Epilepsy in Individuals with Periventricular Nodular Heterotopia: Defining the Clinical Phenotypic Spectrum and Underlying Molecular Causes

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ABSTRACT

Background

Periventricular nodular heterotopia (PVNH) is a common congenital malformation of the brain, characterized by abnormal nodular masses of grey matter around the lateral ventricles. PVNH is known to be associated with seizures, thought to be most often drug-resistant. The existing literature is limited however, and consequently, the epileptology of this condition remains unclear.

Objectives

We aimed to clarify the spectrum of epilepsy phenotypes in PVNH and the significance of more specific patterns of brain malformation to be able to provide clinicians with sound recommendations for diagnostic workup and patient management.

Methods

We recruited individuals with PVNH and a history of seizures and collected clinical data through medical record review, patient interviews and a standardized questionnaire.

Results

One hundred individuals were studied, aged 1 month to 61 years old. Mean age of seizure onset was 7.9 years. Of the 100 patients, 10 had self-limited epilepsy, 35 had a pharmacoresponsive epilepsy course and 55 had ongoing seizures, of which 23 were defined as drug-resistant. Patients were divided based on the presence of PVNH alone (PVNH-Only) or PVNH with other brain malformation (PVNH-Plus). Subgroups were created as follows: 18 PVNH-Only with a single nodule, 21 PVNH-Only multiple nodules, 8 PVNH-Plus single nodule and 53 PVNH-Plus multiple nodules. Focal seizure types were predominant across the cohort.

Generalized seizures were more common in PVNH-Plus compared to PVNH-Only patients. Of PVNH-Only single nodule, there were no patients that had drug-resistant seizures. Amongst PVNH-Plus patients, those with multiple bilateral nodules demonstrated the highest proportion of drug-resistant epilepsy (39%). A review of genetic testing results revealed 8 patients with single-gene variants classified as pathogenic or likely pathogenic (2 of which were *FLNA*), and 5 with copy number variants, 2 of which were pathogenic.

Conclusion

The spectrum of epilepsy phenotypes in PVNH is broad. Epilepsy features are highly variable between patients; however, epilepsy course may be predicted to an extent by the pattern of malformation. A genetic etiology, identified in a small proportion of individuals, is very heterogeneous.

RÉSUMÉ

Contexte

L’hétérotopie nodulaire périventriculaire (l’HNPV) est une malformation congénitale du cerveau qui est caractérisée par des amas nodulaires de matière grise anormales dans la région autour des ventricules latéraux. L’HNPV est associée avec l’épilepsie chez des personnes atteintes de ce maladie, et présentement, l’épilepsie de type HNPV est considérée comme résistantes aux médicaments. La littérature existante est cependant limitée et, par conséquent, l'épileptologie n’est pas bien connu.

Objectifs

Nous avons cherché à clarifier le spectre des phénotypes d'épilepsie chez l’HNPV et l'importance de modèles plus spécifiques de malformation cérébrale afin de pouvoir fournir aux cliniciens des recommandations judicieuses pour le bilan diagnostique et la prise en charge des ces patients.

Méthodes

Nous avons recruté des personnes atteintes de l’HNPV et ayant des antécédents de crises épileptiques, et nous avons recueilli leurs données cliniques par un examen de leur dossier médical et un entretien pour remplir un questionnaire standardisé.

Résultats

Cent individus ont été inclus, âgés de 1 mois à 61 ans. L'âge moyen de ces patients lors de l’apparition de leurs crises était 7,9 ans. Parmi les 100 patients, 10 avaient l’épilepsie résolue, 35 étaient libre de crises sous médicaments épileptiques et 55 avaient des crises en cours au moment de l’entretien, dont 23 étaient définies comme résistantes aux médicaments. Les patients

ont été divisés en fonction de la présence de l'HNPV isolé (PVNH-Only) ou de l'HNPV avec d'autres malformations cérébrales (PVNH-Plus). Les groupes étaient les suivants: 18 PVNH-Only avec nodule unique, 21 PVNH-Only avec plusieurs nodules, 8 PVNH-Plus nodule unique et 53 PVNH-Plus plusieurs nodules. Les crises de type focale étaient les plus courants dans la cohorte. Les crises généralisées étaient plus fréquentes chez les patients PVNH-Plus que chez les patients PVNH-Only. Parmi ceux avec un nodule unique du groupe PVNH-Only, aucun patient n'a présenté de crises résistantes aux médicaments. Parmi les patients PVNH-Plus, ceux avec plusieurs nodules bilatéraux présentaient la proportion la plus élevée d'épilepsie résistante aux médicaments (39 %). Un examen des résultats des tests génétiques a révélé 8 patients avec des variants monogéniques classés comme pathogènes ou probablement pathogènes (dont 2 étaient du gène *FLNA*) et 5 avec des variants du nombre de copies, dont 2 étaient pathogènes.

Conclusion

Le spectre de phénotypes d'épilepsie chez les personnes atteintes de l'HNPV est grand. Les caractéristiques de l'épilepsie associé sont très variables d'un patient à l'autre; cependant, dans une certaine mesure, l'évolution de l'épilepsie peut être prévu par le schéma de malformation. Une étiologie génétique, identifiée chez une faible proportion d'individus, est très hétérogène.

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AUTHOR CONTRIBUTIONS

The candidate contributed to all aspects of the study, including design of the questionnaire, data collection, statistical analyses, results interpretation, and manuscript writing.

Dr. Christelle Dassi and Dr. Saoussen Berrahmoune provided logistical and administrative expertise, and assisted with patient recruitment.

Dr. Marlin Liz Bejaran, Dr. Carlos Eduardo Valera Davila, Dr. Antonella Riva, Dr. Thea Giacomini assisted with data collection, and provided feedback on the manuscript.

Dr. Maria Carme Fons Estupiñà, Dr. Ariadna Borràs Martinez, Dr. Maria Margherita Mancardi, Dr. Mariasevina Severino, Dr. Romina Romaniello, Dr. François Dubeau and Dr. Myriam Srour provided patient contact information for recruitment from their respective clinical and research databases, and provided feedback on the manuscript.

Dr. Kenneth Myers developed the study plan, and was in charge of the study's overall direction. He acquired ethics approval, assisted with patient recruitment, provided expertise and assistance at every step of the study, and assisted with manuscript writing.

THESIS INTRODUCTION

Periventricular nodular heterotopia (PVNH) is among the most common congenital malformations of the brain.[1] PVNH results from the arrest of some migrating neuronal precursor cells to the cortex during cortical development in utero.[2] On magnetic resonance imaging (MRI), PVNH is characterized by the abnormal presence of nodular masses of normal neurons and glial cells within the periventricular region, surrounding the lateral ventricles, and consequently, in many cases, these clusters contribute to awry cortical circuitry and the generation of seizures.[2–5]

Over the past 30 years, numerous studies and case series have revealed the considerable clinical heterogeneity of PVNH depending on the distribution and extent of the malformation.[5–9] The main clinical problem for many who are affected is thought to be drug-resistant epilepsy.[5–7] These studies, however, have been limited by the small number of patients included and biased towards a subset of the population seeking surgical intervention to stop their seizures due to failure to respond to multiple anti-seizure medications (ASM). Furthermore, to date, no large-scale study focused specifically on the epileptology of this disorder has been published. Thus, little is known about the full spectrum of prognostic features, especially in terms of the course of epilepsy in this population. Additionally, PVNH is genetically heterogeneous.[10,11] Certain genetic causes have been identified, including the *FLNA* and *ARFGEF2* genes, although the underlying etiology remains unknown in the majority of cases.[12,13] The many grey areas surrounding this disorder and its presentation render diagnostic counselling and treatment difficult for clinicians. Consequently, a lack of awareness by the patients, their family members or caregivers hinders proper disease management. The findings reported here can serve as a guide for diagnostic work-up and recommendations for treatment plans to assist clinicians with patient care and to promote better health outcomes overall.

THESIS BACKGROUND

Cortical Development

The development of the cerebral cortex is an intricate and tightly controlled process that occurs primarily in utero. The cortex begins to form early in the first trimester from the forebrain.[14] Over the course of the 40 weeks of gestation, the cortex shows a remarkable increase in size and complexity. The process has been characterized into three main steps: (1) progenitor cell proliferation and differentiation, (2) neuronal precursor migration and (3) postmigrational cortical organization and connectivity.[14] Figure A, adapted from a publication by Subramian et al.[14], presents the three main stages of development and demonstrates the key cellular events that occur across the three trimesters of pregnancy. These events are dynamic, occurring sequentially, and sometimes simultaneously, throughout development. At the onset, within the germinal zone of the telencephalon, known as the ventricular zone, neuroepithelial cells (light blue-coloured cells in Figure A) undergo extensive proliferation to establish the pool of progenitor cells.[14] Next, these cells gradually differentiate into radial glial cells which give rise to migrating neuronal precursor cells (orange cells), either directly or indirectly through intermediate progenitor cells (pink cells), and also create a type of scaffold to facilitate their migration toward the eventual cortex.[14,15] These precursor cells are produced in successive waves, whereby early born cells form the deep layers (orange cells with extended processes) and the later born cells (red cells with extended processes) contribute to the more superficial layers of the cortex, resulting in an inside-out formation.[14,16] Once the neuronal precursors have reached their target destination, they undergo maturation, project their axons, form synapses and establish more elaborate networks with other cortical neurons.[14] Interneurons (dark blue cells) migrate to the cortex to integrate into the circuits as well.[14] At this time, rapidly expanding neuronal networks

create a physical stress that contributes to the formation of gyri within the cortical structure.[14] Altogether, this leads to the formation of the well-characterized six-layered cortical structure, containing distinct types of functionally connected neurons organized in highly specialized areas.

Malformations of Cortical Development

Disruption at any of the stages of cortical development can result in a wide range of developmental disorders. These disorders are collectively referred to as malformations of cortical development (MCD). [1,17] Some of these malformations have a clear genetic underpinning, while others are acquired.[10,17] Environmental insults in utero, such as infections, vascular-ischemic events or trauma, may also be causative of the malformation.[18] Advances in neuroimaging technologies, namely high-resolution MRI, along with our improved understanding of normal and pathological brain development, has enabled the identification, delineation and better characterization of these disorders over the past two decades.[19–21] Neuroimaging plays a key role in the diagnoses of MCDs.[21] MCDs are recognized as a heterogeneous group of focal or diffuse congenital anomalies of the cortex, and have been classified based on the earliest stage of cortical development that is affected following the scheme defined by Barkovich et al.[20] Group I comprises malformations associated with abnormal neuronal and glial precursor proliferation or apoptosis, whether due to reduced proliferation or increased apoptosis, elevated proliferation or decreased apoptosis, or abnormal proliferation, causing microcephaly, megalencephaly and cortical dysplasia, respectively.[20] Group II MCDs are secondary to abnormal neuronal precursor migration, and include various grey matter heterotopias, showing neuronal accumulations in abnormal locations, and lissencephalies, where the cortex appears thick by absent or shallow gyri due to few neuronal precursors migrating to the cortex. [20] Group III MCDs are due to abnormal postmigrational development, namely polymicrogyria, characterized by an excessive number of

small gyri, and schizencephaly, demonstrating a cleft from the cortex to the ventricle.[20] These groups are not mutually exclusive.[22] A defect during cortical development may result in more than one morphological subcategory of MCD as well as one type of MCD may result from errors in multiple different developmental mechanisms.[22] Additionally, cortical malformations may occur in association with other extracortical malformations, such as abnormalities of the corpus callosum, hippocampus or cerebellum. [22] The classification scheme for MCDs has been updated several times over the years with greater developments in the field and will continue to evolve with continuous advances in developmental biology, neuropathology, electro-clinical and molecular genetics research.

MCDs have highly variable clinical presentations. Patients with diffuse MCD tend to demonstrate more severe disabilities.[2,22] These children first come to medical attention because of early feeding problems, developmental delay, seizures or other congenital anomalies, such as hydrocephalus, in the first year of life. [2] The most severely disabled children are more likely to have poor developmental and neurological outcomes, as well as a high risk for a reduced life span.[2] Children with these clinical features tend to have severe microcephaly, megalencephaly or lissencephaly.[2] For others, they may only come to medical attention after the onset of focal epilepsy later in life.[2] They may also present with mild-to-moderate learning disability or attention deficit disorder.[2] These less severely affected individuals are more likely to have malformations such as mild forms of grey matter heterotopias or polymicrogyria.[2] As a group, MCDs represent an important cause of drug-resistant epilepsy of variable severity.[18] MCDs can be intrinsically epileptogenic as a result of the abnormal rearrangement of neuronal circuitries within the malformed cortical area or involving areas adjacent to or functionally connected to the

affected area.[18] Overall, the extent of clinical manifestation and the burden of disability depend largely on the severity and etiology of the malformation.

Periventricular Nodular Heterotopia

The grey matter heterotopias (GMH) are a common group of MCDs.[23] The medical term heterotopia refers to the occurrence of normal tissue at an abnormal location. In the context of GMH, it refers to a group of normal neurons and glial cells, forming grey matter, in an abnormal cerebral location.[23] As part of group II MCDs, these heterotopias are secondary to arrested neuronal precursor migration to the cortex. The group is further subdivided into three groups: band heterotopia, subcortical heterotopia and PVNH.[23]

PVNH is the most common type of GMH.[23] PVNH involves round nodules of differentiated neurons and glial cells in the periventricular region, adjacent to the walls or protruding into the lumen of the lateral ventricles.[24] They can be easily detected on MRI, appearing isointense to grey matter.[24] The heterotopic nodules result from a total failure of some neuronal precursors to migrate outward from the ventricular zone, while the remainder of the precursors migrate normally to form the cortex.[24] These nodules vary in number, size, shape and location per patient.[25] The nodules can be unilateral or bilateral, single or multiple, and can be further defined by their location in the frontal, temporal and/or occipital horns of the lateral ventricles.[25] PVNH can occur with other concomitant MCDs, in association with other congenital anomalies of the brain or as part of a multiple congenital anomaly syndrome.[9]

Clinical manifestations are highly heterogeneous, with the main clinical manifestation being epilepsy.[24] Other common neurological features include learning difficulties, namely reading impairment, and developmental delay to varying degrees.[8,24,26] Depending on the underlying cause, patients may present with extra-neurological features such as cardiac

anomalies, coagulopathies, joint hypermobility associated with Ehlers-Danlos syndrome or lung disease. [27,28] Additionally, there are many patients with PVNH who remain asymptomatic, and who are only diagnosed incidentally on MRI.[18]

Epilepsy in Periventricular Nodular Heterotopia

Approximately 80% of patients with PVNH identified on MRI are believed to develop epilepsy during their lifetime.[18] Based on what has been reported in the literature, epilepsy associated with PVNH is frequently drug-resistant, most often refractory focal epilepsy. Early reports of patients with PVNH in the literature throughout the 1980-90s suggest seizure onset in the second decade of life, normal intelligence and normal motor function.[3,29–31] A study of 33 patients by Dubeau et al. described intractable seizures in 82% of the cohort, mainly focal seizures with temporo-parietal-occipital auras.[7] Additional studies over the years have reported considerable variability in epilepsy onset and outcome for patients depending on the extent of their malformation. d'Orsi et al. demonstrated that two distinct electroclinical features exist within their cohort of 16 PVNH patients when separated based on whether periventricular nodules are present alone, known as "simple", or with additional cortical or cerebral malformations, referred to as "plus".[6] The simple group was characterized by focal seizures with onset during the second decade of life and usually becoming less frequent over time.[6] The plus patients tended to have a more severe course and outcome of epilepsy, with an earlier onset of frequent focal seizures in the first decade of life, most often accompanied with sudden drops as well, and becoming drug-resistant early on.[6] Another study by Battaglia et al. showed distinct epileptic outcomes within their cohort of 54 patients subdivided based on imaging data into five groups: bilateral single nodules, bilateral multiple symmetric or asymmetric nodular heterotopia (NH), and unilateral NH with or without extension to the overlying cortex.[5] They concluded that

epilepsy is most likely to be a profound burden for those with bilateral asymmetric and unilateral NH with highly frequent and drug-resistant focal seizures.[5] In the case of patients with bilateral single or symmetric nodules, seizures are likely to be infrequent and/or efficiently controlled by ASM.[5] A more recent study by Liu et al., describing a cohort of 100 Chinese patients with PVNH, of which 70 had epilepsy, in concordance with Battaglia et al.[5] concluded that patients with bilateral symmetric NH have a "benign" course of epilepsy.[32] They also noted, however, that patients with unilateral NH are most likely to have a severe course of epilepsy with worse long-term outcomes, when compared to patients with asymmetric bilateral NH as well as with bilateral symmetric NH.[32] In this way, from what has been reported, the clinical and epilepsy course is thought to be highly heterogenous in this patient population.

