although MDs are heterogeneous, they can still be characterized to identify key features specific to MDs and that many of these overlap with clinical features of MS.

Similarly to what was observed for clinical terms, the results from the content analysis of the MRI features described in the literature highlight many similarities as well as some differences between MDs and PPMS. Terms like 'periventricular', 'cervical spinal cord', and 'corpus callosum' were used frequently throughout the literature to describe the features of MDs 44, 19, and 18 times, respectively (see Figure 4). All these features are known to be common in MS (Garg and Smith 2015; Pongratz et al. 2023). Conversely, cervical spinal cord involvement in MDs are considered rare and only found in LBSL or *DARS2*-related disorders. Our results show that cervical spinal cord involvement in MDs is more common than previously known. For instance, besides *DARS2*-related disorders, most papers from this scoping review describing spinal cord involvement were associated with Leber hereditary optic neuropathy (LHON), Complex III chain disorders. This highlights how MDs should be considered when cervical spinal cord involvement is present in atypical PPMS cases, as this type of involvement is now not uncommon for certain MDs. Key differences between MDs and PPMS was the involvement of gray matter structures like the basal ganglia (counted 34 times) in MDs. This is supported by the fact that gray matter structures have 50% more mitochondria due to a higher metabolic demand than white matter (Plante et al. 2014; Mosharov et al. 2024), thus, it would be reasonable to find such structures more involved in MDs.

When looking at the categories of structures involved (see Figure 4), the cerebrum encompasses 31.5% of the terms used to describe MRI features of MDs mimicking MS. This is consistent with many neurological diseases that affect the white matter of the brain's supratentorial region. It was also found that the 'basal ganglia', 'brainstem', and 'cerebellum' were the next highest categories of structures involved with 14.4%, 12.7%, and 10.6% of the terms belonging to these

categories, respectively. The basal ganglia include many integrative structures namely the caudate nucleus, putamen, pallidum, and thalamus (Grahn et al. 2008; Trufanov et al. 2023). These structures are involved in various functions ranging from integrative processes like conditional reflex activity, to regulating complex motor actions, muscular tone, and inhibitory control over addictive behaviours (Akkermans et al. 2018; Trufanov et al. 2023). This results in a high number of tracts between the basal ganglia and other brain structures like the cortex and thalamus subsequently affecting behavior control, movement, and cognition similar to that of the prefrontal cortex (O'Reilly 2006; Langley et al. 2023). Current literature shows that basal ganglia involvement is more commonly found in MDs over PPMS, therefore the basal ganglia structures being described more in MDs are consistent with what is reported from our scoping review results. Interestingly, it is important to note that recent studies have been looking at the role of fatigue and basal ganglia involvement in relapsing-remitting MS (RRMS) and secondary progressive MS (SPMS) as potential predictors for MS progression (Meijboom et al. 2023; Trufanov et al. 2023). The brainstem and cerebellum were seen to be quite common in MDs mimicking MS, it is also known that the involvement of these 2 categories is common for MS. For example, 81.6% of patients with MS (all forms confounded) have brainstem and/or cerebellar clinical manifestations (Poser et al. 1979; Yang et al. 2022). However, other studies found that cerebellar symptoms are more common in PPMS, while brainstem symptoms are more commonly seen in RRMS (Harding et al. 2014; Sahraian et al. 2018). Regardless of the specification between forms and these 2 categories, the overall clinical manifestations in MS from the involvement of these 2 brain areas are common. Cerebellar symptoms predominantly present as tremors, nystagmus, scanning speech, gait and truncal ataxia, incoordination of voluntary movements, ocular dysmetria, and hypotonia (Weier et al. 2015; Yang et al. 2022),

while brainstem symptoms predominantly present as diplopia, unstable gait, vertigo, facial weakness, and sleep disorders in MS (Habek 2013; Yang et al. 2022). For all these considerations, it can be concluded that MDs should be included in the differential diagnosis of PPMS cases especially when there is involvement of the basal ganglia.

A key limitation of Aim 1 is that scoping reviews are meant to address broad questions that can be answered by identifying trends in the data and gaps in the literature, due to this, specific questions regarding the collected data warrant further studies. A second limitation for Aim 1 is that 'Biochemical features/tests' were not included in this thesis due to time constraints thus, future directions involve reporting the data on 'biochemical features/tests' found for MDs mimicking PPMS as this will guide tests needed for diagnostic purposes.

NGS analyses in participants with atypical PPMS

All 3 subjects with final molecular diagnosis showed pathogenic variants in genes associated with mitochondrial function. The clinical phenotypes, along with genetic information/tests on the variants, coupled with the provided radiological pictures strongly supported the genotype-phenotype relationship. Importantly, 2 of the 3 confirmed cases had variants initially classified as "of unknown significance". Their disease-causing role was confirmed due to expert recommendations, case comparisons, and the use of computational tools.

For the 3 highly probable candidate cases, a total of 4 variants were identified associated with the disease phenotypes seen in our participants and all of them were associated with mitochondrial or metabolic functions. Highlighting how the importance of screening for variants involved in mitochondrial or metabolic functions (Weisfeld-Adams et al. 2015). The classification of these cases as "highly probable" candidate variants highlights the challenges of NGS sequencing for

rare, neurodegenerative disorders. For example, in one case (Participant 4), neither the participant nor his family members were available for biochemical analysis of VLCFAs and segregation analyses to confirm the diagnostic hypothesis, although the identified variants were interpreted as likely pathogenic. A similar issue was encountered for Participant 5 who passed away during the genomic and exome analyses phase, thus preventing any further confirmation. Her family agreed to undergo segregation analysis and further investigations that may allow us to confirm the disease-causing role of the *ZFYVE26* and *GALC* gene variants. For Participant 6, a lack of compelling phenotype-genotype relationship between the participant's clinical features and the *MFF* gene candidate variant warrants further investigations to be of 'confirmed' status.

For the 4 participants with a classification of 'ongoing analysis', further genetic analyses are required to narrow down the list of candidate variants. WES and WGS require routine reanalysis every 6 months as computational/bioinformatic tools and literature are updated. The cases from this study highlight the complex judgements required to identify, classify, and confirm variants responsible for rare diseases. Although ACMG guidelines broadly promote the classification of variants to develop standards for genetic evaluation in clinical applications, it is said that those evaluating specific disease groups (ie., rare genetic diseases) should create their own focused classification of variants and cases that vary according to the gene and disease (Harrison et al. 2019; Richards et al. 2015). Aim 2 of this study highlights how this can be done in a combined clinical and research setting.

