Variable Clinical and Molecular Expressivity of PCDH19 Variants and Girls Clustering Epilepsy

A disorder of cellular "mosaics"

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by

Kristy Louise Kolc

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Children see magic because they look for it

- *Christopher Moore*

Science is nothing but perception.

- *Plato*

Title	Variable Clinical and Molecular Expressivity of PCDH19 Variants and Girls Clustering Epilepsy: A disorder of cellular “mosaics”
Author	Kristy Kolc
Institute	Adelaide Medical School

Academic advisors
Professor Jozef Gecz,
Adelaide Medical School
A/Prof Rachel Roberts,
School of Psychology
Dr Duyen Pham,
Adelaide Medical School
Dr Raman Kumar,
Adelaide Medical School

Adelaide Medical School
University of Adelaide
Adelaide Health and Medical Sciences Building
ADELAIDE SA 5000
E-mail: medicineoffice@adelaide.edu.au
webpage: https://health.adelaide.edu.au/medicine/

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A	Adenine (nucleotide)
ACTH	Adrenocorticotropic hormone
AD	Attention-deficit
ADHD	Attention-deficit hyperactivity disorder
AED	Antiepileptic drug
AKR1C3	Aldo-keto reductase family 1 member C3
ANOVA	Analysis of variance
APA	American Psychiatric Association
AS	Absence seizures
ASD	Autism spectrum disorder
AZD	Acetazolamide
BD	Behavioral disorder
BRIEF	Behavior Rating Inventory of Executive Function
BTCS	Bilateral tonic-clonic seizures
C	Cytosine (nucleotide)
CBD	Cannabidiol
CBZ	Clonazepam
cDNA	Complimentary DNA
CI	Confidence interval
CLB	Clobazam
CoP	Conduct problems
CP	Cytoplasmic
CS	Clonic seizures
CZP	Carbamazepine
DB	Destructive behavior
DD	Developmental delay
DNA	Deoxyribose nucleic acid
DOCS	Dimensional Obsessive-Compulsive Scale
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, fifth edition
DZP	Diazepam
EC	Extracellular cadherin
ED	Executive dysfunction

EEG	Electroencephalogram
EFMR	Epilepsy and mental retardation limited to females
EP	Emotional problems
EtD	Eating disorder
ETX	Ethosuximide
FeS	Febrile seizures
FIAS	Focal impaired awareness seizures
FBTCS	Focal to bilateral tonic-clonic seizures
FMS	Focal motor seizures
FS	Focal seizures
FSIQ	Full scale intelligence quotient
G	Guanine (nucleotide)
GCE	Girls Clustering Epilepsy
gDNA	Genomic deoxyribose nucleic acid
GEFS+	Generalized epilepsy febrile seizures plus
GP	Gabapentin
GTCS	Generalized tonic-clonic seizures
HCS	Hemiclonic seizures
ID	Intellectual disability
ILAE	International League Against Epilepsy
IQ	Intelligence quotient
LEV	Levetiracetam
LTG	Lamotrigine
mRNA	Messenger ribonucleic acid
MS	Myoclonic seizures
MT	Mutant
MZ	Monozygotic
NMD	Nonsense mediated decay
NZ	New Zealand
NZP	Nitrazepam
OCD	Obsessive-compulsive disorder
OMIM	Online Medelian Inheritance in Man
OX	Oxcarbazepine

PCDH19	Protocadherin-19
PB	Phenobarbital
PCR	Polymerase chain reaction
PHT	Phenytoin
PO	Plan/Organize
PP	Peer problems
PRD	Pyridoxine
PRRT2	Proline rich transmembrane protein 2
Pt	Participant
PTC	Premature termination codon
RNA	Ribonucleic acid
SCN1A	Sodium voltage-gated channel alpha subunit 1
SCQ	Social Communication Questionnaire
SD	Social deficits
SDQ	Strengths and Difficulties Questionnaire
SE	Status epilepticus
SIFT	Sorting Intolerant from Tolerant
SRS-2	Social Responsiveness Scale, second edition
SPSS	Statistical Package for the Social Sciences
SV2A	Synaptic vesicle glycoprotein 2A
T	Tyrosine (nucleotide)
TGB	Tiagabine
TPM	Topiramate
TS	Tonic seizures
TCS	Tonic-clonic seizures
UK	United Kingdom
USA	United States of America
VGB	Vigabatrin
VPA	Sodium valproate
XCE	X-linked clustering epilepsy
XCI	X-chromosome inactivation
WM	Working memory
WT	Wild-type

LIST OF CITATIONS

Kolc KL, Sadleir LG, Scheffer IE, et al. A systematic review and meta-analysis of 271 PCDH19-variant individuals identifies psychiatric comorbidities, and association of seizure onset and disease severity. Mol Psychiatry 2018:1.

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ABSTRACT

Girls Clustering Epilepsy is the second most common developmental and epileptic encephalopathy. GCE is due to variants in the X chromosome gene *PCDH19* and is underpinned by cellular mosaicism due to X-chromosome inactivation (XCI) in females or somatic variant in males. The hallmark feature is that seizures occur in clusters and mainly affect females. At the time of thesis submission, GCE was re-named "X-linked clustering epilepsy" (XCE) to accommodate the growing number of affected male cases. Seizures typically present as generalized tonic-clonic and/or focal, which may evolve to bilateral, tonic-clonic. The clinical profile includes variable cognitive impairment and psychiatric features. The prevalence of these comorbidities and cause of the variable clinical expressivity is unknown.

We performed a systematic review and meta-analysis to identify the comorbidities associated with *PCDH19* variants and examine phenotype- and genotype-phenotype associations. Data from 38 peer-reviewed original articles were used and included 271 individual cases. We found that seizure onset ≤ 12 months was significantly associated ($p = 4.127 \times 10^{-7}$) with more severe intellectual disability compared with onset > 12 months. We identified two recurrent variants p.Asn340Ser and p.Tyr366Leufs*10, occurring in 25 (20 unrelated) and 30 (11 unrelated) cases, respectively. *PCDH19* variants were associated with psychiatric comorbidities in approximately 60% females, 80% affected mosaic males, and reported in nine hemizygous males. Executive dysfunction, and hyperactive, autistic, and obsessive-compulsive features were most frequently associated with *PCDH19* variants.

We developed a PCDH19 survey to systematically examine the comorbidities identified in our review using standardized neuropsychiatric assessments. The survey was completed by 122/186 (66%) participants diagnosed with GCE or with a confirmed likely pathogenic *PCDH19* variant. Executive functions were measured using the Behavior Rating Inventory of Executive Function. Psychiatric comorbidities were assessed via the Social Responsiveness Scale or Social Communication Questionnaire, the Strengths and Difficulties Questionnaire, and the Dimensional Obsessive-Compulsive Scale. Genetic, seizure, and developmental information were also collected. Of the 112 evaluated participants (15 males), there were 70 unique variants. Thirty-five variants were novel

and included a newly identified recurrent variant Ile781Asnfs*3. There were no phenotypic differences between published and unpublished cases. Seizures occurred in clusters in 94% individuals, with seizures resolving in 28% at an average age of 17.5 years. Developmental delay prior to seizure onset occurred in 18% of our cohort. Executive dysfunction and autism spectrum disorder (ASD) occurred in approximately 60% of individuals. The ASD profile included features of attention-deficit hyperactivity disorder. Obsessive-compulsive symptomology was observed in 21% individuals. There were no phenotypic differences between heterozygous females and mosaic males. We describe a mosaic male and two hemizygous males with atypical clinical profiles. Earlier seizure onset age and increased number of seizures within a cluster were associated with more severe clinical outcomes. No clinical profile was observed for transmitting males.

