Full Title:	**LeukoSEQ: Whole Genome Sequencing as a First-Line Diagnostic Tool for Leukodystrophies**
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ABBREVIATIONS AND DEFINITIONS OF TERMS

ADEM	Acute Disseminated Encephalomyelitis
CLIA	Clinical Laboratory Improvement Amendments
DUA	Data Use Agreement
HIPAA	Health Insurance Portability and Accountability Act
ICSL	Illumina Clinical Services Laboratory
IRB	Institutional Review Board
MDBP	Myelin Disorders Biorepository Project
MRI	Magnetic Resonance Imaging
NGS	Next Generation Sequencing
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event
SoC	Standard of Care
VUS	Variant of Unknown Significance
WES	Whole Exome Sequencing
WGS	Whole Genome Sequencing

ABSTRACT

Context/Background:

Leukodystrophies, and other heritable disorders of the white matter of the brain, were previously resistant to genetic characterization, largely due to the extreme genetic heterogeneity of molecular causes. While recent work has demonstrated that whole genome sequencing (WGS) has the potential to dramatically increase diagnostic efficiency, significant questions remain around the impact on downstream clinical management approaches versus standard diagnostic approaches.

Objectives: (Primary and Important Secondary Objectives)

Primary

- Estimate the percentage of cases that experience a significant change in diagnostic status as a direct consequence of whole genome sequencing.

Secondary

- Estimate the percentage of cases that experience a significant change in downstream clinical management as a direct consequence of a diagnostic result through whole genome sequencing.

Study Design:

Observational study predicated on positive findings of a randomized crossover study, which demonstrated superior diagnostic efficacy of WGS compared to standard diagnostic testing.

Setting/Participants:

This is a single-site study located at the Children's Hospital of Philadelphia (CHOP). Study recruitment will continue until 100 trios (or duos) have been enrolled.

Study Interventions and Measures:

This is an observational study of subjects who are expected to receive CLIA-certified whole genome sequencing as part of their clinical care. Research-only measures will include both retrospective and prospective review of medical records.

Main Study Outcome Measures:

Clinical utility outcomes will be assessed using medical record review.

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Leukodystrophies are a group of approximately 30 genetic diseases that primarily affect the white matter of the brain[1,2], a complex structure composed of axons sheathed in myelin, a glial cell-derived lipid-rich membrane[1]. Leukodystrophies are frequently characterized by early onset, spasticity and developmental delay, and are degenerative in nature[1]. As a whole,

leukodystrophies are relatively common (approximately 1 in 7000 births or almost twice as prevalent as Prader-Willi Syndrome, which has been far more extensively studied) with high associated health-care costs; however, more than half of the suspected leukodystrophies do not have a definitive diagnosis[3,4], and are generally classified as "leukodystrophies of unknown etiology". Even when a diagnosis is achieved, the diagnostic process lasts an average of eight years[5] and results in test expenses in excess of $8,000 on average per patient, including the majority of patients who never achieve a diagnosis at all [6,7]. These diagnostic challenges represent an urgent and unresolved gap in knowledge and disease characterization, as obtaining a definitive diagnosis is of paramount importance for leukodystrophy patients[8]. The diagnostic workup begins with findings on cranial Magnetic Resonance Imaging (MRI) followed by sequential targeted genetic testing[1], however next generation sequencing (NGS) technologies offer the promise of rapid and more cost effective approaches.

NGS technologies have the potential to revolutionize the diagnostic process for rare diseases, especially those such as the leukodystrophies, which have seen such historically low rates of diagnosis. Our international working group (spearheaded by Drs. Vanderver, Taft, Bonkowsky, Bernard, Schiffmann, and van der Knaap) has extensive and globally recognized expertise in deciphering the genetics underlying unsolved leukodystrophies. They collectively have been the largest contributor to the modern understanding of leukodystrophy etiology and pathobiology. This body of work over the last decades includes the description of Vanishing White Matter Disease (*EIF2B1-5*)[18-20], 4H syndrome (*POL3RA and B*)[21-23], Leukoencephalopathy with Brainstem and Spinal Cord Involvement and Lactate Elevation (*DARS2*)[24,25], Megalencephalic Leukoencephalopathy with subcortical Cysts (*MLC1 and HEPACAM*)[26,27], Leukoencephalopathy with Thalamic involvement and Lactate and Slow Improvement (*EARS2*)[28,29].