It is important to note that WES and WGS have intrinsic limitations and may miss disease-causing variants, such as complex structural variants and repeat expansions, due to the use of short read sequencing (Adams et al. 2024). Long read sequencing (reads 100-300 base pairs) is becoming increasingly available and allows for the detection of large complex structural

variants, variant phases, and methylation changes which short read sequencing does not offer. Longer reads also improve alignment with the reference genome and better detection of structural variants within repetitive elements (ie., segmental duplications or high GC content) (Sedlazeck et al. 2018). Further, haplotype phasing in long read sequencing can be easily done, identifying compound heterozygous mutations and de novo autosomal dominant mutations without sequencing parent genomes (Marhawa et al. 2022). Future directions of this study include the consideration of long-read sequencing for cases which remained unsolved. Recent studies have suggested the use of long-read sequencing coupled with transcriptomics, proteomics, metabolomics, and/or epigenetics to identify the full scope of variant consequences (Sreenivasan et al., 2022; Lunke et al., 2023; Smirnov et al., 2023; Su et al., 2023).

Additionally, while this field evolves, recommendations from the International Rare Diseases Research Consortium (IRDiRC) Diagnostic Scientific Committee (DSC) encourage reverse phenotyping, namely the re-definition of phenotypes associated with identified disease-causing genes; the use of computational tools; data sharing across a network of experts; ongoing refinement of disease classifications; and improving access to care and education for patients (Adams et al. 2024). These recommendations, with the exception of long-read sequencing, were followed for all 10 cases analyzed in this study which helped in classifying cases as ‘confirmed’, ‘highly probable’ or requiring ‘ongoing analysis’.

Another future direction of studies on the genetic causes of mitochondrial MS mimickers involves the close collaboration with laboratories working on mitochondrial function to perform functional and biochemical tests for participants in which candidate variants are identified.

Finally, a notable future direction, especially for cases with inconclusive or ‘ongoing’ WES or WGS analysis is to check the presence of variants in MS susceptibility and MS severity genes,

according to recent publications (Harroud et al. 2023) which identified rs10191329 and rs149097173 in the *DYSF-ZNF638* and *DNM3-PIGC* loci respectively as associated with MS severity. Other MS susceptibility genes like those involved in immune processes (*HLA-DR*, *IL2RA*, *IL4*, *IL6*, *IL12B*, *IL17R*, *IRF5*, *CD24*, *CD58*, and *EVI5)*, vitamin D metabolism (*VDR* and *CYP27B1*), fibrinolysis (*PAI-1*), and other CNS functions (*ApoE* and *DPP*) are important to consider as well (Tizaoui 2018) since their identification can provide further information on cases of PPMS that present atypically.

Conclusion

By conducting a scoping review to better identify and characterize mitochondrial MS mimickers and by using NGS methods in a cohort of participants initially suspected of having PPMS, I have discovered novel variants not previously associated with genetic MS mimickers. I have expanded the clinical phenotypes associated with genes involved in mitochondrial function to include later-onset and mild presentations that have been previously interpreted as PPMS.

My results on the literature review will aid in furthering genetic research and broadening the clinical, radiological and genetic features of MS mimickers, specifically, mitochondrial disorders which are clinically heterogeneous offering their own set of diagnostic challenges.

Finally, my results will support the need to further investigate the pathophysiological mechanisms shared by monogenic mitochondrial diseases and common acquired demyelinating disorders.

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Supplementary Tables and Figures

Supplementary Table 1. Search terms and Database log book. Search terms were entered into MEDLINE (Ovid and Pubmed), Scopus, and Web of Science, as well as, Proquest and Embase. All databases returned results except for Proquest and Embase, for which studies were either non-existent or did not meet the inclusion criteria for this scoping review. A total of 1018 studies were exported for extraction into Covidence software (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia. Available at www.covidence.org).

Date (DD-MM-YY)	Database	Search Terms	Hits
14-Feb-23	PubMed	("Mitochondrial disorder" OR "Mitochondrial disease" OR Mitochondri* OR "Mitochondrial dysfunction" OR "Mitochondrial deficiency" OR "Leber hereditary optic neuropathy" OR LHON OR MELAS OR MERRF OR "Myoclonic epilepsy with ragged red fibers" OR "Mitochondrial encephalomyopathy lactic acidosis and stroke like episodes" OR "MELAS syndrome" OR "Leigh Disease" OR "Leigh Syndrome" OR "POLG-related disease" OR "Optic Atrophy type 1" OR "Pyruvate dehydrogenase deficiency") AND ("Multiple Sclerosis" OR "MS" OR Acquired neuro* OR "Acquired demyelinating disorder") AND (Clinic* OR "clinical evidence" OR "clinical phenotypes" OR "clinical characteristics" OR "clinic* findings" OR "identification" OR "classification" OR "diagnosis" OR "diagnostic criteria") AND (Radiolo* OR "MRI" OR "Magnetic resonance imaging" OR "Radiological evidence" OR "Radiological imaging")	226
23-Apr-23	Web of Science	("Mitochondrial disorder" OR "Mitochondrial disease" OR Mitochondri* OR "Mitochondrial dysfunction" OR "Mitochondrial deficiency" OR "Leber hereditary optic neuropathy" OR LHON OR MELAS OR MERRF OR "Myoclonic epilepsy with ragged red fibers" OR "Mitochondrial encephalomyopathy lactic acidosis and stroke like episodes" OR "MELAS syndrome" OR "Leigh Disease" OR "Leigh Syndrome" OR "POLG-related disease" OR "Optic Atrophy type 1" OR "Pyruvate dehydrogenase deficiency") AND ("Multiple Sclerosis" OR "MS" OR Acquired neuro* OR "Acquired demyelinating disorder") AND (Clinic* OR "clinical evidence" OR "clinical phenotypes" OR "clinical characteristics" OR "clinic* findings" OR "identification" OR "classification" OR "diagnosis" OR "diagnostic criteria") AND (Radiolo* OR "MRI" OR "Magnetic resonance imaging" OR "Radiological evidence" OR "Radiological imaging")	238
08-May-23	Scopus	("Mitochondrial disorder" OR "Mitochondrial disease" OR Mitochondri* OR "Mitochondrial dysfunction" OR "Mitochondrial deficiency" OR "Leber hereditary optic neuropathy" OR LHON OR MELAS OR MERRF OR "Myoclonic epilepsy with ragged red fibers" OR "Mitochondrial encephalomyopathy lactic acidosis and stroke like episodes" OR "MELAS syndrome" OR "Leigh Disease" OR "Leigh Syndrome" OR "POLG-related disease" OR "Optic Atrophy type 1" OR "Pyruvate dehydrogenase deficiency") AND ("Multiple Sclerosis" OR "MS" OR Acquired neuro* OR "Acquired demyelinating disorder") AND (Clinic* OR "clinical evidence" OR "clinical phenotypes" OR "clinical characteristics" OR "clinic* findings" OR "identification" OR "classification" OR "diagnosis" OR "diagnostic criteria") AND (Radiolo* OR "MRI" OR "Magnetic resonance imaging" OR "Radiological evidence" OR "Radiological imaging")	358
13-Feb-23	OVID	exp Mitochondrial Diseases/cl, di, dg, et, ge [Classification, Diagnosis, Diagnostic Imaging, Etiology, Genetics]	186