The penetrance of GCE is incomplete; estimated to be around 80-90%, and might be explained by cellular interference. Cellular interference postulates that the coexistence of *PCDH19* wild-type and variant cells would be pathogenic, whereas a homogenous cell population would be tolerated; an idea supported by the presence of asymptomatic *PCDH19*-negative hemizygous and symptomatic *PCDH19*-mosaic males. The cellular interference hypothesis was tested through analyses of GCE penetrant and non-penetrant female fibroblast cell lines using assays to determine XCI patterns and relative *PCDH19* cDNA expression. Specifically, we hypothesized that XCI and *PCDH19* cDNA expression will be skewed towards complete wild-type or variant expression in non-penetrant females.

We have shown that XCI patterns do not correlate with relative *PCDH19* cDNA expression in fibroblasts, thus invalidating use of this assay to infer *PCDH19* expression. No clear association was observed between penetrance in XCE and the degree of variant and wild-type *PCDH19* mRNA expression in skin fibroblasts. Although we were able to identify three non-penetrant females with 100% wild-type *PCDH19* expression, we were unable to provide support for the mechanism of cellular interference through our finding clinical phenotypes in individuals with markedly skewed XCI. Neuropsychiatric disorders can be very responsive to early intervention; therefore, a better understanding of these comorbidities may help to inform treatment and ultimately lead to better developmental outcomes for individuals affected by GCE. We show that both seizure onset age and activity are associated with clinical outcomes. Clinicians can use this information to

inform prognosis and provide targeted intervention and guidance for patients and their families.

THESIS DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Kristy Kolc

Signed: Date: 5 April 2020

I believe this thesis is properly presented and conforms to the specifications for the degree of sufficient standard to be, prima facie, worthy of examination.

Professor Jozef Gecz
Principal Supervisor

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Chapter 1: **Navigation & overview**

1.1 Navigation

This thesis incorporates a combination of published work (Chapters 2, 3, 4, and 5), material submitted for publication (Chapter 6), and prepared for submission (Chapter 7). Each chapter is formatted as a stand-alone peer-reviewed article and thus some repetition exists with respect to background information across chapters in order to contextualize each study. To minimize repetition, the systematic review and meta-analysis forms the thesis introduction (Chapter 2) and thus provides a 'big picture' overview of the history of GCE and gaps in the knowledge base. The reader is directed to the individual manuscripts for specific literature review and methodologies employed. Formatting of each manuscript has been adjusted to maintain consistency throughout the thesis. Tables and figures appear within each chapter and all references relevant to all chapters are at the end of the thesis.

1.2 Overview

This research explored the heterogeneity associated with GCE. It was observed that variation existed within families with respect to the penetrance or clinical presentation associated with an identical genetic variant. GCE is caused by *PCDH19* pathogenic variants and is expressed in a unique X-linked fashion whereby heterozygous females and mosaic males are affected and hemizygous males are unaffected. The aim of this work was to characterize and identify the cause of the variable penetrance and clinical expressivity associated with *PCDH19* variants. It was also noted that, although seizures were refractory to medication, families describe the cognitive and behavioral problems as the most challenging aspects. A characterization of the cognitive and behavioral deficits associated with *PCDH19* variants had yet to be established. We aimed to characterize the neuropsychiatric profile of GCE and provide an avenue for clinicians and families to access information about *PCDH19* and GCE in real-time.

This research represents the first to systematically characterize the neuropsychiatric profile of GCE using standardized assessments and utilizing a large international cohort. Further, this research is the first to establish a link between seizure frequency within a cluster, the timing of seizure onset, and clinical outcome. Individuals with a *PCDH19* variant were eligible to participate in this research. Our work incorporated findings from both symptomatic and asymptomatic males and females of all ages. This represents a novel approach to understanding GCE, as previous research typically focused on penetrant

individuals. Research exploring the clinical outcomes of GCE focused primarily on electro-clinical features and intellectual disability (ID). There was no research into the specific areas of cognition affected nor was a broad-scale approach to identifying associated psychiatric comorbidities employed. Research in this area focused solely on symptoms associated with ASD. The key strengths of this study are 1) the use of a large international cohort, which grants the power to statistically examine differences between the various symptom presentation subgroups; 2) the use of standardized assessments to target a broad range of neuropsychiatric comorbidities and; 3) the use of patient fibroblast cells from both penetrant and non-penetrant females, which allows for targeted molecular comparisons.

The aim of Study 1 (**Chapter 2)** was to identify the comorbidities frequently associated with GCE in the literature and examine potential associations of clinical and molecular factors with neuropsychiatric outcomes. I was interested in examining the prevalence of each profile, with a view to identifying the most appropriate neuropsychiatric assessments to validate these findings. Associations were tested using a linear regression model, factorial analysis of variance (ANOVA), and Student's *t* tests. For the binary categorical outcome variable, chi-squared tests of independence were performed. Due to the nature of reported data (i.e., raw data not available), certain variables were re-classified to aid the analyses.

In Study 2, **(Chapters 3 and 6)** I conducted similar analyses to that of Study 1, but with a difference in the way data were collected. I developed an online survey in three languages (English, Italian, and French). I collected demographic and clinical information via the Epilepsy Questionnaire (EQ), which was developed based on literature review and discussion with health professionals. I administered the following standardized neuropsychiatric assessments: the Behavior Rating Inventory of Executive function (BRIEF) for the assessment of executive dysfunction; the extended version of the Strengths and Difficulties Questionnaire (SDQ) for the assessment of behavioral difficulties, including ADHD; the Dimensional Obsessive-Compulsive Scale (DOCS) for the assessment of symptoms related to obsessive-compulsive disorder (OCD); and the Social Responsiveness Scale, second edition (SRS-2) for the assessment of symptoms related to ASD. As a French translation of the SRS-2 was not available, I also utilized the Social Communication Questionnaire (SCQ). Using a linear regression model, I validated the

associations from Study 1 and identified an additional association. A characterization of the clinical profile associated with GCE was established and formed the basis for Study 3.

In study 3 **(Chapter 4)** I used the data collected from Study 1 and 2, as well as information gleaned directly from health professionals to develop a *PCDH19* gene page as part of the Human Disease Genes Website Series. This is an international library of websites for professional information about genes and copy number variances and their clinical consequences. The *PCDH19* page will provide a platform for clinicians and families to share information and learn more about *PCDH19*.

