Clinical Research in Genetically Determined Leukoencephalopathies: A Parenting Stress Pilot Study and Rare Disease Database Development

by

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Abstract

Leukodystrophies (LDs) and genetically determined leukoencephalopathies (gLEs) are a heterogeneous group of rare genetic disorders of the cerebral white matter. LDs are primary disorders of the cerebral white matter (i.e. glial cells, myelin sheath), while gLEs include all leukodystrophies and disorders affecting the white matter secondarily to other processes (e.g. vascular, metabolic, etc.). Their incidence is estimated to be around 1:7663 live births. They are primarily affecting the pediatric population. The diagnosis is typically determined by magnetic resonance imaging (MRI) pattern recognition, detailed clinical phenotyping, and genetic testing. Unfortunately, there are currently no known cures for most LDs and gLEs.

Considering that LDs and gLEs are generally devastating diseases and that supportive and preventative care administered by neurologists, physiotherapists, occupational and physical therapists, physiatrists, neuropsychologists, ophthalmologists, clinical geneticists, and others is the main therapeutic avenue for most of these diseases, it is surprising that only a handful of studies look at the impact of these disorders on the patients and their families. In 2018, Dr. Bernard's laboratory, the MyeliNeuroGene lab, published a study identifying specific clinical features such as sialorrhea, gastrostomy, dystonia, and wheelchair use associated with lower quality of life. To further characterize the impact of these diseases, we chose to examine parental stress in LDs and gLEs. Severe parental stress is an important outcome to delineate due to its association with parental depression and potential mismanagement of a child's disease. We performed a cross-sectional pilot study including 55 parents from 36 families to assess the levels of stress in parents with children aged 1 month to 12 years old. 34 mothers and 21 fathers participated by completing the Parenting Stress Index Fourth Edition as well as a demographic questionnaire. All patients underwent detailed clinical phenotyping. Mothers and fathers had no significant differences in stress levels. However, 20% of our parents were found to have high levels of stress while 11% had clinically significant

levels. A child's behavioral difficulties and gross motor function were found to be predictors influencing stress in mothers: these features were not found to influence fathers stress levels. This study was the first to examine parental stress in LDs and gLEs and highlights the need for parental support early in the disease course. We plan to conduct larger, prospective studies to further delineate predictors of parent's stress to be used for future clinical guidance.

In recent years, with the advances in next generation sequencing, the number of known disease-causing genes have increased significantly, opening the door for therapy development. Therefore, rare disease research has begun to pivot from gene discovery to exploration of potential therapies and cures. With impending clinical trials on the horizon, researchers are in urgent need of natural history studies to identify surrogate markers, design therapeutic trials, and define historical control patients. Our group customized a Food and Drug Administration compliant, cloud-based database for rare diseases to increase cohort sizes, delineate surrogate markers, and foster international collaborations. Ninety data entry forms were developed. A customizable clinical letter generator was created to ease the work of clinicians entering data and to assist in the continuity of patient care. This online, rare disease database will be accessible from all over the world, making it easier to collect, share, and disseminate data. We believe this Food and Drug Administration compliant database will be life-changing for patients and families when historical control data is used for emerging clinical trials.

Key words: leukodystrophy, leukoencephalopathy, parental stress, database, natural history studies,

Résumé

Les leucodystrophies (LDs) et les leucoencéphalopathies d'origine génétique (gLEs) forment un groupe hétérogène de maladies génétiques rares de la matière blanche cérébrale. Les LDs sont des maladies primaires de la matière blanche cérébrale (i.e. cellules gliales, gaine de myéline), tandis que les gLEs incluent toutes les leucodystrophies et les maladies touchant la matière blanche secondairement à d'autres mécanismes (e. g. vasculaire, métabolique, etc.). Leur incidence est estimée à 1:7663 naissances vivantes. Elles affectent principalement la population pédiatrique. Le diagnostic est typiquement déterminé par imagerie de résonance magnétique (IRM), un phénotypage clinique détaillé, et des tests génétiques. Malheureusement, il n'existe aucun traitement curatif connu pour la majorité des LDs et gLEs.

Considérant que les LDs et les gLEs sont généralement des maladies dévastatrices et que des soins de support et de prévention offerts par des neurologues, des physiothérapeutes, des ergothérapeutes, des physiatres, des neuropsychologues, des ophtalmologues, des généticiens et par d'autres sont la principale voie thérapeutique pour la majorité de ces maladies, il est surprenant qu'il existe peu d'études se concentrant sur l'impact de ces maladies sur les patients et leurs familles. En 2019, le laboratoire de Dre Bernard, le MyeliNeuroGene lab, a publié une étude identifiant des caractéristiques cliniques spécifiques comme la sialorrhée, la gastrostomie, la dystonie, et l'utilisation de la chaise roulante comme étant associés à une qualité de vie plus faible. Pour caractériser davantage l'impact de ces maladies, nous avons décidé d'examiner le stress parental dans les LDs et gLEs. Le stress parental sévère est un résultat important à souligner en raison de son association avec la dépression parentale et la mauvaise gestion possible de la maladie de l'enfant. Nous avons effectué une étude pilote transversale incluant 55 parents de 36 familles pour évaluer les niveaux de stress chez les parents avec un enfant âgé entre 1 mois et 12 ans. 34 mères et 21 pères ont participé en remplissant l'Index de stress parental ainsi qu'un questionnaire démographique. Tous les patients ont été soumis à un phénotypage clinique détaillé. Il n'y avait pas de différence significative dans les niveaux de stress des mères et des pères. Cependant, nous avons constaté que 20 % de nos parents avaient un niveau de stress élevé, tandis que 11 % avaient un niveau cliniquement significatif.

Les difficultés comportementales et la motricité globale de l'enfant se sont avérées être des facteurs prédictifs du stress chez les mères : ces caractéristiques ne se sont pas avérées affecter le niveau de stress des pères. Cette étude était la première à examiner le stress parental chez les LDs et les gLEs et elle souligne le besoin de soutien parental tôt dans l'évolution de la maladie. Nous prévoyons mener des études prospectives de plus grande envergure afin de mieux définir les prédicteurs de stress parental qui seront utilisés pour l'orientation clinique future.

Ces dernières années, grâce aux progrès réalisés dans le séquençage de nouvelle génération, le nombre de gènes pathogènes connus a considérablement augmenté, ouvrant la voie au développement de thérapies. Par conséquent, la recherche sur les maladies rares a commencé à pivoter de la découverte de gènes à l'exploration de thérapies et de remèdes potentiels. Avec l'imminence des essais cliniques à l'horizon, les chercheurs ont un besoin urgent pour des études sur l'histoire naturelle afin d'identifier des marqueurs de substitution, de concevoir des essais thérapeutiques et de définir des patients témoins historiques. Notre groupe a adapté la base de données LORIS pour qu'elle soit spécifique pour aux maladies rares, conforme aux normes de la Food and Drug Administration, afin d'augmenter la taille des cohortes, de délimiter des marqueurs de substitution et de favoriser les collaborations internationales. Quatre-vingt-dix formulaires de collecte de données ont été élaborés. Un générateur de lettres cliniques personnalisées a été créé pour faciliter le travail des cliniciens qui collectent des données et pour aider la continuité des soins aux patients. Cette base de données en ligne sur les maladies rares sera accessible à partir de partout au monde, ce qui facilitera la collecte, le partage et la diffusion des données. Nous pensons que cette base de données conforme aux exigences de la Food and Drug Administration changera la vie des patients et des familles lorsque les données de contrôle historiques seront utilisées pour les nouveaux essais cliniques.

Mots clés : leucodystrophie, leucoencéphalopathie, stress parental, base de données, études d'histoire naturelle

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I would first and foremost like to thank my supervisor, Dr. Geneviève Bernard, for taking a chance on a very expensive international student. Your belief in me surpassed my own confidence in many ways and at many times. I am incredibly thankful for the time, money, and energy you have invested into me, my future, and my family.

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Thank you to my family for supporting me. With your help I became the first in my family to graduate with a bachelor's degree and now a Master of Science. Thank you to my mom for your incessant, and often annoying, optimism about persevering. Thank you to my Aunt and Uncle, who to this day still have no clue what I do. And finally, thank you to my Grandma Dorothy who taught me how to solve puzzles and never give up.

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Preface

As an original contribution to knowledge, this thesis describes a parental stress pilot study examining clinical feature predictors in patients with genetically determined leukoencephalopathies. It also includes a second, and related, project detailing the development of a rare disease database to be piloted in the natural history study of POLR3-related leukodystrophy. **Chapter 1** is a non-exhaustive literature review and introduction to common terminology that will be used throughout this thesis. **Chapter 2 and 3** are original research papers. **Chapter 2** has been accepted for publication to the *Journal of Child Neurology* on March 20th, 2020. **Chapter 3** will be submitted shortly. **Chapters 2 and 3** contain their own abstract, introduction, methods, results, discussion, conclusion, references, supplementary information, and acknowledgement sections. Each author's contributions to each publication are detailed below. A general discussion and concluding remarks for the entire thesis are found in **Chapter 4**.

Manuscripts Included in This Thesis

Chapter 2

E. Dermer, MD*, **A. Spahr, MSc***, L. T. Tran, MSc, A. Mirchi, MD, F. Pelletier, MD, K. Guerrero, MSc1, S. Ahmed, MD, B. Brais, MD, PhD, N. Braverman, MD, MSc5, D. Buhas, MD, FRCPc, FCCMG, S. Chandratre, MD, FRCPCH, S. Chenier,MD, N. Chrestian, MD, M. Desmeules,MD, M. E. Dilenge, FRCPc, J. Laflamme, MD, A. Larbrisseau, MD, FRCPc, G. Legault, MD, K. Y. Lim, MD, C. Maftei, MD, P. Major, MD, E. Malvey-Dorn, MD, PhD, P. Marois, MD, J. Mitchell, MD, MSc, FRCPc, A. Nadeau, MD, FRCPc, B. Osterman, MD, I. Paradis, MD, D. Pohl, MD, PhD, J. Reggin, MD, E. Riou, MD, G. Roedde, MD, E. Rossignol, MD, MSc, FRCPc, G. S´ebire, MD, PhD, M. Shevell, MD, CM, FRCPc, FCHAS, M. Srour, MD, CM, PhD, M. Sylvain, MD, M. Tarnopolsky, MD, PhD, FRCPc, S. Venkateswaran, MD, FRCPc, M. Sullivan, PhD, and G. Bernard, MD, MSc, FRCPc. Stress in parents of children with genetically determined leukoencephalopathies: A Pilot Study. Journal of Child Neurology. 2020 Jul 28:0883073820938645.

***Authors contributed equal work**

Chapter 3

A. Spahr, Z. Rosli, M. Legault, L.T. Tran, L. Darbelli, C. Madjar, C. Lucia, M. St-Jean, S. Das, A. Evans, G. Bernard. LORIS MyeliNeuroGene Rare Disease Database for Rare Disease Natural History Studies and Clinical Trials Readiness. Intended to be submitted for publication by September 2020 to the Orphanet Journal of Rare Diseases.

Author Contributions

Chapter 1

AS drafted the literature review.

LC and GB reviewed, edited, and provided expertise.

Chapter 2

ED designed the study and collected data.

LT, AM, FP, and KG recruited patients and collected data.

AS analyzed all data, generated all figures, and drafted the manuscript.

MS and GB provided expertise on stress and clinical studies.

All authors read, revised, and approved the final version of this manuscript.

Chapter 3

AS generated all instruments and scoring algorithms, de-identified patient medical history records, entered all patient data and MRIs, and drafted the initial manuscript.

ZR, ML, CM, AE and SD provided expert guidance on the LORIS database system and helped develop some instruments and scoring algorithms.

LT, LD, and GB provided expert guidance in clinical research and study design.

CL and MS helped perform quality control on instruments and de-identified patient medical history records.

Chapter 4

AS drafted the general discussion and concluding remarks.

LC and GB reviewed, edited, and provided expertise.

Contributions to Original Knowledge

1. Validation of the Parenting Stress Index-4th Edition as a measure to quantify parental stress in parents with children affected by genetically determined leukoencephalopathies.
2. Characterization of predictors of parental stress for future clinical guidance.
3. Generation of phenotyping instruments to be used for rare disease natural history studies, including leukodystrophies and genetically determined leukoencephalopathies.

List of Abbreviations

18q	Chromosome 18 q
4H	Hypomyelination, Hypodontia, Hypogonadotropic Hypogonadism
7SK	Small nuclear Ribonucleic Acid 7SK
ABCD1	ATP-binding Cassette, Subfamily D, Member 1 gene
AD	Autosomal dominant
ADDH	Ataxia, Delayed Dentition, and Hypomyelination
ADL	Activities of Daily Living
AIMP1	Aminoacyl-tRNA Synthetase Complex-Interacting Multifunctional Protein 1 gene
ANOVA	Analysis of Variance
AR	Autosomal recessive
ARSA	Arylsulfatase A gene
ASPA	Aspartoacylase gene
ATP7A	ATPase, Copper -Transporting, Alpha Polypeptide gene
CFCS	Communication Function Classification System
CFR	Code of Federal Regulations
CHEO	Children's Hospital of Eastern Ontario
CHU	Centre Hospitalier Universitaire
CMTX	X-Linked Charcot Marie Tooth Disease
CNS	Central nervous system
CSF1R	Colony-Stimulating Factor 1 Receptor gene
CSV	Comma separated values (common file format)
CTX	Cerebrotendinous xanthomatosis
CYP27A1	Cytochrome P450, Subfamily XXVIIA, Polypeptide 1 gene
DARS1	Aspartyl-tRNA Synthetase 1 gene
DARS2	Aspartyl-tRNA Synthetase 2 gene
EDACS	Eating and Drinking Ability Classification System
EIF2B1-5	Eukaryotic Translation Initiation Factor 2B, Subunit one to five genes
ERCC6	Excision Repair Cross-Complementing, Group 6 gene
ERCC8	Excision Repair Cross-Complementing, Group 8 gene
FAHN	Fatty acid hydroxylase-associated neurodegeneration
FAM126A	Family with Sequence Similarity 126, Member A gene
FDA	Food and Drug Administration
FEES	Fiberoptic Endoscopic Evaluation of Swallowing
FUCA1	Fucosidase, Alpha-L, 1
GA-1	Glutaric Aciduria Type I
GALC	Galactosylceramidase
GCDH	Glutaryl-CoA Dehydrogenase
GDS	Global Dystonia Scale
GFAP	Glial Fibrillary Acidic Protein gene
GJA1	Gap Junction Protein, Alpha-1 gene

GJB1	Gap Junction Protein, Beta-1 gene
GJC2	Gap Junction Protein, Gamma-2 gene
GLA	Galactosidase, Alpha gene
GLB1	Galactosidase, Beta-1 gene
gLE	Genetically determined leukoencephalopathy
GLIA	Global Leukodystrophy Initiative
GM1	GM1 Gangliosidosis
GM2	GM2 Gangliosidosis
GM2A	GM2 Activator
GMFCS	Gross Motor Function Classification System
H-ABC	Hypomyelination with Atrophy of the Basal Ganglia and Cerebellum
HBSL	Hypomyelination with Brainstem and Spinal cord involvement and Leg Spasticity
HCAHC	Hypomyelination with Cerebellar Atrophy and Hypoplasia of the Corpus Callosum
HCC	Hypomyelination with Congenital Cataracts
HDLS	Hereditary Diffuse Leukoencephalopathy with Spheroids
HEMS	Hypomyelination of Early Myelinated Structures
HID	Human Imaging Database
HLD	Hypomyelinating leukodystrophy
HSPD1	Heat-Shock 60-KD Protein 1 gene
IDS	Iduronate 2-sulfatase gene
IDUA	Alpha-L-Iduronidase gene
LBSL	Leukoencephalopathy with Brainstem and Spinal Cord Involvement and Lactate Elevation
LD	Leukodystrophy
LONI	LONI Image Data Archive
LORIS	Longitudinal Online Research Information System
LO	Leukodystrophy with Oligodontia
MACS	Manual Ability Classification System
MAS	Modified Ashworth Scale
MELAS	Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like episodes
MitChap60	Heat-shock 60-Kilo Dalton Protein 1 (*HSPD60*-related) gene
MLC	Megalencephalic Leukoencephalopathy with Subcortical Cysts
MLC1	Modulator of volume-regulated anion channel (VRAC) Current 1 gene
MLD	Metachromatic Leukodystrophy
MPS-I	Mucopolysaccharidosis Type I
MPS-II	Mucopolysaccharidosis Type II
MPS-IIIA	Mucopolysaccharidosis Type IIIA
MRI	Magnetic resonance imaging
MT	Mitochondrial
MT-ND1	Complex I, Subunit ND1 gene
MT-ND5	Complex I, Subunit ND5 gene