exp MERRF Syndrome/cl, di, dg, et, ge [Classification, Diagnosis, Diagnostic Imaging, Etiology, Genetics]

exp MELAS Syndrome/cl, di, dg, et, ge [Classification, Diagnosis, Diagnostic Imaging, Etiology, Genetics]

exp Optic Atrophy, Hereditary, Leber/cl, di, dg, et, ge [Classification, Diagnosis, Diagnostic Imaging, Etiology, Genetics]

exp Leigh Disease/cl, di, dg, et, ge [Classification, Diagnosis, Diagnostic Imaging, Etiology, Genetics]

exp Hyperlactatemia/di, et, ge [Diagnosis, Etiology, Genetics]

1 or 2 or 3 or 4 or 5 or 6

exp Multiple Sclerosis/cl, di, dg, et, ge [Classification, Diagnosis, Diagnostic Imaging, Etiology, Genetics]

exp Demyelinating Diseases/cl, di, dg, et, ge [Classification, Diagnosis, Diagnostic Imaging, Etiology, Genetics]

8 or 9

7 and 10

Supplementary Table 2. Exclusion criteria and rationale for literature screened. The exclusion was determined during the screening process and papers that did not focus on the research question were excluded.

Types of Studies Excluded	Rationale
Cell Studies	Not relevant for the purposes of this study that focuses on clinical presentations of mitochondrial disorders that mimic MS.
Traumatic Brain Injury	Traumatic brain injury is not a criterion for inherited leukodystrophies.
Animal Studies	Animal studies are not relevant to the clinical setting.
Cancer	Not associated with inherited leukodystrophies.
Diabetes	Diabetes is not a criterion from inherited leukodystrophies.
Virus Encephalitis	Not associated with inherited leukodystrophies.
Randomized Clinical Trials	Not relevant for clinical diagnostic purposes.
Autism Spectrum Disorders	Not relevant to leukodystrophies.
Cardiomyopathy	Excluded to focus on leukodystrophies.
Myocardial Ischemia	Not associated with inherited leukodystrophies.
Biomarkers of MS	not relevant for mitochondrial disorders that mimic MS; our focus is not in identifying further MS rather to further identify mitochondrial disorders that mimic MS therefore, biomarkers of MDs would be more relevant.
Methodological Papers	Understanding methodology behind, imaging, cellular and genetic studies are irrelevant when dealing with clinical presentations for MDs.
Epilepsia Partialis Continua	mitochondrial diseases that present with EPC do not mimic MS presentation.
LHON papers before 2013	Excessive literature was found on LHON and only pertinent and more chronologically relevant papers after 2013 were included to minimize redundancy. Case reports suggesting LHON were included to identify variants associated with LHON as this will facilitate variant identification.
Proton MR spectroscopy	Excluded to focus on MRI.

Supplementary Table 3. Parameters of the scoping review and the headings used to collect data. These parameters were used for data extraction. Each heading has data extracted from 181 pieces of literature.

Parameters	Heading(s) separated by commas
Article Information	Article number[a], First Author, Journal of publication, Title of article, Year Published, Location published[b]
Study Information	Study Aim(s), Study Setting[c], Methodology/Design[d]
Participant Information	Number of Participants, Disease, Age Group, Age Mean, Number of Females and Males
Disease Information	Genetic Features, Biochemical Tests/Features, Clinical Features, Rare Clinical Features, MRI Features
Study Outcomes	Main Findings, Significant Findings Relevant to Review Studies, Recommendations of the Article, Conclusions, Other Notes.

(a) The article number was the number associated to the article on Covidence during the screening process.

(b) The location of publication was determined based on first author affiliations.

(c) The study setting based on the type of study was selected from 5 options Case report, Review, Research, Essay, or Conference.

(d) The methodology and design were based on 3 options Qualitative, Quantitative or Mixed.

Supplementary Table 4. Genes related to mitochondrial function and their corresponding disorders known to date. Out of the 181 papers evaluated, 76 disorders including 'other mitochondrial disorders' were found with their reported genes, which are listed. The disorders listed are found in either the pediatric population (birth – 17 years of age), the adult (17 + years of age) population, or both. NOTE: Many disorders listed are not MS mimics, however, genes implicated in mitochondrial functions have been found in mitochondrial disorders known to mimic MS.