Study 4 **(Chapter 5)** explores the effectiveness of the anti-epileptic medication Levetiracetam. As seizures in GCE are refractory to treatment, the identification of efficacious treatments is crucial. Through clinical practice, we noted remarkable seizure control with Levetiracetam for several girls with drug resistant GCE. Here, I report the impact of Levetiracetam on girls with GCE in two cohorts: a research cohort obtained through our collaborators in which detailed historical clinical information was available and an international cohort of individuals with *PCDH19* variants who were surveyed via the PCDH19 survey from Study 2.

The final study **(Chapter 7)** explores biological factors associated with penetrance in GCE. I analyzed skin fibroblasts of 13 penetrant and non-penetrant GCE females to determine XCI patterns, relative wild-type to variant *PCDH19* cDNA expression, and protein levels. Where available, biological data were compared to clinical information obtained from the PCDH19 survey in Study 2.

The thesis concludes with a general discussion **(Chapter 8)** which summarises the main findings, incorporates these with recent research in the field, discusses clinical implications, considers study limitations, and presents suggestions for future research.

Chapter 2: **A systematic review and meta-analysis of 271 PCDH19-variant individuals identifies psychiatric comorbidities, and association of seizure onset and disease severity**

2.1 Preamble

This thesis explores the variable clinical and molecular expressivity associated with *PCDH19* variants. To date, there have been no factors identified to explain the variable clinical expression observed among related individuals with identical variants. Further, no biological factors have been identified to explain the variable penetrance. An exploration of such factors could not be undertaken without first characterizing the clinical profile associated with *PCDH19* variants. A challenge for researchers is accessing a cohort that is spread around the world. Trying to evaluate each individual via a face-to-face assessment would not only be time-consuming and costly, it would be near impossible. Moreover, research settings are often constrained by available resources and time, therefore highlighting the need for tools that can be administered quickly and easily. Standardized assessments can be systematically administered by different researchers in different contexts. They can also be administered online, which makes them a quick and easy way to obtain information, particularly if respondents are spread geographically. All the assessments that were incorporated into the PCDH19 survey demonstrated excellent validity and reliability, as well as adequate sensitivity and specificity – some comparable to gold standard clinical assessment. Through the survey, I was able to reach 112 individuals from countries such as the USA, New Zealand, Canada, Italy, France, and Denmark.

The aim of this introduction is to provide an overview of the *PCDH19* literature to support the research questions and aims explored in the subsequent chapters. The introduction is a systematic review and meta-analysis that has been published in the peer-review journal Molecular Psychiatry. I begin by describing GCE and discuss the limited information available with relation to the comorbidities associated with GCE. I highlight the importance of establishing a clinical profile to assist with diagnosis, treatment, and our ability to identify potential associations. This is followed by a systematic review of 38 peer-reviewed original articles and a meta-analysis incorporating studies that included information regarding cognitive function or the degree of impairment, or the presence or type of psychiatric comorbidity. Findings from this work form the basis for the subsequent studies.

2.2 Statement of authorship

Title of Paper	A systematic review and meta-analysis of 271 PCDH19-variant individuals identifies psychiatric comorbidities, and association of seizure onset and disease severity.
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Principal author

Name of Principal Author (Candidate)	Kristy Kolc		
Contribution to the Paper	Major contribution to the research question. Performed the literature review, meta-analysis, and interpretation of data, drafted manuscript, and performed all required revisions.		
Overall percentage (%)	80%		
Certification:	This manuscript reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this manuscript.		
Signature		Date	Feb 14 2020

Co-Author contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Professor Lynette Sadleir		
Contribution to the Paper	Assisted with development of work, data interpretation, and manuscript evaluation.		
Signature		Date	Feb 13 2020

Name of Co-Author	Laureate Professor Ingrid Scheffer		
Contribution to the Paper	Assisted with development of work, data interpretation, and manuscript evaluation.		
Signature		Date	Feb 14 2020

Name of Co-Author	Dr Atma Ivancevic		
Contribution to the Paper	Assisted with data visualisation.		
Signature		Date	Feb 16 2020

Name of Co-Author	Associate Professor Rachel Roberts		
Contribution to the Paper	Supervised development of work, advised on data interpretation, and manuscript editing and evaluation.		
Signature		Date	Feb 15 2020

			

Name of Co-Author	Dr Duyen Pham		
Contribution to the Paper	Supervised development of work and assisted with manuscript editing.		
Signature		Date	

Name of Co-Author	Professor Jozef Gecz		
Contribution to the Paper	Contribution to the research question. Supervised development of work, advised on data collection and interpretation, and manuscript editing and evaluation.		
Signature		Date	Feb 13 2020

2.3 Published manuscript

Title: A systematic review and meta-analysis of 271 PCDH19-variant individuals identifies psychiatric comorbidities, and association of seizure onset and disease severity.

Running Title: CHAPTER 2

List of Authors: Kristy L Kolc[1], Lynette G Sadleir[2], Ingrid E Scheffer[3], Atma Ivancevic[1], Rachel Roberts[1], Duyen Pham[1,4], and Jozef Gecz[1,4,5]

Affiliations: [1]Adelaide Medical School, the University of Adelaide, South Australia, Australia, [2]Department of Paediatrics and Child Health, University of Otago, Wellington, New Zealand, [3]Departments of Medicine and Paediatrics, The University of Melbourne, Austin Health and Royal Children's Hospital and Florey Institute, Victoria, Australia, [4]Robinson Research Institute, the University of Adelaide, South Australia, Australia, [5]Healthy Mothers and Babies, South Australian Health and Medical Research Institute, South Australia, Australia.

Conflict of Interest

The authors declare no conflict of interest.

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Abstract

Epilepsy and Mental Retardation Limited to Females (EFMR) is an infantile onset disorder characterized by clusters of seizures. EFMR is due to variants in the X chromosome gene *PCDH19* and is underpinned by cellular mosaicism due to XCI in females or somatic variant in males. This review characterizes the neuropsychiatric profile of this disorder and examines the association of clinical and molecular factors with neuropsychiatric outcomes. Data were extracted from 38 peer-reviewed original articles including 271 individual cases. We found that seizure onset ≤ 12 months was significantly associated ($p = 4.127 \times 10^{-7}$) with more severe ID compared with onset > 12 months. We identified two recurrent variants p.Asn340Ser and p.Tyr366Leufs*10, occurring in 25 (20 unrelated) and 30 (11 unrelated) cases, respectively. *PCDH19* variants were associated with psychiatric comorbidities in approximately 60% of females, 80% of affected mosaic males, and reported in nine hemizygous males. Hyperactive, autistic, and obsessive-compulsive features were most frequently reported. There were no genotype-phenotype associations in the individuals with recurrent variants or the group overall. Age at seizure onset can be used to provide more informative prognostic counseling.