MT-ND6	Complex I, Subunit ND6 gene
MT-TC	Transfer ribonucleic Acid, Mitochondrial, Cysteine gene
MT-TH	Transfer ribonucleic Acid, Mitochondrial, Histidine gene
MT-TK	Transfer ribonucleic Acid, Mitochondrial, Lysine gene
MT-TL1	Transfer ribonucleic Acid, Mitochondrial, Leucine, 1 gene
MT-TQ	Transfer ribonucleic Acid, Mitochondrial, Glutamine gene
MT-TS1	Transfer ribonucleic Acid, Mitochondrial, Serine, 1 gene
MT-TS2	Transfer ribonucleic Acid, Mitochondrial, Serine, 2 gene
NDAR	National Database for Autism Research
NGS	Next Generation Sequencing
NIH	National Institute of Health
non-HLD	Non-Hypomyelinating leukodystrophy
ODDD	Oculodentodigital Dysplasia
OMIM	Online Mendelian Inheritance in Man gene reference number
OT	Occupational Therapy
PBD	Peroxisomal Biogenesis Disorder
PEX1	Peroxisome Biogenesis Factor 1 gene
PLP	Proteolipid protein
PLP1	Proteolipid protein 1 gene
PMD	Pelizaeus-Merzbacher Disease
PMIM	Phenotype Mendelian Inheritance in Man number
PMLD	Pelizaeus-Merzbacher-like disease
PNS	Peripheral nervous system
POLR1C	Ribonucleic Acid Polymerase I Subunit C gene
POLR3	Ribonucleic Acid Polymerase III
POLR3A	Ribonucleic Acid Polymerase III Subunit A gene
POLR3B	Ribonucleic Acid Polymerase III Subunit B gene
POLR3G	
L	Ribonucleic Acid Polymerase III, Subunit G-Like gene
POLR3-HLD	Ribonucleic Acid Polymerase III-related Hypomyelinating leukodystrophy
POLR3K	Ribonucleic Acid Polymerase III Subunit K gene
PSI-4	Parental Stress Index-Fourth Edition
PT	Physical Therapy
RARS1	Arginyl-tRNA Synthetase 1 gene
RARS1-related	Arginyl-tRNA Synthetase 1 related disorders
REB	Research Ethics Board
RNA	Ribonucleic acid
SLT	Speech and Language Therapy
SASD	Sialic Acid Storage Disease
SD	Standard deviation
SGSH	N-Sulfoglucosamine Sulfohydrolyase gene

SLC16A2	Solute Carrier Family 16 (Monocarboxylic Acid Transporter), Member 2 gene
SLC17A5	Solute Carrier Family 17 (Monocarboxylic Acid Transporter), Member 5 gene
SOX10	Sex Determining Region Y-Box 10 gene
SOX10-related	Sex Determining Region Y-Box 10 related disorders
TACH	Tremor-Ataxia with Central Hypomyelination
TCS	Treacher Collin's Syndrome
tRNA	Transfer ribonucleic acid
TS%ile	Total Stress Percentile
TUBB4A	Tubulin, Beta-4A gene
U6 RNA	U6 spliceosomal Ribonucleic Acid
UFM1	Ubiquitin-Fold Modifier 1 gene
VFSS	Videofluoroscopic Swallow Study
WHO	World Health Organization
XL	X-linked

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Figure 3.12 Test battery of instruments customized for each participant based on time point and age appropriateness

Figure 3.13 Screenshot of the LORIS MyeliNeuroGene dynamic letter generator

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N/A

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Introduction and Thesis Objectives

Leukodystrophies (LDs) and genetically determined leukoencephalopathies (gLEs) are a heterogeneous group of rare genetic disorders of the cerebral white matter. LDs are primary disorders of the cerebral white matter (i.e. glial cells, myelin sheath), while gLEs include all leukodystrophies and disorders affecting the white matter secondarily to other processes (e.g. vascular, metabolic, etc.). LDs typically affect previously healthy children and lead to progressive disabilities and early death[1-3].

LD and gLE incidence is estimated to be around 1:7,663 live births[4]. The diagnosis is typically determined by magnetic resonance imaging (MRI) pattern recognition, clinical phenotyping, and genetic testing[5-8]. Leukodystrophies (LDs) are divided in two main categories according to whether the primary underlying defect involves abnormal myelin deposition during development (hypomyelinating leukodystrophies, HLDs) or abnormal myelin homeostasis (non-hypomyelinating leukodystrophies, non-HLDs)[1]. Unfortunately, there are currently no known cures for most LDs and gLEs[9], which is a phenomenon that is generalizable to most rare diseases since only approximately 5% of all rare diseases have an FDA-approved treatment[10]. For both gLEs and LDs, an interdisciplinary team of health care professionals is often required including a neurologist, physiotherapist, occupational and physical therapist, physiatrist, neuropsychologist, ophthalmologist, clinical geneticist, and others[4,11].

Considering that LDs and gLEs are generally devastating diseases and that supportive and preventive care is the main therapeutic avenue for most of these diseases, it is surprising that there are only a handful of studies looking at the impact of these diseases on the patients and their families. In 2018, our group, the MyeliNeuroGene lab, published a study identifying specific clinical features, sialorrhea, gastrostomy, dystonia, and wheelchair use, that correlated with lower

quality of life[12]. To further characterize the impact of these diseases on patients and their families, we chose to examine parental stress in this patient population. Severe parental stress is an important outcome to delineate due to its association with parental depression and potential mismanagement of the child's disease[13,14].

In recent years, with the advances of next generation sequencing and the identification of numerous disease-causing genes, the leukodystrophy research field has switched from gene discovery to therapeutic development. With the development of novel therapies, the research community has realized that we are not ready to assess if therapies are efficient because we have only little understanding of the natural history of most of these diseases. With this in mind, several years ago, Dr. Bernard, together with numerous international collaborators, has started to collect natural history data and patient-derived outcomes in a prospective fashion. The complex clinical, behavioural, genetic, and imaging datasets that we research prompted our lab to develop a rare disease database.

To address the previously mentioned knowledge gaps, i.e. the impact of LDs and gLEs on patients and families as well as the lack of natural history data, this project included two subprojects. The first subproject is a pilot study analyzing stress in parents of children with LDs and gLEs. This cross-sectional study included 55 parents from 36 families followed at the Leukodystrophy and Neurometabolic Disorders Clinic at the McGill University Health Centre Research Institute.. The second subproject was the development of a rare disease database in collaboration with the Longitudinal Online Research and Imaging System (LORIS) located at the McGill Centre for Integrative Neuroscience. The database developed will be used for all rare diseases studied in Dr. Bernard's lab and will be made available to our local, provincial, national and international collaborators. We opted to design the pilot version of this database to primarily

focus on one of the most common LDs, RNA Polymerase III-Hypomyelinating Leukodystrophy (POLR3-HLD) or 4H leukodystrophy[15].

Chapter 1: Literature Review

1.1 Preface

This chapter provides a non-exhaustive literature review regarding genetically determined leukoencephalopathies, leukodystrophies, POLR3-related leukodystrophy, parental stress, and rare disease databases. Each will be discussed in their own sub-section.

1.2 Leukoencephalopathies

1.2.1 Leukoencephalopathy classification

The term *leuko-* comes from the Greek word *leukos* meaning "clear" or "white". "Encephalopathy" refers to any disorder or disease of the brain. Leukoencephalopathy, therefore, defines disorders of cerebral white matter[1]. Common terms applying to leukoencephalopathies include demyelinating, toxic, infectious, vascular, etc[1]. Multiple sclerosis is one of the most familiar central nervous system (CNS) leukoencephalopathies[16].

The term "genetically determined leukoencephalopathy" (gLE) defines disorders that are inherited and either primarily involve the white matter (leukodystrophies, see below) or secondarily involve the white matter (e.g. primary neuronal, vascular or metabolic disorders)[1]. Genetically determined leukoencephalopathies include diseases such as GM1[17,18] and GM2-gangliosidosis[19] as well as vascular diseases such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)[20] and cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL), etc[21]. A non-exhaustive list of genetically determined leukoencephalopathies is shown in Table 1.1.

Table 1.1 Non-exhaustive List of Genetically Determined Leukoencephalopathies (gLEs): This table includes a non-exhaustive list of gLEs (in alphabetic order)with their inheritance, causative-gene(s) and respective Phenotype MIM (PMIM) disease numbers.

Disease Name	PMIM	Inheritance	Genes
AIMP1-related	260600	AR	*AIMP1*[22]
AIMP2-related	618006	AR	*AIMP2*[23]
Allan-Herndon-Dudley Syndrome	300523	XL	*SLC16A2*[24]
CADASIL	125310	AD	*NOTCH3*[20]
CARASIL	600142	AR	*HTRA1*[21]
Fabry Disease	301500	XL	*GLA*[25]
Glutaric Aciduria Type I (GA-I)	231670	AR	*GCDH*[26]
GM1 and GM2-Gangliosidosis, Infantile onset	230500, 272750	AR	*GLB1*[17,18], *GM2A*[19]
HSPD60-related (MitChap60)	612233	AR	*HSPD1*[27,28]
Menkes Syndrome	309400	XL	*ATP7A*[29]
Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke (MELAS)	540000	MT	*MT-TL1*[30], *MT-TQ*[31], *MT-TH*[32], *MT-TK*[33], *MT-TC*[34], *MT-TS1*[35], *MT-ND1*[36], *MT-ND5*[37], *MT-ND6*[38], *MT-TS2*[39]
MPS-I	607014, 607015, 607016	AR	*IDUA*[40]
MPS-II	309900	XL	*IDS*[37]
MPS-IIIA	252900	AR	*SGSH*[41]
X-Linked Charcot Marie Tooth Disease (CMTX)	302800	XL	*GJB1*[42]

Abbreviations: XL: X-linked; AR: autosomal recessive; MT: mitochondrial inheritance

1.2.2 Leukodystrophy classification

The Greek origin terms *dys-,* meaning defective, and *-trophia,* meaning nourishment, were used together to form "leukodystrophy", referring to disorders with wasting, or malnutrition, of the brain's white matter[1]. A formal definition for leukodystrophies was published in 2015 with the help of a panel of experts from the Global Leukodystrophy Initiative (GLIA)[43], a consortium of clinicians and scientists, private industry stakeholders, and advocacy groups working together to

advance therapeutic strategies for leukodystrophies and gLEs[44]. They defined leukodystrophies as genetically determined disorders affecting the cerebral white matter of the CNS with or without peripheral nervous system (PNS) involvement[1]. Leukodystrophies primarily affect glial cells and the myelin sheath (i.e. oligodendrocyte precursor cells, oligodendrocytes), but may also involve, to a lesser extent, other cell types such as the neuron[1].

Leukodystrophies are often categorized as hypomyelinating (Hypomyelinating Leukodystrophy; HLD) and non-HLD, depending on brain MRI characteristics and whether the pathophysiological mechanism is lack of myelin deposition during development or abnormal myelin homeostasis, respectively[5,6]. In the brains of healthy individuals, white matter is hyperintense compared to gray matter structures on T1-weighted imaging[45]. Conversely, on T2-weighted imaging, white matter appears hypointense compared to gray matter. In hypomyelinating leukodystrophies, the white matter appears hyper-, iso- or slightly hypo-intense on T1 while on T2, it appears mildly hyperintense, compared to grey matter structures[5,6]. In non-HLD, white matter lesions are prominently hypointense on T1 and hyperintense on T2, compared to grey matter structures (Figure 1.1)[5,6].

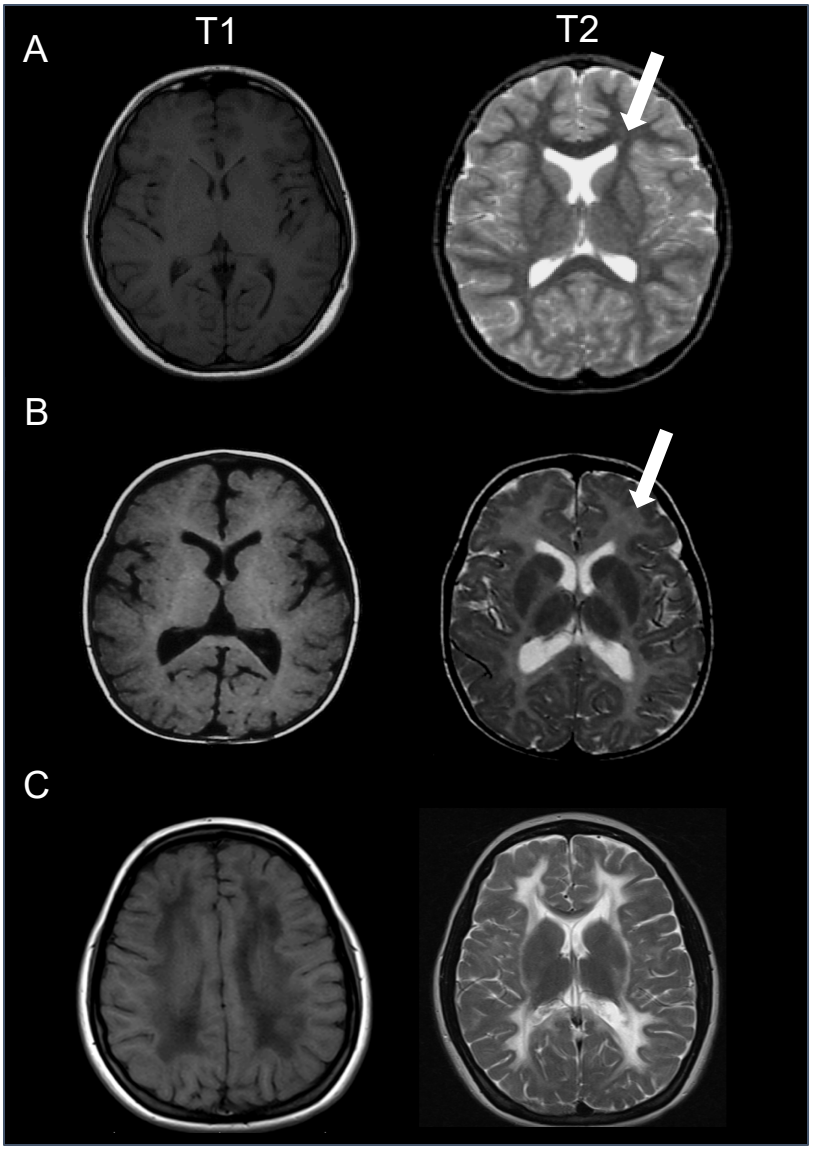

Figure 1.1 Brain MRIs of a healthy individual and individuals with hypomyelinating and non-hypomyelinating leukodystrophies: An axial view of T1 and T2 weighted MRI. Image A shows a healthy brain; B shows a hypomyelinating brain; C shows a non-hypomyelinating brain. The white arrows point to white matter. This figure has been adapted from presentations by Dr. Geneviève Bernard.

1.2.3 Hypomyelinating leukodystrophies

HLDs are a group of neurodegenerative disorders that are characterized by a permanent deficit of the myelin sheath in the CNS: the disorders were described by Drs. Schiffmann and van der Knaap and colleagues in the late 1990's[5,6,46]. The prototypical HLD is Pelizaeus-Merzbacher disease (PMD) (PMIM #312080), caused by X-linked recessive mutations in *PLP1* (OMIM

#300401), encoding proteolipid protein (PLP)[47,48]. Typical clinical manifestations include developmental delay, nystagmus, hypotonia, spasticity, ataxia, and cognitive impairment[49-51]. A non-exhaustive list of Hypomyelinating Leukodystrophies are shown in Table 1.2.

Table 1.2 Non-exhaustive List of Hypomyelinating Leukodystrophies (HLDs): This table includes a non-exhaustive list (in alphabetical order) of hypomyelinating leukodystrophies with inheritance, associated gene(s) or chromosomal abnormality and PMIM disease numbers.