Recent work has been focused on understanding the epileptogenicity of the heterotopic nodules. More extensive imaging and invasive electrophysiology studies, namely using stereo-electroencephalography (SEEG), have provided important insight into the complex nature of the epileptic networks that are patient-specific.[33–35] In some patients, the nodules act as the main epileptogenic zone, and in others they are part of a larger epileptic network through aberrant connections with cortical structures or rather they may not be involved in seizure generation, propagation or synchronization at all.[35] In the past, the response to surgical intervention has not been favorable, however it is now possible to achieve seizure freedom. SEEG has become an indispensable tool for surgical workup to help reveal the epileptic zone and the relationship between the heterotopia and overlying cortex. SEEG-guided radiofrequency thermocoagulation (SEEG RF-TC) has also been gaining more and more traction in epilepsy surgery in general as a less invasive and safer technique when possible.[36,37] As reported by Mirandola et al. in their study on SEEG in PVNH, SEEG RF-TC demonstrated an excellent outcome in patients with

single nodules, multiple unilateral or bilateral NH.[37] Additionally, for patients with more complex malformations, SEEG RF-TC alone can provide temporary absence or reduction of seizure frequency, and additional traditional resective surgery is often necessary to achieve seizure freedom.[37]

Genetics of Periventricular Nodular Heterotopia

The genetic etiology of PVNH is heterogeneous, and in most cases remains unknown. The most frequent cause of bilateral symmetric PVNH is single nucleotide variants or deletions in the *FLNA* gene on chromosome Xq28, also known as classical X-linked PVNH, leading to disrupted or loss of function of the encoded filamin A protein.[12] Filamin A is an actin-binding protein that is involved in the regulation and organization of the cytoskeleton with diverse functions, including cell protrusion and motility, vessel wall integrity and coagulation.[38] *FLNA* expression is upregulated during cortical development in utero and is subsequently downregulated in the adult.[38] Pathogenic variants in *FLNA* are most commonly inherited and detected in females, while demonstrating high prenatal lethality in males.[38] Extracerebral manifestations of *FLNA* mutations include persistent ductus arteriosus in newborns, cardiac valve disease, chronic obstructive lung disease, Ehlers-Danlos like syndrome and oto-palato-digital syndrome.[28,38] Other rare genetic causes of PVNH have been associated with various biallelic variants, such as in *ARFGEF2*, *DCHS1* and *FAT4*, as well as heterozygous variants in *NEDD4L*, *ERMARD* and *MAP1B* genes.[13,39–42] Many of these variants have been associated to syndromic forms of PVNH, namely PVNH and microcephaly caused by pathogenic variants in *ARFGEF2* and PVNH associated with toe syndactyly, cleft palate and developmental delay due to pathogenic variants in *NEDD4L*.[13,40] Various rare forms of PVNH associated with chromosomal imbalances, such as microdeletions at 1p36, 22q11 and 7q11.23, and 5p anomalies,

have also been reported.[43,44] Recently, an article by Cellini et al. highlighted a high incidence of large-scale genomic rearrangements and small copy number variants in PVNH patients, revealing a list of 30 different causative genomic imbalances, often involving multiple chromosomes, gathered from previously published reports and novel findings using array comparative genomic hybridization.[11] Their data also points to the involvement of genes in the regulation of vesicle-mediated transport and encoding plasma membrane receptor complexes.[11] *ARFGEF2* encodes brefeldin-inhibited guanine exchange factor 2 protein which is involved in vesicle transport between the trans-Golgi apparatus and the cell membrane.[13] Filamin A is also implicated in vesicle trafficking, associating with the Golgi membranes during budding and transport of vesicles.[45] Thus, it is believed that PVNH comprises a group of disorders with several subtypes associated with abnormalities in genes involved in neuronal precursor proliferation and migration, as well as in genes involved in vesicle-mediated trafficking yet to be resolved.

THE STUDY OBJECTIVES

The overarching goals of this study are to develop a comprehensive picture of the phenotypic spectrum of epilepsy in individuals with PVNH and to identify causative gene defects. We plan to recruit a sufficiently large cohort of participants to constitute a representative sample of the population. Our primary aim is to clarify epilepsy patterns and features in this population. Clinical variables of interest include age of seizure onset, seizure types and frequency, response to ASMs, to surgery or to alternative therapies, and overall course of epilepsy in this population. Our second aim is to elucidate likely molecular pathways contributing to the underlying pathophysiology of epilepsy in PVNH through a review of test results from those who have previously undergone genetic testing.

THE STUDY HYPOTHESIS

From what is already known about PVNH, this disorder is extremely heterogeneous regarding both clinical presentation and genetic causes. In this regard, we suspect that the course of epilepsy in this population is more variable than previously reported. We expect a greater percentage of patients with PVNH to have self-limited epilepsy, with onset of seizures in early adolescence and decreasing in frequency over time, rather than refractory epilepsy that is persistent throughout their lifetime.

THE STUDY

The Phenotypic Spectrum of Epilepsy Associated With Periventricular Nodular Heterotopia

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*Supplemental material is incorporated into the Appendix.

Abstract

Background: Periventricular nodular heterotopia (PVNH) is a congenital brain malformation often associated with seizures. We aimed to clarify the spectrum of epilepsy phenotypes in PVNH and the significance of specific brain malformation patterns.

Methods**:** In this retrospective cohort study, we recruited people with PVNH and a history of seizures, and collected data via medical record review and a standardized questionnaire.

Results**:** One hundred individuals were included, aged 1 month to 61 years. Mean seizure onset age was 7.9 years. Ten patients had self-limited epilepsy and 35 pharmacoresponsive epilepsy. Fifty-five had ongoing seizures, of whom 23 met criteria for drug-resistance. Patients were subdivided as follows: isolated PVNH ("PVNH-Only") single nodule (18) or multiple nodules (21) and PVNH with additional brain malformations ("PVNH-Plus") single nodule (8) or multiple nodules (53). Of PVNH-Only single nodule, none had drug-resistant seizures. Amongst PVNH-Plus, 55% with multiple unilateral nodules were pharmacoresponsive, compared to only 21% with bilateral nodules. PVNH-Plus with bilateral nodules demonstrated the highest proportion of drug-resistance (39%).

A review of genetic testing results revealed eight patients with pathogenic or likely pathogenic single-gene variants, two of which were *FLNA*. Five had copy number variants, two of which were pathogenic.

Conclusions**:** The spectrum of epilepsy phenotypes in PVNH is broad, and seizure patterns are variable; however, epilepsy course may be predicted to an extent by the pattern of malformation.

Overall, drug-resistant epilepsy occurs in only a minority of affected individuals. Genetic etiologies are identified in only a small proportion of individuals, and are very heterogeneous.

Introduction

Periventricular nodular heterotopia (PVNH) is a congenital brain malformation resulting from the arrest of migrating neuronal precursor cells to the cortex during development in utero.[24] On magnetic resonance imaging (MRI), PVNH is recognized by the abnormal presence of grey matter nodules within the periventricular region.[24] Clinical presentation is variable, but the most common feature is epilepsy; patients often first present to medical attention at the time of seizure onset.[24] Other neurological features include learning difficulties and varying degrees of developmental impairment.[8,24,26] Patients may also have extra-neurological features such as cardiac anomalies, coagulopathies, joint hypermobility, or lung disease.[27,28] Lastly, some asymptomatic patients are only diagnosed incidentally on MRI.[18]

PVNH is frequently associated with drug-resistant epilepsy.[7,8,29–32] There is considerable variability in reported epilepsy onset and outcomes for patients depending on the extent of malformation, namely the number and distribution of heterotopic nodules, as well as the presence or absence of other concomitant brain anomalies.[5,6] The full spectrum of epileptology remains unclear, however. In this study, we analyzed clinical epilepsy features in individuals with PVNH and seizures to better characterize the epilepsy phenotypic spectrum.

Materials & Methods

Recruitment and inclusion criteria

Participant recruitment was supported through liaison with *PVNH Support & Awareness*, an international PVNH patient support group. Additional patients were recruited through screening of the McGill University Health Centre archives between 2018 and 2021. The remaining patients were identified through a review of authors' respective clinical and research databases and recruited accordingly. Recruitment was ongoing from September 2020 to February 2022. Inclusion criteria required that patients have (1) a confirmed PVNH diagnosis from MRI and (2) history of at least one clinical or electrographic seizure.

Data collection

Medical records were obtained from the subjects directly or their respective medical care facilities, including reports from EEG and neuroimaging studies, and clinic notes from neurology, genetics, and neurosurgery if applicable. Following recruitment, each patient or their caregiver completed a standardized questionnaire in person or by telephone. There was no follow-up with patients after the interview. Patients were subdivided into four groups according to extent of brain malformation: (1) "PVNH-Only" isolated single nodule, (2) "PVNH-Only" isolated multiple nodules, (3) "PVNH-Plus" single nodule with other brain malformations and (4) "PVNH-Plus" multiple nodules with other brain malformations. Those with multiple nodules were further separated based on unilateral or bilateral distribution. Data on additional brain malformations were also recorded.

Data collected via the questionnaire included: antenatal and birth history, developmental milestones, epilepsy features, treatment responses, personal and family medical histories. Epilepsy phenotypic data collected included: age of seizure onset, common seizure types, EEG patterns, epilepsy course, and response to seizure treatment. Epilepsy course was classified at the time of interview as: "self-limited" (seizure-free for a minimum of 2 years and anti-seizure medication (ASM) discontinued), "pharmacoresponsive" (seizures under control with ongoing pharmacotherapy), "ongoing"(seizures not controlled with current ASM but had not undergone two adequate ASM trials), or "drug-resistant" (failed at least two adequate trials of ASM). Seizure types were classified according to the International League Against Epilepsy (ILAE) 2017 classifications.[46] Medications were classified as effective if patients or caregivers reported at least partial improvement in seizure control, and ineffective if there was no improvement in seizure control.

Statistical and genetic analyses

IBM SPSS® Statistics software was used to conduct all analyses. Two-sided Pearson Chi-square tests were used to determine whether different demographic, clinical and epilepsy features were associated with patient subgroups and their specific radiologic features, namely number and distribution of PVNH. Two-sided independent samples *t*-tests were used to detect differences in mean age, age at seizure onset, number of seizure types and ASM trialed between patient subgroups. A $p < 0.05$ was used for establishing statistical significance.

When reviewing genetic testing results, we used American College of Medical Genetics and Genomics criteria and Varsome to classify variants.[47,48]

Ethics

This study is part of the Neurodevelopmental Disorders Biobank (NDD Biobank), approved by the McGill University Health Centre Research Ethics Board (2018-3937). Informed verbal or written consent was obtained from patients or their parents/legal guardians (in the case of minors and incapable adults).

Results

Cohort characteristics and clinical features

Cohort characteristics and clinical features are listed in Table 1. MRI examples of the different PVNH subgroups are in Figure 1. The cohort included 100 patients mean age at study 18.6 years. Ages of patients ranged from 1 month to 61 years. Prior to study, one patient had died, aged 17 years, from sudden unexpected death in epilepsy (SUDEP). There was a slight female predominance, consisting of 58% (58/100) of the cohort. The average age at which patients obtained their PVNH diagnosis was 10.9 years. Three patients were diagnosed in utero. Thirty-three patients reported a history of seizures in their immediate family. Delayed achievement of early developmental milestones occurred in 43% (42/98), ranging from mild to severe. Difficulties with reading, writing, comprehension and/or communication were reported in 62% (56/91). Autism spectrum disorder had been diagnosed in 15 patients.

Neuroradiological findings

MRI reports identified heterotopic nodules in the subependymal/periventricular region as the sole abnormality (PVNH-Only) in 39 patients, and nodules along with other structural brain abnormalities (PVNH-Plus) in 61. Within the PVNH-Only group, 18 patients had a single

nodule, and 21 had multiple nodules, of whom 81% (17/21) had bilateral nodule distribution. In the PVNH-Plus group, the most common additional abnormalities were corpus callosum agenesis/dysgenesis (28%, 17/61), ventricular abnormalities (20%, 12/61), polymicrogyria (18%, 11/61), cysts (16%, 10/61) and hippocampal abnormalities (13%, 8/61). Other findings included cortical dysplasia, band heterotopia, schizencephaly, dysgyria, cerebellar malformations, hamartomas, white matter disease, and findings consistent with septo-optic dysplasia, namely optic nerve anomalies, septum pellucidum anomalies, small pituitary gland and ectopic neurohypophysis. Within the PVNH-Plus group, there were eight patients with a single heterotopic nodule. The remainder (87%, 53/61) had multiple nodules, with the majority (62%, 33/53) showing a bilateral distribution.