Introduction

Epilepsy and Mental Retardation Limited to Females (EFMR; OMIM #300088) was first described in 1971 by Juberg and Hellman as an early onset seizure disorder triggered by febrile illness, and with female-limited expression.[1] The causative gene was identified in 2008 by Dibbens et al. in a study that involved six new EFMR families, as well as the original EFMR family reported by Juberg and Helman.[2] In the same year EFMR was further characterized as a neurological disorder with a markedly varied neuropsychiatric profile including ID, and aggressive, autistic, or obsessive features.[3] In 2009 Depienne et al. identified *PCDH19* variants in sporadic cases with an infantile developmental and epileptic encephalopathy resembling Dravet Syndrome (DS).[4] Males have since been identified who are cellular mosaics for the *PCDH19* gene with a similar clinical profile to affected females,[4-6] thus challenging the dogma that this is a disorder limited exclusively to females. The hallmark feature of *PCDH19*-associated epilepsy is that seizures occur in clusters. We therefore proposed "Girls Clustering Epilepsy" (GCE) as a name to facilitate clinical identification of this disorder.[7] Seizures typically present as generalized tonic-clonic and/or focal seizures, which may evolve to bilateral, tonic-clonic seizures. An additional unifying feature of GCE is cellular mosaicism, either due to XCI in females or early somatic variant and, as such, somatic mosaicism in males.

GCE is associated with a reduction or remission of seizures during adolescence.[3, 8, 9] Unfortunately, neuropsychiatric dysfunction remains, often exacerbating with age and becoming the most prominent and disabling feature in some patients.[10-12] ID ranging from mild to profound is present in approximately 70% of cases.[13] The prevalence of psychiatric comorbidities is unknown, however, reports suggest that ASD is a common feature in both females[14-16] and males.[17] Intriguingly, no association has been established between the severity of epilepsy and ID.[9, 10] Here we conduct a comprehensive and systematic review of the GCE literature, specifically focusing on the neuropsychiatric profile and examine if associations exist between age at seizure onset, variant type, variant location, or mode of inheritance and cognitive function or psychiatric comorbidity. Determining factors that contribute to clinical outcome will be useful for prognostic counseling.

Method

Inclusion Criteria

Studies were included if they met the following criteria: 1) reported the cDNA or protein change, 2) were peer-reviewed and written in English, and 3) were original cases only. To be included in the meta-analysis, information regarding cognitive function or the degree of impairment, or the presence of or type of psychiatric comorbidity was also required.

Search Strategy

A computerized search of public databases Embase, PubMed, Google Scholar, and Scopus from January 2008 to August 2017 was conducted. Search terms were as follows: pcdh19, pcdh 19, protocadherin19, and protocadherin 19. Full text articles of abstracts were then selected, retrieved, and assessed for eligibility considering established criteria detailed above. Inclusion was based on final consensus between two authors. Authors were contacted via email if further information or clarification was required. The reference lists of all articles selected for review and the full texts of potentially relevant studies were also examined.

Data Extraction

All relevant data were extracted from selected articles and imported into a Statistical Program for the Social Sciences (SPSS) dataset. Data were cleaned and cross-checked to ensure that no individual was recorded more than once.

Excluding Duplicates

To minimise bias through the reporting of the same individual from multiple publications, we used the following information to identify duplicates: variant (cDNA or protein change) and age at seizure onset. As additional measures, we also used the age at study and inheritance information to further confirm the likelihood that a case was the same across two or more publications. The supplementary file “raw data” lists all reviewed cases with a double asterisk against cases identified as duplicates. Once a potential duplicate was identified, the most recent duplicated information for that individual was included in the review and all references were assigned to that individual. For example, case 68 and 172 in the raw data are flagged as potential duplicates. Both cases are reported as having a *de novo*

c.2097dupA variant and an age at seizure onset of 7 months. In addition, the age of the individual when reported satisfies the expected change based on publication dates (11 months; 2010 and 2 years 2 months; 2012). If there was a slight discrepancy between suspected duplicates, caution was taken, and the suspected duplicate was removed. For example, case 110 and 187 in the raw data file are flagged as potential duplicates. Both cases are reported as having the c.1298T>C variant, however with an age at seizure onset of 9 months and 7 months, respectively. As additional information such as age at study and inheritance satisfies the assumption of a duplicate, the case was only included once in this review.

Data Coding

Initially, certain variables were re-classified to aid analyses. Seizure onset was classified as follows: (0) *"early"* (≤ 12 months) or (1) *"late"* (> 12 months). Variant type was classified as follows: (0) *"truncating"* or (1) *"missense"*. Other variants were too infrequent and, as such, were excluded from the analyses. Variant location was classified as (0) *"early"* (EC1 to EC3) or (1) *"late"* (EC4 to cytoplasmic) and inheritance as (0) *"sporadic"* or (1) *"familial"*. The first dependent variable (cognitive function) was scored on a scale: (0) *"normal"*, (1) *"borderline"*, (2), *"mild ID"*, (3) *"moderate ID"*, or (4) *"severe/profound ID"*, with higher scores indicating increased ID severity. Information regarding the degree of ID was extracted from reports only if explicitly stated and where a report indicated a range, i.e., moderate to severe ID, classification was based on the more severe category. Given that the number of levels of cognitive function exceeded four, this variable was upgraded to continuous.[18] The second dependent variable (psychiatric comorbidity) was scored as follows: (0) *"no psychiatric features reported"*, (1) *"psychiatric features reported"*. Reports that did not cover this aspect of the clinical profile were coded "N/A" and excluded from the analyses.

Missing Data

Full-scale intelligence quotient (FSIQ) scores were rarely reported. Generally, reports involved reference to the classification of normal, borderline, mild ID, moderate ID, severe ID, or profound ID. As such, this classification was adopted for analytical purposes. There were some instances where developmental quotients were provided. Although early developmental quotient testing has been shown to correlate with later IQ,[19] they are not the

same. Therefore, a classification could not be attributed to these cases and, as such, they were excluded from the analysis. If data were missing from any of the other variables in the meta-analysis, the case was excluded to prevent an over or under-estimation of the true nature of any association. In total, 131 cases were represented in the meta-analysis.

Data Analyses

Continuous data were analyzed using SPSS version 24 and followed significant effects ($p \leq .05$) using a linear regression model, factorial ANOVA, and Student's *t* tests. For the binary categorical outcome variable, chi-squared tests of independence were performed. Descriptive statistics, scatterplots, and histograms were generated for all variables used in the analyses to ensure data met criteria for the use of parametric tests. While the normality assumption within the levels of certain independent variables was not met, there were more than 30 cases in each group and, as such, parametric tests could be utilized.[20] Furthermore, non-parametric tests yielded the same results for all parametric tests performed.

PCDH19 Reference Sequences

All PCDH19 cDNA and protein were based on the following reference sequences, which represent the longest isoform of the PCDH19 mRNA and protein: NM_001184880 and NP_001171809 (https://www.ncbi.nlm.nih.gov/).