Disease Name	PMIM	Inheritance	Genes
18q- syndrome	601808	Sporadic, AD	18q[52]
Cockayne Syndrome	133540, 216400	AR	*ERCC6*[53], *ERCC8*[54]
EPRS1-related	617951	AR	*EPRS1*[55]
Fucosidosis	230000	AR	*FUCA1*[56]
Hypomyelination of Early Myelinating Structures (HEMS)	N/A	XL	*PLP1*[57]
Hypomyelination with Atrophy of the Basal Ganglia and Cerebellum (H-ABC)	612438	Sporadic, AR	*TUBB4A*[58], *UFM1*[59]
Hypomyelination with Braintem and Spinal cord involvement and Leg spasticity (HBSL)	615281	AR	*DARS1*[60]
Hypomyelination with Congenital Cataracts (HCC)	610532	AR	*FAM126A*[61]
Oculodentodigital Dysplasia (ODDD)	164200	AD	*GJA1*[62]
Pelizaeus-Merzbacher Disease (PMD)	312080	XL	*PLP1*[63,64]
Pelizaeus-Merzbacher-like (PMLD)	608804	AR	*GJC2*[65]
POLR3-related (POLR3-HLD, 4H)	607694, 614381, 616494	AR	*POLR3A*[66], *POLR3B*[67], *POLR1C*[68], *POLR3K*[69]
RARS1-related	616140	AR	*RARS1*[70]
Sialic Acid Storage Disease (SASD)	604369	AR	*SLC17A5*[71]
SOX10-related	609136	Sporadic	*SOX10*[72]

Abbreviations: XL: X-linked; AR: autosomal recessive; AD: autosomal dominant;

1.2.4 Non-hypomyelinating leukodystrophies

In non-HLDs, myelin deposition in the brain occurs normally until myelin homeostasis is interrupted[1,73]. One example of non-HLD is Vanishing White Matter Disease (VWM) (PMIM #603896) caused by mutations in the genes *EIF2B1*, *EIF2B2*, *EIF2B3*, *EIF2B4*, and *EIF2B5* (OMIM #606686, 606454, 606273,606687, 603945)[74]. Another example of a non-HLD is Metachromatic Leukodystrophy (MLD) (PMIM #250100) caused by mutations in the *ARSA* (OMIM #607574) gene which encodes for the enzyme arylsulfatase A[75-79]. This lysosomal enzyme is responsible for breaking down sulfatides, and if impaired result in the accumulation of toxic levels of sulfatides in the nervous system and deregulation of myelin homeostasis[80]. A non-exhaustive list of non-Hypomyelinating Leukodystrophies are shown in Table 1.3.

Table 1.3 Non-exhaustive List of non-Hypomyelinating Leukodystrophies (non-HLDs): This table includes a non-exhaustive list (in alphabetical order) of non-HLDs with their inheritance, associated genes and PMIM disease numbers.

Disease Name	PMIM	Inheritance	Genes
Adrenoleukodystrophy	300100	XL	*ABCD1*[82,83]
Aicardi-Goutières syndrome	225750, 610333, 610181	AD, AR	*TREX1*[94], *RNASEH2A*[95], *RNASEH2B*[95], *RNASEH2C*[95]
Alexander Disease	203450	Sporadic, AD	*GFAP*[86]
Autosomal Dominant Leukodystrophy with Autonomic Disease	169500	AD	*LMNB1*[93]
Canavan Disease	271900	AR	*ASPA*[84]
Cerebroretinal microangiopathy with calcifications and cysts	612199	AR	*CTC1*[96]
Cerebrotendinous xanthomatosis (CTX)	213700	AR	*CYP27A1*[85]
Hereditary Diffuse Leukoencephalopathy with Spheroids (HDLS)	221820	AD	*CSF1R*[88]
Krabbe	245200	AR	*GALC*[81]
Leukoencephalopathy with Brainstem and Spinal Cord Involvement and Lactate Elevation (LBSL)	611105	AR	*DARS2*[87]
Megalencephalic Leukoencephalopathy with subcortical Cysts (MLC)	604004	AR	*MLC1*[89], *HEPACAM*[90]
Metachromatic Leukodystrophy	250100	AR	*ARSA*[75-79]
Mitochondrial Leukoencephalopathies	301020. 618225, 618228	XL, AR	*NDUFA1*[97], *NDUFV1*[98], *NDUFS2*[99], etc.
Peroxisome Biogenesis Disorders	214100, 614866, 614882	AR	*PEX1*[100], *PEX2*[101], PEX3[102], etc.
Vanishing White Matter Disease	603896	AR	*EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5*[74,91,92]

Abbreviations: XL: X-linked; AR: autosomal recessive; AD: autosomal dominant

1.2.5 Alternative Classification Methods

There are alternative classification systems for leukodystrophies, which is why the terminology is conflicting in the literature. The most recently proposed system by van der Knaap et al. defines leukodystrophies as genetically determined disorders of the cerebral white matter and

therefore includes LDs and gLEs and further divides them into five main categories based mainly on cell type; myelin disorders (i.e. oligodendrocyte defects), astrocyte disorders, neuronal or axonal defects, microglia defects, and disorders due to vascular pathology[73]. The advantage of this classification is its inclusiveness and association with a pathogenesis process. The disadvantage of this classification is its reliability on pathological studies, which are not available or well-defined for all diseases.

1.2.6 MRI-Pattern Recognition and Diagnosis of White Matter Disorders

Diagnosing white matter diseases can be difficult due to their phenotypic and genotypic heterogeneity, as seen above. LDs and gLEs can occur at any age, whether inherited or acquired[81,82]. Over the past decade, brain MRI has proven to be the "foundational investigation"[44] when patients are suspected to be affected by white matter disorders[6,44,83-86].

A normal, healthy, myelinated brain has hyperintense (i.e. brighter) signal compared to gray matter structures on T1-weighted images. On T2-weighted images, the inverse is true, where the white matter is hypointense relative to gray matter structures[45,87]. In hypomyelinating leukodystrophies, the white matter is hyper-, iso- or slightly hypointense on T1 and hyperintense on T2, compared to grey matter structures. In non-HLDs, the white matter is significantly hypointense on T1 and hyperintense on T2, compared to grey matter structures. (Figure 1.1)[45,87].

Myelination begins in utero and progresses through adult life. However, the majority of myelin formation occurs in the first two years of life.[45,87,88]. Therefore, in a child younger than two years, two MRIs spaced by at least 6 months are needed to differentiate delayed myelination and permanent hypomyelination[44].

MRI pattern recognition goes beyond the differentiation of HLD, myelination delay and non-HLD. Indeed, different diseases have different MRI patterns, i.e. characteristic involvement or preservation of specific structures[6]. This is beyond the scope of this thesis, but as an example, the MRI pattern of POLR3-related leukodystrophy is very specific and allows a trained eye to differentiate this disorder from other HLDs (REF, see below for details).

1.2.7 POLR3-HLD / 4H Leukodystrophy

POLR3-HLD, an autosomal recessive disease, is named after the causal mutated genes encoding for subunits of the protein complex RNA polymerase III that is responsible for transcribing noncoding RNA from DNA[89-91]. The term 4H arises from the common clinical features seen in these patients: hypomyelination, hypodontia, and hypogonadotropic hypogonadism[92]. Prior to the genetic discovery of POLR3-HLD, it was described as five distinct diseases with overlapping phenotypes[15]. These 5 diseases were as follows: (1) leukodystrophy with oligodontia (LO)[93,94], (2) ataxia, delayed dentition, and hypomyelination (ADDH)[95,96], (3) hypomyelination, hypodontia, hypogonadotropic hypogonadism (4H syndrome)[97-99], (4) hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum (HCAHC)[100,101], and (5) tremor-ataxia with central hypomyelination (TACH)[66,67,102,103].

POLR3-HLD is defined by a variety of neurological and non-neurological clinical features, as well as a specific MRI pattern[66-68,92]. Patients typically present with developmental delay, most often motor delay. Other neurological features include motor regression, cerebellar signs, pyramidal signs, and cognitive delay/regression. Non-neurological features include dental abnormalities (e.g. hypodontia, oligodontia, neonatal teeth, etc.), hypogonadotropic hypogonadism (i.e. delayed or arrested puberty), myopia, and short stature with or without growth hormone deficiency[104,105]. Characteristic MRI patterns include diffuse hypomyelination with

relative preservation of specific white matter structures such as the dentate nucleus, optic radiations, ventrolateral thalamus, globus pallidus and, in some patients, the corticospinal tracts at the level of the posterior limb of the internal capsule[5,84]. These features can be seen in Figure 1.2. Other MRI signs include a thinning corpus callosum as well as cerebellar atrophy[44,92]. A POLR3-HLD MRI scoring system was developed in 2017 to assess hypomyelination and atrophy[106]. This scoring system was designed as a potential outcome measure for future clinical trials.

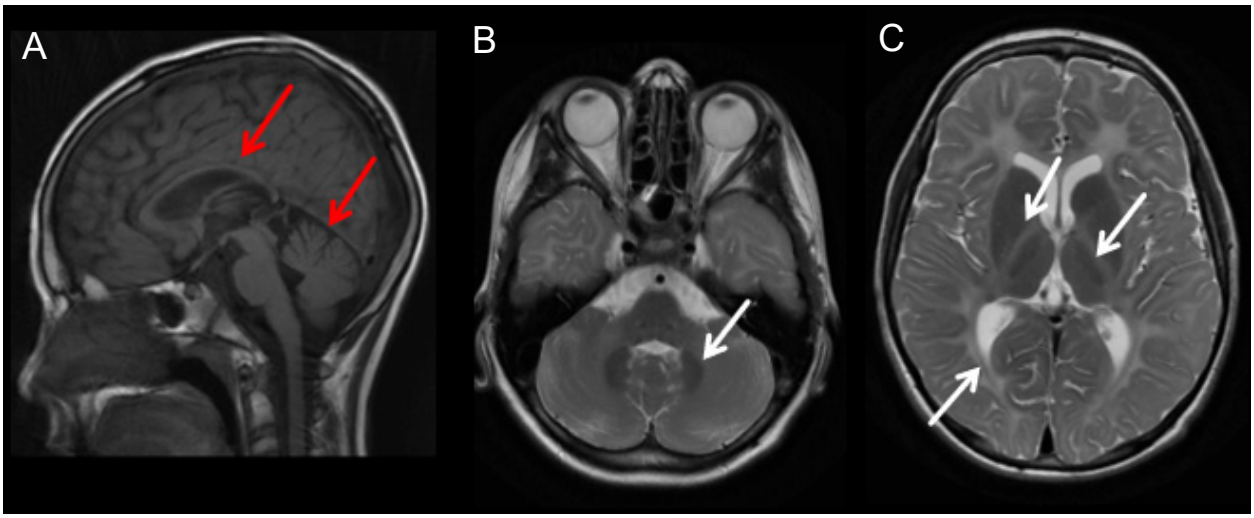

Figure 1.2 Brain MRI characteristics seen in patients with POLR3-related leukodystrophy: A) sagittal MRI of the brain, T1-weighted sequence, at the level of the midline. The red arrows highlight mild superior cerebellar atrophy and thin corpus callosum. B) axial T2-weighted image at the level of the pons showing hypomyelination of the cerebellum, pons and superior cerebellar peduncles, with relative preservation of the dentate nucleus (white arrow). C) axial T2-weighted image at the level of the basal ganglia showing diffuse hypomyelination with relative preservation of the optic radiations, anterolateral nucleus of the thalamus and globus pallidus (white arrows). This figure has been adapted from presentations by Dr. Geneviève Bernard.

Our understanding of the pathophysiological mechanisms of this disease remains incomplete. Functional studies are currently being conducted with the hopes of elucidating how mutations in these genes lead to POLR3-HLD. Previous studies have shown there are three RNA polymerases, aptly named RNA polymerase I, II, and III, each with their own unique functions and targets[90]. RNA polymerase III transcribes all tRNAs, 5S ribosomal RNA, 7SK RNA, U6 RNA, and other small noncoding RNA[107]. RNA polymerase III has 17 subunits, with POLR3A and

POLR3B being the two largest subunits and forming the catalytic core[91]. POLR1C is a shared subunit between RNA polymerase I and III[91]. These three subunits are highlighted because the vast majority of patients affected by POLR3-HLD have mutations in either *POLR3A, POLR3B,* or *POLR1C*[15,68,108]. Recently, however, mutations in *POLR3K* have been found in patients with POLR3-related leukodystrophy[69].

POLR3A mutations are most often found in French-Canadian individuals whereas *POLR3B* mutations are typically seen in individuals of European descent[15]. Patients affected by POLR3-HLD caused by *POLR3B* variants have an earlier onset but a milder progression relative to patients with *POLR3A* or *POLR1C* variants[92]. Typically, patients with *POLR3B* mutations are more prevalent than patients with *POLR3A* mutations, 49% and 41% respectively[15,92,108-112]. POLR3-HLD caused by variants in *POLR1C* is seen the least frequently with only about 5% relative incidence[15,68]. Patients with *POLR1C* mutations have a more severe phenotype, compared to patients with *POLR3A* or *POLR3B* mutations[113]. No genotype phenotype correlation can be done with patients affected by mutations in *POLR3K* because only 2 patients have been published[69].

Since the discovery of the POLR3-related causal genes in 2011[66,67], 2015[68] and 2018[69], the disease spectrum has expanded quite significantly. Patients with Wiedemann Rautenstrauch syndrome, a neonatal progeria syndrome, have been reported with mutations in *POLR3A*[114-117]. A severe infantile form[118], a striatal form[119-121] and later onset forms consisting of spastic ataxia and spastic paraparesis have also been reported[122,123]. Regarding the MRI characteristics, patients without hypomyelination have been reported[111,120,124] as well as patients with specific basal ganglia involvement[118,119,121].

Biallelic pathogenic variants in *POLR1C* have been reported to cause Treacher-Collins syndrome (TCS)[125]. Our group reported, in 2015, a series of patients with POLR3-HLD with

biallelic variants in *POLR1C*[68] and in 2019 we published the clinical spectrum of POLR3-HLD caused by mutations in *POLR1C*[113]. It was found that a proportion of patients (5/23, 22%) had a combination of hypomyelinating leukodystrophy and abnormal craniofacial development reminiscent of Treacher-Collins syndrome and one patient with POLR3-HLD also had typical craniofacial abnormalities indicative of TCS[113].

Biallelic variants in *POLR3GL* have been found in three patients affected by endosteal hyperostosis, oligodontia, short stature, and facial dysmorphissms[124].Additionally, an individual with biallelic pathogenic variants in *POLR3GL* suffering from a neonatal progeroid syndrome, known as Wiedemann-Rautenstrauch syndrome, was published in 2020[126]. Previously patients affected by Wiedemann-Rautenstrauch syndrome were thought to be caused by biallelic variants in *POLR3A*[114-116]. These findings further expand the phenotypic spectrum of POLR3-related disorders.

1.3 Characterizing Parental Stress in Parents of Children with gLEs

For both gLEs and LDs, an interdisciplinary team of health care professionals is required to improve quality of life including a neurologist, physiotherapist, occupational and physical therapist, physiatrist, neuropsychologist, ophthalmologist, clinical geneticist, and others[11]. Surprisingly, few studies have systematically examined parental stress, nor its effects, in this patient population despite the severity and progressive nature of these diseases. In other studies characterizing stress, parents of children with neurodevelopmental disorders or functional impairments experience greater levels of stress compared with parents of healthy children[127-129]. Factors such as pediatric depression and maladaptive behaviours have also been associated with greater parenting stress in disorders such as cerebral palsy and epilepsy[128,130]. Studies have shown that increased parenting stress is correlated with several negative outcomes including parental

depression, maladaptive parenting practices, and potential mismanagement of a child's symptoms and disease[13,14]. Through anticipatory and supportive child/family care, these factors can be addressed and potentially alleviated[9,131]. Therefore, it is imperative that stress is characterized in this patient population to provide better guidance on best clinical practices.

The World Health Organization defines health as physical, mental, and social well-being whereas quality of life is an individual's perception of their health within the context of their culture and social value system[132]. In 2018, our lab and collaborators published a study characterizing the quality of life of patients with gLEs[12]. This study found that clinical features such as sialorrhea, gastrostomy, dystonia, and wheelchair-use correlated with lower health-related quality of life[12]. Parents were also identified with specific emotional functioning problems, namely trouble sleeping and excessive worrying about the prognosis of their child's progressive disease[12]. In a natural progression from the health-related quality of life, our lab has studied parental stress in a cohort of parents with children who have LDs and gLEs, followed at the Leukodystrophy and Neurometabolic Disorders Clinic at the McGill University Health Centre Research Institute. The manuscript can be found in **Chapter 2.**

1.4 Development of the LORIS MyeliNeuroGene Rare Disease Database

The Centers for Disease Control and Prevention define the natural history of a disease as the progression of a disease over time without treatment[133]. For genetically determined diseases, this may include age at disease onset, clinical manifestations such as developmental disability and their evolution, and age of death[133]. Unfortunately, natural history data is lacking for many rare diseases, such as LDs and gLEs[134,135]. To adequately store and homogenize diverse natural history data such as demographic data, diagnosis and genetic data, clinical phenotyping, behavioral data,

imaging data, and patient and parent reported outcomes, we developed a novel rare disease database using Longitudinal Observation Research and Imaging System (LORIS)[136].

LORIS is a browser-based data management system for multi-center studies that was originally developed for the National Institute of Health (NIH) MRI study of Normal Brain Development[137] launched by Dr. Alan C. Evans at the Montreal Neurological Institute at the McGill Centre for Integrative Neuroscience[138]. It facilitates data acquisition, storage, processing, and dissemination. It features a cloud-based design that allows easier collaboration with local and international researchers that can log-in from anywhere in the world to confidentially share patient data[136]. Data entry is quality controlled across modalities by validating ranges of acceptable values, authenticating proper entry (numerical vs strings), and authorizing users. Results can be easily queried, aggregated, and retrieved as CSV files for further analysis and dissemination.