In the last several years, the pace of discovery has increased at a remarkable rate due to the use of whole genome sequencing (WGS) and whole exome sequencing (WES). This includes the discovery of a novel leukodystrophy, Hypomyelination with Brain Stem and Spinal Cord Involvement and Leg Spasticity (HBSL), which is caused by mutations in *DARS*[30], as well as the discovery that Hypomyelination with Atrophy of the Basal Ganglia and Cerebellum (H-ABC) is caused by *de novo TUBB4A* mutations[31]. Finally, our group has performed the first cohort analysis of WES in leukodystrophy, with a diagnostic efficacy in the unsolved patients of nearly 50%, decreasing the overall proportion of unsolved leukodystrophy cases from 50% to approximately 25%.

Despite these advances in diagnostic efficacy, there are still significant issues with respect to implementation of NGS in clinical settings. First, sample cohorts demonstrating diagnostic efficacy are generally small, retrospective, and susceptible to ascertainment bias, ultimately rendering them poor candidates for utility analyses (to determine how efficient a test is at producing a diagnosis). Second, historic sample cohorts have not been examined prospectively for information about impact on clinical management (whether the test results in different clinical monitoring, a change in medications, or alternate clinical interventions).

To address these issues, the study team conducted an investigation of patients with suspected leukodystrophies or other genetic disorders affecting the white matter of the brain at the time of initial confirmation of MRI abnormalities, with prospective collection of patients randomly received on a "first come, first served" basis from a network of expert

clinical sites. Subjects were randomized to receive early (1 month) or late (6 months) WGS, with Standard of Care (SoC) clinical analyses conducted alongside WGS testing. An interim analysis performed in May 2018 assessed these study outcomes for a cohort of thirty-four (34) enrolled subjects. Two of these subjects were resolved before complete enrollment and were retained as controls. Nine subjects were stratified to the Immediate Arm, of which 5 (55.6%) were resolved by WGS and 4 (44.4%) were persistently unresolved. Of the 23 subjects randomized to the Delayed Arm, 14 (60.9%) were resolved by WGS and 5 (21.7%) by SoC, while the remaining 4 (17.4%) remained undiagnosed. The diagnostic efficacy of WGS in both arms was significant relative to SoC ($p<0.005$). The time to diagnosis was significantly shorter in the immediate WGS group ($p<0.05$). The overall diagnostic efficacy of the combination of WGS and SoC approaches was 26/34 (76.5%; 95% CI = 58.8% to 89.3%) over <4 months, greater than historical norms of <50% over more than 5 years.

1.2 Rationale for Approach

We anticipate that whole genome sequencing will produce measurable downstream changes in diagnostic status and clinical management, as defined by disease-specific screening for complications or implementation of disease-specific therapeutic approaches.

1.3 Compliance Statement

This study will be conducted in full accordance with all applicable Children's Hospital of Philadelphia Research (CHOP) Policies and Procedures and all applicable Federal and state laws and regulations, including 45 CFR 46, and the HIPAA Privacy Rule. Any episode of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report unanticipated problems involving risks to research subjects or others. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

This study seeks to determine whether WGS results in changes to diagnostic status and clinical management in subjects affected by undiagnosed genetic disorders of the white matter of the brain.

2.1 Primary Objective (or Aim 1)

Estimate the percentage of cases that experience a significant change in diagnostic status as a direct consequence of whole genome sequencing.

The primary objective of this study is to evaluate changes in diagnostic status in the study cohort for patients who received WGS as part of clinical care. Differences in diagnostic status will be measured at disclosure of initial results or disclosure of reanalyzed results.

Types of change in management are defined in Section 5.1.4.

2.2 Secondary Objective (or Aim 2)

Estimate the percentage of cases that experience a significant change in downstream clinical management as a direct consequence of a diagnosis through whole genome sequencing (subjects with a diagnostic result).

The secondary objective of this study is to evaluate changes in clinical care in subjects who received a diagnosis through WGS. Differences in clinical care will be evaluated 1 year following disclosure of results.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

This is an observational study of subjects who are expected to receive CLIA-certified whole genome sequencing as part of their clinical care.