Epilepsy

Epilepsy and EEG features are summarized in Table 2 and Table 3, with individual details for the cohort in Supplemental Table S1. The average age of seizure onset across all groups was 7.9 years, ranging from the first day of life to 41 years. Average ages of onset per subgroup were relatively similar given the large standard deviations. Almost half of the patients (47%, 47/100) had experienced more than one seizure type since onset. Within the PVNH-Only group, patients with multiple nodules tended to have a greater diversity of seizure types compared to those with a single nodule. Focal seizures were most common in PVNH-Only, in comparison with PVNH-Plus where bilateral-onset (possibly generalized) seizures were more common. Seizure types included focal motor (tonic, clonic, myoclonic, automatisms), focal non-motor (cognitive, emotional, sensory, autonomic), bilateral tonic-clonic, absences (typical, atypical, eyelid myoclonia), bilateral motor (tonic, clonic, myoclonic), and epileptic spasms. In terms of EEG,

the majority of patients had abnormal findings (95%, 92/99 – report unavailable for one patient). EEG features included focal interictal epileptiform discharges (IEDs) in 40, multifocal IEDs in 34 and generalized IEDs in 11. Non-epileptiform abnormalities, most commonly focal or diffuse slowing, were reported in 25 patients. Non-epileptiform abnormalities were almost three times more frequent in PVNH-Plus compared to PVNH-Only. There were otherwise no remarkable differences in seizure semiology across the four subgroups.

Epilepsy course details are summarized in Table 2 and Table 3. Of the 100 patients, 10 (10%) had a self-limited course, remaining seizure-free and no longer requiring ASM. For these patients, the average age of seizure onset and offset was 1.6 and 3.5 years, respectively. The majority of these patients (60%, 6/10) had infrequent focal motor seizures. The four other patients had bilateral-onset seizures (tonic-clonic, typical absences and epileptic spasms) that were most often frequent at onset, occurring multiple times per day or week, then controlled with ASM and no longer requiring pharmacotherapy. On average, patients had been off medication for almost three years at the time of interview.

Thirty-five patients (35%) were classified as pharmacoresponsive at the time of interview. On average, patients had been seizure-free for 4.5 years (range 1 month-27 years) with their current ASM regimen. Four had undergone surgery and reported improved seizure control post-operatively. Classification as pharmacoresponsive was most common amongst PVNH-Only patients with a single nodule (61%, 11/18) (Table 3). Amongst PVNH-Plus patients with multiple nodules, those with unilateral distribution demonstrated a significantly higher

proportion of pharmacoresponsiveness (55%, 11/20) compared to those with bilaterally-distributed nodules (21%, 7/33; $p < 0.05$) (Table 3).

Fifty-five patients (55%) had uncontrolled seizures, with at least one episode within the past month at the time of interview. Thirty-two of these were classified as "ongoing," as they had not undergone two adequate ASM trials at the time of interview. The remaining 23 patients had an epilepsy course meeting criteria for drug-resistance. The average age of the patients with drug-resistant epilepsy was 27.1 years (range 4 to 61 years). The average age of seizure onset for these patients was 8.9 years, more specifically 13.4 years for PVNH-Only and 7.7 years for PVNH-Plus. Bilateral-onset seizures were most common in this group, with over 91% (21/23) having had at least one bilateral-onset seizure type. These patients had all experienced more than one seizure type, with an average of 2.4 seizure types reported. There were no patients with a single nodule in PVNH-Only group that had a drug-resistant course. Among the different subgroups, the prevalence of drug-resistant epilepsy was highest in those with multiple bilateral PVNH (39%, 13/33) (Table 3).

Treatment for seizures

Patient response to seizure treatment is summarized in Table 4. Most patients in the cohort had, or currently used ASM. PVNH-Only patients tended to undergo fewer medication trials, on average 2.5 ASMs compared to PVNH-Plus with 3.6 ASMs, to obtain better seizure control or complete seizure remission. Clobazam had the greatest reported efficacy for patients in both PVNH-Only (76%, 13/17) and PVNH-Plus groups (64%, 16/25). Levetiracetam and valproic acid were also commonly used, and response rates were positive in both groups, in particular for

PVNH-Only patients. Of the 35 patients with a pharmacoresponsive epilepsy course, 69% (24/35) were currently on ASM monotherapy. The other patients were on polytherapy, most often a combination of two ASMs. Within this group, levetiracetam was most commonly reported as an effective treatment for seizure control, providing seizure freedom in 14 cases. In over half of these, it was beneficial when prescribed as monotherapy.

Besides classical ASMs, alternative therapies were attempted in 14 patients: vagal nerve stimulation in four, ketogenic diet in five, and combinations of the two in five more patients. The majority of patients (71%, 10/14) reported no benefit from these therapies. Seizure freedom was not achieved in any of these patients. Twenty-three patients had been evaluated for surgical treatment, of whom twelve underwent surgery, the majority with PVNH-Plus (86%, 12/14). Surgeries included focal resections: temporal in eight patients and frontal in two. Radiofrequency thermocoagulation of periventricular nodules was employed in four patients, of whom one had undergone a prior posterior right temporal lobe resection. Overall, two patients achieved seizure freedom post-surgery with ongoing pharmacotherapy, seven reported improved seizure control with reduced frequency, intensity and/or duration, and three had no clear benefit.

Genetic testing results

We were able to obtain genetic testing results from 44 patients, including 17 single gene sequencing, 19 clinical gene panels, six whole exome sequencing, and 20 chromosomal microarray (Table 5). Eight patients had single gene variants that were classified as pathogenic or likely pathogenic, including two with *FLNA*. An additional five patients had at least one copy number variant identified, including three that were pathogenic.

Discussion

Multiple studies have highlighted the manifestations of epilepsy in PVNH and have reported considerable heterogeneity in epilepsy features and outcomes. A common theme in these studies is the impression that epilepsy in this population is most often drug-resistant.[35–37] However, most of these studies have been limited by small numbers of patients and skewed towards the subset of individuals seeking surgery to treat their seizures. To our knowledge, this is the largest clinical study aimed at characterizing the broader epileptology associated with PVNH.

Our data was analyzed as a whole and in subgroups based on whether PVNH occurred alone (PVNH-Only) or in association with other brain malformations (PVNH-Plus), and further by nodular distribution. This classification approach was in keeping with previous studies that have reported variability in epilepsy onset, course, and treatment response for patients depending on the extent of brain malformation.[5,6] Our data indicate that epilepsy typically presents near the end of the first decade of life. There were no significant differences in age of seizure onset between PVNH-Only and PVNH-Plus. These findings are in contrast with what has been previously reported, namely that seizures begin most often in the second decade of life, most notably for PVNH-Only, and markedly earlier for PVNH-Plus patients.[3,7,30] The discordance may be explained by our greater sample size and less biased recruitment strategy when compared to previous studies.

In concordance with previous studies, focal seizure types were commonly reported amongst our cohort.[3,5,7] Patients with more extensive malformation tended to have more bilateral-onset seizure types, likely reflecting a greater capacity for recruitment of bilateral pathologic networks.

This subset of our cohort also tended to present with multiple different seizure types, consistent with previous reports.[5,6] EEG studies revealed a greater propensity for patients with more extensive malformations to have interictal non-epileptiform slowing in addition to IEDs, compared to those with isolated PVNH whose EEGs primarily showed focal or multifocal spikes or sharp waves.

The course of epilepsy is highly variable, but only 23% of patients met criteria for drug-resistant epilepsy and 45% had seizures that were controlled, on or off ASM. These findings contradict what has been previously reported, where epilepsy has been most often described as persistent throughout life and difficult to treat.[7,8,32,35] There was no incidence of drug resistance in patients with an isolated, single nodule; one possible explanation is that PVNH was an incidental finding in some of these patients, not actually having a causative role in their seizures. Of those with PVNH-Plus, approximately 60% had seizures that were responsive to treatment or resolved, while 40% had drug-resistant epilepsy. Further, patients with a unilateral nodular distribution showed a relatively good response to ASM polytherapy, and only a small proportion (10%) had drug-resistance. This differs from previous studies reporting that the course of epilepsy in unilateral PVNH patients is significantly worse than others, with a high prevalence of drug-resistant seizures.[5,32] Again, the discordance may be a product of differences in relative sample sizes, however it may also be a consequence of selection bias in these studies.

Surgery was an uncommon therapeutic route in our cohort. Few patients had trialed alternative therapies, and those that had tended not to derive significant clinical benefit. Clobazam appeared to be a favourable choice of ASM in both subgroups of our cohort, most often when prescribed

as an adjunctive therapy. Large prospective studies are needed to identify a more comprehensive list of effective therapeutic options.

Once PVNH is identified on MRI, patients and caregivers must be made aware of the increased likelihood of seizures and counseled that the range of possible outcomes is broad. Previous studies have indicated that approximately 80% of patients with PVNH develop epilepsy in their lifetime.[18] This may be an overestimation given that people who are affected can be asymptomatic and remain undiagnosed. At presentation, MRIs should be reviewed and reported by experienced neuroradiologists for patients to be properly defined by their distribution of PVNH. Based on our findings and previous reports, prognostic counseling and treatment options will differ depending on the brain malformation pattern. Our data support previous findings that patients with an isolated, single nodule are more likely to have self-limited epilepsy.[5,32] These patients tend to have fewer seizure types and pharmacoresponsive epilepsy. Others with isolated, bilateral PVNH are likely to experience a longer course of epilepsy and require ASM polytherapy. Patients with PVNH and other coexisting malformations will present with multiple different seizure types, and more frequently experience bilateral-onset seizures. More specifically, those with unilateral PVNH are more likely to be responsive to ASM than patients with bilateral distribution. Our findings suggest that among this subgroup of patients, seizures may be difficult to control initially but seizures are not necessarily more likely to be drug-resistant. This is an important point to emphasize as it is a cause for concern for patients and caregivers when they receive their diagnosis.

Some of our patients had interesting genetic findings that may shed light on the molecular underpinnings of PVNH. *CASK* (OMIM 300172) encodes calcium/calmodulin-dependent serine protein kinase; heterozygous pathogenic variants are associated with a phenotype of X-linked intellectual disability with microcephaly and pontine/cerebellar hypoplasia.[49,50] Although brain malformations are a well-recognized feature, PVNH has not previously been reported. Interestingly, our patient #8 with a pathogenic *CASK* missense variant had isolated PVNH and self-limited focal epilepsy, with autism spectrum disorder but otherwise normal development. The milder phenotype in our patient may have occurred because she carries a missense variant, whereas most patients reported in the literature have deletions or truncation variants.[49,50]

An 8-year-old girl, patient #38, had a *de novo* 3q26 deletion affecting *TBL1XR1* (OMIM 608628), which encodes transducin-beta-like 1 receptor 1. She had moderate global developmental impairment, self-resolving suspected focal impaired awareness seizures, and migraines. EEG showed occasional-to-frequent focal spikes over the left central-temporal-parietal region. MRI revealed bilateral PVNH as well as a Chiari I malformation associated with a syrinx affecting almost the entire spinal cord, requiring surgical intervention. Heterozygous missense pathogenic variants in *TBL1XR1* have been associated with Pierpont syndrome and intellectual disability with autistic features;[51,52] brain MRI can be normal but may also show atrophy, enlarged ventricles, abnormal gyration, delayed myelination, Chiari I malformation, and choroid plexus papilloma.[53–55] Our patient's findings further expand the spectrum of structural brain abnormalities that may be seen with *TBL1XR1* haploinsufficiency.

Patient #53 had a maternally-inherited pathogenic variant in *ABCC8* (OMIM 600509), encoding ATP-binding cassette, subfamily C, member 8 (ABCC8). Pathogenic variants in this gene are associated with primarily neonatal diabetes mellitus and hypoglycemia. However, there is high expression of the gene in the brain, and some patients have developmental delay, epilepsy, and neonatal diabetes (DEND) syndrome.[56] The clinical significance of the variant in this patient is unclear, as she did not have a personal or family history of diabetes or hypoglycemia. ABCC8 dysfunction may play a broader role in epilepsy but more research is necessary to explore this possibility.

A 16-year-old male, patient #100, with macrosomia, autism, and focal epilepsy, had a *de novo* pathogenic variant in *PHF21A* (OMIM 608325). This gene encodes BHC80, a component of a BRAF35/histone deacetylase complex that mediates repression of neuron-specific genes; heterozygous pathogenic variants are associated with a syndromic form of intellectual disability that may involve craniofacial abnormalities, epilepsy, hypotonia, and neurobehavioural abnormalities.[57] Patient #71 had a *de novo* pathogenic variant in *GABRB2* (OMIM 600232), encoding a GABA receptor subunit; pathogenic variants in this gene are primarily associated with developmental and epileptic encephalopathy.[58] For both *PHF21A* and *GABRB2*, individuals with pathogenic variants typically have normal brain MRI, so in these cases the PVNH found in our patients may have been coincidental. The clinical relevance of the remaining pathogenic variants to PVNH was unclear. In some cases, as with the patient with a pathogenic variant in *DMD* (OMIM 300377; dystrophin), an association with brain malformations appears very unlikely based on what is known about the genes' function and expression. As well, the

significance of the heterozygous *CEP152* variant is unclear given that biallelic pathogenic variants in this gene are generally required to cause disease.

Our cohort likely presents a representative picture of the spectrum of epilepsy in people with PVNH. Collaborating with a patient support group enabled recruitment of a large, international cohort, and limited the inherent biases that come from recruiting patients only from neurology clinics in tertiary centres. However, there is still a degree of ascertainment bias related to recruiting families who elect to use social media and join an online support group. Our data should also be considered with some caution, given that this is a retrospective study and clinical data were obtained through interviews in combination with a thorough review of medical records.

Conclusion

The data from this large cohort of individuals with PVNH reveal important findings on the spectrum of epilepsy features that can occur in association with this brain malformation. Our work provides insight on common electro-clinical features, epilepsy course, and molecular factors that may be involved. This information should allow clinicians who treat people with PVNH to be able to provide better counseling and management to the patients and their caregivers.

THESIS SUMMARY

Discussion

Over the past 30 years, developments in neuroimaging technology have enabled the detection and diagnosis of PVNH by the presence of grey matter nodules along the walls of the lateral ventricles. PVNH is believed to result from an arrest of neuronal precursor migration from the periventricular region to the cortex during development, and has been associated with various distinct clinical-radiological syndromes and genetic characteristics. The resulting disorganized cortical structure and the presence of ectopic neuron and glial cell aggregates has been linked to abnormal electrical activity and seizures and the majority of those affected are thought to develop refractory focal epilepsy.[3,5,7,30] There have been numerous studies that have investigated the electroclinical picture of epilepsy in patients with PVNH; however, they have been limited by the small number of patients studied and skewed towards a subset of the population seeking surgery for epilepsy. Consequently, despite the progress that has been made, the epilepsy phenotypic spectrum has not been well characterized and the epileptology remains unclear.