A unique feature of LORIS is the ability for students, researchers, project managers, and principal investigators to create their own data entry forms without the need for a computer scientist or developer. Alternatively, with the help of a computer scientist, scoring algorithms can be developed to automatically score and validate patient progress. In addition, LORIS' "system architecture" is organized around a "subject profile"[136]. This allows project managers to easily filter information for large cohorts, projects, and subprojects. Each subject profile consists of "timepoints" that store a battery of instruments and data collected during a specific visit. This allows for longitudinal analysis, which is imperative for natural history data collection.

Data in the LORIS ecosystem is typically divided into two categories: (1) non-imaging (demographic, phenotyping and behavioural data, and patient and family reported outcomes) and (2) imaging datasets[136]. All non-imaging data is collected by "instruments" or questionnaires.

Imaging data is also stored with each subject's corresponding timepoint for longitudinal analysis with an imaging browser available within LORIS for radiological review and quality control.

We elected to develop the MyeliNeuroGene database using LORIS and to pilot it using a subset of the data gathered on POLR3-HLD. Clinical trials in rare diseases often rely on historical controls, and in the case of POLR3-HLD potential therapeutics will solely leverage historical natural history data entered into the LORIS database to serve as the control arm of a clinical trial, a trend supported by several studies[139-142]. The manuscript to be submitted to The Orphanet Journal of Rare Disease can be found in **Chapter 3**.

1.5 Conclusions

The MyeliNeuroGene lab was founded in 2011 and has since conducted numerous studies characterizing LDs and gLEs, planning for future clinical trials. **Chapter 2** is a study which examined parental stress and analyzed predictors for clinical guidance. Our lab focuses many resources on POLR3-HLD and has begun to elucidate the pathophysiology of this disease and to develop therapies. However, without natural history data, clinical trials evaluating treatment efficacy will be unable to commence. **Chapter 3** is a study detailing database development. The MyeliNeuroGene LORIS database will facilitate natural history studies by recording and organizing vast volumes of diverse clinical and imaging data allowing the elucidation of disease progression.

Chapter 2 Parental Stress Manuscript

A Pilot Study Analyzing Stress in Parents of Children with Genetically Determined Leukoencephalopathies

2.1 Preface

Few studies have investigated parental stress in LDs and gLEs. As a natural progression from our lab's quality of life study published in 2018, the work in this chapter focuses on characterizing parental stress using the Parental Stress Index-4th Edition and delineating predictors for stress for future clinical guidance.

2.2 Title Page

Stress in Parents of Children With Genetically Determined Leukoencephalopathies: A Pilot Study

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2.3 Abstract

Genetically determined leukoencephalopathies comprise a group of rare inherited white matter disorders. The majority are progressive diseases resulting in early death. We performed a cross-sectional pilot study including 55 parents from 36 families to assess the level of stress experienced by parents of patients with Genetically determined leukoencephalopathies, aged 1 month to 12 years. Thirty-four mothers and 21 fathers completed the Parenting Stress Index 4th Edition. One demographic questionnaire was completed per family. Detailed clinical data was gathered on all patients. Statistical analysis was performed with total stress percentile score as the primary outcome. Mothers and fathers had significantly higher stress levels compared to the normative sample; 20% of parents had high levels of stress whereas 11% had clinically significant levels of stress. Mothers and fathers had comparable total stress percentile scores. We identified pediatric behavioral difficulties and gross motor function to be factors influencing stress in mothers. Our study is the first to examine parental stress in this population and highlights the need for parental support early in the disease course. In this pilot study, we demonstrated that using the Parenting Stress Index-4th Edition to assess stress levels in parents of patients with genetically determined leukoencephalopathies is feasible, leads to valuable and actionable results, and should be used in larger, prospective studies.

Keywords: leukodystrophy, pediatric, behavior, neurodevelopment, outcome; quality of life, parental stress, genetically determined leukoencephalopathies

2.4 Introduction

Leukodystrophies and genetically determined leukoencephalopathies comprise a group of rare inherited white matter disorders primarily affecting the pediatric population.[1,2] Leukodystrophies are genetic diseases characterized by abnormal cerebral white matter and are classified as hypomyelinating and non-hypomyelinating diseases, depending on whether the primary underlying defect is insufficient myelin deposition during development or abnormal myelin homeostasis, respectively. Genetically determined leukoencephalopathies include all leukodystrophies, caused by primary myelin defects, and inherited white matter diseases caused by other mechanisms.[1,2] Specifically, the classification of genetically determined leukoencephalopathy is determined when neuronal, vascular, or systemic manifestations overshadow white matter abnormalities.[1] The majority of genetically determined leukoencephalopathies are progressive diseases for which no known curative therapy is available. They lead to motor, cognitive, and behavioral dysfunction and often early death.[1,2] Their consequences are broad-ranging, and affect patients, their families, caretakers and the health care system.[3]

Due to the significant and often progressive limitations experienced by patients affected by gLEs, the parents or caregivers must constantly adapt to their child's needs. Surprisingly, no previous studies have systematically examined parental stress in this patient population. Studies examining parental stress in other diseases have demonstrated that parents of children with neurodevelopmental disorders or functional impairments experience greater stress compared to parents of healthy children.[4-9] In these patient populations, factors such as a child's depression[10] and maladaptive behaviors[5] are associated with greater stress in the parents and reduced familial quality of life.[7] It is vital to understand the contribution of these various factors to parental stress to address them in the child/family anticipatory and supportive care plan.[11] Indeed, increased

parental stress has been correlated to several negative outcomes including parental depression[12], maladaptive parenting practices, and potential mismanagement of a child's symptoms and disease.[13] Moreover, it has been shown that proper identification and management of parents that are under high levels of stress can have beneficial impacts on a family's quality of life and on the child's behavior.[14]

In this cross-sectional pilot study, we characterized stress experienced by parents of children affected by genetically determined leukoencephalopathies, along with demographic and clinical factors correlating with higher parental stress and demonstrated the feasibility of the Parenting Stress Index-4th Edition for future larger prospective studies.

2.5 Materials and Methods

Participants. The study sample consisted of 55 parents of 36 families with genetically determined leukoencephalopathies. In accordance with the Parenting Stress Index 4th Edition (PSI-4) Manual, parents were eligible to complete the questionnaire if their child was between 1 month and 12 years of age. All parents were either primary or secondary caregivers. All patients were followed at the Montreal Children's Hospital of the McGill University Health Center in Montreal, Quebec, Canada. Patients were enrolled in the study between 2016 and 2018. Patients were included if they had a molecular diagnosis of gLE or evidence of gLE based on the brain MRI pattern without an identified molecular diagnosis.[15] Written informed consent was obtained from all research participants. This study was approved by the Research Ethics Board of the McGill University Health Center Research Institute (11-105-PED, 2019-4972). Exclusion criteria included the inability of the parents to understand English or French.

Clinical history and examination. Electronic and paper charts were reviewed for each patient. The following clinical features were recorded: patient's age, diagnosis, time from

diagnosis to questionnaires, sialorrhea, and presence of significant behavioral difficulties. Clinical notes were also used to evaluate the degree of the child's disability, using the Gross Motor Function Classification System (GMFCS),[16] Manual Ability Classification System (MACS),[17] Eating and Drinking Ability Classification System (EDACS),[18] and Communication Function Classification System (CFCS),[19] where higher scores indicate increased disability (Table 1).[20]

Functional Scale	Level 1	Level 2	Level 3	Level 4	Level 5
GMFCS	Walks without limitation	Walks with some limitations	Uses assistive mobility devices irregularly	Uses assistive mobility devices regularly	No independent mobility
MACS	Handles objects independently	Independent but with reduced quality	Often requires external help	Can only perform certain actions	Can only perform basic actions
EDACS	Eats and drinks independently, safely, and efficiently	Eats and drinks independently and safely, but with reduced quality	Limitations to safety and efficiency, with some risk of choking	Significant limitations to safety, high risk of choking	Unable to eat or drink without feeding tube
CFCS	Sends and receives info with unfamiliar individuals	Sends and receives info with reduced quality	Sends and receives info with familiar individuals	Has difficulty sending and receiving info with familiar individuals	Rarely sends or receives info with familiar individuals

Table 2.1: Summary of Functional Scales Abbreviations: GMFCS: Gross Motor Function Classification System; MACS: Manual Ability Classification System; EDACS: Eating and Drinking Ability Classification System; CFCS: Communication Function Classification System; info: information

Parenting Stress Index 4th Edition (PSI-4). The PSI-4 evaluates the magnitude of stress in a parent-child system for children aged 1 month to 12 years.[21] The test is designed to be completed in approximately 20 minutes. The inter-rater and intra-rater reliability coefficients are reported as 0.96 or higher.[21] The PSI-4 has been validated for completion by parents of children in numerous patients' population, including United States and French-Canadian.[22-27] The PSI-4 includes 120

statements focusing on three major domains affecting stress: child characteristics, parental characteristics and situational/demographic life stress. Each statement is rated on a five-point Likert scale, in which the parent or caregiver is asked to rate the degree to which they agree or disagree (1 = Strongly Agree to 5 = Strongly Disagree). A raw score is generated for each subscale. From these sub-scores, a total score is generated, which is then converted to a total stress percentile. The total stress percentile is calculated based on an age-matched normal distribution of parents with healthy children.[21] A higher total percentile score indicates higher stress levels as compared to the normative sample. Total stress scores above the 85th percentile are considered high, and scores above the 90th percentile are considered clinically significant, requiring intervention.[21] The PSI-4 also includes a Defensive Responding score, which is a validated score allowing identification of parents that are responding defensively and therefore misrepresenting their total stress as being lower than it is. As per the PSI-4 Manual's instructions, the total score can only be calculated if no more than the following number of statements are missing: (1) 3 from either the child or parental characteristics subscales, (2) 1 from a single subscale, and (3) 5 from the entire PSI-4.

Protocol. The participants completed the PSI-4 and clinical data were collected within a maximum of 6 months from the date the PSI-4 was filled for patients with stable diseases and within one month for patients with rapidly progressive diseases. Clinical data were collected through electronic and/or paper medical records, or through a standardized clinical evaluation performed by the treating physician. One standardized demographic questionnaire was completed per family at the same time as the PSI-4 questionnaire. The PSI-4 was completed by one or both parents. The PSI-4 was completed by hand or electronically via PARIconnect, the PSI-4's official means of electronic answering. French-speaking families were provided with an official translated

version of the PSI-4. Participants were given as much time as necessary to fully complete the questionnaires.

Statistical Methods. To assess parental stress using the PSI-4 questionnaire, clinical variables were statistically analyzed with total stress percentile scores as the primary outcome. For the purpose of this analysis, mothers and fathers were studied separately to overcome mother and father pairs reporting on the same child. Defensive Responders were included to improve the power of our analysis. Divorced and separated parents were grouped together and compared against married and common law parents. Clinical characteristics were presented by either median or inter-quartile range for continuous variables and number or percentage for categorical variables. The primary outcome, total stress percentile score, was reported by mean and standard deviation. The mean total stress percentile was compared between mothers and fathers with an independent 2-tailed Welch *t* test. The mean total stress percentile for mothers was compared between various groups using independent 2-tailed and 1-tailed *t* test or analysis of variance when indicated and appropriate. The same statistical methodology was used for mothers and fathers. Spearman correlation coefficients and P values were calculated to study correlations between the total stress percentile and Gross Motor Function Classification System, Manual Ability Classification System, Eating and Drinking Ability Classification System, and Communication Function Classification System scores. All analyses were performed using SAS for Windows version 9.4 (By SAS Institute Inc., Cary, NC, USA).

2.6 Results

Fifty-five parents of 36 patients were included in this cross-sectional pilot study. There was a larger proportion of male patients (n=24) compared to female (n=12). Most children were aged 8-12 years (39%) or 1-4 years (33%), while 11% were between 1-12 months old and 17% between

5-7 years old. Most patients recruited had a known molecular diagnosis (69%) as compared to no molecular diagnosis (31%). For those with a molecular diagnosis, the study included 15 molecular diagnoses, with the most prevalent being POLR3-related leukodystrophy (11%). All results are shown in Table 2.

55 parents answered the PSI-4 and demographic questionnaires. The majority of parents reported being married or common law (81% of mothers, 90% of fathers), as opposed to divorced/separated or single (19% of mothers and 10% of fathers) shown in Table 2. Fathers had a higher rate of employment (89%) as compared to mothers (55%). More than half of the parents involved in the study (58% of mothers, 53% of fathers) did not have a university level education.

Questionnaires filled by parents	**N = 55**	**Percentage**
Mothers	34	62%
Fathers	21	38%
Family demographic	**N (Mother) / N (Father)**	**% (Mother) / % (Father)**
Married/common law	25 / 17	81% / 90%
Separated/divorced	4 / 1	13% / 5%
Single	2 / 1	6% / 5%
Unemployed	14 / 2	45% / 11%
University education	13 / 9	42% / 47%
Missing demographic data	3 / 2	9%(3/34) / 10%(2/21)
Children Patients	**N = 36**	**Percentage**
Male	24	67%
Female	12	33%
Child age group (range)	**N = 36**	**Percent**
Infants (1-12 months)	4	11%
Toddlers (1-4 years)	12	33%
Young child (5-7 years)	6	17%
Child (8-12 years)	14	39%

Clinical features	**N = 36**	**Percent**
sialorrhea	21	58%
behavioral difficulties	7	19%
Scales	**N = 36**	**Median (IQR)**
GMFCS*	36	3.5 (2,4)
MACS*	22	3 (2,3)
EDACS*	27	3 (2,4)
CFCS*	31	4 (3,5)
Molecular diagnosis	**N = 36**	**Percentage**
POLR3-related leukodystrophy	4	11%
Allan-Herndon-Dudley syndrome	2	6%
Metachromatic leukodystrophy	2	6%
MPS-I	2	6%
Adrenoleukodystrophy	2	6%
Zellweger-PBD	2	6%
Mitochondrial leukoencephalopathy	2	6%
TUBB4A-related or H-ABC	2	6%
FAHN	1	3%
Glutaric aciduria-1	1	3%
GM1 gangliosidosis	1	3%
MPS-II	1	3%
MPS-IIIA	1	3%
Pelizaeus-Merzbacher Disease	1	3%
Pelizaeus-Merzbacher Like Disease	1	3%
No molecular diagnosis	11	31%

Table 2.2: Parent demographics, child clinical characteristics and molecular diagnosis: *Median (quartile range). Abbreviations: N: number of observations; GMFCS: Gross Motor Classification System; MACS: Manual Ability Classification System; EDACS: Eating and Drinking Ability Classification System; CFCS: Communication Function Classification System; POLR3: DNA-directed RNA polymerase III PBD: Peroxisome Biogenesis Disorders; H-ABC: Hypomyelination with Atrophy of the Basal ganglia and Cerebellum; FAHN: Fatty Acid Hydroxylase-associated Neurodegeneration; MPS: mucopolysaccharidosis;

A total score above the 50th percentile indicates higher stress than half the normative sample; scores higher than 85% and 90% are high stress levels and clinically significant stress levels, respectively, as described by the PSI-4 Manual. The average total stress percentile across

our entire sample was 65 ± 22.3, significantly higher than the median normative sample (t(54)=4.8736, P<0.001). No significant difference was found between mother and father's mean total stress percentile score (TS%ile) (t(52)=-0.05362, P=0.59). 20% (11/55) of parents had high levels of stress (>85%ile) and 11% (6/55) had clinically significant levels of stress (>90%ile). These results are shown in Table 3.