3.1.1 Screening Procedures

Candidates with a suspected leukodystrophy or other suspected genetic disorder affecting the white matter of the brain will be provided with information about the study by a qualified member of the study team. Interested candidates will be invited to enroll in CHOP IRB 14-011236 to facilitate collection and review of medical records. At a minimum, these records must include a copy of the most recent neurologic exam and brain MRI. Additional notes, reports, test results, imaging studies, etc. will be requested if available.

Candidates who meet basic eligibility requirements will then be independently reviewed by Dr. Genevieve Bernard, MD, a pediatric neurologist at McGill University with significant expertise in MRI pattern recognition. Dr. Bernard may receive abstract clinical summaries, as well as radiology studies that have been coded with a unique study identifier generated by the CHOP study team. Dr. Bernard will not be able to link these study identifiers to PHI.

MRIs will be analyzed according to a publicly available non-computational algorithm accessible to even non-expert child neurologists and geneticists[46]. Candidates may be enrolled irrespective of the clinician's presumptive diagnosis (even if the clinician feels there is an unambiguous diagnosis based on MRI and initial clinical assessment), as long as no confirmatory testing has been performed. Each candidate's MRIs must be reviewed by at least two of the four experts. If necessary, candidates will be reclassified with respect to their pre-test diagnostic probabilities.

3.1.2 Follow-Up Period (12 Months FOLLOWING REPORT)

Subjects enrolled in the study will concurrently undergo WGS as part of their clinical care, with samples sent to the Illumina Clinical Services Laboratory (ICSL) for clinically-validated testing.

3.1.3 Early Termination and Withdrawal

Subjects may withdraw from the study at any time and for any reason. This would result in removal of any identifying information from recorded databases.

3.2 Study Duration, Enrollment and Number of Sites

3.2.1 Duration of Study Participation

Study participation will last for 12 months following the disclosure of results.

3.2.2 Total Number of Study Sites/Total Number of Subjects Projected

This is a single-site study located at the Children's Hospital of Philadelphia (CHOP). Study recruitment will continue until 100 trios (or duos) have been enrolled.

3.3 Prospective Study Population (WGS Cohort)

3.3.1 Inclusion Criteria & Initial Candidate Assessment

These criteria have been selected in order to ensure that selected cases fit the definition of a leukodystrophy [33] and allow accurate calculations of diagnostic efficacy based on time to diagnosis from initial identification of a white matter disorder. The heritable leukodystrophies affect males and females equally, with the exception of Pelizaeus Merzbacher and ALD, which are X-linked. We expect therefore that males and females will be equally represented in the population of patients with unclassified leukodystrophies. The age of presentation is variable, ranging from infancy to adulthood. All ethnicities are equally represented in these disorders, and we expect ethnicities to be represented based on U.S. census data of population distribution.

3.3.2 Inclusion Criteria – All Candidates

1) Abnormalities of the white matter signal on neuroimaging (MRI) with T2 hyperintensity which must be diffuse or involve specific anatomical tracts consistent with a genetic diagnosis;
2) No pre-existing genetic diagnosis;
3) A clinical decision has been made to perform WGS;
4) Less than 18 years of age (exception for affected sibling of the proband);
5) Availability of both biologic parents for blood sampling;
6) Availability of both biological parents to provide informed consent;
7) Concurrently enrolled in CHOP IRB 14-011236 (Myelin Disorders Biorepository Project)

3.3.3 Exclusion Criteria – All Candidates

1) Candidates with acquired disorders, including infection, acute disseminated encephalomyelitis (ADEM), multiple sclerosis, vasculitis or toxic leukoencephalopathies;
2) Patients who have had previous next-generation genetic sequencing*, including WES or WGS;
3) Those with no third-party payer insurance, unable to receive standard of care diagnosis and therapeutic approaches;

4) Candidates who have already received a diagnosis.

***Note:** Karyotype or microarray testing that did not yield a definitive diagnosis should not be considered as an excluding factor.

3.3.4 Inclusion Criteria - Parents

1) Males or females 18 years or older;
2) Child with a suspected leukodystrophy or other suspected genetic disorder affecting the white matter of the brain.

3.3.5 Exclusion Criteria - Parents

1) Inability to provide consent;
2) Pregnant women and fetuses will not be included in this study.

Candidates who do not meet all of the enrollment criteria may not be enrolled. Violations of these criteria will be reported in accordance with IRB Policies and Procedures.