In terms of the etiology and pathogenesis of PVNH, pathogenic variants in the *FLNA* and *ARFGEF2* genes, among others, have been identified as responsible for X-linked dominant and autosomal recessive forms of bilateral PVNH, respectively.[12,13] PVNH is a genetically heterogeneous disorder that may occur secondary to abnormalities in genes involved in neuronal precursor migration, such as the *FLNA* gene which encodes an integral cytoskeletal protein.[12] More recent studies by Cellini et al. suggest that dysregulation or disruption of gene products involved in vesicle-medicated trafficking are likely to be causative of PVNH as well.[11] Both *FLNA* and *ARFGEF2* encoded proteins are similarly implicated in vesicle trafficking.[13,45] Thus,

understanding the genetic mechanisms underlying impairments in neuronal migration and vesicle trafficking may help to elucidate undiscovered causes of PVNH.

In this study, we analyzed the epilepsy features of a large population of pediatric and adult patients with PVNH and seizures to be able to clarify the spectrum of epilepsy phenotypes associated with this pattern of brain malformation. This is the first large scale study of its kind to date. We recruited 100 patients with PVNH with a history of seizures, with ages ranging from 1 month to 61 years, and over 60% in the pediatric age group. Of the 100 patients, 39 had isolated PVNH (PVNH-Only) and 61 had PVNH with other brain malformations (PVNH-Plus). The average age of seizure onset was 7.9 years, while slightly earlier for PVNH-Plus compared to PVNH-Only patients. Considering the large standard deviations observed, it is important to note that patients may present with seizures at any time up until their mid-to-late teenage years. Focal seizure types were common across the cohort; however, PVNH-Plus patients tended to present with a greater number of seizure types, and were more likely to have apparently generalized seizure semiologies. These findings add to the existing literature about seizure types in PVNH which has previously been more commonly associated with focal epilepsy. PVNH-Plus patients also tended to demonstrate a higher prevalence of interictal non-epileptiform abnormalities on EEG, which is likely related to the greater extent of their brain malformations in addition to the heterotopic nodules. Epilepsy course, which was of greatest interest to us, was variable across all subgroups. In contrast to our hypothesis, only 10 out of the 100 patients had a self-limited course of epilepsy, and there were no consistent correlation with specific radiological features. Thirty-five percent of our cohort had seizures controlled with their current ASM regimen at the time of interview. Patients with a single nodule of isolated PVNH had the highest proportion (61%) of pharmacoresponsiveness across the cohort. Additionally, none of these patients had drug-

resistant epilepsy, which, together, indicates that seizures are not likely to be a major burden for these patients. It was not possible to interpret findings for patients with multiple unilateral and bilateral nodules in the PVNH-Only given the discrepancy in the number of patients per subgroup. PVNH-Plus patients with multiple unilateral NH demonstrated a higher prevalence of pharmacoresponsiveness (55%) compared to drug-resistance (10%), which contradicts previous reports stating that these patients are likely to have a significantly worse course of seizures compared to other patients.[5,32] On the other hand, PVNH-Plus patients with multiple bilateral NH had the highest proportion of drug-resistance (39%) across all subgroups, as well as a lower prevalence of pharmacoresponsiveness relative to other subgroups (21%). Out of the entire cohort, these patients are most likely to have a more difficult course of epilepsy throughout their lifetime, with more frequent seizures and multiple seizure types, often apparently generalized, and will need to be counselled appropriately. Overall, only 23 patients had a seizure course deemed drug-resistant, and therefore, refractory epilepsy is expected to affect a minority of patients. Our findings suggest that epilepsy course in patients with PVNH is likely to be more indolent than what has been previously reported. In terms of treatments for seizures, virtually all patients had trialed ASM, but few had trialed any alternative therapies or undergone surgery. On average, PVNH-Plus patients had trialed a greater number of ASM compared to PVNH-Only patients, which is consistent with our findings that patients in this group tend to have a more difficult-to-treat course. Medications with GABAergic (γ-aminobutyric acid) mechanisms of action, namely clobazam and valproic acid, had the highest reported effectiveness amongst patients in PVNH-Only and PVNH-Plus groups. Levetiracetam also showed benefit in a subset of patients in each group. There was no particular trend or significant finding in medication effectiveness when both groups were compared, however.

Our findings for the epilepsy phenotyping portion of the study demonstrate that certain epilepsy features can be predicted to an extent by the pattern of a patient's malformation. Those with a single nodule of isolated PVNH are likely to have fewer seizure types, predominantly focal seizures, that are more easily controlled with existing ASM or resolve over time. Patients with bilateral isolated NH will have a longer course of epilepsy, with a greater number of seizure types, including apparently generalized seizure semiologies, that may take more time and multiple medications trialed before obtaining seizure freedom. Patients with multiple unilateral NH and other brain malformations are likely to have multiple seizure types, often a combination of focal and apparently generalized seizures, that tend to be responsive to ASM therapy. Patients with multiple bilateral NH and other brain malformations are likely to have multiple seizure types, predominantly apparently generalized semiologies, that may be more difficult-to-treat with ASM therapy, compared to other patients. Drug-resistance is not the most common outcome for these patients however, and this finding should be properly explained to patients and their parents or caregivers when receiving their diagnosis. After speaking with many patients, parents, and caregivers who participated in this study, one of their main concerns was the likelihood of developing drug-resistant epilepsy after reading about this in the existing literature online. Our findings add more nuance to what has been previously reported about this condition, and can hopefully help to better guide clinicians with disease counseling and patient management.

With the genetic analyses, our hope was to identify causative genetic defects for future research. As discussed in the manuscript, there were a few interesting findings from the genetic testing results that were available to us. The presence of PVNH in patient #8 with a hemizygous, missense mutation in *CASK* adds to the phenotype of intellectual disability, microcephaly and pontine/cerebellar hypoplasia that has been previously associated with pathogenic variants in this

gene.[49,50] Similarly, the finding of PVNH in patient #38 with a *de novo* deletion in the *TBL1XR1* gene adds to the list of brain abnormalities previously reported in patients with variants in *TBL1XR1*.[54,55,59] These genes have not been previously reported to be associated with PVNH; however, there is the possibility that these variants, along with those identified in *ABCC8, PHF21A*, *GABRB2, CEP152,* and *DMD,* may be unrelated and incidental findings. That being said, more research is needed to further explore their possible involvement in the pathophysiology of PVNH-related epilepsy. Some additional genetic findings, not part of the manuscript, have been included here for further discussion (see Table 6). Three patients, patient #8, #53 and #83, have heterozygous variants in the *CNTNAP2* gene. The variants are reported as damaging for patient #8 and #53, according to Sorting Intolerant From Tolerant (SIFT) program used to predict whether an amino acid substitution affects protein function[60], and in all three cases are classified as of uncertain significance but with minor pathogenic evidence, according to ACMG 2015 guidelines for interpretation of sequence variants[47] using VarSome platform for variant discovery[48]. *CNTNAP2* encodes contactin-associated protein 2, a neuronal cell adhesion molecule involved in many important aspects of brain development, including neuronal precursor cell migration, axon guidance and synapse formation.[61] Heterozygous variants in this gene are thought to represent a susceptibility factor for epilepsy, intellectual disability, autism and other neuropsychological disorders, and the spectrum of associated disease severity is known to be rather large.[61,62] Given the role of *CNTNAP2* in vital cell function, and the detection of variants in this gene multiple patients, it may be that *CNTNAP2* acts as a contributor or modifier of other genetic defects, and may play a role in the pathophysiology of epilepsy in PVNH. Two patients, patient #53 and #87, harbour variants in *SPTAN1*, and the variant in patient #87 is reported to be damaging to protein function, according to SIFT. *SPTAN1* encodes alpha-spectrin

protein which is cytoskeletal protein that acts as a scaffold to stabilize the cell's plasma membrane and to organize intracellular organelles.[63] Cytoskeletal structure is known to be important for proper cell proliferation and migration, and therefore in the case of damaged protein function, cell migration may be disrupted and fail, as in PVNH. Mutations in this gene have been previously associated with early infantile epileptic encephalopathy, also known as Ohtahara syndrome, which is also typically associated with various brain abnormalities, including malformations of cortical development.[64]. As with the other genes mentioned, these findings may be solely coincidental; further research is necessary to investigate the possible involvement of these genes more precisely in the pathogenesis of PVNH. Our hope with the genetic analyses was that insight derived could potentially open new avenues for diagnostic genetic testing in patients with PVNH and help specialists to provide more accurate genetic counseling. Additionally, if successful, these discoveries could have the potential to drive the development of targeted therapies and new treatment strategies for patients with PVNH.

Limitations

As a final note, it is important to recognize that this is a retrospective study with various implicit biases compared to a prospective design. Our data are observational in nature and primarily descriptive, and therefore, only associations and not causations can be inferred.

In order to avoid selection bias by recruiting patients solely from the neurology clinics at the McGill University Health Centre, we collaborated with *PVNH Support and Awareness* group to recruit more participants internationally. We acknowledge, however, that there is a degree of bias related to recruitment through the PVNH-specific support group which only operates online, through social media, and is limited to those who elect to join the group.

Our data was collected through a review of patient medical records and an interview with the patient or their parent/caregiver. The combination of approaches was used to minimize recall bias that may be posed by patient interviews, especially in a context where events, as in the event of a seizure or repeated seizures, may have led to cognitive impairment or may have occurred many years prior to the interview. We are aware that medical chart reviews are subject to their own information and misclassification biases as well.

THESIS CONCLUSION

To conclude, we gathered clinical data from 100 patients with PVNH and a history of seizures to be able to provide insight on common electro-clinical features for clinicians that can help improve their ability to provide proper prognostic counseling when they encounter these patients in their practice. In doing so, we also wanted to provide greater awareness about the disease, management strategies and possible outcomes for patients, their families, and informal caregivers alike. With the genetic analyses, we also hope to have laid down some groundwork for future research to identify common molecular pathways that are disrupted in this condition. All together, we hope that the outcome of this work will allow for improved diagnosis, treatment, and management of epilepsy in patients with PVNH, and lead to better patient care overall.

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TABLES

Table 1. Demographic and Clinical Characteristics of the study population, divided into PVNH-Only and PVNH-Plus groups based on neuroradiological features

	Total	PVNH-Only	PVNH-Plus	*p*-value
Patients, *n*	**100**	**39**	**61**	
Pediatric, *n* (%)	61 (61)	25 (64)	36 (59)	0.611
Adult, *n* (%)	39 (39)	14 (36)	25 (41)	0.611
Age, *years*				
Time of interview				
Mean (SD)	18.6 (14.4)	18.0 (12.1)	19.0 (15.7)	0.729
Range	60.9[0.08-61]	43.9[0.08-44]	60.7[0.33-61]	
PVNH diagnosis				
Mean (SD)	10.9 (11.5)	12.3 (10.6)	10.0 (12.1)	0.315
Seizure onset				
Mean (SD)	7.9 (7.9)	9.6 (7.8)	6.9 (7.9)	0.103
Range	41[0.001-41]	34[0.02-34]	41[0.001-41]	
Elapsed time				
Mean (SD)	10.6 (10.4)	8.4 (8.2)	12.1(11.4)	0.090
Range	48.9[0.08-49]	30.9[0.08-31]	48.9[0.08-49]	
Sex, *n* (%)				
Female	58 (58)	23 (59)	35 (57)	0.875
Male	42 (42)	16 (41)	26 (43)	0.875
Birth history, *n* (%)				
Pregnancy complications	32 (32)	13 (33)	19 (31)	0.819
Poor condition at birth	13 (13)	2 (5)	11 (18)	0.061
Development, *n* (%)				
Delay in achievement of early milestones	42/98 (43)	13/38 (34)	29/60 (48)	0.369
Difficulties with reading, writing, comprehension and/or communication	56/91 (62)	19/37 (51)	37/54 (69)	0.139
Autism spectrum disorder	15 (15)	6/39 (15)	9/61 (15)	0.931
Family history, *n* (%)				
PVNH	2 (2)	0	2 (3)	0.253
Seizures	33 (33)	14 (36)	19 (3)	0.622

Table 2. Epilepsy features of study patients, comparing PVNH-Only and PVNH-Plus groups

	Total *n*=100	**PVNH-Only *n*=39**	**PVNH-Plus *n*=61**	***p*-value**
Age at sz onset, Mean (SD), *years*	7.9 (7.9)	9.6 (7.8)	6.9 (7.9)	0.103
Common seizure types [a], *n* (%)				
Focal	58 (58)	23 (59)	35(57)	0.875
Generalized	59 (59)	17 (44)	42 (69)	**0.012**
Focal-to-BTC	16 (16)	9 (23)	7(11)	0.123
Number of seizure types, Mean (SD)	1.7 (0.8)	1.5 (0.7)	1.7 (0.8)	0.196
EEG Findings [b], *n* (%)	***n*=99**	***n*=38**		
Normal	7 (7)	4 (10)	3 (5)	
Abnormal	92 (93)	34 (89)	58 (95)	0.260
Epileptiform	82 (83)	34 (89)	51 (84)	0.326
Nonepileptiform	24 (24)	5 (13)	20 (33)	**0.042**
Epilepsy course, *n* (%)				
Self-limited	10 (10)	4 (10)	6 (10)	0.946
Pharmacoresponsive	35 (35)	15 (38)	20 (33)	0.562
Seizures ongoing	32 (32)	15 (38)	17 (28)	0.268
Drug-resistant	23 (23)	5 (13)	18 (29)	0.053

Abbreviations: BTC: bilateral tonic-clonic; EEG: electroencephalogram

[a] More than 1 seizure type may occur in the same participant

[b] More than 1 abnormal finding on EEG may occur in the same participant

$p < 0.05$ statistical significance, **in bold**

Table 3. Epilepsy features of PVNH-Only and PVNH-Plus patients, divided based on number, single versus multiple, and distribution of nodular heterotopia, unilateral versus bilateral