Stress	**Mean (TS%ile ± SD)**	***P* (TS%ile > 50%)**	**N >85%ile**	**N >90%ile**
Mothers (n=34)	66 ± 26.4	<0.001	29%	18%
Fathers (n=21)	63 ± 13.7	<0.001	5%	0%
All parents recruited (n = 55)	65 ± 22.3	<0.001	20%	11%
Child's Behavior	**Difficult (TS%ile ± SD)**	**Normal (TS%ile ± SD)**	**Inconclusive**	***P***
Mothers (n=34)	91 ± 11.5 (n=7)	58 ± 25.1 (n=26)	(n=1)	<0.001[a]
Fathers (n=21)	64 ± 10.7 (n=4)	62 ± 14.9 (n=16)	(n=1)	0.39
Motor Function Correlation	**Spearman Correlation (mother/father)**	**p (mother/father)**	**N (mother/father)**	
GMFCS	-0.56649 / 0.10442	0.0005 / 0.6524	34 / 21	

Table 2.3: Primary outcome of parents and significant TS%ile correlations: [a]Levene's test was significant suggesting a violation of the equal variance assumption so a Welch's t-test was performed. The total stress percentile score of mothers and fathers as well as statistics of all parents are presented. The mother and father's total stress percentile versus the child's clinically noted behavior difficulties was analyzed by 1-tailed t-test. When children had either normal or difficult behavior, both parents agreed upon the behavior and brought it to the physician's attention. The motor function correlation shows the mother and father's total stress percentile vs. the GMFCS. Correlation found using continuous variables Spearman correlation. Abbreviations: GMFCS: Gross Motor Function Classification System;

Several clinical and demographic variables were analyzed, as listed in Table 4. No significant correlations were found between the father's total stress percentile and any clinical or demographic features. The mothers' total stress percentiles were significantly higher if their child had behavior difficulties (Welch's 1-tailed *t*-test, (t(22)=-5.0117, P <0.001)). The mothers' total stress percentiles show a moderate negative correlation with the GMFCS scale (Spearman coefficient -0.56649, P <0.001, n=34) shown in Table 3.

Clinical/Demographic features	Mother	Father
Behavior difficulties	$p<0.001^a$	n.s.
GMFCS	$p<0.001^a$	n.s.
MACS	n.s.	n.s.
EDACS	n.s.	n.s.
CFCS	n.s.	n.s.
Time from diagnosis to questionnaires	n.s.	n.s.
sialorrhea	n.s.	n.s.
Marital status	n.s.	n.s.
Employment	n.s.	n.s.
University degree	n.s.	n.s.

Table 2.4: **Clinical and demographic correlations analyzed:** [a]To account for multiple independent comparisons, a Bonferroni-Holm correction was conducted at an alpha level of 0.005 (0.05/10) and found that behavior difficulties and GMFCS were significant in mother's TS%ile. n.s.= not significant ($p>0.005$).

2.7 Discussion

This cross-sectional pilot study is the first to investigate stress in parents of children with genetically determined leukoencephalopathies. The primary outcome, total stress percentile as measured by the Parenting Stress Index 4th Edition, has been previously validated in several populations.[4,21,28,29] Considering that a higher total stress percentile score indicates higher stress levels than parents of healthy children, this study demonstrates that both mothers (66±26.4) and father's (63±13.7) of patients with genetically determined leukoencephalopathies experience greater stress levels than the median parents' population. The clinical and demographic variables analyzed in this study represent those that have been found in the literature to be most clinically impactful on parental stress, such as a child's functional ability and behavior.[5,6,10,30,31]

The negative correlation between mothers' total stress and the Gross Motor Function Classification System is surprising. We hypothesize that the expectation of a child's progressive impairment may have a greater impact on maternal stress than a child's actual functional impairment once the disease has already progressed and that this result is a reflection of our study design, i.e. cross-sectional, where mothers of children with greater motor disabilities had more time to cope with the disease. A larger prospective study is required to reach any definitive conclusion.

This study also found that mothers' total stress is higher when their children have clinically noted behavioral difficulties, a finding seen in other stress and quality of life studies.[5,31-34] The same result was not found in fathers included in this study. We hypothesize that since mothers typically assume a greater role in caregiving responsibility, they experience higher levels of stress than fathers. Of note, most studies demonstrating correlations between caregiver stress and the child's behavior have studied patients with cerebral palsy[5], mucopolysaccharidosis diseases[31], and epilepsy[32].

The proportion of highly stressed parents scoring greater than the 85th percentile in our population was 20%. Studies involving other patient populations have demonstrated larger proportions of highly stressed parents using the Parenting Stress Index-4th Edition questionnaire. For example, proportions have been shown to be as high as 45%[5] and 100%[6] in cerebral palsy, 63%[32] in intractable epilepsy, 45%[10] in childhood epilepsy, and 51%[29] in children with brain tumors. Our lower proportion of highly stressed parents may be explained by parents of patients with genetically determined leukoencephalopathies underreporting or underestimating their levels of stress, which has been observed in clinic. This is further supported by the fact that 11% of the parents (4 mothers and 2 fathers) were labeled as defensive responders using the Parenting Stress

Index-4th Edition questionnaire, indicating that they are framing their stress as lower than it is. Furthermore, additional factors such as culture, societal acceptance towards individuals with functional disabilities, and universal access to healthcare in Canada may also explain the lower proportions of highly stressed parents when compared to studies performed in other countries. It is also important to note that our patients are also followed at a quaternary health care center using a multidisciplinary approach, in a Leukodystrophy Center of Excellence, as well as receive support resources from the Québec and Canadian governments, which may also account for lower stress levels when compared to patients who do not receive comprehensive care and support.

The limitations in our study are inherent to the fact that it is a pilot trial aiming at assessing the value of using the Parenting Stress Index-4th Edition in parents of patients with genetically determined leukoencephalopathies. It should be noted that the Parenting Stress Index-4th Edition is intended to be used with parents of children ranging from 1 month to 12 years. Therefore, adolescent stresses and other hardships (e.g. physical manipulation, transfers, etc.) may not have been fully captured in this population. Our small sample size is likely to explain the lack of statistically significant correlations between parental stress and clinical and demographic variables such as sialorrhea, time from diagnosis to questionnaires, Manual Ability Classification System, Eating and Drinking Ability Classification System, Communication Function Classification System, marital status, employment status, and university education. Our small sample size also prevented us from looking at confounding factors such as spousal relationship, socioeconomic status, and other personal factors such as individual resilience, susceptibility to stress and family support structures. Another limitation of this study is its cross-sectional nature. In our experience, parental stress evolves throughout the course of the disease and is at its highest at the time of the diagnosis and, in the majority of patients with a neurodegenerative disease course, at end of life.

A larger cohort of parents followed longitudinally for an extended period of time is required to overcome these limitations and allow for the identification of modifiable factors that influence parental stress and could be addressed as part of a comprehensive management plan.

2.8 Concluding Remarks

Parental stress is an understudied but very important aspect of the care for patients with genetically determined leukoencephalopathies. We successfully measured stress in parents of children with genetically determined leukoencephalopathies for the first time using the Parenting Stress Index-4th Edition. We have identified two main factors associated with increased stress in mothers of children with genetically determined leukoencephalopathies, which, with detailed parental counseling, psychological support early after the initial diagnosis, and treatment of children with behavioral difficulties could potentially alleviate the stress experienced by these mothers. The lack of statistically significant data associated between other variables and parental stress is most likely due to the small sample size in this pilot study and highlights a need for a larger prospective study to identify modifiable factors.

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2.10 Author Contributions

ED and AS contributed equally to the work described in this paper. M Sullivan and GB contributed to the study conception and design. ED, LTT, AM, FP, KG, SA, BB, NB, DB, SC, S Chenier, NC, MD, MED, JL, AL, GL, KYL, CM, PM, EMD, P Marois, JM, AN, BO, IP, DP, JR, ER, GR, E Rossignol, GS, MS, M Srour, M Sylvain, MT, SV, and GB collected and reviewed

participant data. AS performed statistical analysis. ED, AS, and GB drafted the manuscript and figures. All authors contributed to the manuscript's revision.

2.11 Declaration of Conflicting Interests

The authors declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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2.14 Ethical Approval

Written and informed consent was obtained from all research participants. This study was approved by the Research Ethics Board of the McGill University Health Center Research Institute (11-105-PED, 2019-4972).

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Chapter 3 Rare Disease Database Manuscript

LORIS MyeliNeuroGene Rare Disease Database for Rare Disease Natural History Studies and Clinical Trials Readiness

3.1 Preface

Rare disease research is beginning to pivot from gene discovery to exploration of potential therapies and clinical trials. To developed well-designed therapeutic trials, natural history studies are needed to delineate disease progression and identify biomarkers and surrogate markers of disease progression. We have built and customized a LORIS database to conduct natural history studies of many rare diseases such as LDs and gLEs. In this manuscript, we also summarize the U.S. Food and Drug Administration's requirements for digital records to be considered reliable and trustworthy.

3.2 Title Page

The LORIS MyeliNeuroGene Rare Disease Database for Rare Disease Natural History Studies and Clinical Trials Readiness

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3.3 Abstract

Background: With advances in next generation sequencing, the number of known disease-causing genes has increased significantly, opening the door for therapy development. Rare disease research has therefore pivoted from gene discovery to the exploration of potential therapies. With impending clinical trials on the horizon, researchers are in urgent need of natural history studies to help them identify surrogate markers, design therapeutic trials, and define historical control patients. **Results**: We customized a browser-accessible multi-modal (e.g. demographic, clinical, behavioral, genetics, imaging, and patient-determined outcomes) database to increase cohort sizes,

identify surrogate markers, and foster international collaborations. Ninety instruments were developed. A customizable clinical letter generator was created to ease the work of clinicians entering data and assist in continuity of patient care. **Conclusions**: Small cohorts and underpowered studies are a major challenge for rare disease research. This online, rare disease database will be accessible from all over the world, making it easier to share and disseminate data. FDA compliant databases will be life-changing for patients and families when historical control data is used for emerging clinical trials.

Keywords: leukodystrophy, rare diseases, information management systems, databases, registry, natural history, outcome measures, clinical trials, biomarkers

3.4 Background

According to the World Health Organization (WHO), the definition of a rare disease is one that affects every 1 in 2,000 people or less. Global prevalence of these approximately 8,000 rare genetic disorders is estimated to affect between 150-350 million people[1-7]. Historically, rare diseases have been notoriously difficult to diagnose due to their heterogeneous phenotypes and genotypes[8]. Since only around 5% of all rare diseases have an FDA-approved treatment, many orphan diseases utilize off-label indications of medications approved for other purposes[9]. However, an incredible amount of advancement has been accomplished over the last decade using rapidly evolving genetic technologies, including with the most recent use of next generation sequencing (NGS), to identify genes causing these diseases[6]. The description of novel rare disease entities and the identification of novel disease-causing genes have opened the door for studies investigating disease pathogenesis and potential therapeutic approaches[6]. Rare disease research has therefore begun to pivot from gene discovery towards investigating potential therapeutics[6].

With impending clinical trials on the horizon, rare disease researchers are realizing a tremendous need for natural history data[10,11]. The goal of a natural history study is to recruit patients for longitudinal analysis of natural disease progression[12]. The data gathered is used to help identify surrogate markers, determine the best outcome measures to be used in potential therapeutic trials, and can serve as the control arm of a clinical trial[13-16]. Natural history studies result in incredible amounts of information being collected, including clinical, behavioral, sociodemographic, genetic, imaging, and patient and family reported outcomes.

This diversity and quantity of data can be difficult to manage, so rare disease researchers must begin to utilize information management systems, or databases, to facilitate natural history studies. Rare disease research relies heavily on international collaboration and data sharing in order to recruit large enough patient populations to obtain adequate statistical power[6,17]. Therefore, utilizing an online database can uniquely benefit rare disease research more than other disease research where significant patient populations are more prevalent.

If rare disease databases are going to be successful in future clinical trials, they must adhere to local and international regulations for electronic records. Title 21 Code of Federal Regulations (CFR) Part 11 published in 1997, from the U.S. Food and Drug Administration, outlines what is considered trustworthy, reliable record keeping. These regulations apply to any FDA-regulated industry, such as pharmaceutical companies, medical device manufacturers, biotechnological companies, and clinical research organizations. We chose to adhere to all general requirements that will be detailed below in the Methods section.

There are a variety of different databases available to aid researchers such as RedCap[18], Deduce[19], HID[20], DFBIdb[21], LONI[22], MIND[23], NeuroLOG[24], etc. We elected to customize the Longitudinal Online Research and Imaging System (LORIS)[25] to help organize data and make

international collaboration easier when conducting multi-site natural history studies because of its strong track record and the fact that it is open source. Here, we detail below how our group used LORIS and 21 CFR Part 11 guidelines to set up workflows and developed the LORIS MyeliNeuroGene database for Rare Diseases to lead us to clinical trial preparedness in the coming years.

3.5 Methods

3.5.1 Title 21 Code of Federal Regulations Part 11 Compliance (Part 11 Compliance)[26]

To adhere to Part 11 Compliance regulations, the LORIS MyeliNeuroGene database has been customized to include additional security measures such as biometrics, digital signatures, and time stamped audit trails. There is a gap in scientific literature detailing workflow and database development. As such, we will summarize the general requirements of Part 11 Compliance below and how they were implemented into our database.

Training Verification: Users are required to have their credentials (e.g. education, training, experience) verified before performing tasks within the database. Written policy must be signed holding users accountable and responsible for their electronic signatures (discussed further below). This written policy must be stored, and a hard copy sent to the Office of Regional Operations (HFC-100), 5600 Fishers Lane, Rockville, MD 20857.

Biometrics: This is a method of verifying an individual's identity based on a measurement of the individual's physical features (i.e. fingerprints, etc.) or repeatable action that are unique to that person. In our case, we chose to use a unique pin separate from an authorized user's password for 2-factor authentication.

Closed system: The LORIS architecture is a closed environment, meaning that access to the system is controlled by the same people who are responsible for the content of the electronic

records. This includes the researchers and principal investigator. Operational audits on the system are done on a routine basis. Time stamp audit trails are tracked for each authorized user to trace creation, modification, or deletion of any instrument, visit, or other electronic record. User access is hierarchical, meaning some users do not have full access to the database and may only have "read" or "write" access only. The database also must ensure that no user has the same pin or password, and that pins and passwords are periodically checked and changed to prevent unauthorized use. If unauthorized use occurs, there are immediate system security notifications. Per Canadian predicate rules, records must be stored for 25 years after study completion. United States record retention rules state storage must be for a minimum of 10 years.

Quality Control: Processing pipelines must ensure data fits specific parameters and types. This is discussed in depth under the Methods section "LORIS Database and Workflow".

Electronic signature: This includes any combination of text, graphic, data, audio, or other information that is represented in digital form by the database. Electronic signatures must include printed names of the signers, dates and times, meanings (e.g. approval, creation, reviewing), and an internal audit trails. These signatures are legally binding. Authority checks are completed every month to ensure only authorized users may sign, input, output, or modify records.

Digital signature: A digital signature combines the electronic signature and its corresponding cryptographic authentication, usually a pin and/or password that is used to verify the identity of the signer. It cannot be copied or pasted to or from another document, making it inexorably linked to the signed document. To not become cumbersome, continuous signing periods only require the first to be 2 factor authenticated with a biometric identification and password.

External Auditing: It is highly recommended that after database development a third-party auditor inspects the system and documentation put in place. Auditors alert parties of any gaps or shortcomings and can advise developers of what needs to be changed for full compliance with FDA policy.

3.5.2 LORIS Database and Workflow

Architecture: LORIS is a web-based data and project management software that stores demographic, clinical, behavioral, genetic, imaging, and patient-related outcomes accessible from any computer browser connected to the internet[25]. Multiple sites can enter, organize, and validate data under one management framework. Longitudinal data is organized around the "Subject Profile". Clinical examination, imaging data, outcome measures, and metadata are organized by "Visits". All stored information is de-identified and can be queried by an authorized user. Source documentation can be uploaded and affiliated with each visit. Quality control is ensured by automated scoring of clinical, behavioral and patient-reported outcomes, validating data types (string vs numerical), and requiring double data entry where necessary.

Workflow: To properly set up our rare disease database, we first began by drafting a data dictionary in the form of an Excel sheet. This Excel outlined all of the data entry forms, or instruments, that would be developed in the LORIS Instrument Builder module detailed below. After instrument creation, participant enrollment and data entry can begin with data query and exportation following after. An overview of the workflow can be found in Figure 3.1.