4 STUDY EVALUATIONS AND MEASUREMENTS

4.1 Study Outcome Measures

4.1.1 Prospective Collection of Clinical Utility Data

Clinical utility outcomes will be assessed using a combination of medical record review 1 year prior to (retrospective) and 1 year following (prospective) disclosure of results. An appropriately trained member of the study team will review this data for documented changes in care and clinical state, included changes in medical morbidities, surgeries, pharmacologic management of complications and implementation of disease specific therapies. The following data elements will be abstracted from patient medical records for analysis.

- Diagnostic status (primary): Results were diagnostic, non-diagnostic (negative or variant of unknown significance (VUS) with inconsistent phenotype), uncertain (previously and/or not previously reported VUS identified and requires follow-up).
- Implications of WGS: confirmed a diagnosis within the differential diagnosis prior to testing, established a new diagnosis that was not previously suspected, produced a diagnosis unrelated to the clinical indication for testing [includes incidental findings and/or secondary findings], ruled out a suspected diagnosis/ diagnoses, eliminated the need for additional tests or evaluations, contributed to the overall diagnostic evaluation of this patient.
    - Diagnostic changes in management if diagnostic result (secondary): Referrals to New Healthcare Providers (inc. Specialty)
    - Disease-specific treatment or medication Changes (inc. Type and Detail)
    - Disease-specific screening (implemented new screening, screening implemented for suspected dx was justified)
    - Decisions on palliative care

- Participation in research study (Type)
- Family planning, counseling, or support (Type)

4.1.2 Outcome Measure Definitions

The following types of change in management will be considered.

- Recommended changes in future disease monitoring, as documented in medical records or based on changes to providers seen or interventions recommended;
- Changes in the detection and/or management of target complications (ex. aspiration pneumonia) as documented in medical records or based on changes to providers seen or interventions recommended;
- Recommended changes in monitoring for secondary complications (ex. cancer susceptibility) as documented in medical records or based on changes to providers seen or interventions recommended;
- Changes to condition specific management (specific therapy related to the etiology of the diagnosis) as documented in medical records or based on changes to providers seen or interventions recommended;
- Changes to specific supportive interventions (supportive care informed by a better understanding of the diagnosis) as documented in medical records or based on changes to providers seen or interventions recommended;
- Changes to palliative or end-of-life care or a significant change to expected outcomes (informed by a better understanding of the prognosis) as documented in medical records or based on changes to providers seen or interventions recommended.

4.2 Safety Evaluation

As a minimal-risk observational study, the investigators do not intend to implement safety evaluations. Results of clinical testing performed concurrently to study enrollment will be disclosed by licensed practitioners in accordance with established clinical guidelines. Data collection from medical records and presents minimal risks to subjects.

5 STATISTICAL CONSIDERATIONS

5.1 Analysis Plan and Sample Size Determination

5.1.1 Analysis for Aim 1

The proportion of subjects who have a change in diagnosis status and the conditional proportion of subjects who were impacted by WGS will be calculated for the cohort, with 95% confidence intervals (95% CI). Individuals who were recruited to the previous (randomized crossover) version of this protocol will be included.

5.1.2 Analysis for Aim 2

The frequency and conditional proportion of subjects who received downstream changes in clinical management after a diagnostic result through WGS will be calculated for the cohort, with 95% confidence intervals (95% CI). The type of downstream clinical changes will be reported. Individuals who were recruited to the

previous (randomized crossover) version of this protocol and received a diagnosis through WGS will be included.

5.1.3 Sample Size Determination

5.1.3.1 ***Aim 1:*** *Due to the rapid changes in standard of care, many of the control arm were ineligible for participation as a historical control, since they received WGS through clinical care. These changes to standard of care also hindered study enrollment sample size goals. The total sample size that was achieved is N=80 (including the subjects enrolled in the randomized control arm). Power calculations are no longer needed for comparison to control arm.*

5.1.3.2 ***Aim 2 Refer to 5.1.2.***

5.1.4 Missing Data

The proportion of subjects who were lost to follow-up will be reported.

6 SAFETY MANAGEMENT

The major research-specific risk associated with this observational study is the inadvertent disclosure of protected health information (PHI) from medical records collected during the follow-up period. If any unanticipated problems related to the research involving risks to subjects or others happen during the course of this study (including SAEs) they will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

7 STUDY MANAGEMENT

7.1 Data Collection and Management

Subject medical records will be kept in locked file cabinets in the principal investigator's office. Only the approved study staff will be able to access these records. Demographic information will be kept in a password-protected REDCap database accessible only by approved study staff. While we do not anticipate the need to regularly collect parental medical records, this would be handled in a similar fashion.