Results

Thirty-eight studies, with a total of 297 cases met inclusion criteria. After excluding duplicates, there were a total of 271 individuals comprising 12 (4.4%) males and 259 (95.6%) females. Two males were excluded from the meta-analysis as they harbored hemizygous *PCDH19* variants and exhibited a phenotype that was not characteristic of GCE. All ten mosaic males are included in all descriptive and statistical analyses and did not differ significantly from females on the outcome measures tested (cognitive function: $t_{(193)} = -0.33$, $p = .745$; psychiatric comorbidity: $\chi^2_{(1,\, n=230)} = 1.99$, $p = .158$). The mean age at time of study ($n = 235$) was 13.0 years (*SD* = 12.1, *range* = 1 to 79 years). The average age at seizure onset ($n = 219$) was 11.9 months (*SD* = 9.0, *median* = 10 months, *range* = 1 to 70 months, see Supplementary Fig. 2.1), with seizure onset precipitated by fever in 81.1% of cases where this information was available (see Supplementary Table 2.1).

PCDH19 Variant

The *PCDH19* gene is located at Xq22.1, and its coding sequence consists of six exons. The gene encodes a 1,148 amino acid protein with typical features of the δ2-protocadherin sub-family, with 23 amino acid signal peptides, six conserved cadherin repeats in the extracellular cadherin domain (EC), a transmembrane domain, and conserved motifs (CM1-CM2) in the C-terminal region.[2, 21] The first exon encodes the EC and transmembrane domains, as well as a small portion of the C-terminal region. While the rest of the C-terminal region is encoded by exons 2-6, the second, and likely third, exon is subjected to alternative splicing. Exon 5 and 6 encode for CM1 and CM2, respectively. The majority of reported GCE variants were observed in the EC domain of the protein encoded by exon 1 (86.7%; Fig. 2.1). Of the reported variants in this region, almost half were located in the EC3 and EC4 domains (20.3% and 23.2%, respectively; see Supplementary Table 2.2). Missense variants were the most frequently reported type of GCE variant (45.4%), followed by frameshift (27.3%), and nonsense (19.6%; see Supplementary Table 2.3). In total, 145 unique germline *PCDH19* variants were identified in GCE, both in large families as well as singleton cases (see Supplementary Table 2.4 for a complete list).

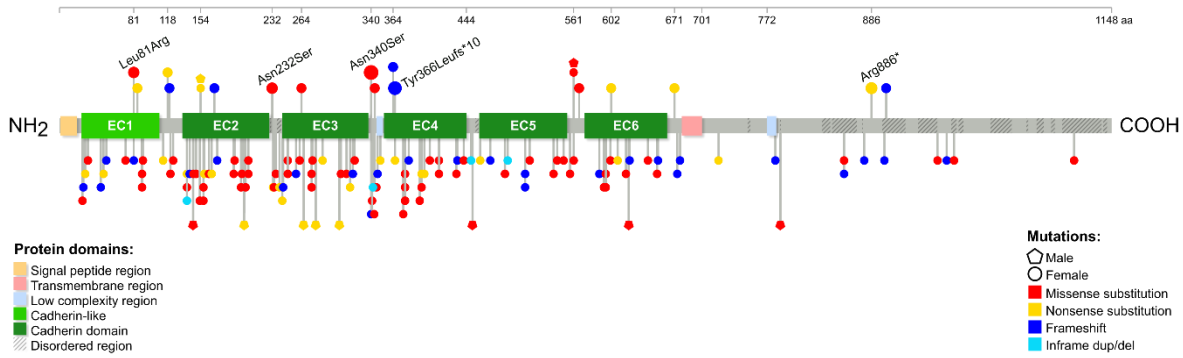

Figure 2.1 Lollipop plot illustrating all reviewed GCE variants (*n* = 271). Lollipop size is exponentially proportional to the number of times the variant has been observed. Recurrent (i.e., seen more than once in unrelated individuals) variants are located above the protein and labelled if they occur more than twice.

Mode of inheritance. GCE was originally recognized as a familial disorder.[1, 3, 22] However, in recent years a significant number of sporadic cases have been identified due to next generation sequencing, with over half of reported GCE cases arising *de novo* (50.2%; Supplementary Table 2.5). Interestingly, there were a considerable number of maternally inherited variants (18.7%). The penetrance of GCE has been estimated as greater than 90%, however, recent reports of asymptomatic carrier mothers[11, 12, 23] would suggest this is somewhat lower. The difficulty in determining penetrance lies in the definition. Some studies define unaffected individuals by a complete absence of symptoms,[10, 11] while others

refer to an individual as being unaffected if they only had a brief history of infantile seizures.[3, 11, 24] Based on the inclusion of all reports where a variant has been maternally inherited, we estimated the penetrance of GCE to be 80%. This may still be conservative given this number is based only on reported cases in which mothers have been tested.

Recurrent variants. Previous reports have identified p.Asn340Ser to be a recurrent variant.[5, 22] The present study has validated and extended this further by identifying 25 (20 unrelated) GCE cases (Table 2.1). Another recurrent variant (p.Tyr366Leufs*10) reported in 30 (11 unrelated) GCE cases was also identified (Table 2.2). Of the 25 patients with a p.Asn340Ser variant, almost half (40.0%, 10/25) had normal or borderline cognitive function, 28.0% (7/25) had mild or moderate ID, with the remaining 32.0% either unclear (6/25) or not reported (2/25; Figure 2.2a). Further, psychiatric comorbidities primarily included autistic or hyperactive features, or both, with over half of cases reported as having no psychiatric comorbidity (55.0%, 11/20). Psychiatric reports were not specified for five cases. Of the 30 patients with a p.Tyr366Leufs*10 variant, just over a quarter (26.7%, 8/30) had normal cognitive function, 23.3% (7/30) had mild or moderate ID, and 26.7% (8/30) had severe or profound ID, with the remaining 23.3% either not reported (5/30) or not specifying the degree of ID (2/30; Figure 2a). Psychiatric comorbidities were predominantly hyperactive (36.7%, 11/30), with one third of cases reported as having no psychiatric comorbidity (33.3%, 10/30). Psychiatric reports were not specified for three cases.

Neuropsychiatric Profile

Cognitive function. The cognitive profile associated with GCE (*n* = 195) was found to be highly heterogeneous, ranging from normal cognitive function (28.2%), to borderline (5.1%), mild (27.2%), moderate (22.1%), or severe to profound (17.4%) ID (see Supplementary Table 2.6). We observed that development prior to the onset of seizures was reported to be delayed in approximately 15% of cases.