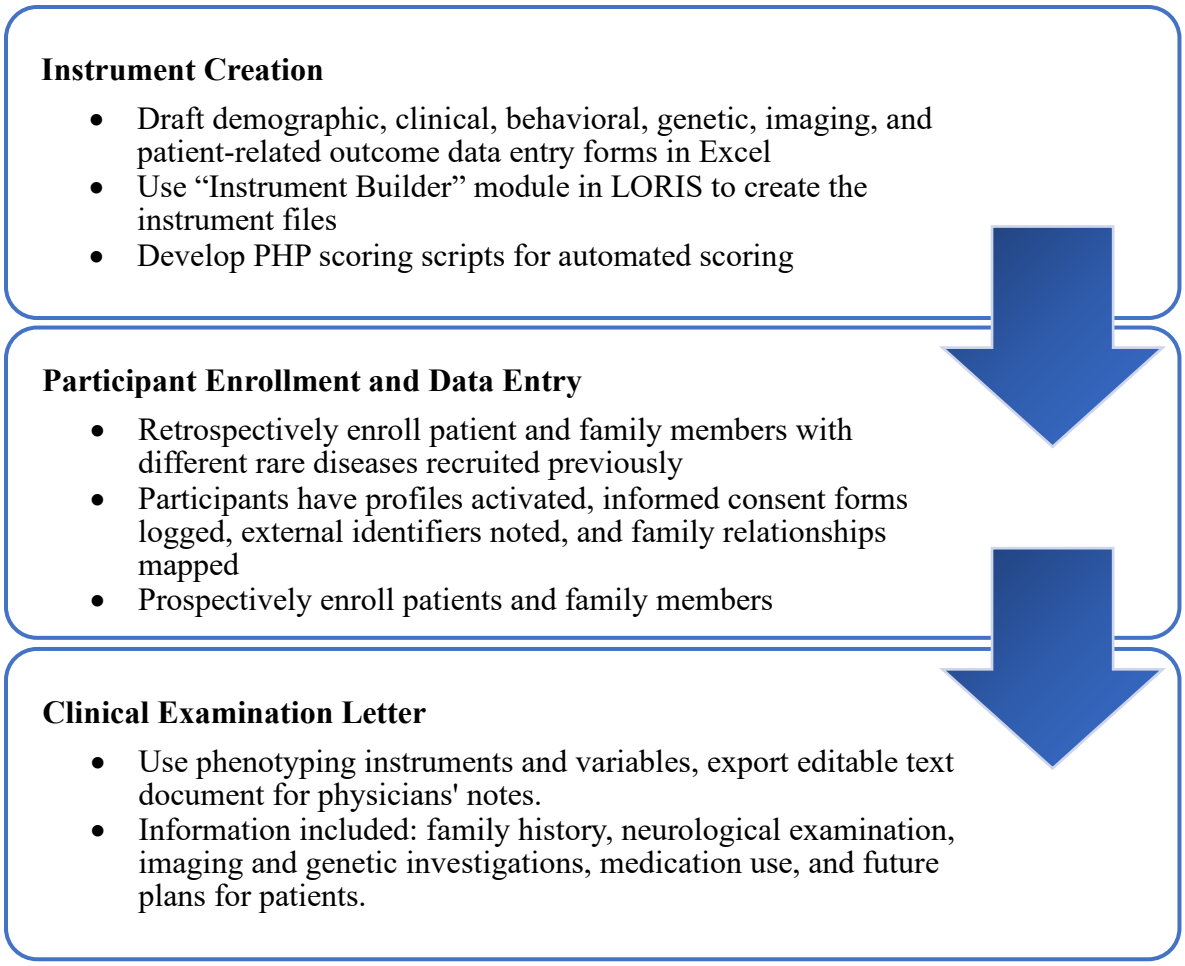

Figure 3.1 Database development workflow to create instruments, scoring algorithms, enroll patients, enter data, and output information into a clinical examination letter

Instrument Builder: Within LORIS are different modules to help researchers with no computer science or programming experience required. The Instrument Builder module aids in the creation of demographic, clinical phenotyping, behavioral, genetic, imaging, and patient-related outcome measures. Each instrument can be customized with specific information such as a "Header", "Label", and "Scored Field" that give the instrument title, background information, and automatically calculated scoring respectively (Figure 3.2 and 3.3).

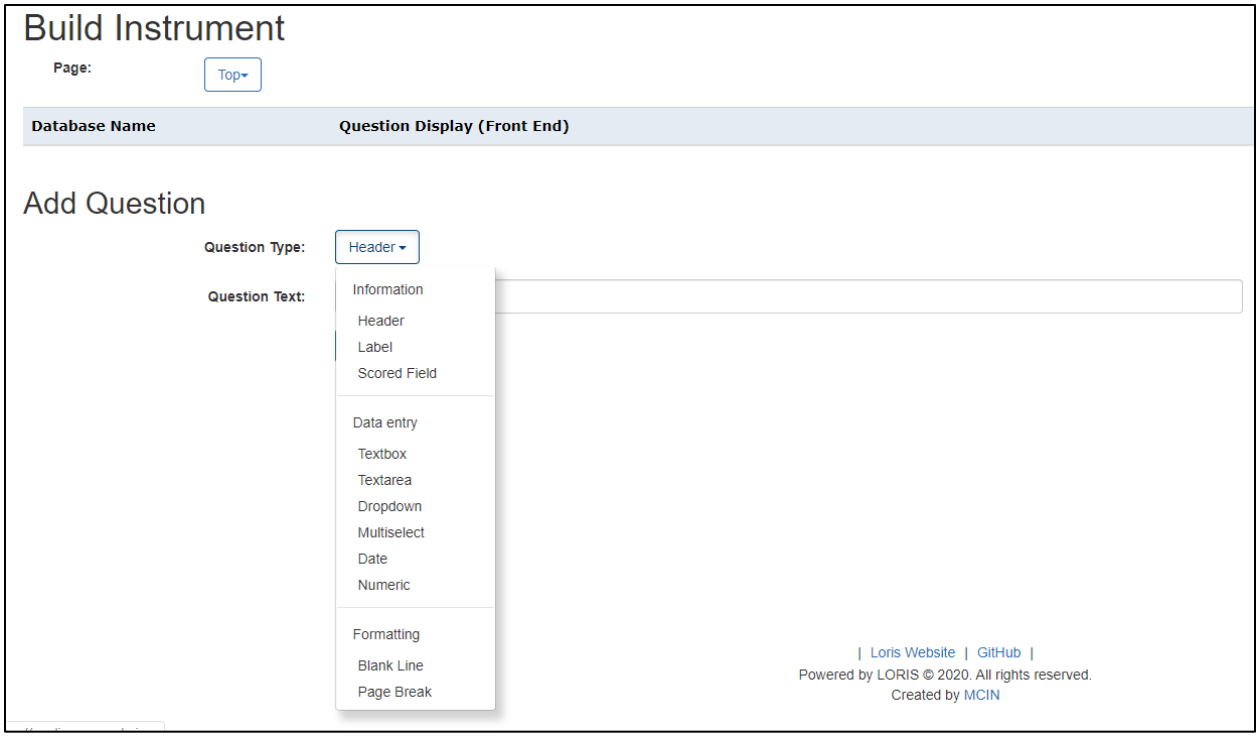

Figure 3.2 Question types for the LORIS instrument builder module

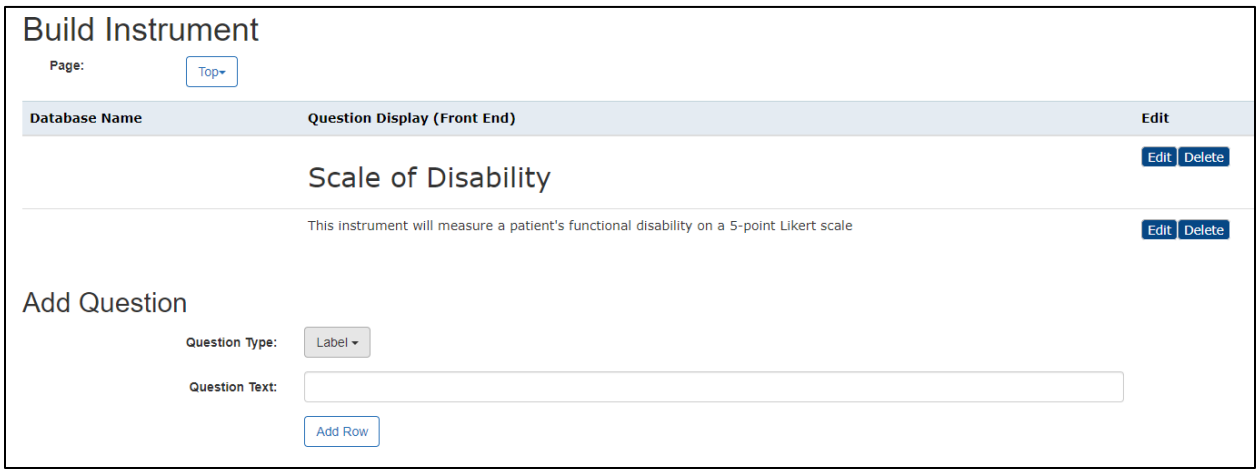

Figure 3.3 Formatting with the use of headers and labels

Data entry can be standardized using a "Textbox", "Text area", "Dropdown", "Multiselect", "Date", and "Numeric" question entry (Figure 3.2). Each question is assigned a variable name "Question Name", for calculations and data querying, and "Question Text" which asks the pertinent question at hand (Figure 3.4). For Dropdown questions, instrument specific options can be added for every question (Figure 3.4).

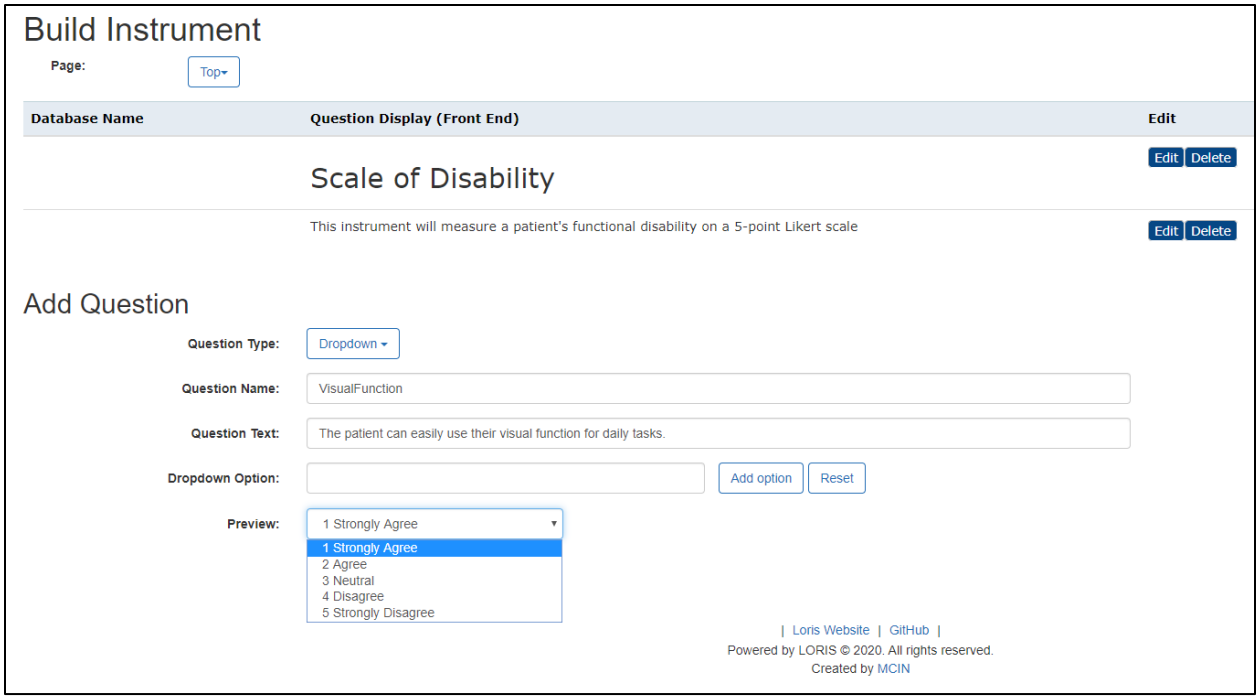

Figure 3.4 Standardizing data entry with dropdown menus

Instrument Creation: Instruments were first planned and drafted using Excel in a data dictionary. Columns consisted of Question Names, type of question (e.g. Numeric, Dropdown, etc.), Question Text, Question Options (available choices), and Formulas (for later calculations). Each row represented one question. Using the data dictionary Excel and the Instrument Builder module on LORIS, each instrument was created. The following instruments were created: demographic forms, clinical phenotyping (i.e. spasticity an dystonia measures, gross and fine motor, eating, and drinking function, ataxia, intelligence, disability, swallowing evaluations etc.), behavioral , genetic, imaging (i.e. MRI analyses), and patient-related outcomes (i.e. health-related quality of life, parental stress, pain characterization, etc.) . Instruments were then uploaded onto GitHub for quality control.

Scoring Algorithms: After instrument completion, a PHP scoring script was developed for instruments that required them. Automatic scoring reduces human error and dramatically decreases

time spent on calculations. Scoring scripts were also uploaded onto GitHub for additional quality control.

Instrument Implementation: After any edits to instruments or scoring scripts, Pull Requests on GitHub were approved and available on an insulated LORIS staging server where beta testing occurred. After testing was completed, instruments were pushed to the LORIS production server for instrument pipeline completion and data entry.

Participant Enrollment: Before data entry could be completed, Subject Profiles must be entered. Our group has consented more than 1,000 patients and family members with different rare diseases since 2011, and patient and family recruitment is ongoing. To create a new profile, "Date of Birth", "Sex", "Site" (in the case of a multi-site study), and "Project" must be entered. Projects can be separated into different studies such as natural history, imaging, genetic, or even clinical trials assessing therapeutics (Figure 3.5).

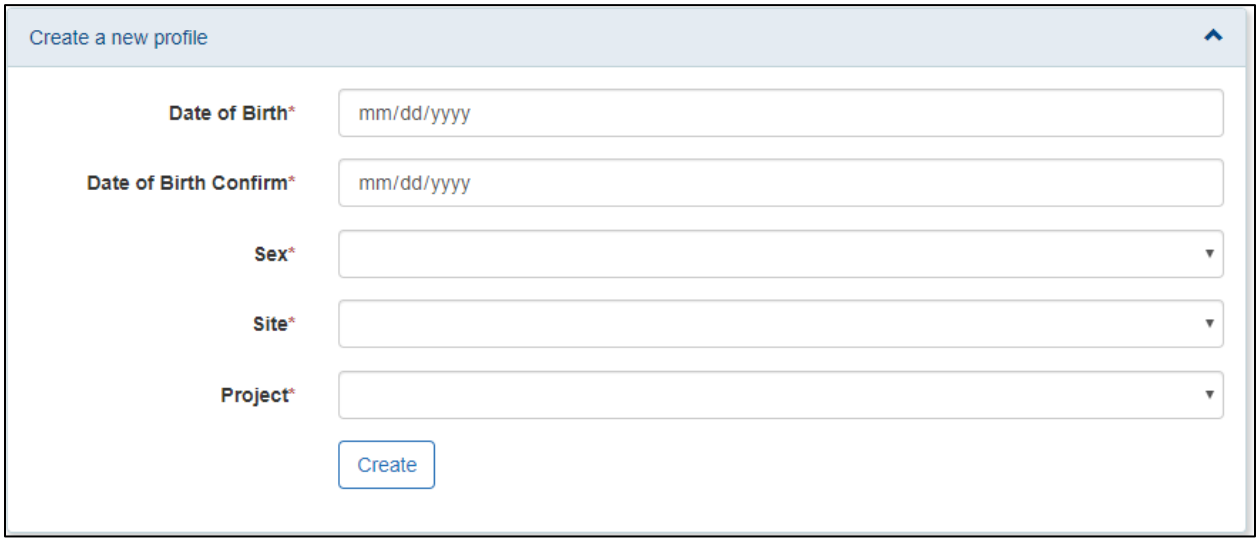

Figure 3.5 Creating a new candidate profile with date of birth, sex, site, and project

A new Subject Profile, or candidate, generates two identifier codes, a DCCID and a PSCID which are unique LORIS identifiers (Figure 3.6).

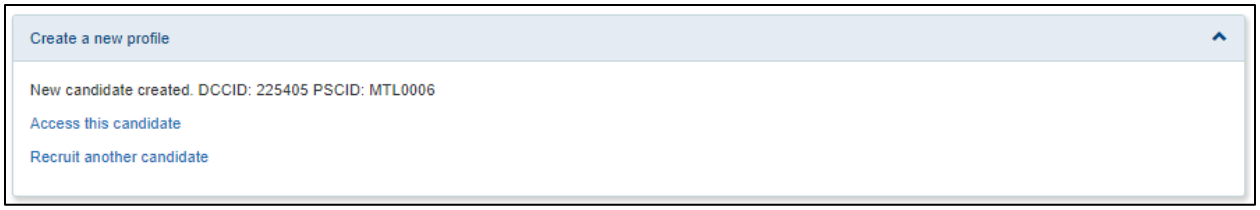

Figure 3.6 Generation of unique LORIS identifiers DCCID and PSCID

After creation of the Subject Profile, each candidate was edited to be activated in the study, designated for which informed consent form was signed, and mapped to any external identifier codes. Under "Participant Status", we tracked the participant's status in the study (e.g. Active, Death, Lost to Follow-up, etc.). Comments can be entered with both time, date, and author history tracked in the internal audit trail (Figure 3.7).