Genes reported	Associated disorders to reported genes
AARS2, KARS, LARS2	AARS-L/Ovarioleukodystrophy
ACAD9	ACAD9 deficiency
ACO2	ACO2 deficiency
OPA1	Autosomal dominant optic atrophy
GFAP	Alexander's disease
C10ORF2, FARS2, NARS2, PARS2, POLG1, TWINKLE	Alper's syndrome
AMACR	Alpha-methylacyl-CoA racemase deficiency
MARS2	Autosomal recessive spastic ataxia with leukoencephalopathy
APTX	Ataxia-oculomotor apraxia type 1
BTHS, TAZ	Barth Syndrome
BTD	Biotinidase deficiency
BCS1L	Björnstad syndrome
BOLA3	BOLA3 deficiency
NOTCH3	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)
IARS2	Cataracts, growth hormone deficiency, sensory neuropathy, sensorineural hearing loss, skeletal dysplasia syndrome (CAGSSS)
FDX1L	Cerebrotendinous Xanthomatosis
AIFM1, DNM2, DTNC1H1, POLG1, SURF1, GDAP1, GARS, KARS, Mitofusin, LMNA, MFN2, MPZ, TRPV4	Charcot-Marie tooth diseases
ADCK3, CoQ10, CoQ2, CoQ4, CoQ8A, CoQ8B, CoQ9, PDSS1, PDSS2	CoQ10 deficiency
GFM1	COXPD1 deficiency
MRPS16	COXPD2 deficiency
TSFM	COXPD3 deficiency
TUFM	COXPD4 deficiency
AIFM1	COXPD6 deficiency

DNAJC19	Distal cardiomyopathy with ataxia syndrome
DCTN1	Distal hereditary motor neuropathy type 7
DMD	Duchenne Muscular Dystrophy
ECHS1	ECHS1 deficiency
MFF	Encephalopathy due to defective mitochondrial and peroxisomal fission 2
DGOUK, POLG1, MPV17	Encephalohepatopathy
ETHE1	Ethylmalonic Encephalopathy
DNM1L, TARS2	Fatal infantile encephalomyopathy
VARS2	Fatal mitochondrial encephalocardiomyopathy
FBXL4	FBXL4 deficiency
ABC7, FXN, ISCU	Friedreich ataxia
BCS1L	Gracile Syndrome
HIBCH	HIBCH deficiency
GNE, MYH2	Hereditary inclusion body myopathy
HSD17B10	HSD10 disease
ACO2, FARS2, ATL1, C12orf65, HSPD1, SPG13, SPG20, PNPLA6, SPAST, SPG43, SPG55, SPG7, SPG74, REEP1, SPG31, KIF5A	Hereditary spastic paraplegia (HSP)
SARS2	Hyperuricemia, pulmonary hypertension, and renal failure in infancy and alkalosis syndrome (HUPRA)
SLC25A15	Hyperornithinemia–hyperammonemia-homocitrullinuria syndrome
MPV17, POLG1	Infantile Hepatic Mitochondrial DNA Depletion
C10ORF2, TWINKLE, POLG1	Infantile-onset spinocerebellar ataxia
MT-ND4, MT-TL1	Kearns-Sayre syndrome (KSS)
DARS2	Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL)

C12orf65, C20orf7, C8Orf38, COX10, COX15, COXFA4, DNAJC30, EFG1, FARS2, FOXRED1, IARS2, LRPPR1, LRPPRC, MT-ATP6, MT-CO1, MT-CO3, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND5, MT-ND6, MT-TK, MT-TL1, MT-TV, NDUFA1, NDUFA10, NDUFA11, NDUFA12, NDUFA2, NDUFAF2, NDUFAF5, NDUFAF6, NDUFB6, NDUFS1, NDUFS2 NDUFS3, NDUFS4, NDUFS7, NDUFS8, NDUFV1, PDSS2, PET100, POLG1, SCO1, SCO2, SDHA, SUCLG1, SURF1, TACO1, TTC19, URCRQ	Leigh syndrome
DNAJC30, MT-ATP6, MT-CO1, MT-CO2, MT-CO3, MT-CYB, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6, MT-RNR, MT-TK	Leber hereditary optic neuropathy (LHON)
MCOLN1	Lysosomal storage disease
EARS2	Leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL)
POLG1	Myocerebrohepatopathy spectrum (MCHS)
POLG1, SSBP1, MPV17, SUCLA1, SUCLA2, RRM2B, TK2, TYMP	mtDNA depletion syndrome (MDS)
SERAC, TMEM70	3-methylglutaconic aciduria with deafness-encephalopathy-Leigh-like syndrome (MEGDEL)
FASTKD2, MT-CO1, MT-CO2, MT-CO3, MT-CYB, MT-ND1, MT-ND3, MT-ND4, MT-ND5, MT-ND6, MT-TC, MT-TH, MT-TK, MT-TL1, MT-TS1, MT-TS2, MT-TV, MT-TW, POLG1	Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS)
POLG1	Myoclonic epilepsy myopathy sensory ataxia (MEMSA)
FDX2	Mitochondrial myopathy, episodic, with or without optic atrophy and reversible leukoencephalopathy (MEOAL)
MT-ND5, MT-TF, MT-TH, MT-TK, MT-TL1, MT-TP, MT-TS1, MT-TS2, POLG1	Myoclonic epilepsy with ragged red fibers (MERRF)
CARS2	Mitochondrial Epileptic Encephalopathy
MT-ATP6, MT-CO3, MT-ND4	Mitochondrial HSP
YARS, PUS1, MT-ATP6	Mitochondrial myopathy, lactic acidosis and sideroblastic anemia (MLASA)
MCOLN1	Mucolipidosis type IV (MLIV)
HCCS	Microphthalmia with linear skin defects syndrome (MLS)

ECFG1, LIG3, POLG1, RRM2B, TYMP	Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)
CAPN3, DES, GMPPB, LMNA	Muscular Dystrophy
MT-ATP6	Neuropathy, ataxia, retinitis pigmentosa syndrome (NARP)
MPV17	Navajo neurohepatopathy
C19orf12	Neurodegeneration with brain iron accumulation (NBIA) and Mitochondrial membrane protein associated neurodegeneration (MPAN)
DNM1L	Optic atrophy 5
SSBP1 gene c.113G>A	Optic Atrophy 13 with retinal and foveal abnormalities
PC	PC deficiency
RARS2	Pontocerebellar hypoplasia type 6
DLAT, DLD, LIAS, MPC1, PDH, PDHA1, PDHA2, PDHB, PDHX, PDP1	Pyruvate dehydrogenase deficiency (PDHD)
ANT1, C10orf2 (PEO1), MT-TI, MT-TL1, MT-TL2, MT-TS2, POLG1, POLG1 2, RNAse H1 gene, RNAse H2 gene, RRM2B, TWINKLE	Progressive external opthalmoplegia (PEO)
CLPP, HARS2, HSD17B4, LARS2	Perrault Syndrome
POLG1	Sensory ataxic neuropathy, dysarthria, and ophthalmoparesis (SANDO)
SDH, SDHA, SDHAF1,SDHB	Succinate dehydrogenase deficient leukoencephalopathy
DDP	X-linked deafness–dystonia syndrome