Withdrawal from the study is permitted at any time and would result in removal of any identifying information from recorded databases.

7.2 Confidentiality

No PHI will be used for future study without first obtaining IRB approval. A data use agreement (DUA) will be executed between the provider of the data and any recipient researchers (including those at CHOP) before sharing a limited dataset. Fully de-identified data may be shared on a case-by-case basis.

7.3 Regulatory and Ethical Considerations

7.3.1 Risk Assessment, General Considerations

This observational study is considered to involve no greater than a minimal amount of risk. The primary risk of study participation involves breach of confidentiality due to collection, analysis, and/or retention of PHI from subject medical records.

7.3.2 Psychological Harms

All genetic testing done in this study is part of the child's clinical care. We do not anticipate any psychological harms. This protocol may give false hope; the principal investigator and genetic counselors will attempt to avoid this.

7.3.3 Discrimination

To the fullest extent possible, the investigators will not disclose to third parties any information about the participants without their expressed consent.

7.3.4 Confidentiality of Medical Information and Samples

Upon enrollment in CHOP IRB 14-011236 for preliminary assessment of eligibility, candidates will receive a unique alphanumerical study identifier (ex. LS_078) generated by study staff. The key will be located in a password-protected REDCap database, accessible only by approved study staff. Names are maintained in this database in order to provide patients with clinically relevant information generated from this study. Withdrawal from the study would result in removal of any identifying information from recorded databases and destruction of any blood samples.

7.3.5 Potential Benefits

Study participation is not intended to confer any direct benefit to subjects. The investigators are confident that the knowledge gleaned from this study will support wider implementation and access to whole genome sequencing.

7.3.6 Risk-Benefit Assessment

This is a no more than minimal risk protocol, where risks encountered are the same as those encountered in routine clinical care with the addition of those risks related to medical record review.

7.4 Recruitment Strategy

Candidates will generally be identified during their routine clinical care at CHOP and other outside institutions. Information about the study has been posted on ClinicalTrials.gov (NCT02699190), and may be shared with clinicians and/or investigators at CHOP and other outside institutions who routinely encounter patients with suspected leukodystrophies or other genetic disorders affecting the white matter of the brain.

Referring providers will ask the study candidate's family to complete a REDCap-based 'Referral Survey' used by the CHOP Leukodystrophy Center to collect basic contact and clinical information from individuals/families interested in scheduling a clinical appointment and/or participating in a research study.

7.5 Informed Consent/Assent and HIPAA Authorization

Informed consent will typically be documented in the context of a subject's clinical evaluation at CHOP. The subject will be given a thorough explanation of the study, including the purpose, procedures, risks and benefits of participation, confidentiality, procedures for withdrawal, and contact information for study personnel. Families will be informed that their medical care at that institution will not be affected if they choose not to participate in the proposed research. If the subject is interested in enrolling, a qualified member of the study team will ensure that informed consent is fully documented using approved versions of the informed consent form (ICF).

Methods other than a face-to-face consent interview may be performed if an eligible subject is unable to visit a participating study site. In this scenario, study staff will ensure that this approach allows for an adequate exchange of information and documentation, and a method to ensure that the signer of the consent form is the person who plans to enroll as a subject in the clinical investigation or is legally authorized to provide a signature on behalf of the subject. The ICF will be sent to the subject by postal mail, facsimile, or email, and the consent interview will then be conducted by telephone or a CHOP-approved video conferencing tool such as WebEx, BlueJeans, or Microsoft Teams after ensuring that the subject has received a copy of the informed consent form for reference during the discussion.

Following the consent discussion, the subject will sign and date the consent form and return the document to an authorized member of the Study Team by postal mail, facsimile, email, or by uploading it to a CHOP-approved file hosting service such as Box, OneDrive, Google Drive, etc.

A subject may also be invited to sign the informed consent form electronically using an eletronic signature via Adobe Sign or DocuSign, or any other cloud-based electronic signature platform that has been approved for use by the CHOP Research Institute. In such a case, forms will be sent for signature via secure email. Upon completion of all required fields, the document will be automatically returned to a designated member of the study team for review and final signature. A fully executed copy will then be uploaded to the subject's research chart in REDCap and returned to the subject for future reference.