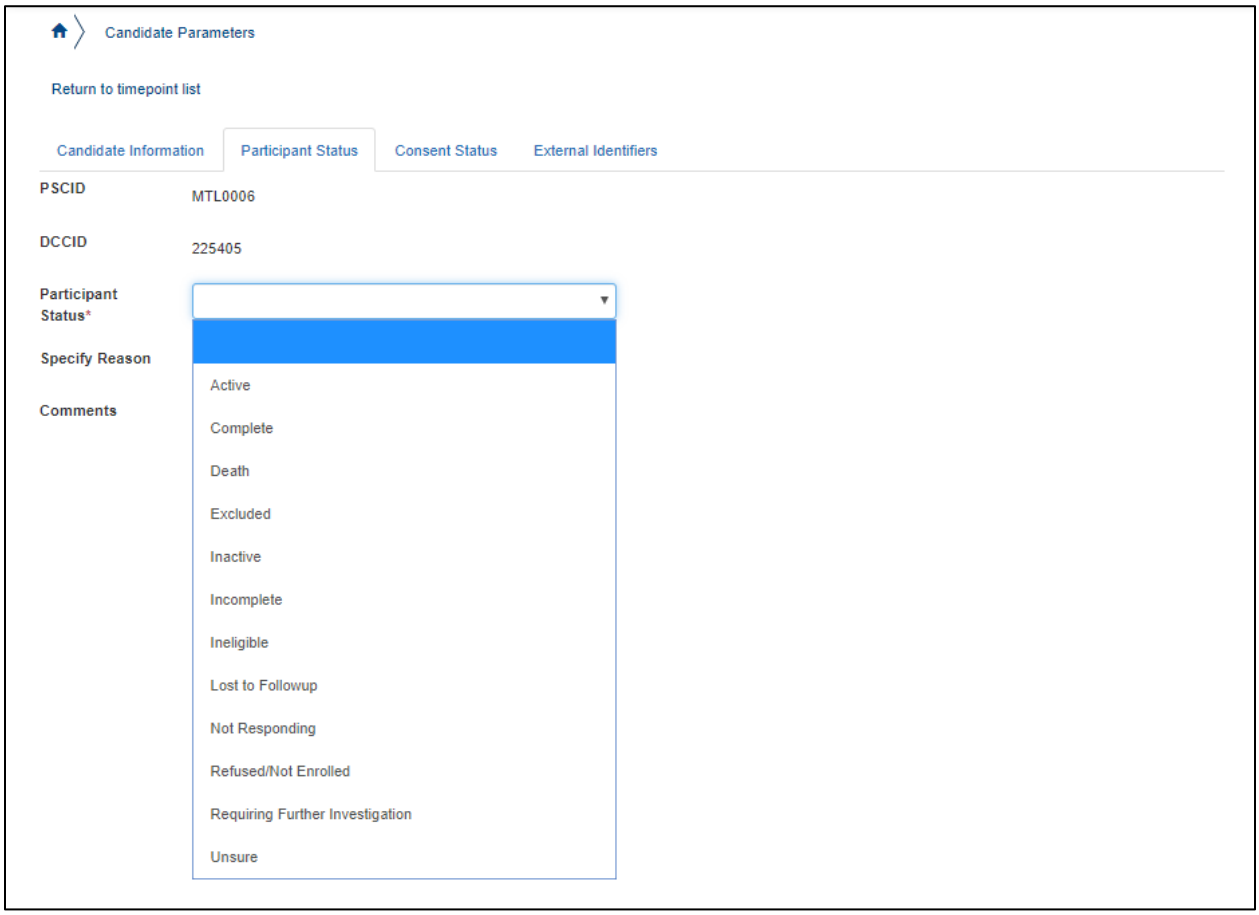

Figure 3.7 Specifying participant status in study

"Consent Status" tracks the latest signed Research Ethics Board (REB) approved informed consent form. Finally, mapping the "External Identifier" is crucial for future correspondence with family doctors and other collaborators (Figure 3.8).

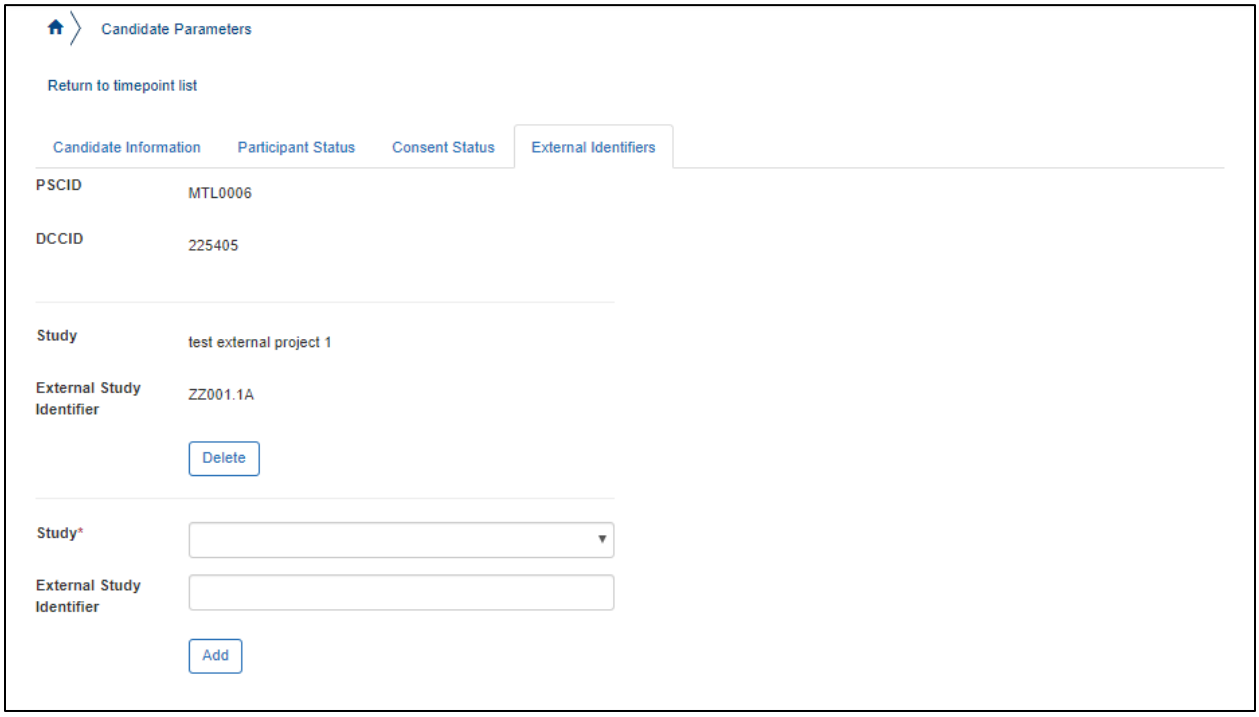

Figure 3.8 Mapping external identifiers from collaborators

Data Entry: "Create time point" allows for data entry of clinical, behavioral, and patient determined outcomes that were created during the Instrument Creation process (Figure 3.9). It also enables uploading of any imaging data collected. We customized our time points to correspond to the age of the patient. For instance, a participant's birth date would be time point T000, and a follow-up appointment 6 months later would be time point T006. A prenatal examination 1 month before a T000 examination would be designated as T-001. Time point creation can be seen in Figure 3.9, 3.10, and 3.11.

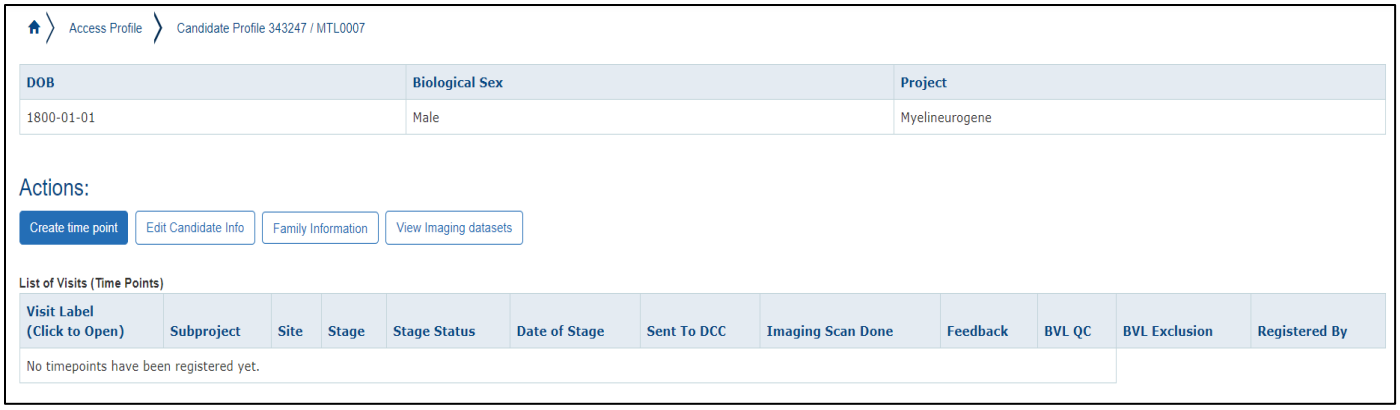

Figure 3.9 Creating longitudinal time points for patient visits

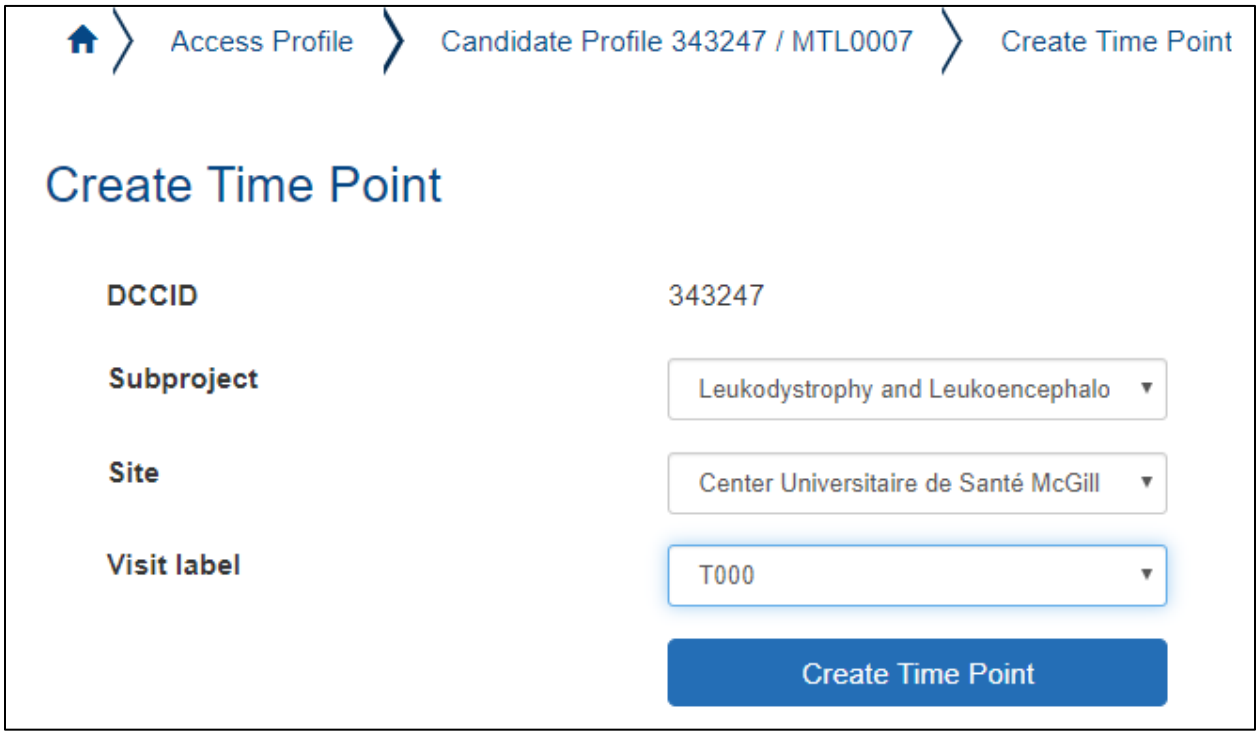

Figure 3.10 Associating time points with subprojects and study sites

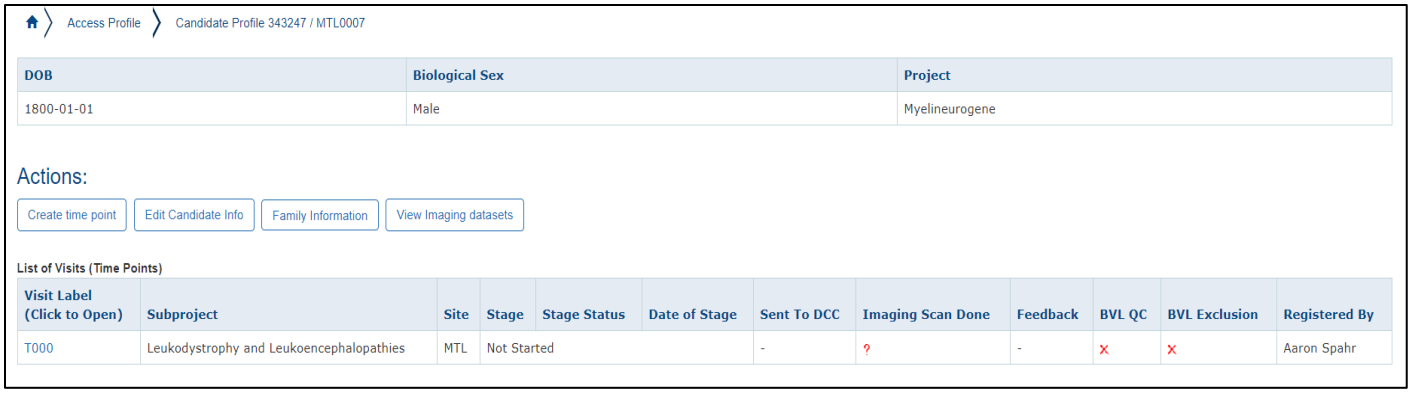

Figure 3.11 Visualizing time point information in the LORIS Candidate Profile

Selecting time point T000 opens a page for all instruments developed to work on our database (Figure 3.12). Time points can be customized so that only specific instruments are available to participants at specific ages. Entering multiple visits allows for prospective tracking.

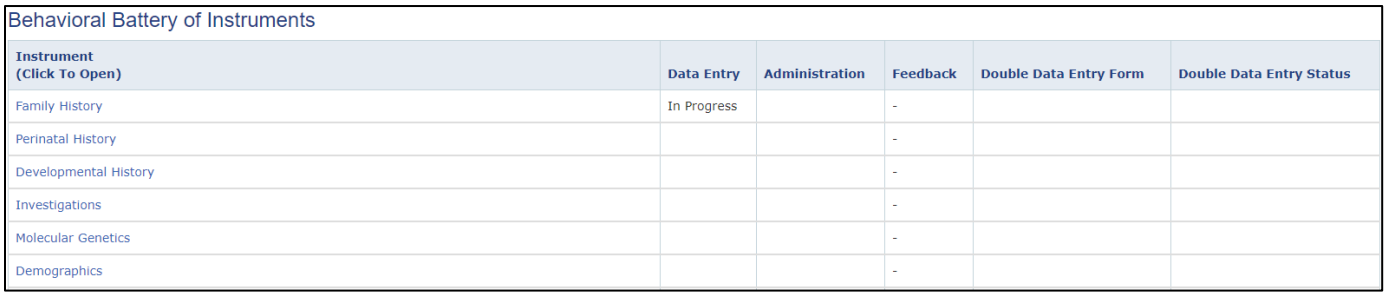

Figure 3.12 Test battery of instruments customized for each participant based on time point and age appropriateness

Family Information: We have further customized LORIS to include Family Relationship information. Linking de-identified individuals allows us to link a given patient's disease characteristics to his/her parents reported measures such as parental stress or patient/parents/sibling's quality of life. It also allows us to organize family genetic results when next generation sequencing (NGS) investigations are being conducted.

3.6 Results

Within LORIS, data entry forms, or instruments, can be created using the "Instrument Builder" module. Using the workflow found in the Methods section, 90 LORIS instruments were created. Detailed phenotyping including family history, perinatal history, developmental history, clinical evolution, time to event, neurological examination, neuropsychological assessment, etc. were developed in conjunction with other parent- and patient-reported outcomes such as quality of life, disability, and stress. Of the 90 instruments, 62 had scoring algorithms developed to aid in data processing. The resulting instruments are summarized in Table 3.1.