C7orf10, DCI, APOPT1/COA8, ATP5F1A, ATP5F1D, ATP5F1E, ATP5MK, ATPAF2, BCS1L, CYC1, COA3, COA5, COA6, COA7, COL4A2, COX14, COX20, COX4I1, COX4I2, COX5A, COX6A1, COX6A2, COX6B, COX6B1, COX7A1, COX7B, COX8A, DHDPSL, GPAM, IBA57, ISCA2, IVD, LACTB, LYRM5, LYRM7, MCCC2, MICU1, MT-ATP8, MT-TE, MTFMT, MTRE, MZM1L, NDUFA13, NDUFA3, NDUFA4, NDUFA5, NDUFA6, NDUFA7, NDUFA8, NDUFA9, NDUFAB1, NDUFAF1, NDUFAF3, NDUFAF4, NDUFB1, NDUFB10, NDUFB11, NDUFB2, NDUFB3, NDUFB4, NDUFB5, NDUFB7, NDUFB8, NDUFB9, NDUFC1, NDUFC2, NDUFS5, NDUFS6, NDUFV2, NDUFV3, NSUN3, NUBPL, OXCT1, OXCT2, PET117, PHYH, PITRM1, POLR3A, SLC25A3, SUOX, SURF1, TP1, UQCC2, UQCC3, UQCRB, UQCRC2,	Other mitochondrial-related disorders*

*Other mitochondrial-related disorders include mitochondrial complex deficiencies and disorders which were listed as ‘mitochondrial disorder(s)’ in articles with no further specifications.

Supplementary Table 5. List of included studies with their journal and year of publication from most recent to least recent. 181 papers were included in the final scoping review. All titles, journal names, and year of publication were collected from Covidence during the 'Selecting studies' stage of Aim 1.

Journal	Title	Year
Pediatric Neurology	Natural History of SURF1 Deficiency: A Retrospective Chart Review	2023
Scientific Reports	Analysis of the entire mitochondrial genome reveals Leber's hereditary optic neuropathy mitochondrial DNA mutations in an Arab cohort with multiple sclerosis	2022
JAMA Neurology	Adult-onset genetic central nervous system disorders masquerading as acquired neuroinflammatory disorders: A review	2022
European Journal of Paediatric Neurology	Mitochondrial diseases mimicking autoimmune diseases of the CNS and good response to steroids initially	2022
Nature Communications	Specialist multidisciplinary input maximises rare disease diagnoses from whole genome sequencing	2022
Acta Neuropathologica Communications	A case of primary optic pathway demyelination caused by oncocytic oligodendrogliopathy of unknown origin	2022
Annals of Clinical and Translational Neurology	Visual memory failure presages conversion to MELAS phenotype	2022
Movement Disorders Clinical Practice	Biallelic loss-of-function NDUFA12 variants cause a wide phenotypic spectrum from Leigh/Leigh-like syndrome to isolated optic atrophy	2022
BMC Neurology	Magnetic resonance imaging negative myelopathy in Leber's hereditary optic neuropathy: a case report	2022
Frontiers In Neurology	Case report: Long term follow-up of two patients with LHON caused by DNAJC30:c.152G>A pathogenic variant-case series	2022
Neurological Sciences	Genetic causes of acute encephalopathy in adults: Beyond inherited metabolic and epileptic disorders	2022
Journal of Neuroimaging	Neuroimaging in cerebellar ataxia in childhood: A review	2022
Cells	Mitochondrial neurodegeneration	2022
Frontiers In Genetics	Biallelic COA7-Variants leading to developmental regression with progressive spasticity and brain atrophy in a Chinese patient	2021
Multiple Sclerosis and Related Disorders	Mitochondrial mutations in multiple sclerosis patients with atypical optic neuropathy	2021
Multiple Sclerosis and Related Disorders	Multifocal cavitating leukodystrophy-A distinct image in mitochondrial LYRM7 mutations	2021

Multiple Sclerosis and Related Disorders	Late-onset Leber's hereditary optic neuropathy presenting with longitudinally extensive myelitis harbouring the m.14484T > C mutation: Extending the genotype-phenotype spectrum	2021
Case Reports in Ophthalmology	Mitochondrial neurogastrointestinal encephalopathy disease: A rare disease diagnosed in siblings with double vision	2021
Postgraduate Medicine	Genetic diseases mimicking multiple sclerosis	2021
Autoimmunity Reviews	Pediatric inflammatory demyelinating disorders and mimickers: How to differentiate with MRI?	2021
American Journal of Neuroradiology	Imaging patterns characterizing mitochondrial leukodystrophies	2021
American Journal of Medical Genetics	ECHS1 disease in two unrelated families of Samoan descent: Common variant - rare disorder	2021
Pediatric Radiology	Magnetic resonance imaging of the brainstem in children, part 2: Acquired pathology of the pediatric brainstem	2021
Neurology Genetics	LBSL: Case series and DARS2 variant analysis in early severe forms with unexpected presentations	2021
JIMD Reports	HSD10 disease in a female: A case report and review of literature	2021
Journal of Neurology	Leber hereditary optic neuropathy plus dystonia, and transverse myelitis due to double mutations in MT-ND4 and MT-ND6	2020
Radiographics	Primary mitochondrial disorders of the pediatric central nervous system: Neuroimaging findings	2020
European Journal of Ophthalmology	Optic atrophy in children: Current causes and diagnostic approach	2020
Clinical Neurology and Neurosurgery	POLG-associated ataxias can represent a substantial part of recessive and sporadic ataxias in adults	2020
Journal of Clinical Investigation	Dominant mutations in mtDNA maintenance gene SSBP1 cause optic atrophy and foveopathy	2020
Multiple Sclerosis and Related Disorders	Optic nerve atrophy and whole and regional brain atrophy in Leber's hereditary optic neuropathy with multiple sclerosis-like disease with m.11778G>A mutation	2020
JAMA Neurology	Myelin oligodendrocyte glycoprotein antibody–associated demyelination comorbid with Leber hereditary optic neuropathy	2019
Mitochondrion	Complete elimination of a pathogenic homoplasmic mtDNA mutation in one generation	2019
Journal of Pediatric Neurosciences	Non-multiple-sclerosis-related typical and atypical white matter disorders: Our experience in the last 2 years in both children and adults from a tertiary care center in India	2019
BMJ Case Reports	Harding's disease: An important MS mimic	2019