	PVNH-Only						PVNH-Plus					
				Multiple						Multiple		
	Single *n*=18	**Multiple *n*=21**	***p*-value**	**Unilat *n*=4**	**Bilat *n=17***	***p*-value**	**Single *n*=8**	**Multiple *n*=53**	***p*-value**	**Unilat *n=20***	**Bilat *n=33***	***p*-value**
Age at sz onset, Mean (SD), years	8.2(8.3)	10.7(7.3)	0.344	12(4.0)	11(9.0)	0.807	6.7(7.7)	7.0(8.0)	0.919	5(5.8)	8(9.0)	0.287
Common seizure types [a], *n* (%)												
Focal sz	9 (50)	14 (67)	0.291	4 (100)	10 (59)	0.116	4 (50)	30 (57)	0.729	11 (55)	19 (58)	0.854
Generalized sz	5 (28)	12 (57)	0.065	1 (25)	11 (65)	0.149	6 (75)	36 (68)	0.687	12 (60)	24 (73)	0.336
Focal-to-BTC sz	6 (33)	3 (14)	0.159	1 (25)	2 (12)	0.496	1 (12)	7 (13)	0.956	4 (20)	3 (9)	0.256
Number of sz types, Mean (SD)	1.2(0.4)	1.8 (0.7)	**0.004**	1.7(0.9)	1.8(0.8)	0.876	1.6(0.5)	1.7(0.9)	0.563	1.5(0.9)	1.8(0.8)	0.223
EEG Findings [b], *n* (%)	***n* = 17**											
Normal	2 (12)	2 (10)		1 (25)	1 (6)		0	3 (6)		1 (5)	2 (6)	
Abnormal	15 (88)	19 (90)	0.536	3 (75)	16 (94)	0.241	8 (100)	50 (94)	0.490	19 (95)	31 (94)	0.871
Epileptiform	15 (88)	19 (90)	0.536	3 (75)	19 (94)	0.241	8 (100)	43 (81)	0.179	15 (75)	28 (85)	0.374
Nonepileptiform	2 (12)	3 (14)	0.535	0	3 (18)	0.364	1 (12)	19 (36)	0.190	7 (35)	12 (36)	0.920
Epilepsy course, *n* (%)												
Self-limited	3 (17)	1 (5)	0.222	0	1 (6)	0.619	2 (25)	4 (8)	0.122	0	4 (12)	0.105
Pharmacoresponsive	11 (61)	4 (19)	**0.007**	2 (50)	2 (12)	0.080	2 (25)	18 (34)	0.615	11 (55)	7 (21)	**0.012**
Sz ongoing	4 (22)	11 (52)	0.054	1 (25)	10 (59)	0.223	1 (12)	16 (30)	0.298	7 (35)	9 (27)	0.553
Drug-resistant	0	5 (24)	**0.027**	1 (25)	4 (23)	0.950	3 (38)	15 (28)	0.595	2 (10)	13 (39)	**0.021**

Abbreviations: BTC; bilateral tonic-clonic; bilat: bilateral distribution; EEG: electroencephalograpm; sz: seizure; unilat: unilateral distribution; [a] More than 1 seizure type may occur in the same participant; [b] More than 1 abnormal finding on EEG may occur in the same participant; $p < 0.05$ statistical significance, in **bold**

Table 4. Response to treatments for seizures for PVNH-Only and PVNH-Plus groups

	PVNH-Only	PVNH-Plus	*p*-value
Surgery, *n* (%)	2 (5)	12 (20)	**0.047**
Seizure free	0	3 (25)	
Improved seizure control	2 (100)	5 (42)	
No effect	0	4 (33)	
Alternative therapies, *n* (%)	4 (11)	10 (17)	0.425
Improved seizure control, *n* (%)			
Ketogenic diet	0/2 (0)	0/3 (0)	
Vagal nerve stimulation	1/1 (100)	1/3 (33)	
Combination therapy	0/1 (0)	2/4 (50)	
Anti-seizure medications, *n* (%)	37 (95)	58 (95)	0.931
Improved seizure control, *n* (%)			
Clobazam	13/17 (76)	16/25 (64)	0.754
Valproic acid	9/12 (75)	10/22 (45)	0.097
Levetiracetam	13/22 (59)	16/35 (46)	0.352
Lamotrigine	5/7 (71)	10/17 (59)	0.562
Carbamazepine	6/9 (67)	10/23(44)	0.238
Topimarate	2/6 (33)	8/19 (42)	0.702
Oxcarbazepine	2/4 (50)	3/10 (30)	0.48
Zonisamide	4/5 (80)	1/4 (25)	0.099
Lacosamide	1/2 (50)	4/6 (67)	
Clonazepam	1/2 (50)	4/9 (44)	
Vigabatrin	1/1 (100)	5/13 (39)	
Eslicarbazepine	1/1 (100)	2/3 (67)	
Brivaracetam	1/1 (100)	1/3 (33)	
Cannabidiol	1/1 (100)	2/2 (100)	
Phenytoin	1/2 (50)	0/4 (0)	
Perampanel	0/1 (0)	1/3 (33)	
Ethosuximide	0/1 (0)	2/5 (40)	
Gabapentin	0	1/2 (50)	
Rufinamide	0	0/2 (0)	
Phenobarbitol	0	1/4 (25)	
ACTH	0	2/3 (67)	
Number of ASM trialed, Mean (SD)	2.5 (2.2)	3.6 (2.5)	**0.043**

Abbreviations: ACTH: adrenocorticotropin hormone; ASM: anti-seizure medication
$p < 0.05$ statistical significance, in **bold**

Table 5. Genetic Testing Results

SINGLE GENE PATHOGENIC OR LIKELY PATHOGENIC VARIANTS									
ID	**Sex**	**Gene**	**Variant**	**Zygosity**	**Inheritance**	**Testing**	**Variant type**	**gnomAD count**	**Interpretation**
5	F	*FLNA* NM_001110556.2	c.6022+1 G>C	Hemizygous	Unknown	*FLNA* seq	Splicing	0	Pathogenic (PVS1, PM2)
8	F	*CASK* NM_003688.3	c.1819 A>G p.T607A	Hemizygous	Unknown	NGS panel	Missense	0	Pathogenic (PM1, PM2, PP2)
12	F	*FLNA* NM_001110556.1	c.221 G>A p.G74Q	Hemizygous	*De novo*	*FLNA* seq	Missense	0	Pathogenic (PM1, PM2,PP3)
53	F	*ABCC8* NM_000352.6	c.1306 C>T p.P436S	Heterozygous	Maternal	NGS panel	Missense	0	Pathogenic (PM1, PM2, PP2, PP3)
55	M	*CEP152* NM_001194998.2	c.2034 T>G p.Y678*	Heterozygous	Unknown	NGS panel	Nonsense	83 ($2.7x10^{-4}$)	Likely pathogenic (PVS1,PP3)
71	M	*GABRB2* NM_021911	c.542 A>T p.Y181F	Heterozygous	*De novo*	WES	Missense	0	Pathogenic (PM2,PP3)
80	M	*DMD*	63 kb deletion exons 49-51	Heterozygous	Unknown	*DMD* seq	Intra-genic	N/A	Pathogenic
100	M	*PHF21A* NM_016621.3	c.1032_1035-delAACA p.T345RfsX28	Heterozygous	*De novo*	NGS panel	Frame-shift	0	Pathogenic (PVS1,PM2, PP5)

Table 5 Continued. Genetic Testing Results

COPY NUMBER VARIANTS					
ID	**Sex**	**Genomic rearrangement**	**Genes affected**	**Inheritance**	**Interpretation**
27	F	17p11.2 duplication (197 kb) Chr17:19144143-19341370 (hg19)	*EPN2, B9D1, MIR1180, MAPK7, MFAP4, RNF112*	Maternal	Uncertain significance
38	F	3q26 deletion (1.6 Mb) Chr3:175518649-177090617 (hg19)	*NAALADL2,* ***TBL1XR1***	*De novo*	Pathogenic
54	F	Xp22 duplication (992 kb) ChrX:18421809-19414777 (hg18)	***CDKL5****, RS1, PPEF1, PHKA2, ADGRG2,* ***PDHA1****, MAP3K15*	Unknown	Uncertain significance
		Xp22 duplication (53 kb) ChrX:110449481-110503056 (hg18)	*PAK3, CAPN6*	Unknown	Uncertain significance
77 **	M	17p11.2 deletion (3.9 Mb) Chr17:16590716–20463302 (hg19)	*CCDC144A, USP32P1, FAM106C, KRT16P2, KRT17P1, BC1D27P, TNFRSF13B, LOC284191, MPRIP, PLD6, FLCN, COPS3, NT5M, MED9, RASD1, PEMT, SMCR2,* ***RAI1****, SMCR5, SREBF1, MIR33B, TOM1L2, DRC3, ATPAF2, GID4, DRG2, LOC105371566, MYO15A, ALKBH5, LLGL1, FLII, MIEF2, TOP3A, SMCR8, SHMT1, EVPLL, LINC02076, KRT17P2, KRT16P1, LGALS9C, USP32P2, FAM106A, CCDC144B, TBC1D28, ZNF286B, FOXO3B, TRIM16L, FBXW10, TVP23B, PRPSAP2, SLC5A10, FAM83G, BC112347, GRAP, SNORD3B, SNORD3D, LOC102724624, GRAPL, SNORD3A, SNORD3C, EPN2, B9D1, MAPK7, MFAP4, RNF112, SNORA59B, SLC47A1, ALDH3A2, SLC47A2, ALDH3A1, ULK2, AKAP10, SPECC1, CCDC144CP, FAM106B, NOS2P3, LGALS9B, KRT16P3*	Unknown	Pathogenic
96	M	Xp22.31 deletion (601 kb) ChrX:6612412-7213158 (hg19)	*PUDP, MIR4767,* ***STS***	Maternal	Pathogenic
		16p13.11 duplication (775 kb) Chr16:15512480-16287900 (hg19)	*BMERB1, MARF1, MIR484, NDE1, MYH11, CEP20, ABCC1, ABCC6*	Maternal	Uncertain significance

** Patient #77 has been published previously as patient #1 in Capra et al (2014)[65]

Table 6. Additional Genetic Findings (not included in Table 5)

ID	Gene	Variant	Type	Testing modality	Zygosity	Inheritance	gnom AD	VarSome
1	*DYPD*	c. 274 C>G (p. P92A)	Missense	NGS panel	Heterozygous	Unknown	$3.9x10^{-5}$	VUS**
8	*CNTNAP2*	c.3577 G>T (p. A1193S)	Missense	NGS panel	Heterozygous	Unknown	$3.9x10^{-6}$	VUS**
	ST3GAL5	c.581 G>A (p.R194H)	Missense	NGS panel	Heterozygous	Unknown	$2.8x10^{-5}$	VUS
9	*COL18A1*	c.979 C>T (p.R327W)	Missense	NGSCNV	Heterozygous	Paternal	$1.0x10^{-4}$	VUS
		c.1687 G>A (p.A563T)	Missense	NGSCNV	Heterozygous	Paternal	$4.0x10^{-4}$	VUS
		c.515 G>A (p.R172H)	Missense	NGSCNV	Heterozygous	Maternal	$4.4x10^{-4}$	VUS
13	*CPLANE1*	c.2138 C>T (p.S713L)	Missense	NGS panel	Heterozygous	Unknown	$1.9x10^{-5}$	VUS
	TMEM216	c. 140T>C (p.V47A)	Missense	NGS panel	Heterozygous	Unknown	$5.9x10^{-4}$	VUS**
29	*RPGRIP1L*	c. 2399A>C (p.N800T)	Missense	NGS panel	Heterozygous	*De novo*	$2.2x10^{-4}$	VUS
53	*SPTAN1*	c.3167 C>T (p. T1056I)	Missense	NGS panel	Heterozygous	Maternal	0	VUS
	RELN	c.6193 G>A (p.V2065I)	Missense	NGS panel	Heterozygous	Maternal	$1.3x10^{-4}$	VUS
	HCN4	c.73 A>G (p. I25V)	Missense	NGS panel	Heterozygous	Paternal	0	VUS
	VPS13A	c.1438 A>T (p.T480A)	Missense	NGS panel	Heterozygous	*De novo*	$6.3x10^{-5}$	VUS

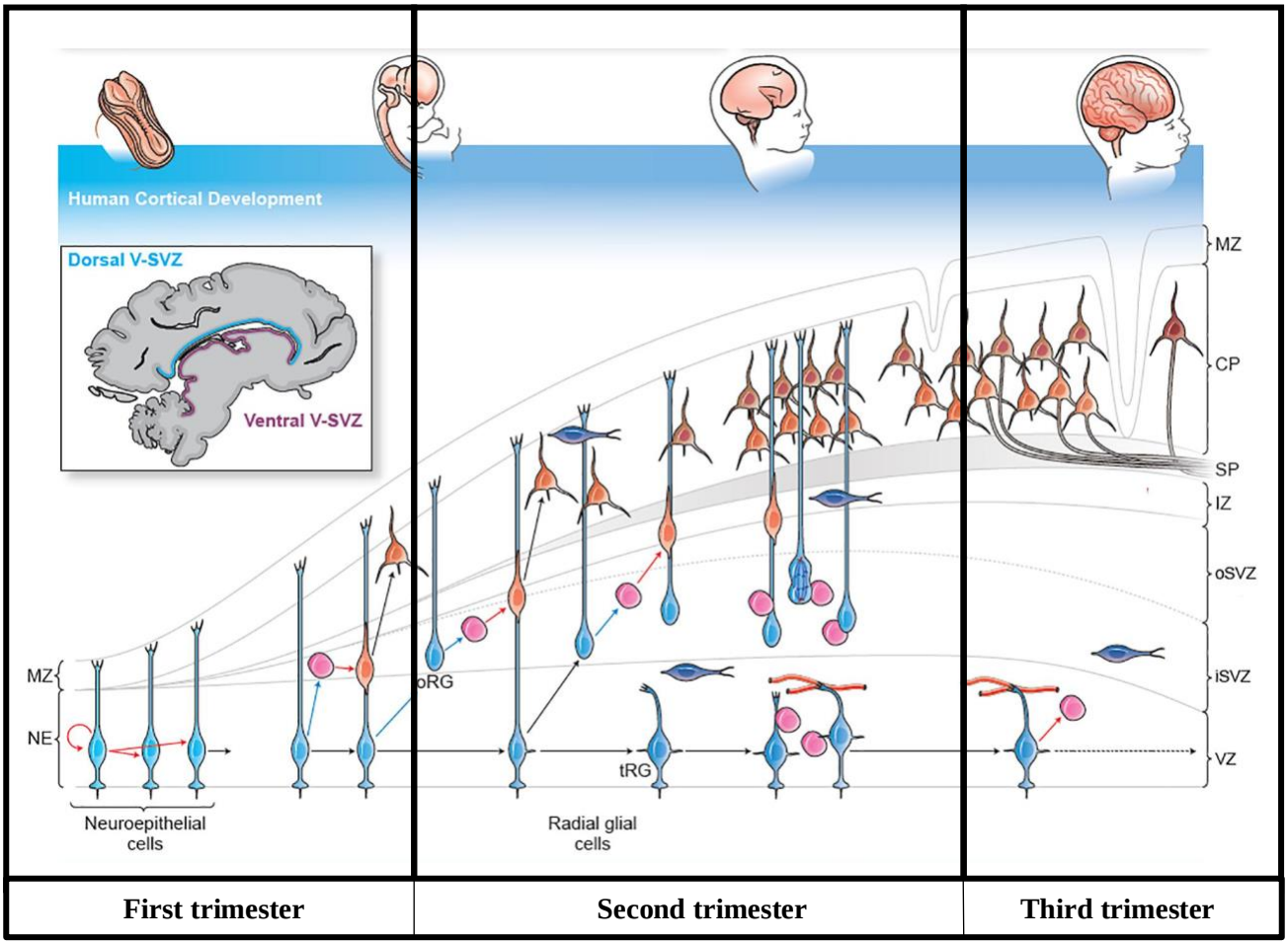
Table 6 Continued. Additional Genetic Findings (not included in Table 5)