Subjects who turn 18 while enrolled in the study will be asked to sign the currently approved version of the consent form at their next study visit after the subject reaches the age of majority (or, if cognitively impaired, their legally authorized representative).

All reasonable efforts will be made to accommodate non-English speaking subjects. If a Limited English Proficiency (LEP) subject is able to visit a participating study site for enrollment, a certified clinical interpreter will generally be available to facilitate the 'Short Form Consent' process, which has been approved for use in this study. The study team will document the interpreter's employee ID number.

If a 'Short Form Consent' form is not available in a particular subject's native language, the study team will submit an amendment to the CHOP IRB. The amendment will include a

translated version of 'Short Form Consent' form in the language needed, along with a certificate of translation, and will need to be approved prior to enrolling the subject.

The study team will obtain assent from adults with diminished capacity and children older than 7 years of age whenever possible. If subjects under the age of 18 years are unable to provide assent, because they are either too young (less than 7 years of age), or their disease interferes with their ability to provide assent, this reason will be documented on the consent/assent forms.

Upon occasion, subjects may become lost to follow-up. This applies in particular to re-consent in patients over the age of 18. A subject will be considered lost to follow up if 3 attempts by phone or email on separate days and separate times and hard copy letter if address is current are unsuccessful in reaching the subject.

7.5.1 Short Form Consent Process Requiring Telephonic Interpretation Services

If a certified interpreter is not physically available at the time of a site visit, or if a LEP subject is unable to travel to a participating site, an authorized telephonic interpretation service (e.g., 'InterpreTalk') will be used to facilitate the 'Short Form Consent' process.

A member of the study team will share an approved 'Short Form Consent' document in a language native to the subject prior to the consent interview. Upon conclusion of the consent interview, the subject will sign the 'Short Form Consent' form, and an impartial witness/interpreter who speaks both the subject's native language and English will be asked to sign the 'Short Form Consent' form and Study Summary Document". The witness/interpreter's involvement (when not present in person) will be documented.

7.5.2 HIPAA Attestation for the Use of Decedent PHI

A waiver of HIPAA Authorization has been requested to use clinical data obtained from deceased individuals, as this research will also utilize the PHI of decedents. In accordance with 45 CFR 164.512(i)(1)(iii), the use or disclosure being sought is solely for research on the protected health information of decedents, the protected health information being sought is necessary for the research, and, at the request of the covered entity (CHOP), documentation of the death of the individuals about whom information is being sought can be provided. Since decedents are no longer human subjects, their data/samples included in this research will not be included in the enrollment numbers for this study and research activities using these samples/data will not be reported as part of the continuing review.

7.6 Payment to Subjects/Families

There will be no payment or gifts to subjects or families as part of this protocol.

8 PUBLICATION

As this study may involve findings of novel genes implicated in disease, identification of a specific individual from the diagnosis could be discerned in publications or presentations although this is unlikely in a cohort of 100 participants and their biological parents. It is doubtful that any demographic information could identify an individual.