Multiple Sclerosis	Relapsing remitting multiple sclerosis in progressive external ophthalmoplegia: A report of two cases	2019
Canadian Journal of Ophthalmology	Severe sequential visual loss in MS co-diagnosis of Leber's hereditary optic neuropathy	2019
Molecular Genetics & Genomic Medicine	Identification of a novel splice site mutation in the SERAC1 gene responsible for the MEGDHEL syndrome	2019
Practical Neurology	Genetic chameleons: remember the relapsing disorders	2019
Mitochondrion	The m.11778 A>G variant associated with the coexistence of Leber's hereditary optic neuropathy and multiple sclerosis-like illness dysregulates the metabolic interplay between mitochondrial oxidative phosphorylation and glycolysis	2019
Indian Journal of Radiology and Imaging	Imaging of dentate nucleus pathologies; A pictorial essay	2018
Multiple Sclerosis and Related Disorders	Mitochondrial leukoencephalopathies: A border zone between acquired and inherited white matter disorders in children?	2018
Journal of Neuroophthalmology	MRI of the optic nerves and chiasm in patients with Leber hereditary optic neuropathy	2018
Neurology Genetics	ACO2 homozygous missense mutation associated with complicated hereditary spastic paraplegia	2018
BMJ Case Reports	Leber's hereditary optic neuropathy misdiagnosed as optic neuritis and lyme disease in a patient with multiple sclerosis	2018
Brain	A novel complex neurological phenotype due to a homozygous mutation in FDX2	2018
Journal of Neurology, Neurosurgery, and Psychiatry	Practical approach to the diagnosis of adult-onset leukodystrophies: An updated guide in the genomic era	2018
Journal of Neurology, Neurosurgery, and Psychiatry	Spinal cord involvement in adult-onset metabolic and genetic diseases	2018
BMC Neurology	Subclinical Leber's hereditary optic neuropathy with pediatric acute spinal cord onset: More than meets the eye	2018
Mitochondrion	The frequency of mitochondrial polymerase gamma related disorders in a large Polish population cohort	2018
Neurologic Clinics	Common clinical and imaging conditions misdiagnosed as multiple sclerosis: A current approach to the differential diagnosis of multiple sclerosis	2018
Journal of International Advanced Otology	Repeated attacks of dizziness caused by a rare mitochondrial encephalomyopathy	2018
PLOS One	Plasma metabolomics reveals a diagnostic metabolic fingerprint for mitochondrial aconitase (ACO2) deficiency	2017

Cerebellum	Hereditary spastic paraplegia: clinical and genetic hallmarks	2017
Acta Neurologica Belgica	C10ORF2 mutation associated with progressive external ophthalmoplegia and clinically isolated syndrome	2017
International Ophthalmology	Baseline demographics, clinical features, and treatment protocols of 240 patients with optic neuropathy: Experiences from a neuro-ophthalmological clinic in the Aegean region of Turkey	2017
Parkinsonism and Related Disorders	Evolution and novel radiological changes of neurodegeneration associated with mutations in C19orf12	2017
Human Genetics	Common genetic etiology between "multiple sclerosis-like" single-gene disorders and familial multiple sclerosis	2017
Brain	LYRM7 mutations cause a multifocal cavitating leukoencephalopathy with distinct MRI appearance	2016
International Ophthalmology Clinics	Visual findings in chiasmal syndromes	2016
Journal of Neurology	Multiple sclerosis and chronic progressive external ophthalmoplegia associated with a large-scale mitochondrial DNA single deletion.	2016
Neuropediatrics	Absent thalami caused by a homozygous EARS2 mutation: Expanding disease spectrum of LTBL	2016
JAMA Neurology	Clinical, genetic, and radiological features of extrapyramidal movement disorders in mitochondrial disease	2016
Journal of Child Neurology	Methionyl-tRNA formyltransferase (MTFMT) deficiency mimicking acquired demyelinating disease	2016
Neurology Genetics	Mitochondrial cytopathy with common MELAS mutation presenting as multiple system atrophy mimic	2016
Neurology	Teaching neuroimages: Longitudinally extensive transverse myelitis in MELAS	2016
Journal of The Neurological Sciences	Sporadic hereditary motor and sensory neuropathies: Advances in the diagnosis using next generation sequencing technology	2015
Ophthalmologica	White matter changes in two Leber's hereditary optic neuropathy pedigrees: 12-Year follow-up	2015
Journal of Medical Genetics	Disturbed mitochondrial and peroxisomal dynamics due to loss of MFF causes Leigh-like encephalopathy, optic atrophy and peripheral neuropathy	2015
Canadian Journal of Ophthalmology	Leber hereditary optic neuropathy and multiple sclerosis: the mitochondrial connection	2015
Orphanet Journal of Rare Diseases	The hyperornithinemia-hyperammonemiahomocitrullinuria syndrome	2015