ID	Gene	Variant	Type	Testing modality	Zygosity	Inheritance	SIFT	gnomAD	VarSome
53	*SLC19A3*	c.901 A>G (p.I301V)	Missense	NGS panel	Heterozygous	*De novo*	Tolerated	$2.4x10^{-4}$	Likely benign
	FKTN	c.200 A>G (p.N67S)	Missense	NGS panel	Heterozygous	*De novo*	Tolerated	0	VUS
	CNTNAP2	c.1636 A>G (p.N546D)	Missense	NGS panel	Heterozygous	*De novo*	Tolerated	$8.7x10^{-5}$	VUS**
	TMEM67	c.2307 A>C (p.L769P)	Missense	NGS panel	Heterozygous	*De novo*	n/a	0	VUS**
55	*CEP152*	c.2034 T>G (p.Y678*)	Nonsense	NGS panel	Heterozygous	Unknown	n/a	$2.7x10^{-4}$	Pathogenic
	DHDDS	c.356 G>A (p.R119Q)	Missense	NGS panel	Heterozygous	Unknown	Damaging	$4.7x10^{-5}$	VUS**
	GSS	c.301 G>A (p.A101T)	Missense	NGS panel	Heterozygous	Unknown	Tolerated	$1.20x10^{-5}$	VUS**
	TMEM240	c.201 C>A (p.D67E)	Missense	NGS panel	Heterozygous	Unknown	Tolerated	0	VUS
83	*CNTNAP2*	c.479 G>A (p.R160H)	Missense	n/a	Heterozygous	Unknown	Damaging	$4.4x10^{-4}$	VUS**
	TSC2	c.4523 C>G (p.P1508R)	Missense	n/a	Heterozygous	Unknown	Damaging	**0**	VUS**
87	*SPTAN1*	c.4153 C>T (p.R1385W)	Missense	n/a	Heterozygous	*De novo*	Damaging	$2.4x10^{-5}$	VUS
	PLCB1	c.1536 C>T (p. D512D)	Synonymous	n/a	Heterozygous	Paternal	n/a	$2.4x10^{-5}$	Likely benign
97	*RELN*	c.1307C>G (p.A436G)	Missense	NGS panel	Heterozygous	Unknown	Tolerated	0	VUS

Abbreviations: CNV: copy number variants; NGS: next-generation sequencing; VUS: variant of unknown significance.
VUS** with minor pathogenic evidence

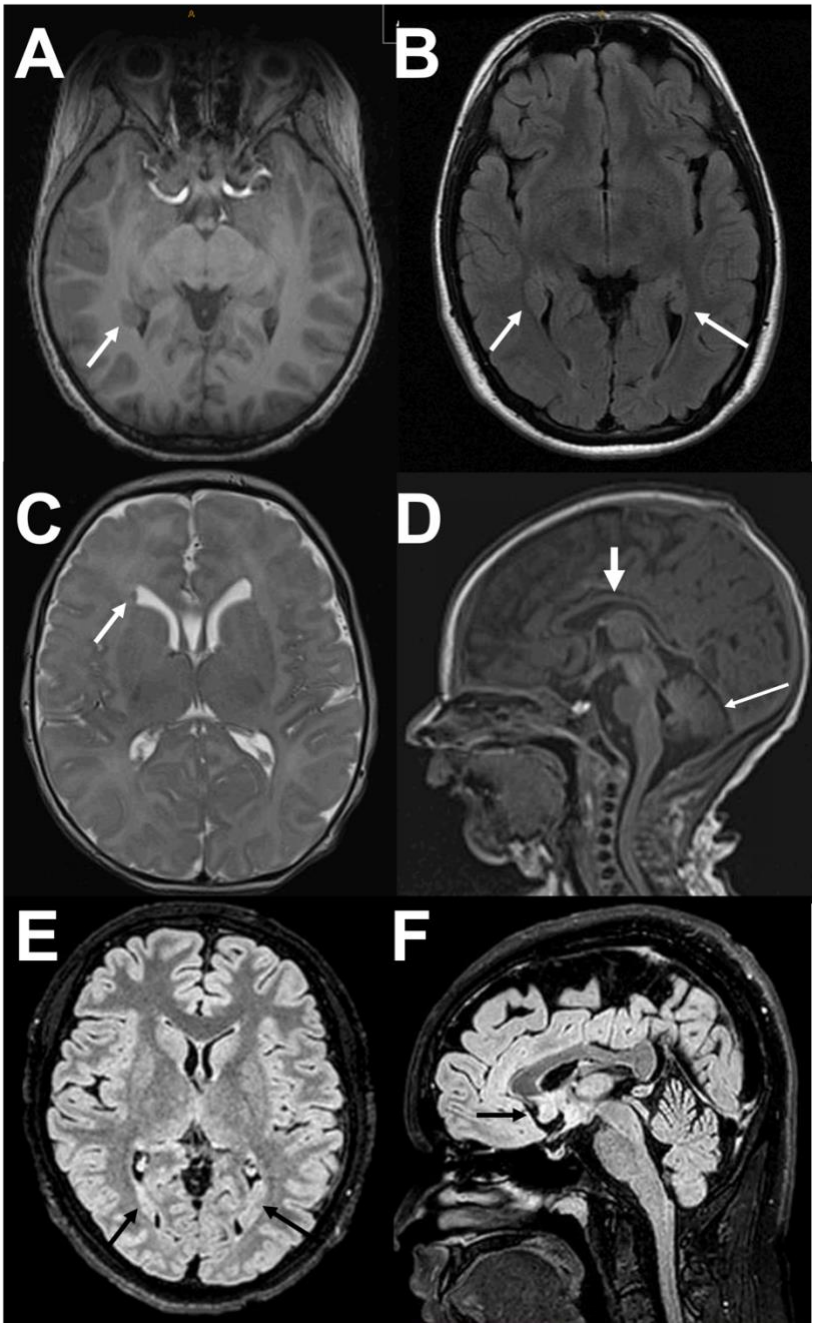
FIGURES

Figure A. Cortical Development in Utero



Key cellular events contributing to cortical development across the three trimesters of pregnancy: (1) neuro-epithelial cells undergo extensive proliferation to generate the pool of progenitor cells within the ventricular zone [left], (2) some neuroepithelial cells differentiate into radial glial cells, directly or indirectly via intermediate progenitor cells, and give rise to migrating neuronal precursor cells, which migrate outward to the eventual cortex [middle], (3) neuronal precursors have arrived at their final destination, undergo maturation, extend their axons and form synapses with interneurons and other cortical neurons, contributing to a physical stress that leads to gyri formation [right].

Figure 1. Examples of Brain Malformation Patterns.



(A) Patient #3 at age 9 y (PVNH-only with single nodule). Axial T1-weighted sequence shows a single NH in the right posterior lateral ventricle (arrow). (B) Patient #39 at age 23 y (PVNH-only with multiple nodules). On axial FLAIR sequence NH are seen bilaterally in the posterior lateral ventricles (arrows). (C, D) Patient #35 on first day-of-life (PVNH-plus with single nodule). In (C), axial T2-weighted sequence shows a single NH in the right anterior region of the lateral ventricle (arrow). In (D), sagittal T1 shows thin corpus callosum (thick arrow) and mild cerebellar hypoplasia involving vermis and cerebellar hemispheres (thin arrow). (E, F) Patient #43 at age 18 y (PVNH-plus with multiple nodules). In (E), axial FLAIR sequence shows bilateral NH in the posterior lateral ventricles (arrows). In (F), sagittal FLAIR shows a hypothalamic hamartoma (arrow)

APPENDIX

Centre universitaire de santé McGill
McGill University Health Centre

X HME MCH X HGM MGH X HRV RVH
X HNM MNH X ITM MCI X CL LC

L'Hôpital de Montréal pour enfants
The Montreal Children's Hospital
Centre universitaire de santé McGill
McGill University Health Centre

ID # _____

Epilepsy in People with Periventricular Nodular Heterotopia Questionnaire

**This questionnaire is to be completed by affected competent adults or by parents/legal guardians of affected minor (0-18 years old) or affected incapacitated persons*

**Indicate by √ all applicable per question or statement where options □ have been listed and/or provide further details if necessary*

❖ Pregnancy and Birth History

- Prenatal complications: □ **Yes** □ **No**
  - ☐ Viral illness/infections
  - ☐ Diabetes
  - ☐ Hypertension
  - ☐ Bleeding
  - ☐ Proteinuria (protein in urine)
  - ☐ Edema (swelling in hands, feet or ankles)
  - ☐ Polyhydramnios (excessive amniotic fluid)
  - ☐ Oligohydramnios (low amniotic fluid)
  - ☐ Miscarriage
  - ☐ Intrauterine growth restriction (IUGR)
  - ☐ Other: _____
    - How many weeks gestation: _____
    - Prescribed medication, if any: _____
- Birth:
  - Gestation: _____ weeks
  - Presentation:
    - ☐ Cephalic
    - ☐ Breech
    - ☐ Shoulder
    - ☐ Transverse
    - ☐ Oblique
  - Type of delivery
    - ☐ Normal vaginal
      - ☐ Spontaneous
      - ☐ Induced
      - ☐ Use of forceps
    - ☐ Caesarian section
      - ☐ Elective for: _____
      - ☐ Emergency for: _____
    - ☐ Vacuum extraction

- Condition at birth:
  - ☐ Good
  - ☐ Poor
  - ☐ Resuscitation needed
    - ☐ Suction
    - ☐ Ventilation
    - ☐ Intubation
    - ☐ Drugs given: _____
- Neonatal problems: □ **Yes** □ **No**
  - ☐ Early death
  - ☐ Seizures
    - ☐ Febrile seizures
    - ☐ Other type: _____
      - Onset at how many days: _____
      - Treatment: _____
  - ☐ Ventilation
  - ☐ Hypotonia (low muscle tone)
  - ☐ Feeding difficulties
  - ☐ Other: _____

❖ Development and Education

- Early developmental milestones:
  - Sit independently – Age: _____
  - Walk independently – Age: _____
  - Talking - single words – Age: _____
  - Talking - two words together – Age: _____
  - Toilet trained – Age: _____
    - Regression: □ **Yes** □ **No**
      - Skill(s) lost: _____
      - What age(s): _____
- Developmental impairment: □ **Yes** □ **No**
  - Specify: _____
  - Identified at what age: _____
- Educational attainment:
  - Homeschooled: □ **Yes** □ **No**
    - ☐ Elementary school
    - ☐ High school
    - ☐ CEGEP/College/Technical school
    - ☐ University
    - ☐ Higher graduate degree
    - ☐ Never attended school
- Learning difficulties: □ **Yes** □ **No**
  - ☐ Reading
  - ☐ Writing
  - ☐ Comprehension
  - ☐ Communication (speech/language problems)
  - ☐ Autism
    - Identified at what age(s): _____
- Past employment/ Current occupation:

  _____

_____

❖ PVNH Diagnosis

- Age at diagnosis: _____
- Specify diagnosis (e.g. PVNH with Ehlers Danlos syndrome), if necessary:
  _____
- Mutation identified:
  _____
- Specify events/factors leading to diagnosis (e.g. seizures, family member diagnosed, developmental delay, incidental etc):
  _____
  _____

❖ Investigations

- Have you ever had:
  - ☐ EEG
    - Dates: _____
    - Place: _____
    - Findings:
      _____
      _____
      _____
  - ☐ MRI Scan
    - Dates: _____
    - Place:
      _____
    - Findings:
      _____
      _____
      _____
  - ☐ Genetic testing
    - Dates: _____
    - Place: _____
    - Results: _____
      _____
      _____

❖ Seizure History

- When did the seizures begin:
  Age: _____
- Seizures frequency:
  - ☐ Daily
  - ☐ Multiple times per day
  - ☐ One per week
  - ☐ Less than one per week
  - ☐ Multiple times per week
  - ☐ Very infrequent
  - ☐ Controlled by medication
- Relation to time of day:
  - ☐ After waking (first 2 hours)

- ☐ Last morning
- ☐ Afternoon
- ☐ Evening
- ☐ During sleep
- ☐ No relation

- **Epilepsy syndrome**

  Specify if known (confirmed or suspected):

  _____

- **Seizure types**
  - Type (1): _____

    Age at onset: ____
    Duration: _____
    Frequency:
    _____
    Investigations (hospitalizations, medical interventions):
    _____
  - Type (2), if applicable:

    Age at onset: ____
    Duration: _____
    Frequency:
    _____
    Investigations:
    _____
  - Type (3), if applicable: _____

    Age at onset: ____
    Duration: _____
    Frequency:
    _____
    Investigations:
    _____

    - Have you experienced drop attacks: □ **Yes** □ **No**
      - ☐ Dropped to the ground
      - ☐ Dropped objects
      - ☐ Head dropped unexpectantly
        - How often does this happen:
          _____
        - Total number of episodes: _____
        - Did you lose consciousness: □ **Yes** □ **No**

- Seizure incidence
  - Changes over time: □ **Yes** □ **No**
    - ☐ Increase in frequency over time
    - ☐ Decrease in frequency over time
    - ☐ Increase in intensity of seizures over time
    - ☐ Decrease in intensity of seizures over time
    - ☐ Increase in duration of seizures over time
    - ☐ Decrease in duration of seizures over time
      - When did improvement/deterioration occur:
        _____
  - When was the last seizure: _____

- Have seizures ever been so frequent that you did not recover in between seizures: □ **Yes** □ **No**
  When: _____
- Have you ever had a long period free of seizures: □ **Yes** □ **No**
  When: _____
  How long did it last: _____

- **Precipitants**
  - Specify if there are any triggers, precipitants or situations that are more likely to cause seizures:
    - ☐ Sleep deprivation (late nights, getting less sleep than usual)
    - ☐ Increased amount of alcohol in previous 24 hours
    - ☐ Bright lights (flashing television or computer, disco lights)
    - ☐ Physical stress
    - ☐ Emotional stress
    - ☐ Fever
    - ☐ Noise or startle
    - ☐ Other: _____
- **Aura**
  - Before a seizure, do you experience: □ **Yes** □ **No**
    - ☐ Distorted or altered vision
      - ☐ Objects appear bigger than should be
      - ☐ Objects appear smaller than should be
    - ☐ Distorted or altered hearing
      - ☐ Sounds appear louder than should be
      - ☐ Sounds appear softer than should be
  - Specify any warnings before seizures occur:
    _____
- **Ictal**
  - During a seizure, do you **lose consciousness**: □ **Yes** □ **No**
    - ☐ Go completely blank
    - ☐ Retain some awareness of surroundings
    - ☐ Stop any action or continue moving automatically "like a robot"

      How long does it last:
      _____
  - During a seizure, do you experience:
    - Queasy feeling in stomach □ **Yes** □ **No**
    - Mouth watering or drooling □ **Yes** □ **No**
    - Pounding heart □ **Yes** □ **No**
    - Dizziness or vertigo □ **Yes** □ **No**
    - Tongue biting □ **Yes** □ **No**
    - Chewing or swallowing □ **Yes** □ **No**
    - Laughing □ **Yes** □ **No**
    - Passing stool □ **Yes** □ **No**
    - Wetting yourself □ **Yes** □ **No**
    - Shortness of breath (go blue) □ **Yes** □ **No**
  - During a seizure, do you experience **sensory disturbances**:
    □ **Yes** □ **No**