9 REFERENCES

1. Costello DJ, Eichler AF, Eichler FS. Leukodystrophies: classification, diagnosis, and treatment. *The neurologist* 2009; **15**(6): 319-28.
2. Boespflug-Tanguy O, Labauge P, Fogli A, Vaurs-Barriere C. Genes involved in leukodystrophies: a glance at glial functions. *Current neurology and neuroscience reports* 2008; **8**(3): 217-29.
3. Bonkowsky JL, Nelson C, Kingston JL, Filloux FM, Mundorff MB, Srivastava R. The burden of inherited leukodystrophies in children. *Neurology* 2010; **75**(8): 718-25.
4. van der Knaap MS, Breiter SN, Naidu S, Hart AA, Valk J. Defining and categorizing leukoencephalopathies of unknown origin: MR imaging approach. *Radiology* 1999; **213**(1): 121-33.
5. Vanderver A, Hussey H, Schmidt JL, Pastor W, Hoffman HJ. Relative incidence of inherited white matter disorders in childhood to acquired pediatric demyelinating disorders. *Seminars in pediatric neurology* 2012; **19**(4): 219-23.
6. Richards J, Korgenski EK, Srivastava R, Bonkowsky JL. Costs of the diagnostic odyssey in children with inherited leukodystrophies. *Neurology* 2015; **85**(13): 1167-70.
7. Richards J, Korgenski EK, Taft RJ, Vanderver A, Bonkowsky JL. Targeted leukodystrophy diagnosis based on charges and yields for testing. *Am J Med Genet A* 2015; **167A**(11): 2541-3.
8. Kohlschutter A, Eichler F. Childhood leukodystrophies: a clinical perspective. *Expert review of neurotherapeutics* 2011; **11**(10): 1485-96.
9. Bamshad MJ, Ng SB, Bigham AW, et al. Exome sequencing as a tool for Mendelian disease gene discovery. *Nature reviews Genetics* 2011; **12**(11): 745-55.
10. Ng SB, Nickerson DA, Bamshad MJ, Shendure J. Massively parallel sequencing and rare disease. *Human molecular genetics* 2010; **19**(R2): R119-24.
11. Makrythanasis P, Nelis M, Santoni FA, et al. Diagnostic Exome Sequencing to Elucidate the Genetic Basis of Likely Recessive Disorders in Consanguineous Families. *Hum Mutat* 2014.
12. Taylor RW, Pyle A, Griffin H, et al. Use of whole-exome sequencing to determine the genetic basis of multiple mitochondrial respiratory chain complex deficiencies. *JAMA : the journal of the American Medical Association* 2014; **312**(1): 68-77.
13. Srivastava S, Cohen JS, Vernon H, et al. Clinical Whole Exome Sequencing in Child Neurology Practice. *Annals of neurology* 2014.
14. Fogel BL, Lee H, Deignan JL, et al. Exome Sequencing in the Clinical Diagnosis of Sporadic or Familial Cerebellar Ataxia. *JAMA neurology* 2014.
15. Yang Y, Muzny DM, Reid JG, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *The New England journal of medicine* 2013; **369**(16): 1502-11.
16. Beaulieu CL, Majewski J, Schwartzentruber J, et al. FORGE Canada Consortium: outcomes of a 2-year national rare-disease gene-discovery project. *American journal of human genetics* 2014; **94**(6): 809-17.

17. de Ligt J, Willemsen MH, van Bon BW, et al. Diagnostic exome sequencing in persons with severe intellectual disability. *The New England journal of medicine* 2012; **367**(20): 1921-9.

18. Schiffmann R, Fogli A, Van der Knaap MS, Boespflug-Tanguy O. Childhood Ataxia with Central Nervous System Hypomyelination/Vanishing White Matter. In: Pagon RA, Adam MP, Bird TD, Dolan CR, Fong CT, Stephens K, eds. GeneReviews. Seattle (WA); 1993.

19. van der Knaap MS, Barth PG, Gabreels FJ, et al. A new leukoencephalopathy with vanishing white matter. *Neurology* 1997; **48**(4): 845-55.

20. Leegwater PA, Vermeulen G, Konst AA, et al. Subunits of the translation initiation factor eIF2B are mutant in leukoencephalopathy with vanishing white matter. *Nature genetics* 2001; **29**(4): 383-8.

21. Timmons M, Tsokos M, Asab MA, et al. Peripheral and central hypomyelination with hypogonadotropic hypogonadism and hypodontia. *Neurology* 2006; **67**(11): 2066-9.

22. Bernard G, Chouery E, Putorti ML, et al. Mutations of POLR3A encoding a catalytic subunit of RNA polymerase Pol III cause a recessive hypomyelinating leukodystrophy. *American journal of human genetics* 2011; **89**(3): 415-23.

23. Daoud H, Tetreault M, Gibson W, et al. Mutations in POLR3A and POLR3B are a major cause of hypomyelinating leukodystrophies with or without dental abnormalities and/or hypogonadotropic hypogonadism. *Journal of medical genetics* 2013; **50**(3): 194-7.

24. van der Knaap MS, van der Voorn P, Barkhof F, et al. A new leukoencephalopathy with brainstem and spinal cord involvement and high lactate. *Annals of neurology* 2003; **53**(2): 252-8.

25. Scheper GC, van der Klok T, van Andel RJ, et al. Mitochondrial aspartyl-tRNA synthetase deficiency causes leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation. *Nature genetics* 2007; **39**(4): 534-9.

26. Leegwater PA, Yuan BQ, van der Steen J, et al. Mutations of MLC1 (KIAA0027), encoding a putative membrane protein, cause megalencephalic leukoencephalopathy with subcortical cysts. *American journal of human genetics* 2001; **68**(4): 831-8.