PLOS One	A clinical, neuropathological and genetic study of homozygous A467T POLG-related mitochondrial disease	2015
Annals of Neurology	Magnetic resonance imaging spectrum of succinate dehydrogenase related infantile leukoencephalopathy	2015
Brain	Differential diagnosis of Mendelian and mitochondrial disorders in patients with suspected multiple sclerosis	2015
Journal of Neurology, Neurosurgery and Psychiatry	A practical approach to diagnosing adult onset leukodystrophies	2014
Multiple Sclerosis (Houndmills, Basingstoke, England)	A case of neuromyelitis optica harboring both anti-aquaporin-4 antibodies and a pathogenic mitochondrial DNA mutation for Leber's hereditary optic neuropathy: clinical commentary	2014
Neurology	Novel (ovario) leukodystrophy related to AARS2 mutations	2014
Egyptian Journal of Radiology and Nuclear Medicine	Role of brain magnetic resonance spectroscopy in the evaluation of suspected mitochondrial diseases in children: Experience in 30 pediatric cases	2014
Clinical and Experimental Neuroimmunology	Topics at the 7th congress of the Pan-Asian committee for treatment and research in multiple sclerosis held at Taipei in 2014	2014
Journal of Neurology, Neurosurgery, and Psychiatry	MRI in Leber's hereditary optic neuropathy: The relationship to multiple sclerosis	2014
American Journal of Human Genetics	Mutations in APOPT1, encoding a mitochondrial protein, cause cavitating leukoencephalopathy with cytochrome c oxidase deficiency	2014
Glia	Mitochondrial dysfunction in central nervous system white matter disorders	2014
American Journal of Medical Genetics	HIBCH deficiency in patient with phenotypic characteristics of mitochondrial disorders	2014
Multiple Sclerosis	A case of neuromyelitis optica harboring both anti-aquaporin-4 antibodies and a pathogenic mitochondrial DNA mutation for Leber's hereditary optic neuropathy	2014
Neurotherapeutics	Neuromuscular and systemic presentations in adults: Diagnoses beyond MERRF and MELAS	2013
Journal of Clinical Neuroscience	POLG mutations associated with remitting/relapsing neurological events	2013
Neurology	NUBPL mutations in patients with complex I deficiency and a distinct MRI pattern	2013
Seminars In Neurology	Young-onset dementia	2013
Journal of Neuropathology and Experimental Neurology	Early-onset cataracts, spastic paraparesis, and ataxia caused by a novel mitochondrial tRNAGlu (MT-TE) gene mutation causing severe complex I deficiency: A clinical, molecular, and neuropathologic study	2013

Journal of Neurology	Impaired information-processing speed and working memory in leukoencephalopathy with brainstem and spinal cord involvement and elevated lactate (LBSL) and DARS2 mutations: A report of three adult patients	2013
Journal of Child Neurology	Masquerades of acquired demyelination in children: Experiences of a national demyelinating disease program	2013
Neurology	Clinical features of MS associated with Leber hereditary optic neuropathy mtDNA mutations	2013
Journal of Neurology	Adult-onset leukodystrophies from respiratory chain disorders: do they exist?	2013
Current Pain and Headache Reports	Migraine and neurogenetic disorders	2013
Clinical Neuroradiology	Differential diagnosis of white matter lesions: nonvascular causes-part II	2013
Metabolic Brain Disease	Mimicry between mitochondrial disorder and multiple sclerosis	2012
Journal of Magnetic Resonance Imaging	Lesions masquerading as acute stroke	2012
Journal of Neurology	Patterns of white matter diffusivity abnormalities in Leber's hereditary optic neuropathy: A tract-based spatial statistics study	2012
Molecular Genetics and Metabolism	Mitochondrial DNA depletion syndrome: New descriptions and the use of citrate synthase as a helpful tool to better characterise the patients	2012
Brain	What is influencing the phenotype of the common homozygous polymerase-gamma mutation p.Ala467Thr?	2012
Archives of Neurology	Novel infantile-onset leukoencephalopathy with high lactate level and slow improvement	2012
Nature Reviews Neuroscience	Will the real multiple sclerosis please stand up?	2012
Cerebellum	Characterizing POLG ataxia: Clinics, electrophysiology and imaging	2012
Critical Reviews in Biochemistry and Molecular Biology	Defects in mitochondrial DNA replication and human disease	2011
Folia Neuropathologica	Increased reactive oxygen species (ROS) production and low catalase level in fibroblasts of a girl with MEGDEL association (Leigh syndrome, deafness, 3-methylglutaconic aciduria)	2011
Archives Neurology	Large kindred evaluation of mitofusin 2 novel mutation, extremes of neurologic presentations, and preserved nerve mitochondria	2011
Multiple Sclerosis	Leber's hereditary optic neuropathy associated with a multiple-sclerosis-like picture in a man	2011
European Journal of Neurology	Progressive multifocal leukoencephalopathy: A review of the neuroimaging features and differential diagnosis	2011

Journal of Child Neurology	Leukoencephalopathy with brain stem and spinal cord involvement and high lactate: A genetically proven case without elevated white matter lactate	2011
Journal of Clinical Neuroscience	A novel mitochondrial DNA deletion producing progressive external ophthalmoplegia associated with multiple sclerosis.	2011
Irish Journal of Medical Science	Late-onset progressive visual loss in a man with unusual MRI findings: MS, Harding's, Leber's or Leber's Plus?	2010
Methods (San Diego, Calif.)	The clinical diagnosis of POLG disease and other mitochondrial DNA depletion disorders	2010
Archives of Neurology	POLG1 variations presenting as multiple sclerosis	2010
Journal of Neurology	Heterogeneous patterns of tissue injury in NARP syndrome	2010
Archives of Neurology	Is it ADEM, POLG, or both?	2010
The Lancet Neurology	Sporadic ataxia with adult onset: classification and diagnostic criteria	2010
BMC Neurology	POLG1 p.R722H mutation associated with multiple mtDNA deletions and a neurological phenotype	2010
Journal of Child Neurology	Leukoencephalopathy with brainstem and spinal cord involvement and normal lactate: A new mutation in the DARS2 gene	2010
Brain & Development	Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation in the first Polish patient	2010
Journal of Child Neurology	A de novo mutation in the adenosine triphosphatase (ATPase) 8 gene in a patient with mitochondrial disorder	2010
The New England Journal of Medicine	Case 9 -2010: A 37-year-old woman with paresthesias and ataxia	2010
The New England Journal of Medicine	Case records of the Massachusetts General Hospital. Case 12-2009. A 46-year-old man with migraine, aphasia, and hemiparesis and similarly affected family members	2009
Biochimica Et Biophysica Acta	DNA polymerase gamma and mitochondrial disease: understanding the consequence of POLG mutations	2009
Neuromuscular Disorders	Demyelinating disease of central and peripheral nervous systems associated with a A8344G mutation in tRNALys	2009
Blood Pressure	Recurrent posterior reversible encephalopathy syndrome in mitochondrial disorder	2009
Journal of Medical Genetics	DARS2 mutations in mitochondrial leucoencephalopathy and multiple sclerosis	2009
Clinical Neurology and Neurosurgery	A novel TYMP mutation in a French-Canadian patient with mitochondrial neurogastrointestinal encephalomyopathy	2009