- ☐ Pins and needles, tingling or electric shocks
  - Specify: _____
  - Where: _____
  - Spread to other areas: _____
  - How long does it last: _____
- ☐ Objects appear distorted
  - ☐ Bigger than should be
  - ☐ Smaller than should be
- ☐ Sounds appear distorted
  - ☐ Louder than should be
  - ☐ Softer than should be
- ☐ Feeling that everything is in slow motion
  - How long does it last: _____
- ☐ Feeling that everything is sped up
  - How long does it last: _____

- During a seizure, do you experience **psychic symptoms**:
  □ **Yes** □ **No**
  - ☐ Déja vu (unfamiliar objects or surroundings appear familiar)
  - ☐ Jamais vu (familiar objects or settings appear unfamiliar)
  - ☐ Sudden emotional changes
    - ☐ Intense fear
    - ☐ Rage or anger
    - ☐ Euphoria, happiness or pleasure
      - How long does it last: _____
- During a seizure, do you experience **mnestic or perceptual hallucinations**: □ **Yes** □ **No**
  - ☐ Flashbacks
    - How long does it last: _____
  - ☐ Dream-like state (feeling you are in a dream but you are awake)
    - How long does it last: _____
  - ☐ See or hear things that are not there
    - Specify: _____
  - ☐ Unusual smells or tastes
    - Specify: _____
- During, do you experience **changes in tone and posture**:
  □ **Yes** □ **No**
  - ☐ Eyes roll back or look in one particular direction
    Specify: _____
    Which direction do they turn: _____
    Able to control movement: □ **Yes** □ **No**
    How long does it last: _____
  - ☐ Head turns in a particular direction
    Which direction: _____
    Able to control movement: □ **Yes** □ **No**
    How long does it last: _____
  - ☐ Arm(s) or leg(s) stiff in particular position
    Specify: _____

Bent or straight position: _____
How long does it last: _____

- During a seizure, do you experience **unusual movements**:
  □ **Yes** □ **No**
  - ☐ Jerking or twitching movements of face, arm/ leg
    - Specify: _____
    - Both sides or one side of body: _____
    - Jerking is repeated and regular or infrequent and irregular: _____
    - How long does it last:_____
  - ☐ Eyelids twitch or repeated blinking
    - Specify: _____
    - Both or one side of body: _____
    - How long does it last: _____
  - ☐ Licking or smacking of lips
  - ☐ Fiddling with fingers
  - ☐ Stepping or bicycling movements with your legs

- **Post-Ictal**
  - After a seizure, do you experience:
    - Confusion or drowsiness □ **Yes** □ **No**
      Specify: _____
      How long does it last: _____
    - Headache □ **Yes** □ **No**
      Describe: _____
      How long does it last: _____
    - Longer lasting effects on vision, sensation or muscle tone □ **Yes** □ **No**
      Specify: _____
      How long do they last: _____
    - Other: _____

❖ **Anti-epileptic/anti-seizure treatment**

- Indicate all medications that have been tried, duration of trial and specify whether seizures <u>improved</u>, <u>worsened</u> or there was <u>no effect</u>

	IMPROVED	**NO EFFECT**	**WORSENED**
- Levetiracetam (Keppra)	☐	☐	☐
- Carbamazepine (Tegretol)	☐	☐	☐
- Clobazam (Frisium)	☐	☐	☐
- Valproic Acid (Depakene)	☐	☐	☐
- Oxcarbazepine (Trileptal)	☐	☐	☐
- Clonazepam (Rivotril)	☐	☐	☐
- Lamotrigine (Lamictal)	☐	☐	☐

- Topiramate (Topamax)	☐	☐	☐
- Ethosuximide (Zarontin)	☐	☐	☐
- Vigabatrin (Sabril)	☐	☐	☐
- Rufinamide (Banzel)	☐	☐	☐
- Brivaracetam (Brivlera)	☐	☐	☐
- Zonisamide (Zonegran)	☐	☐	☐
- Stiripentol	☐	☐	☐
- Acetazolamide (Diamox)	☐	☐	☐
- Cannabidiol CBD	☐	☐	☐
- Other: _____	☐	☐	☐

- Specify any adverse side effects or secondary symptoms during trial:
  _____
  _____
- Current treatment plan:
  Medication(s) and dose(s):
  _____
  _____
  _____
  Adverse side effect or secondary symptoms from current medication:
  _____
  _____
- Have you undergone evaluation for surgery? □ **Yes** □ **No**
  Specify assessment:
  _____
  _____
- Have you undergone surgery to remove affected parts? □ **Yes** □ **No**
  Type of surgery: _____
  When: _____
  Outcome:
  - ☐ No change in seizure frequency/intensity/duration
  - ☐ Reduction of seizure frequency/intensity/duration
  - ☐ Seizure free
  - ☐ Other:

    _____

  Specify any side effects of intervention: _____
  _____
  _____
  Specify any complications post-surgery:
  _____
  _____
  Results post-surgery:
  - ☐ EEG

Date: _____
Place: _____

- ☐ MRI Scan
  Date: _____
  Place: _____

- Have you tried lifestyle changes or alternative therapies: □ **Yes** □ **No**
  - ☐ Ketogenic diet
  - ☐ Vagal nerve stimulation
  - ☐ Modified Atkins diet
  - ☐ Low glycemic index treatment
  - ☐ Other: _____
    Duration of trial: _____
    Specify outcome: _____
    _____

❖ Medical History

- Have you had:
  - Infection of the brain □ **Yes** □ **No**
    - ☐ Meningitis
    - ☐ Encephalitis
    - ☐ Other: _____
  - Serious head injury (caused loss of consciousness) □ **Yes** □ **No**
    Describe:
    _____
    _____
- Have you experienced/ Do you experience:
  - Muscle cramps □ **Yes** □ **No**
    - ☐ At rest
    - ☐ With exercise
    - ☐ From prolonged walking
      When did it start: _____
      How often does it happen: _____
      How long does it last: _____
  - Jerks/ involuntary limb movements: □ **Yes** □ **No**
    Does the limb twist into a funny position: □ **Yes** □ **No**
  - Twitches □ **Yes** □ **No**
  - Blank spells □ **Yes** □ **No**
  - Headaches/Migraines □ **Yes** □ **No**
    Where: _____
    Describe: _____
    Intensity (1=no pain; 10= excruciating pain): _____
    Aggravating factors:_____
    Alleviating factors: _____
    Noise sensitivity: □ **Yes** □ **No**
    Light sensitivity: □ **Yes** □ **No**
    Nausea/vomiting: □ **Yes** □ **No**
    Associated changes in visions/speech:
    _____
  - Changes in vision (lasting minutes) □ **Yes** □ **No**
    - ☐ Sparkling lights

- ☐ Shapes
- ☐ Holes in vision
- ☐ Other: _____

- Numbness or tingling (lasting minutes) □ **Yes** □ **No**
  - ☐ Face
  - ☐ Hands

- Are you currently followed or have been followed by a specialist in the past for other illnesses/conditions?
  - Respiratory issue □ **Yes** □ **No**
    - ☐ Asthma
    - ☐ Breathing problems
    - ☐ Persistent cough
    - ☐ Recurrent pneumonia/bronchitis
    - ☐ Oxygen dependent
    - ☐ Oxygen desaturation
    - ☐ Other: _____
  - Cardiovascular disease or anomalies □ **Yes** □ **No**
    - ☐ Murmur
    - ☐ Tachycardia
    - ☐ Bradycardia
    - ☐ Chest pain or SOB
    - ☐ Lightheadedness
    - ☐ Dizziness
    - ☐ Fainting
    - ☐ High blood pressure
    - ☐ Low blood pressure
    - ☐ Pulmonary hypertension
    - ☐ Valve abnormalities
    - ☐ Aneurysm
    - ☐ Other: _____
  - Gastrointestinal disease or issues □ **Yes** □ **No**
    - ☐ Chronic constipation
    - ☐ Diarrhea
    - ☐ Reflux
    - ☐ Bowel malrotation
    - ☐ IBS
    - ☐ IBD
    - ☐ Celiac disease
    - ☐ Chrohn's disease
    - ☐ Other: _____
  - Psychiatric issues □ **Yes** □ **No**
    - ☐ Depression
    - ☐ Anxiety
    - ☐ Bipolar disorder
    - ☐ Schizophrenia
    - ☐ Other: _____
  - Behavioral issues □ **Yes** □ **No**
    Specify: _____
  - Dysmorphism/ malformations □ **Yes** □ **No**
    - ☐ Microcephaly

- ☐ Shortened digits
- ☐ Hyperflexible joints
- ☐ Cysts
- ☐ Kidney malformation
- ☐ Other: _____

- Other medical problems: □ **Yes** □ **No**
  - ☐ Fainting
  - ☐ Diabetes
  - ☐ Cancer
    Specify: _____
  - ☐ Alzheimer's disease
  - ☐ Dementia
  - ☐ Other: _____

- Have you had any medical interventions (e.g. hospitalization, surgery, rehabilitation, medication) to treat/correct or remediate aforementioned illness/condition: □ **Yes** □ **No**
  Specify: _____
  _____
- List any past and all current medications (not including anti-seizure medication):
  _____
  _____
  _____

❖ Family Medical History

- Indicate whether any of your immediate family members (include your relation) have or had any of the following illnesses or conditions:

□ PVNH	□ Epilepsy	□ Seizures
□ Febrile convulsions	□ Blank spells	□ Jerks
□ Developmental delay	□ Autism	□ Intellectual disability
□ Behavioral problems	□ Anxiety	□ Depression
□ Neurological disease	□ Still births	□ Consanguinity
□ Sudden unexpected death (specify age)		□ Spontaneous miscarriages

□ Other: _____

Family Pedigree

TABLE S1. Detailed list of epilepsy and electroencephalography features of each participant

No./Sex /Age(y)	Subgroup	NH distribution	Sz onset (y)	Sz types	EEG	Epilepsy course	Effect-ive ASM	Ineffect-ive ASM	Surgery	Alt
1/F/50	PVNH-Plus other cerebral malformations	Bilateral foci	41	FIA-M (automatisms)	Focal IED	Pharmaco-responsive	LTG, ZNS	LEV	Right anterior temporal lobe resection	
2/F/4	PVNH-Plus other cerebral malformations	Single focus	0.42	GS FIA-NM	Multifocal IIAE & Generalized nonepileptiform abnormalities	Drug-resistant	VPA	LEV, CBZ, CLB, CLZ, TPM, VGB, RFM		
3/M/10	PVNH-Only	Single focus	9	F-BTC FIA-M (myoclonic)	Focal IED	Sz ongoing	LEV	CLB		
4/F/5	PVNH-Only	Bilateral foci	0.5	GS	Multifocal IIAE & Generalized non epileptiform abnormalities	Self-limited	VGB			
5/F/61	PVNH-Plus other cortical malformations	Bilateral foci	12	FIA FA-M (myoclonic) GM (clonic)	Generalized IED	Drug-resistant	VPA, TPM	LEV, PHT		
6/F/3	PVNH-Only	Single focus	3	FIA-M (clonic)	Focal IED	Pharmaco-responsive	CLB			
7/F/43	PVNH-Only	Unilateral foci	16	FA-NM (cognitive) FIA-NM GTC	Focal IED	Drug-resistant	CLB, CBZ	LEV, VPA		
8/F/8	PVNH-Only	Single focus	3	FIA	Focal IED	Self-limited				
9/M/5	PVNH-Only	Bilateral foci	4	GTC GA GM (myoclonic)	Generalized IED	Sz ongoing	CLB, VPA, ZNS, CBD	LEV, TPM		
10/M/15	PVNH-Plus other cerebral malformations	Bilateral foci	10	GTC	Multifocal IED	Pharmaco-responsive	LEV			
11/F/13	PVNH-Only	Single focus	9	FIA-M (myoclonic)	Focal IED	Pharmaco-responsive	LEV			
12/F/18	PVNH-Plus other brain malformations	Bilateral foci	0.5	GA GTC	Focal IED	Self-limited	CBZ			
13/F/5	PVNH-Plus other brain malformations	Bilateral foci	0.033	FIA-M (tonic)	Focal nonepileptiform abnormalities	Self-limited	LEV			
14/M/23	PVNH-Plusother brain malformations	Single focus	12	GA	Multifocal IED	Drug-resistant	CLB	LEV, VPA, LTG, TPM, LCM, ESL, PER, OXC, BRIV	RFTC HN	
15/F/29	PVNH-Plus other brain malformations	Bilateral foci	12	GA GTC	Focal IED	Drug-resistant	LTG, OXC, CLZ, PER	LEV, CBZ, TPM, VGB	Right temporal resection, RFTC, Right cortico-amygdalec-tomy	VN

16/F/32	PVNH-Plus other cerebral malformations	Bilateral foci	24	FA-NM (cognitive)	Focal nonepileptiform abnormalities	Sz ongoing	LEV, CLZ	ZNS		KD
17/F/12	PVNH-Only	Bilateral foci	5	GTC	Generalized IED	Sz ongoing	LEV			
18/M/4	PVNH-Plus other brain malformations	Bilateral foci	4	GTC	Generalized nonepileptiform abnormalities	Pharmaco-responsive	LEV			
19/M/51	PVNH-Plus other cerebral malformations	Single focus	13	GA GTC	Focal IED	Drug-resistant	LEV, CLB, TPM	PHB, PHT	Left anterior temporal lobe resections	
20/F/6	PVNH-Plus other cerebral malformations	Bilateral foci	1.25	GA FIA-M (clonic) GTC	Normal EEG	Sz ongoing	LTG	LEV		
21/F/44	PVNH-Only	Bilateral foci	34	FIA-NM (cognitive) FA-NM (sensory) F-BTC	Generalized IED	Drug-resistant	LEV,CLB, ZNS, ESL	CBZ, LTG, TPM, LCM, PER		VN
22/F/0.42	PVNH-Plus other brain malformations	Unilateral foci	0.25	GS	Focal IED & nonepileptiform abnormalities	Pharmaco-responsive	ACTH, VGB			
23/M/9	PVNH-Plus other cerebral malformations	Bilateral foci	7	GA (eyelid myoclonia)	Focal IED & Generalized nonepileptiform abnormalities	Sz ongoing	LEV	TPM		
24/F/43	PVNH-Only	Bilateral foci	12	FIA-NM (cognitive) GTC	Focal IED	Pharmaco-responsive	CBZ			
25/F/16	PVNH-Only	Bilateral foci	9	FIA-M (clonic) GTC	Focal IED	Sz ongoing	LEV, ZNS, LCM			
26/F/34	PVNH-Only	Single focus	34	GTC	Normal EEG	Pharmaco-responsive	LEV			
27/F/38	PVNH-Only	Unilateral foci	15	FA-M (Jacksonian)	Normal EEG	Sz ongoing		TPM		
28/M/33	PVNH-Plus other cortical malformations	Unilateral foci	12	GTC	Focal nonepileptiform abnormalities	Pharmaco-responsive	CBZ, CLB			
29/F/13	PVNH-Plusother cortical malformations	Bilateral foci	1.5	FIA-NMGTC	Multifocal IED & Focal nonepileptiform abnormalities	Pharmaco-responsive	LEV, LCM	CBZ, VPA, CLB		
30/F/4	PVNH-Plus other cerebral malformations	Bilateral foci	0.083	GTC F-BTC FA-NM (emotional)	Generalized IED & nonepileptiform abnormalities	Drug-resistant	TPM, VBG, ACTH, CBD, GAB	LEV, OXC, CLB		KD & VN
31/M/14	PVNH-Plus other cortical malformations	Unilateral foci	13	FIA-NM	Normal EEG	Sz ongoing	OXC			
32/M/16	PVNH-Only	Single focus	14	GA atypical GTC	Generalized IED	Sz ongoing	LEV			
33/F/ 0.083	PVNH-Only	Single focus	0.022	FIA-M (clonic)	Multifocal IED	Pharmaco-responsive	CBZ, PHT	LEV		