27. Lopez-Hernandez T, Ridder MC, Montolio M, et al. Mutant GlialCAM causes megalencephalic leukoencephalopathy with subcortical cysts, benign familial macrocephaly, and macrocephaly with retardation and autism. *American journal of human genetics* 2011; **88**(4): 422-32.

28. Steenweg ME, Vanderver A, Ceulemans B, et al. Novel infantile-onset leukoencephalopathy with high lactate level and slow improvement. *Archives of neurology* 2012; **69**(6): 718-22.

29. Steenweg ME, Ghezzi D, Haack T, et al. Leukoencephalopathy with thalamus and brainstem involvement and high lactate 'LTBL' caused by EARS2 mutations. *Brain : a journal of neurology* 2012; **135**(Pt 5): 1387-94.

30. Taft RJ, Vanderver A, Leventer RJ, et al. Mutations in DARS cause hypomyelination with brain stem and spinal cord involvement and leg spasticity. *American journal of human genetics* 2013; **92**(5): 774-80.

31. Simons C, Wolf NI, McNeil N, et al. A de novo mutation in the beta-tubulin gene TUBB4A results in the leukoencephalopathy hypomyelination with atrophy of the basal ganglia and cerebellum. *American journal of human genetics* 2013; **92**(5): 767-73.

32. Vanderver A, Hathout Y, Maletkovic J, et al. Sensitivity and specificity of decreased CSF asialotransferrin for eIF2B-related disorder. *Neurology* 2008; **70**(23): 2226-32.

33. Vanderver A, Schiffmann R, Timmons M, et al. Decreased asialotransferrin in cerebrospinal fluid of patients with childhood-onset ataxia and central nervous system hypomyelination/vanishing white matter disease. *Clinical chemistry* 2005; **51**(11): 2031-42.

34. Rice G, Patrick T, Parmar R, et al. Clinical and molecular phenotype of Aicardi-Goutieres syndrome. *American journal of human genetics* 2007; **81**(4): 713-25.

35. Rice GI, Bond J, Asipu A, et al. Mutations involved in Aicardi-Goutieres syndrome implicate SAMHD1 as regulator of the innate immune response. *Nature genetics* 2009; **41**(7): 829-32.

36. Mochel F, Engelke UF, Barritault J, et al. Elevated CSF N-acetylaspartylglutamate in patients with free sialic acid storage diseases. *Neurology*; **74**(4): 302-5.

37. Mochel F, Sedel F, Vanderver A, et al. Cerebellar ataxia with elevated cerebrospinal free sialic acid (CAFSA). *Brain : a journal of neurology* 2009; **132**(Pt 3): 801-9.

38. Gorospe JR, Singhal BS, Kainu T, et al. Indian Agarwal megalencephalic leukodystrophy with cysts is caused by a common MLC1 mutation. *Neurology* 2004; **62**(6): 878-82.

39. Schiffmann R, Boespflug-Tanguy O. An update on the leukodsytrophies. *Curr Opin Neurol* 2001; **14**(6): 789-94.

40. Singhal BS. Leukodystrophies: Indian scenario. *Indian J Pediatr* 2005; **72**(4): 315-8.

41. Srikanth SG, Chandrashekar HS, Nagarajan K, Jayakumar PN. Restricted diffusion in Canavan disease. *Childs Nerv Syst* 2007; **23**(4): 465-8.

42. Valk J. Indian Agarwal megalencephalic leukodystrophy with cysts is caused by a common MLC1 mutation. *Neurology* 2004; **63**(11): 2197; author reply

43. Linnankivi T, Lundbom N, Autti T, et al. Five new cases of a recently described leukoencephalopathy with high brain lactate. *Neurology* 2004; **63**(4): 688-92.

44. van der Knaap MS, Linnankivi T, Paetau A, et al. Hypomyelination with atrophy of the basal ganglia and cerebellum: follow-up and pathology. *Neurology* 2007; **69**(2): 166-71.

45. Vanderver A, Simons C, Helman G, et al. Whole exome sequencing in patients with white matter abnormalities. *Annals of neurology* 2016; **79**(6): 1031-7.

46. Schiffmann R, van der Knaap MS. Invited article: an MRI-based approach to the diagnosis of white matter disorders. *Neurology* 2009; **72**(8): 750-9.