Table 2.1 *PCDH19* variant: p.Asn340Ser (c.1019A>G)

#	Age at study	Seizure onset			Subsequent seizure types	Fever sensitivity$^{+/-}$	Seizure offset	Inheritance	Language delay$^{+/-}$	Intellectual disability	Psychiatric comorbidity	Reference
		Age	Type	Fever$^{+/-}$								
1	3.5y	9m	GTCS	+	FS, SE	+	Controlled	*De novo*	+	Mild	None	4
2	6y	8m	FS	NS	GTCS	+	Ongoing	*De novo*	+	Mild	BD	4
3	44y	8m	TCS	NS	-	NS	17y	Unknown	NS	Normal	NS	8a
4	16y	6m	FS	+	TCS, SE	+	NS	Maternal	NS	Mild	No autistic traits*	8, 25a
5	11y	10m	FS	+	-	+	NS	*De novo*	+	Moderate	No autistic traits*	8, 25
6	7.5y	12m	FBTCS	+	-	+	Ongoing	*De novo*	NS	Normal	None	9
7	6y	13m	TCS	NS	NS	NS	Ongoing	#Maternal	NS	Moderate	Autism	11b
8	3y	17m	TCS	NS	NS	NS	NS	#Maternal	+	NS	NS	11b
9	NS	12m	NS	NS	NS	NS	14y	*De novo*	NS	Normal	None	11b
10	5y	13m	FS	NS	FBTCS	+	Ongoing	*De novo*	NS	Normal	None	26c
11	5y	25m	GTCS	-	None	-	25m	*De novo*	NS	Normal	None	26c
12	NS	NS	NS	NS	NS	NS	NS	Unknown	NS	NS	NS	22
13	32y	15m	NS	-	FS, GTCS, SE	-	NS	Unknown	NS	Mild	ASD	27
14	5.5y	10m	FS	NS	-	NS	NS	*De novo*	NS	Borderline	Psychosis, EtD, no autistic traits*	25
15	10y	5m	FS	NS	-	NS	NS	*De novo*	NS	DQ 72 (NS)	Autistic traits*	25
16	8y	12m	FS	NS	-	NS	NS	#Maternal	NS	Normal	No autistic traits*	25
17	8y	11m	CS	+	NS	+	5.5y	#Maternal	-	Normal	None	23d
18	NS	N/A	N/A	N/A	N/A	N/A	N/A	NS	-	Normal	None	23d
19	6y	15m	HCS	+	TCS, FS, TS, SE	+	Ongoing	Unknown	NS	Moderate	Autistic, hyperactive	16
20	8y	5m	FS, TS	+	-	+	Ongoing	*De novo*	NS	DQ 44 (7y4m)	NS	28
21	5.5y	8m	TCS, FS	+	-	+	Ongoing	Maternal	NS	DQ 38 (4y7m)	Autistic, hyperactive	28
22	NS	NS	NS	+	NS	+	NS	*De novo*	NS	NS	NS	5^
23	9y	7m	GTCS	NS	FS	+	Ongoing	*De novo*	NS	Yes	Autism, aggression	24
24	3y	8m	GTCS	NS	FS, MCS	+	Ongoing	Maternal	NS	Yes	Autism, aggression	24
25	7y	18m	GTCS	NS	-	+	Ongoing	*De novo*	NS	Normal	None	24

Abbreviations: *GTCS*, generalized tonic-clonic seizure; *FS*, focal seizure; *SE*, status epilepticus; *BD*, behavioral disturbances; *NS*, not specified; *TCS*, tonic-clonic seizure; *FBTCS*, focal-to-bilateral seizure; *ASD;* autism spectrum disorder; *MCS*, myoclonic seizure; *EtD*, eating disorder; *CS*, clonic seizure; *N/A*, not applicable; *HCS*, hemiclonic seizure; *TS*, tonic seizure
#asymptomatic female; [abcd]familial relationships ([a]mother/daughter; [b]mother/daughters; [c]twins; [d]mother/daughter)
*Information obtained from the author
^Reported as p.Asn370Ser

Table 2.2 *PCDH19* variant: p.Tyr366Leufs*10 (c.1091dupC or c.1091_1092insC)

#	Age at study	Seizure onset			Subsequent seizure types	Fever sensitivity$^{+/-}$	Seizure offset	Inheritance	Language delay$^{+/-}$	Intellectual disability	Psychiatric comorbidity	Reference
		Age	Type	Fever$^{+/-}$								
1	23y	18m	TCS	NS	NS	NS	NS	Paternal	NS	Profound	Hyperactive	1, 2, 29a
2	22y	NS	NS	NS	NS	NS	NS	Paternal	NS	Normal***	None	1, 2, 29a
3	21y	NS	NS	NS	NS	NS	NS	Paternal	NS	Normal***	None	1, 2, 29a
4	20y	NS	NS	NS	NS	NS	NS	Paternal	+	Mild	Hyperactive	1, 2, 29a
5	22y	NS	NS	NS	NS	NS	NS	Paternal	NS	Normal***	None	1, 2, 29a
6	18y	NS	NS	NS	NS	NS	NS	Paternal	NS	Normal***	None	1, 2, 29a
7	8y	NS	NS	NS	NS	NS	NS	Paternal	NS	Normal***	None	1, 2, 29a
8	14y	NS	NS	NS	NS	NS	NS	Paternal	NS	Severe**	Hyperactive	1, 2, 29a
9	12y	NS	FS	NS	AS, GTCS	NS	NS	Paternal	NS	Normal***	None	1, 2, 29a
10	11y	NS	NS	NS	NS	NS	NS	Paternal	NS	Normal	None	1, 2, 29a
11	14y	NS	NS	NS	FS, GTCS	NS	NS	Paternal	NS	Profound	Hyperactive	1, 2, 29a
12	8y	NS	NS	NS	NS	NS	NS	Paternal	NS	Moderate	Hyperactive	1, 2, 29a
13	6y	NS	NS	NS	NS	NS	NS	Paternal	NS	Moderate**	Hyperactive	1, 2, 29a
14	6y	NS	NS	NS	NS	NS	NS	Paternal	NS	Severe	Hyperactive	1, 2, 29a
15	5y	NS	NS	NS	NS	NS	NS	Paternal	NS	Severe	Hyperactive	1, 2, 29a
16	2y	NS	NS	NS	NS	NS	NS	Maternal	NS	Mild	None	1, 2a
17	2y	NS	NS	NS	NS	NS	NS	Maternal	NS	NS	None	1, 2a
18	≥5y	4m	GTCS	-	AS, FS, SE	NS	Ongoing	Maternal	+	Severe	BD, hyperactive, impulsive	2, 29, 30a
19	NS	7m	NS	NS	NS	NS	NS	Maternal	NS	NS	NS	2, 29, 30a
20	NS	14m	NS	+	NS	NS	NS	Paternal	NS	NS	NS	2, 29, 30a
21	14y	7m	GTCS	NS	-	+	11y	Paternal	+	Severe	Hyperactive	26^
22	NS	NS	NS	NS	NS	NS	NS	*De novo*	NS	NS	NS	22
23	7.5y	17m	FS	NS	-	NS	NS	*De novo*	NS	Mild	Autistic traits*	25
24	9y	2m	FS	NS	-	NS	NS	*De novo*	NS	NS	No ASD*	25
25	3y	6m	FBTCS, FS	NS	-	-	Ongoing	*De novo*	+	Normal	AD, hyperactive, No ASD*	25, 31
26	13y	17m	TCS	-	-	+	12y	*De novo*	NS	Moderate	Autistic traits	16
27	8y	5m	FS	+	TS	+	Ongoing	Unknown	NS	Mild	Impulsive	28^
28	1y	6m	FS	NS	GTCS	+	Ongoing	*De novo*	NS	Yes	None	24
29	4y	11m	GTCS, FS	NS	FS	+	Ongoing	*De novo*	NS	Yes	AD	24
30	7y	7m	NS	NS	GTCS, SE	+	NS	Unknown	NS	Severe	Autistic traits*	32