34/F/12	PVNH-Only	Single focus	10	GTC	Focal IED	Pharmaco-responsive	CLB			
35/M/1	PVNH-Plus other cerebral malformations	Single focus	0.003	FIA-M (clonic)	Multifocal IED	Self-limited	LEV			
36/M/4	PVNH-Only	Single focus	2.5	FIA-M (myoclonic)	Focal IED & Generalized nonepileptiform abnormalities	Self-limited	CLB			
37/M/39	PVNH-Only	Single focus	21	FIA-M (clonic)	Focal IED	Sz ongoing	OXC, CLB	CBZ, VPA	Right anterior temporal lobe resection	
38/F/8	PVNH-Plus other cerebral malformations	Bilateral foci	4.5	GA	Focal IED	Self-limited				
39/M/38	PVNH-Only	Bilateral foci	23	FIA-NM (cognitive) FA-NM (cognitive) GTC	Multifocal IED & Generalized nonepileptifom abnormalities	Sz ongoing	OXC, LEV, CLB	CBZ, PHT	RFTC HN	
40/F/46	PVNH-Plus other cerebral malformations	Bilateral foci	34	GA FIA-M (tonic) GTC	Multifocal IED	Drug-resistant	LTG, ESL	LEV, CBZ, CLB, VPA, CLZ, TPM		
41/M/41	PVNH-Plus other cortical malformations	Unilateral foci	32	FA-M (automatisms) F-BTC	Generalized nonepileptiform abnormalities	Pharmaco-responsive	CBZ			
42/F/28	PVNH-Plusother cortical malformations	Unilateral foci	19	FIA-M (myoclonic) GAGTC GS	Generalized IED	Sz ongoing	CLB, VPA, LCM, ESL	PER, BRIV	Right frontal lobe resection	
43/M/23	PVNH-Plus other cerebral malformations	Bilateral foci	19	GA GTC	Multifocal IED	Sz ongoing	LEV, LCM, CLB	TPM		
44/F/34	PVNH-Plus other brain malformations	Single focus	21	GA GTC	Focal IED	Pharmaco-responsive	BRIV, CLB, LCM	LEV, CBZ	Right temporal lobe resections	
45/M/39	PVNH-Only	Bilateral foci	23	FIA-NM (cognitive) GTC	Multifocal IED	Pharmaco-responsive	CBZ	LEV		
46/F/29	PVNH-Plus other brain malformations	Unilateral foci	2	FIA-M (tonic)	Focal IED	Pharmaco-responsive	LTG, CLB	CBZ	Frontal lobe resections	KD
47/F/34	PVNH-Plus other cerebral malformations	Bilateral foci	16	FIA-NM (cognitive) GTC	Focal IED	Drug-resistant	CLB, LTG, CBZ	LEV, VPA, TPM	RFTC HN	
48/F/55	PVNH-Plus other brain malformations	Bilateral foci	18	FIA-M (automatisms)	Focal IED	Drug-resistant	LEV, LTG, CBZ	TPM		
49/M/50	PVNH-Plus other brain malfromations	Unilateral foci	15	FIA-NM GTC	Generalized nonepileptiform abnormalities	Pharmaco-responsive	CBZ	CLZ, LTG, PHT, PHB	Right cortico-amygdalo-hippo-campec-tomy	

50/M/50	PVNH-Plus other cerebral malformations	Bilateral foci	9	FIA-M (automatisms) FIA-NM (cognitive, sensory) GTC	Generalized IED	Drug-resistant	CLB, OXC, TPM	VGB, PHT, PHB, GAB		
51/F/17	PVNH-Plus other brain malformations	Unilateral foci	5	F-BTC FIA-NM (behavioral arrest)	Generalized IED & nonepileptiform abnormalities	Sz ongoing	CLB	LEV		
52/F/9	PVNH-Plus other cerebral malfornations	Unilateral foci	5.5	FIA-M (tonic)	Focal IED	Pharmaco-responsive	LEV			
53/F/11	PVNH-Only	Single focus	0.583	F-BTC	Generalized IED	Pharmaco-responsive	LEV, TPM	CLB		
54/F/26	PVNH-Only	Bilateral foci	3	GM (tonic) GA	Generalized nonepileptiform abnormalities	Drug-resistant	VPA	LEV, CLB, LTG, ESM		KD & VN
55/M/21	PVNH-Plusother cerebral malformations	Bilateral foci	1	F-BTCGA	Focal nonepileptiform abnormalities	Drug-resistant	CLB	CBZ, OXC, VPA, CLZ		KD & VN
56/M/18	PVNH-Plus other cerebral malformations	Bilateral foci	6	FIA-NM (cognitive) GTC GA	Generalized IED	Sz ongoing	VGB, CBZ	ZNS		KD & VN
57/F/25	PVNH-Plus other cerebral malformations	Bilateral foci	17	FIA-M (tonic)	Generalized IED	Sz ongoing	CLB, TPM	LEV, CBZ		KD
58/F/ DECD (17)	PVNH-Plus other cerebral malformations	Bilateral foci	0.25	GTC GS GA	Generalized nonepileptiform abnormalities	Drug-resistant		CBZ, VPA, LTG, VGA, TPM, OXC, LEV, CLZ, CLB		VN
59/M/21	PVNH-Only	Bilateral foci	10	GTC GM (myoclonic, atonic) GA (eyelid myoclonia)	Generalized IED & nonepileptiform abnormalities	Drug-resistant	LTG, TPM, CLB	VPA, OXC, CLZ, LEV, ZNS, CBZ		KD
60/F/22	PVNH-Plus other cortical malformations	Bilateral foci	5	GTC GM (myoclonic) GS	Focal nonepileptiform abnormalities	Drug-resistant		LEV, OXC, VPA, LTG		VN
61/M/20	PVNH-Only	Bilateral foci	4	GTC GA (typical, myoclonic)	Generalized IED	Drug-resistant	VPA, LEV, CLB	CBZ, OXC, TPM, VGB		KD
62/F/14	PVNH-Plus other cerebral malformations	Bilateral foci	0.333	GM (myoclonic, tonic) GS	Multifocal IED	Drug-resistant	VPA, CLZ	LEV, LTG, VGB, CBZ, ESM, OXC		KD & VN
63/M/17	PVNH-Only	Bilateral foci	16	FA-M (automatisms) GA	Normal EEG	Sz ongoing	BRIV, ZNS	LEV, OXC		

64/F/0.5	PVNH-Plus other cerebral malformations	Bilateral foci	0.1667	FA-NM (emotional)	Multifocal IED	Sz ongoing		VPA, OXC, ZNS		
65/F/0.33	PVNH-Plus other brain malformations	Unilateral foci	0.33	GS	Generalized nonepileptiform abnormalities	Sz ongoing	VGA			
66/F/15	PVNH-Only	Bilateral foci	9	FIA-NM (sensory, autonomic)	Focal IED	Sz ongoing	LTG	LEV		
67/M/11	PVNH-Only	Unilateral foci	9	F-BTC FIA-NM (sensory)	Multifocal IED	Pharmaco-responsive	LEV, CLB			
68/M/15	PVNH-Plus other cerebral malformations	Unilateral foci	7	GTC	Focal IED & nonepileptiform abnormalities	Sz ongoing	TPM			
69/F/11	PVNH-Plusother cerebralmalformations	Unilateral foci	0.33	FIA-M F-BTCGS	Focal IED	Drug-resistant	OXC	CBZ, VPA, TPM, CLB		
70/M/13	PVNH-Plus other brain malformations	Single focus	2	FIA-M F-BTC	Generalized IED	Sz ongoing	ESM, VPA			
71/M/9	PVNH-Only	Bilateral foci	0.67	FIA-M (myoclonic)	Generalized IED	Sz ongoing	CLZ, VPA	CLB		
72/M/6	PVNH-Plus other cortical malformations	Single focus	2.75	GTC (febrile)	Multifocal IED	Self-limited				
73/M/7	PVNH-Plus other cortical malformations	Unilateral foci	0.25	GS	Multifocal IED	Self-limited	LEV, CLZ, PHB			
74/M/12	PVNH-Only	Unilateral foci	8	FIA-M (clonic)	Focal IED	Pharmaco-responsive	VPA, CLB			
75/F/7	PVNH-Only	Single focus	5	F-BTC	Multifocal IED	Pharmaco-responsive	VPA			
76/F/19	PVNH-Plus other cerebral malformations	Unilateral foci	5	F-BTC	Focal IED	Pharmaco-responsive	CBZ, LEV, LTG		Right temporal resection	
77/M/14	PVNH-Plus other brain malformations	Bilateral foci	4	FIA-M (tonic) GM (tonic)	Multifocal IED & Generalized nonepileptiform abnormalities	Drug-resistant	CBD	CLB, CLZ, VPA, LEV, ESM, RFM		
78/M/2	PVNH-Plus other brain malformations	Unilateral foci	0.75	GS	Multifocal IIAE & generalized nonepileptiform abnormalities	Self-limited	VGB			
79/F/16	PVNH-Plus other cortical malformations	Unilateral foci	12	FIA-M	Focal IED	Pharmaco-responsive	CBZ, TPM	LEV		
80/M/11	PVNH-Plus other cerebral malfornations	Single focus	2	FIA-NM GA	Multifocal IED	Pharmaco-responsive	VPA			
81/F/10	PVNH-Plus other brain malformations	Bilateral foci	0.583	FIA-M (tonic) GTC	Multifocal IED	Pharmaco-responsive	VPA			
82/F/19	PVNH-Only	Bilateral foci	10	FIA-M	Multifocal IED	Sz ongoing	CBZ, LTG, CLB			

83/F/13	PVNH-Plus other brain malformations	Unilateral foci	5	FIA-M (myoclonic)	Focal IED	Sz ongoing	CBZ, CLB	VPA, LTG, ESM		
84/M/7	PVNH-Only	Bilateral foci	6	FIA-M	Multifocal IED	Self-limited				
85/F/12	PVNH-Only	Single focus	2	GTCGA (atypical)	Multifocal IED	Pharmaco-responsive	LEV, VPA			
86/M/22	PVNH-Only	Single focus	3.5	F-M F-BTC	Multifocal IED	Pharmaco-responsive	VPA			
87/F/7	PVNH-Plus other brain malformations	Unilateral foci	0.42	GS FA-M	Multifocal IED	Drug-resistant		VPA, CLB, VGB, LEV, CBZ, TPM, LTG		
88/F/16	PVNH-Only	Single focus	4	FIA-M	Multifocal IED	Self-limited	VPA			
89/M/8	PVNH-Plus other cerebral malformations	Unilateral foci	4	GM (atonic)	Multifocal IED	Pharmaco-responsive	ESM			
90/F/12	PVNH-Only	Single focus	8	F-BTC	Multifocal IED	Pharmaco-responsive	LEV			
91/F/7	PVNH-Plus other cerebral malformations	Bilateral foci	1.5	FIA GTC GS	Multifocal IED	Drug-resistant		VGB, ACTH, VPA, CLB, LCM		
92/F/23	PVNH-Plus other cerebral malformations	Bilateral foci	10	FIA	Multifocal IED	Pharmaco-responsive	VPA, LEV			
93/M/6	PVNH-Plus other cerebral malformations	Bilateral foci	3	F-BTC	Multifocal IED	Sz ongoing	VPA, LTG, CLB	LEV		
94/F/33	PVNH-Plus other brain malformations	Bilateral foci	13	GM (myoclonic) GA (typical)	Generalized IED & nonepileptiform abnormalities	Sz ongoing	VPA, CLB, LTG			
95/M/1.6	PVNH-Plus other cerebral malformations	Bilateral foci	0.5	F-M	Normal EEG	Self-limited				
96/M/7	PVNH-Plus other brain malformations	Unilateral foci	0.75	GTC (atypical febrile) GS GM (myoclonic)	Multifocal IED	Pharmaco-responsive	LEV, CLB, TPM	VGB		
97/M/3	PVNH-Plus other brain malformations	Bilateral foci	3	GTC	Focal IED & nonepileptiform abnormalities	Pharmaco-responsive	LEV			
98/F/16	PVNH-Only	Bilateral foci	15	F-BTC	Focal IED	Sz ongoing	LTG			
99/F/21	PVNH-Only	Single focus	8	F-BTC	N/A	Sz ongoing	VPA			
100/M/16	PVNH-Only	Single focus	12	GTC	Normal EEG	Pharmaco-responsive	LTG	LEV, CLB		

ACTH, adrenocorticotrophic hormone; Alt, alternative therapies; ASM, anti-seizure medication; BRIV, brivaracetam; CBD, cannabidiol; CBZ, carbamazepine; CLB, clobazam; CLZ, clonazepam; ESM, ethosuximide; ESL, eslicarbazepine; FA, focal aware; FIA, focal impaired awareness; F-M, focal motor; F-NM, focal nonmotor; F-BTC, focal-to-bilateral tonic-clonic; GA, generalized absence; GAB; gabapentin; GM, generalized motor; GS, generalized spasms; GTC, generalized tonic-clonic; IED, interictal epileptiform abnormality;KD, ketogenic diet; LEV, levetiracetam; LCM, lacosamide; LTG, lamotrigine; OXC, oxcarbazepine; PER, perampanel; PHB,

phenobarbitol; PHT; phenytoin; RFM, rufinamide; RFTC, radiofrequency thermocoagulation of heterotopic nodules; Sz, seizure; TPM, topimarate; VGB, vigabatrin; VN, vagal nerve stimulation; VPA, valproate; ZNS, zonisamide