Table 3.1: Developed Instruments of the MyeliNeuroGene Loris Database

Instrument	Purpose
Family History	Inheritance pattern
Perinatal History	Disease Onset/Progression
Developmental History	Disease Onset/Progression
Investigations	Diagnostic Odyssey
Demographics	Sociodemographic variables
Clinical Presentation	Disease Onset/Progression
Primary Diagnosis	Disease Onset/Progression
Gross Motor Function Measure - 88	Measure changes in motor function
Leiter-3 Intelligence Scale	Measure changes in intelligence
Neuropsych Examinations	Measure changes in cognition
Rehabilitation	PT, OT, SLT, etc. used
Clinical Evolution	Disease Onset/Progression
Time to Event	Disease Milestones
Clinical Examination	Disease Onset/Progression
Swallowing Scales	VFSS and FEES evaluation
MRI Analyses	Disease Onset/Progression
Modified Ashworth Scale (MAS)	Measure changes in spasticity
Fahn Marsden Scale (F-M)	Measure changes in dystonia
Global Dystonia Scale (GDS)	Measure Changes in dystonia
Guy's Neurological Disability Scale (GNDS)	Measure disability and ADL
Gross Motor Function Classification System (GMFCS)	Characterize gross motor function
Communication Function Classification System (CFCS)	Characterize communication function
Manual Ability Classification System (MACS)	Characterize fine motor function
Eating and Drinking Ability Classification System (EDACS)	Characterize eating function
Scale for the Assessment and Rating of Ataxia	Measure changes in ataxia
Non-communicating Children's Pain Checklist - Revised	Measure parent reported pain
Parent Reported Stress Questionnaires	Measure parental stress
Health-Related Quality of Life Questionnaires	Measure patient's quality of life

Abbreviations: PT: physical therapy; OT: occupational therapy; SLT: Speech and language therapy; VFSS: Video fluoroscopic swallow study; FEES: Fiberoptic endoscopic evaluation of swallowing; MRI: Magnetic resonance imaging; ADL: Activities of daily living

One thousand patients and family members with rare diseases have been included onto LORIS and given their unique identifiers. This includes activation of enrollment, informed consent designation, external identifier logging, and family relationship mapping.

In addition, a dynamic letter generator has been developed to assist in forwarding patient information to other physicians. The tool compiles the patient's data, entered via the phenotyping instruments, into a Clinical Examination Letter. In place of the database field names, highlighted in yellow in Figure 3.13, an instance of the letter renders the patient data for the corresponding field. The Clinical Examination Letter can be exported as an editable word document that details patient information, such as family history, clinical evolution, time to event and future plans for investigations. This letter can then be sent to the referring physicians for continuity of care. This letter has the advantage of not duplicating the clinician who enters the data's work; as the clinician sees the patient and enters the data in the LORIS MyeliNeuroGene database, the clinical note is populated.

DATE OF THE VISIT: {DateFromLastVisit}
REFERRING MD: Dr. {ClinicalPresentation/Q02Presentation}
CC: {ClinicalPresentation/Q02Presentation}
RE: {NameOfPatient
MCH#: {MCHNUMBER
D.O.B.: {DoB}

CONSULTATION FROM THE LEUKODYSTROPHIES AND NEUROMETABOLIC DISORDERS CLINIC

Dear Dr. {ClinicalPresentation/Q02Presentation},

Thank you for referring this {DOB} year/month-old {ClinicalPresentation/Q04Presentation, | Q03Presentation}-handed, to the Leukodystrophies and Neurometabolic Disorders clinic. The patient was seen on {DateFromLastVisit}. The patient came with OneOrBoth parents.

You referred the patient for {ClinicalPresentation/Q01Presentation}.

Family History: {NameOfPatient is the {FamilyHistory/Q02FamilyHistory} of {FamilyHistory/Q03FamilyHistory} children. They have {FamilyHistory/Q05FamilyHistory} sisters and {FamilyHistory/Q04FamilyHistory} brothers.

The mother is {FamilyHistory/Q01MotherHistory} years old and is {FamilyHistory/Q02MotherHistory} healthy. {FamilyHistory/Q03MotherHistory}. She has {FamilyHistory/Q04MotherHistory} had miscarriages. {If YES Q04MotherHistory=>FamilyHistory/Q05MotherHistory, Q06MotherHistory, Q07MotherHistory, Q08MotherHistory}. She works as a{FamilyHistory/Q09MotherHistory}. The maternal family is from {FamilyHistory/Q10MotherHistory}. The mother's last name is {MotherLastName. The maternal grandmother is from {FamilyHistory/Q03ExtendedFamily} and is {FamilyHistory/Q01HistoryDisease} healthy. The maternal grandfather is from {FamilyHistory/Q04ExtendedFamily} and is {FamilyHistory/Q02HistoryDisease} healthy. The patient has {FamilyHistory/Q11MotherHistory} maternal aunts and {FamilyHistory/Q12MotherHistory} maternal uncles and has {FamilyHistory/Q13MotherHistory} cousins on their mother's side of the family.

Figure 3.13: Screenshot of the LORIS MyeliNeuroGene dynamic letter generator: Yellow highlights customizable variables for the clinical letter generator. Black highlighted variables represent information that is not stored in LORIS and must be filled in by the physician.

3.7 Discussion

Most patients affected with rare diseases, from mildly to severely affected, support data sharing to promote research, healthcare, and knowledge transfer[17]. We have built and customized a LORIS database and detailed our workflow to aid rare disease researchers to create their own

information management system, electronic health records, or database. There is a major need and benefit to sharing data in rare disease research. De-identifying and sharing information allows rare disease researchers to efficiently study disorders by collaborating and minimizing redundant studies[27].

In addition to the clinical phenotyping instruments and dynamic letter generator, we have outlined, for the first time, the methodology to become Title 21 Code of Federal Regulations Part 11 Compliant, which is a requirement to use electronic records as historical controls in clinical trials in the United States[28,29]. To our knowledge, our manuscript is the first to outline the requirements to adhere to 21 Code of Federal Regulations Part 11 Compliance (Part 11) and is one of the few to summarize the general requirements from the FDA to aid researchers in adherence. Future work will leverage the tools developed in this project to delineate the natural history of several rare diseases and will hopefully be used by clinicians and researchers around the globe.

An exportable dynamic letter generator has also been developed to save time when examining patients referred to clinic. Patients with a rare disease who come to the Montreal Children's Hospital undergo a battery of tests that can take up to two days to complete. All information is stored in the LORIS MyeliNeuroGene Database and can be exported in the form of a Clinical Examination Letter detailing all results, impressions, and plans to help treat the patients. This letter is then sent back to the referring physician for continuity of care. When this letter is written by hand it takes several hours and introduces numerous chances for human error. Exporting the letter from quality-controlled instruments reduces this error and saves researchers and physicians time.

3.8 Conclusions

A major obstacle in rare disease research is overcoming small cohorts. Developing an online database that international collaborators can access and contribute to from all over the world is invaluable for increasing cohort sizes, discerning surrogate markers, and improving natural history data. Using this FDA compliant natural history data to validate outcome measures will be life-changing for patients and families because it will lead to historical control data that can be used in emerging clinical trials.

3.9 Declarations

Ethics approval and consent to participate: Written and informed consent was obtained from all research participants. This study was approved by the Research Ethics Board of the McGill University Health Center Research Institute (11-105-PED, 2019-4972)

Consent for publication: Not applicable

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: Our group, the MyeliNeuroGene Lab, is a collaborator with Dr. Alan Evan's research group, the McGill Centre for Integrative Neuroscience, who developed LORIS, a free and open-source web-accessible database solution for multi-modal data and multi-site studies.

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Author's contributions: AS, ZR, ML, SD, AE, GB were responsible for the conception and design of this project. AS developed, all of the instruments with expert guidance from GB, ZR, and ML. AS, ZR, and ML developed the scoring scripts. CL and MS helped perform quality control on the scoring scripts. CM developed the imaging platform. AS retrospectively entered over 1,000 participants and was responsible for initial data entry. AS drafted the initial manuscript. ZR, ML, CM, SD, AE, and GB provided feedback and approved the current submitted version.

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Author's information: GB is a pediatric neurologist and clinician scientist leading the Leukodystrophy and Neurometabolic Disorders Clinic at the McGill University Health Centre Research Institute.

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Chapter 4: General Discussion and Conclusion

4.1 Preface

Genetic leukoencephalopathies encompass all white matter disorders with genetic etiology, including leukodystrophies[1]. The vast majority of these diseases are progressive and lead to motor, cognitive, and behavioral dysfunction and often early death[9]. Their consequences are broad-ranging, and affect patients, their families, caretakers, and the health care system. As part of the research in the MyeliNeuroGene lab, this thesis has detailed two projects, a pilot study characterizing parental stress in parents of children affected by gLEs and the development of the LORIS MyeliNeuroGene Rare Disease Database.

4.2 Characterizing Parental Stress in Parents of Children with gLEs

Parental stress is an understudied but very important aspect of the care for patients with gLEs. In this project, the Parenting Stress Index 4th Edition (PSI-4)[143] was chosen to quantitatively measure parental stress. We elected to use the PSI-4 because of its validity and reliability in English and French populations, and its ability to measure stress in our specific patient population; parents of children. All patients included in this cross-sectional study were followed clinically and recruited at the Montreal Children's Hospital of the McGill University Health Center,. This pilot study was conducted as a proof-of-concept for a future, longitudinal study examining parental stress over a child's disease course. We found that 20% of parents had high levels of stress while 11% had clinically significant levels of stress, as indicated by the PSI-4 Manual[143]. Comparing parents, mothers and fathers had comparable TS%ile scores. We identified that a child's behavioral difficulties and gross motor function are influencing maternal stress. These same factors did not predict paternal stress.

Our group hypothesized that there would be a greater prevalence of highly stressed parents. An information bias[144] could explain the decrease in prevalence of highly stressed parents. Three common types of information bias are misclassification bias, observer bias, and recall bias[144]. From an examiner point of view, the possibility of misclassification bias is low. All patients received a molecular diagnosis, or they were diagnosed by brain MRI pattern indicative of a gLE. A standardized clinical evaluation was also performed by the same physician. On the responder's side, parents may have misclassified their stress, but we believe this possibility is low with the inclusion of the Defensive Responder score included in the PSI-4 Manual. Observer bias could be possible since parents are seen in a Leukodystrophy Center of Excellence. The multi-disciplinary approach may affect parent's responses as they would answer the questionnaire after seeing a

physician. Finally, it is possible that parents were not accurately remembering stressful events in their past, indicating a recall bias, but once again the Defensive Responder score is a reliable and valid instrument to detect significant decreases in parental stress self-reports.

There are several alternatives to the PSI-4 that measure parental stress, but none are as applicable to our study population and cited as often as the PSI-4. The Stress Profile is an psychosocial stress questionnaire, but does not specifically address stress factors associated with children, instead focusing only on life and work stress[145]. The Caregiver Strain Questionnaire is a free, 21 item instrument focusing on objective and subjective stressors, but specifically analyzes children with behavioural problems, like autism, and not functional disabilities[146,147]. The Parenting Daily Hassles Scales is a free, 20 item questionnaire focusing on the frequency and intensity of daily caregiving, but fails to address other stressors such as life stress or support structures[148]. The Questionnaire on Resources and Stress is another free, 52 item instrument focusing on parents who care for ill and disabled family members, but not specifically for children[149]. Finally, the Parental Stress Scale is another free instrument with 18 items that characterizes stressors and rewards of parenting, but fails to address specific life stressors[150].

The PSI-4 model makes several assumptions to quantitatively characterize stress. One assumption is that all stressors are additive and equal, for which studies have backed up[143,151,152]. However, there is evidence in the literature that points to unequal additive effects from education and occupation on perceived stress[153]. Another assumption is that a parent's level of stress is the best indicator of dysfunctional parenting[154]. Dissenting opinions argue that dysfunctional parenting is better predicted by education level and sociodemographic factors, both of which had no statistical significance in our study[155].

Alternative disability measures exist such as the Functional System Score and Expanded Disability Status Scale[156,157], predominately used for multiple sclerosis. However, these instruments are more useful for detailed disability characterization and require trained neurologists for specific system analyses[157]. Other disability measures such as the Barthel Index of Activities of Daily Living[158] and the Guy's Neurological Disability Scale[159,160], which can be parent or patient reported outcomes, also did not meet our criteria for a simple functional disability measure that could also be scored retrospectively. Instead, the GMFCS, MACS, EDACS, and CFCS were chosen for their easy to use (1-5 levels) classification system. These functional scales were initially developed for the cerebral palsy research community, however they have been validated in other gLEs such as Aicardi-Goutières Syndrome[161] and adapted for Metachromatic Leukodystrophy[162]. Our group has also published a study with preliminary validation of the GMFCS and MRI severity[106] further indicating the usefulness of these scales in our patient population.

There are many future directions that this study could segue to. First and foremost, our group is already collecting prospective data to characterize stress over a family's diagnostic odyssey, or the time to get their child's diagnosis and throughout disease progression. A challenge with most rare disease research is achieving adequate statistical power for a study. In the future, we have estimated a total cohort size of 128, including both mothers and fathers, will give us 80% power to detect moderate effect sizes, $d = 0.5$, with alpha probabilities of 0.05. We've also begun collecting more detailed information such as who is the primary or secondary caregiver. Additionally, we are now collecting a larger number of potentially modifiable factors such as parent and child sleep quality as well as family support structures. Finally, now that we have been able to demonstrate that our methodology is sound for our gLE patient population, we have started collecting data on parents of children aged 13-18 years with the Stress Index for Parents of

Adolescents[163]. Longitudinal data collection will be stored in the MyeliNeuroGene LORIS database. Data processing and analysis for a prospective study will be forthcoming. With a larger cohort, our statistical power will allow for the analysis of any potential effect modifiers. Linear regression analysis including potential covariates such as age of the parent, education level, marital status, income level, etc. will be analyzed to better delineate the magnitude and direction of the results seen in this study.

Parental stress is an understudied and important aspect of the continued care for patients affected by gLEs. This project has examined, for the first time, parental stress in this population and identified maladaptive behavior and gross motor function to be correlated with mother's parental stress. Surprisingly, no significant clinical features were correlated with father's parental stress and there were no statistical differences between maternal and paternal stress levels.

4.3 Development of the LORIS MyeliNeuroGene Rare Disease Database

Acquiring and organizing clinical data to conduct the parental stress study and previous research projects led our group to search for more efficient methods of data acquisition and storage. We chose to customize and develop a LORIS database for our lab's rare disease research and the broader rare disease research community. We developed 90 data entry forms storing sociodemographic, clinical, behavioral, genetics, imaging, and patient determined outcomes. We also developed an exportable dynamic clinical letter generator that uses the variables, and entered data, to save clinicians and scientists time when examining patients referred to clinic. We are currently piloting this database by conducting a natural history study of POLR3-HLD, or 4H leukodystrophy. LORIS was chosen because of its ability to acquire, store, and process large amounts of diverse longitudinal data. However, there are many alternatives to acquiring and storing data which will be detailed below.

Research Electronic Data Capture (REDCap)[164], a web-based application developed at Vanderbilt University, and Duke Enterprise Data Unified Content Explorer (DEDUCE)[165] were designed to support translational research projects but unfortunately lack capabilities to view imaging data which is imperative for studying many rare diseases, including leukodystrophies. On the other hand, neuroimaging data management systems such as the Human Clinical Imaging Database (HID)[166] and DFBIdb[167] were not designed to store clinical, behavioural, nor parent/patient reported outcomes. The Mind Research Network[168] most closely resembles LORIS with imaging and non-imaging capabilities. However, since LORIS is open source and based out of the Montreal Neurological Institute and is affiliated with McGill University, we chose to collaborate with them.

LORIS databases were designed for multi-center prospective studies[136]. The complexity of these studies can grow at an alarming rate as more variables, candidates, and sites are added. Authorized user's access rights must be managed and updated on a regular basis to ensure security. All of this can be overwhelming and expensive for certain research groups. Although many processes can be completed without computer science training, certain tasks, such as server management and developing scoring algorithms may require additional training and certification not already gained through the biomedical sciences. Mitigation strategies include detailed record keeping, protocol development and collaboration with developers.

Future directions with our LORIS MyeliNeuroGene Rare Disease Database will incorporate an automated data processing pipeline that outputs figures for analysis. An example of this would be plotting patient information on public CDC growth charts. Development is also being planned for an automated pedigree, or family tree, since family relationship information is already being tracked on LORIS. Finally, we will integrate the Online Mendelian Inheritance of Man's

(OMIM) API so that patients will have OMIM and PMIM numbers associated with their candidate profile for further reference and analysis.

4.4 Concluding Remarks

We have identified two main factors associated with increased stress in mothers of children with gLEs, which, with detailed parental counseling and psychological support early after the initial diagnosis, could potentially alleviate the stress experienced by these mothers. Future directions will work towards a larger, prospective study to identify modifiable factors.

Developing the online LORIS MyeliNeuroGene Rare Disease Database, that international collaborators can access and contribute to from all over the world, will be invaluable for increasing cohort sizes, discerning surrogate markers, and improving the quality of acquired natural history data. Using this natural history data will be instrumental and life changing to inform patients and family members of expected disease course and to be able to evaluate response to emerging therapeutic strategies.

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