Abbreviations: *FBTCS*, Focal to bilateral seizure; *FS*, focal seizure; *NS*, not specified; *AD*, attention-deficit; *GTCS*, generalized tonic-clonic seizure; *AS*, absence seizure; *DB*, destructive behavior; *SE*, status epilepticus; *TS*, tonic seizure; *TCS*, tonic-clonic seizure

[a]Original EFMR family

*Information obtained from author; **Reliable observer reports; ***Based on level of education attained; ^Reported as c.1300_1301insC

Psychiatric comorbidities. Of the 213 cases where psychiatric information was provided, autistic features were most prominent (19.7%) followed by hyperactive and/or attention-deficit (11.7%), and behavioral disturbances (6.1%). Many reports described individuals with multiple psychiatric comorbidities (21.6%) that predominantly included combinations of autistic, aggressive, hyperactive, and/or obsessive features (see Supplementary raw data https://www.nature.com/articles/s41380-018-0066-9#Sec23 for a complete list).

Genotype-Phenotype Association

To determine if age at seizure onset, variant type, variant location, and mode of inheritance were associated with cognitive function, a linear regression was performed. Age at time of study was also included in the model as a covariate to control for any confounding effects that age may have on the severity of reported ID.[33] Of all the variables tested in the model, only age at seizure onset was significantly associated with cognitive function (p = 4.127 x 10^{-7}; Figure 2.2b). Specifically, individuals with an early seizure onset had an average ID severity that was 1.3 units greater than individuals with a late seizure onset, holding other predictors in the model constant (estimate = 1.30, 95% CI: 0.80, 1.80; Table 2.3). A factorial ANOVA was then performed to ascertain whether variant type or variant location were associated with seizure onset. No significant associations were found (Table 2.4).

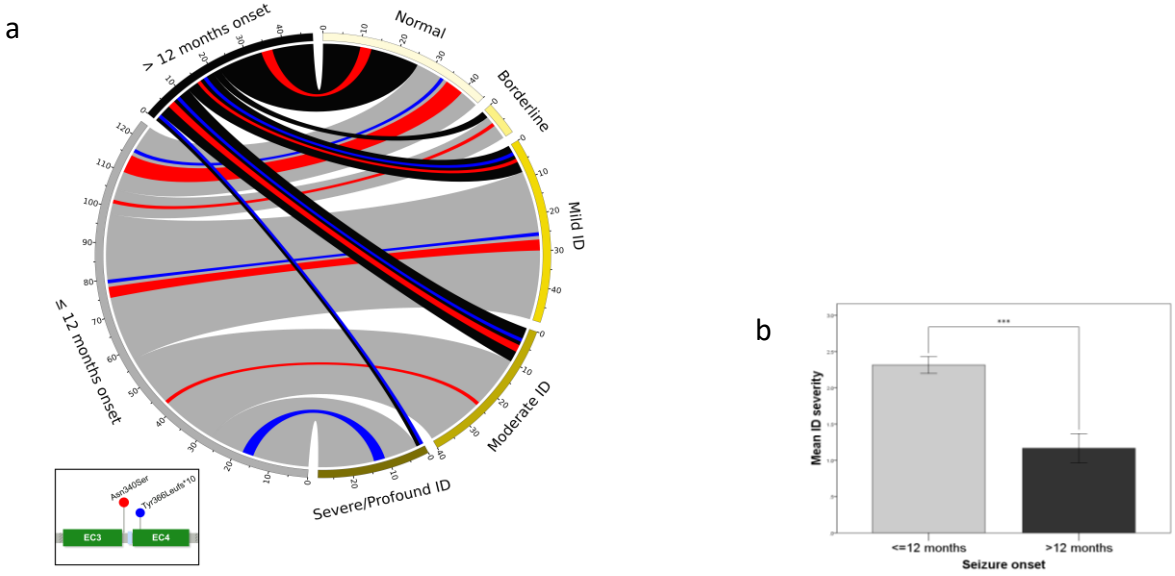

Figure 2.2 Genotype-phenotype association. (a) Circos plot illustrating the variable cognitive profile of GCE (n = 155) against age at seizure onset: ≤12 months (n = 124) and >12 months (n = 48). Recurrent variants p.Asn340Ser (n = 17) and p.Tyr366Leufs*10 (n = 23) are highlighted in red and blue, respectively. Axes show the number of individuals in each category. Illustration is representative of cases where relevant information was available. (b) Bar graph (±SEM) illustrating the association of age at seizure onset and ID severity (values from unadjusted linear model) ***p = 3.090 x 10^{-7}.

Table 2.3 Estimated marginal means (controlling for covariates)

Age at seizure onset	*Mean ID severity*	*Standard Error*
≤12 months ("early")	2.2	0.1
>12 months ("late")	0.9	0.2

NB: Scale: (0) *"normal"*, (1) "*borderline"*, (2), *"mild ID"*, (3) *"moderate ID"*, and (4) *"severe/profound ID"*

Non-Significant Associations

To determine if any predictor variable was associated with the presence of a psychiatric comorbidity a series of Pearson's chi-squared tests of independence were performed. There was a trend towards earlier seizure onset being associated with the presence of a psychiatric comorbidity, $\chi^2_{(1,\, n=205)} = 3.01$, $p = .083$, however this was not statistically significant. All other tested associations were non-significant (Table 2.4).

Table 2.4 Non-significant associations

	Psychiatric comorbidity	*Age at seizure onset*
Variant Type		
Truncating versus missense	$\chi^2_{(1,\, n=210)} = 0.46$, $p = .497$	$F_{(195)} = 0.41$, $p = .523$
Variant Location		
Early versus late	$\chi^2_{(1,\, n=216)} = 0.08$, $p = .778$	$F_{(195)} = 0.49$, $p = .487$
Inheritance		
Sporadic versus familial	$\chi^2_{(1,\, n=193)} = 0.75$, $p = .385$	