Journal of Medical Genetics	Novel POLG1 mutations associated with neuromuscular and liver phenotypes in adults and children.	2009
Neuropediatrics	Two cases with progressive cystic leukoencephalopathy	2009
Seminars In Neurology	Neuroimaging of Toxic and Metabolic Disorders	2008
Practical Neurology	Leber's hereditary optic neuropathy associated with multiple sclerosis: Harding's syndrome	2008
Clinical Neurology and Neurosurgery	Leber's optic neuropathy associated with disseminated white matter disease: a case report and review	2008
Mitochondrion	Neuroimaging of mitochondrial disease	2008
Journal of Inherited Metabolic Disease	Leukoencephalopathies associated with inborn errors of metabolism in adults	2008
Journal of The Neurological Sciences	Leukoencephalopathy with brain stem and spinal cord involvement and high lactate: A genetically proven case with distinct MRI findings	2008
Neurology	Multiple sclerosis-like disorder in OPA1-related autosomal dominant optic atrophy	2008
Human Mutation	Molecular and clinical genetics of mitochondrial diseases due to POLG mutations	2008
Neuropediatrics	MR spectroscopy and serial magnetic resonance imaging in a patient with mitochondrial cystic leukoencephalopathy due to complex I deficiency and NDUFV1 mutations and mild clinical course	2008
Journal of Clinical Neuroscience	Etiological profile of presumptive optic neuritis in China	2008
European Journal of Pediatrics	Multiple oxidative phosphorylation deficiencies in severe childhood multi-system disorders due to polymerase gamma (POLG1) mutations	2007
European Journal of Neurology	White matter changes in Leber's hereditary optic neuropathy: MRI findings	2007
Lancet Neurology	Brainstem signs with progressing atrophy of medulla oblongata and upper cervical spinal cord	2007
Neuroophthalmology	Clinical features of genetically proved Leber hereditary optic neuropathy in China	2007
Brain	Phenotypic spectrum associated with mutations of the mitochondrial polymerase gamma gene	2006
Hong Kong Journal of Psychiatry	Bipolar mood disorder secondary to mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS): A case report	2006
NMR In Biomedicine	Identification of diffuse and focal brain lesions by clinical magnetic resonance spectroscopy	2006
Brain	The spectrum of clinical disease caused by the A467T and W748S POLG mutations: a study of 26 cases	2006

Chinese Medical Journal	Clinical and laboratory survey of 65 Chinese patients with Leigh syndrome	2006
Current Opinion in Neurology	Cerebral small vessel diseases: Cerebral microangiopathies	2005
Biochimica Et Biophysica Acta	Clinical and molecular findings in children with complex I deficiency	2004
Journal of Neuroophthalmology	41st Annual meeting of the Japanese neuro-ophthalmology society, Kyoto, Japan, December 12-13, 2003	2004
Neurologist	Familial multiple sclerosis and other inherited disorders of the white matter	2004
Neurology	Juvenile form of Alexander disease with GFAP mutation and mitochondrial abnormality	2004
Neurologist	Diagnostic approach in patients with symmetric imaging lesions of the deep gray nuclei	2003
European Neurology	Lack of association between Leber's hereditary optic neuropathy primary point mutations and multiple sclerosis in Iran.	2003
Seminars in Pediatric Neurology	Mitochondrial disorders and ataxia	2003
Neurology	Diagnostic criteria for respiratory chain disorders in adults and children.	2002
Acta Neurologica Scandinavica	Visual recovery in a man with the rare combination of mtDNA 11778 LHON mutation and a MS-like disease after mitoxantrone therapy	2002
European Journal of Ophthalmology	Ladies with Leber's hereditary optic neuropathy: an atypical disease	2002
European Journal of Paediatric Neurology	White matter abnormalities in Leber's hereditary optic neuropathy due to the 3460 mitochondrial DNA mutation	2002
Neurological Sciences	Neurological manifestations of gastrointestinal disorders, with particular reference to the differential diagnosis of multiple sclerosis	2001
Neurogenetics	Leber hereditary optic neuropathy: clinical and molecular genetic findings	2001
Opthalmologica	Leber's hereditary optic neuropathy mutations in Korean patients with multiple sclerosis	2001
Annals of Neurology	A SURF1 gene mutation presenting as isolated leukodystrophy	2001
American Journal of Ophthalmology	Leber hereditary optic neuropathy, progressive visual loss, and multiple-sclerosis-like symptoms	2001
Neurological Sciences	The differential diagnosis of multiple sclerosis: Classification and clinical features of relapsing and progressive neurological syndromes	2001
Journal of Neuroophthalmology	A multiple sclerosis-like illness in a man harboring the mtDNA 14484 mutation	1999
Neuroradiology	Mitochondrial diseases in children: neuroradiological and clinical features in 17 patients	1999

European Neurology	Follow-up in carriers of the 'MELAS' mutation without strokes	1998
Biochemical and Biophysical Research Communications	A novel mitochondrial DNA point mutation in the tRNA(Ile) gene: studies in a patient presenting with chronic progressive external ophthalmoplegia and multiple sclerosis	1997
Journal of The Neurological Sciences	Leber's hereditary optic neuropathy with the 11778 mtDNA mutation and white matter disease resembling multiple sclerosis: clinical, MRI and MRS findings	1995
Journal of Neurology, Neurosurgery, and Psychiatry	Bilateral simultaneous optic neuropathy in adults: clinical, imaging, serological, and genetic studies	1995
Journal of Neurology, Neurosurgery, and Psychiatry	Lebers plus - neurological abnormalities in patients with Lebers hereditary optic neuropathy	1995
Acta Neurologica Scandinavica	Leber's hereditary optic neuropathy associated with a disorder indistinguishable from multiple sclerosis in a male harbouring the mitochondrial DNA 11778 mutation	1995
Biochemical and Molecular Medicine	Leber's hereditary optic neuropathy (LHON)-related mitochondrial DNA sequence changes in Italian patients presenting with sporadic bilateral optic neuritis	1995
Journal of Neurology	Multiple sclerosis and mitochondrial myopathy: an unusual combination of diseases	1994
European Journal of Neurology	Diagnostic problems in "clinically definite" multiple sclerosis patients with normal CSF and multiple MRI abnormalities	1994
American Journal of Medical Genetics	Genetic disorders that masquerade as multiple sclerosis	1994
American Journal of Neuroradiology	Mitochondrial disorders: analysis of their clinical and imaging characteristics	1993
Journal of Inherited Metabolic Disease	Inborn errors and demyelination: MRI and the diagnosis of white matter disease	1993