PCDH19 Mosaic Males

There have been ten reported cases of *PCDH19* variants causing a GCE-like phenotype in males. *PCDH19* variants were initially thought to only affect females, however, in 2009 Depienne and colleagues[4] described a *SCN1A*-negative male diagnosed with DS as having a *de novo* deletion on chromosome Xq22.1 that spanned the entire *PCDH19* gene. Using fluorescence *in situ* hybridization, the mosaic status of this *PCDH19*-variant male was confirmed, with a "normal" *PCDH19* allele detected in 53% of skin fibroblasts. A second case was described by Thiffault et al., (2016).[6] Sanger sequencing revealed an exon 1 protein truncating variant in a mosaic status that was associated with focal myoclonic as well as TCS, at the age of 9 months. Two additional mosaic males were recently reported by Terracciano et al. (2016).[5] The first, a 4-year-old boy, presented with an afebrile hypotonic seizure at the age of 9 months. The second, a 3.5-year-old boy, presented with a 24 h cluster of febrile seizures at the age of 10 months. Multigene panel revealed an exon 1 nonsense and missense substitution in each case, respectively. Recently, a male mosaic for a *PCDH19*

missense variant believed to affect the canonical splice donor site in the first intron (c.2147+2 T>C) was reported[34], as well as an additional five males.[17] All six males exhibited a clinical profile corresponding to the female phenotype. Nine of the ten reported *PCDH19* mosaic males have been described as having comorbid psychiatric features.[4-6, 17, 34] For example, the case described by Thiffault and colleagues[6] involved a young boy with behavioral disturbances (i.e., aggression and rigidity) that became evident by the age of 3 years. At the time of the study he had been diagnosed with ADHD, anxiety, OCD, and oppositional defiant disorder. All cases of affected males with a normal complement of sex chromosomes have arisen *de novo*, suggesting that a somatic *PCDH19* variant during early development resulted in a mixed population of *PCDH19* variant and wild-type cells, and therefore cellular expression resembling that of an affected female.

PCDH19 Transmitting Males

It is generally considered that hemizygous or "transmitting" males are unaffected or asymptomatic.[1, 2, 29] While epilepsy has not been reported in these males, there is some evidence to suggest that there is a mild phenotype associated with this transmitting status in males. The first indication of such arose from the observations of Scheffer et al. in 2008.[3] In this study, five males were all described as inflexible, having rigid, controlling personalities, and obsessive interests and traits (e.g., obsessively repeating details in conversation). Such characteristics are particularly common in ASD (e.g., inflexibility) and OCD (e.g., repetitive behaviors). In addition, some transmitting fathers of affected daughters have varying degrees of ID.[4, 16] Lastly, *PCDH19* variants have been reported in a male with autism[35], a male with Asperger's syndrome[16], and two males with ID.[36] These reports suggest that a psychiatric profile may be evident in some transmitting males and that *PCDH19* is involved in other neurodevelopmental disorders.

Discussion

This review is the first to systematically characterize the reported neuropsychiatric profile of GCE and examine any associations between clinical and molecular factors and neuropsychiatric outcomes. We have demonstrated that an earlier seizure onset is significantly associated with more severe ID. We have also shown that there is no association between the type and location of a *PCDH19* variant and seizure onset and confirmed that onset is often precipitated by fever. Although the association of early seizure

onset with more severe ID may simply reflect the underlying severity of the disorder in that individual, it is also possible that the early seizure activity may be contributing to adverse cognitive and behavioral outcomes. There are "critical periods" of development during which the brain undergoes changes that are crucial to the formation of certain behaviors and various cognitive processes.[37, 38] Functional changes in frontal cortical brain regions, in particular, coincide with cognitive and behavioral alterations known to occur during early development.[37, 39] We have observed that a majority of first seizures in GCE occur at a median age of 10 months. It is at this time that the frontal cortex shows an increase in glucose metabolism,[40] with total brain volume increasing by 101% in the first year of life.[41] There is also a rapid elaboration of new synapses in the first 2 years of life that corresponds to an increase in cortical grey matter.[42, 43] The frontal cortex is involved in a diverse range of functions that can be broadly referred to as "cognition".[44] Injury to this region has been associated with deficits in executive functions (i.e., attention), as well as psychiatric conditions including schizophrenia, depression, and OCD.[44] It is therefore reasonable to speculate that seizure activity within the first 12 months of life may be more likely to disrupt neural development and lead to cognitive dysfunction.

Given the clinical similarities involving age at seizure onset and fever sensitivity shared by GCE and DS, we investigated whether a similar association between ID severity and age at seizure onset has been demonstrated in the Dravet literature. Brunklaus et al., (2012) demonstrated an association between early focal seizures with impaired awareness ≤24 months (yes/no) and worse developmental outcome.[33] Patients with DS with the highest seizure burden are reported to also suffer from more comorbidities.[45] Recently it has been shown that, in children with *SCN1A* variants, early seizure onset related to DS rather than GEFS+.[46] Conversely, we recently defined a new profound *SCN1A* developmental and epileptic encephalopathy far more severe than DS, that is associated with an even earlier (6-12 weeks) seizure onset.[47]

Considering this question from a different perspective, McIntosh et al., 2010 investigated whether seizure onset in DS triggered by vaccination (called vaccination proximate) had a more severe clinical outcome than patients whose seizure onset was not related to vaccination (vaccination distal). The two groups differed significantly in the average age at seizure onset, with onset being earlier by approximately 8 weeks in the vaccination proximate group.[48] As there are anecdotal reports of vaccination triggering seizures in GCE,

it would be interesting to ascertain whether GCE demonstrates a similar association between vaccination and age at seizure onset.

We also identified two recurrent variants, p.Asn340Ser and p.Tyr366Leufs*10. Both recurrent variants are found at a similar location within the *PCDH19* gene, suggesting that this region may be vulnerable to or selected for genetic variant. Recurrent variants provide some scope for determining a genotype-phenotype association. We have been able to utilize the recurrence of these two variants to demonstrate for the first time that there is no association between these specific *PCDH19* variants and the type and/or severity of symptoms, at least at a qualitative level. However, it is feasible to postulate that a milder phenotype may be associated with the p.Asn340Ser variant. Additional cases would help draw a more definitive conclusion. Heterogeneity is typically observed among related individuals, suggesting that other mechanisms, such as hormones[49, 50], XCI[51] or other genetic or environmental factors may be the underlying explanation for the variable clinical expressivity associated with GCE. One interesting finding that emerged was the absence of any paternally inherited p.Asn340Ser variants. Complete pedigree information regarding these cases will help determine if this is, in fact, a true observation. Additionally, the annotation of p.Tyr366Leufs*10 varied in the literature. As such, there may be additional variants that have been reported across multiple individuals and families that have not been correctly identified. Such additional recurrent cases will allow for more detailed quantitative analyses.

This review has revealed that the neuropsychiatric profile of GCE varies considerably across individuals and within families. Current reports concerning psychiatric comorbidities in GCE are incomplete. This review provides some insight into the type of psychiatric comorbidities that likely exist in association with *PCDH19* variants. In line with previous reports[11, 14, 52] we observed that autistic features were most prominent. A novel finding to emerge was that hyperactivity was frequently observed. This finding is reflected in a recent animal model study showing that heterozygous female *Pcdh19* knockout mice show hyperactivity in social interactions, under stress and with advancing age.[53] Overall, features associated with ADHD, ASD, and OCD were observed in GCE at rates much higher than those observed in the general population.[54] Although these rates are comparable to those reported among individuals with ID[55], 25% of reviewed cases had normal cognitive function in association with psychiatric comorbidities. These results should be considered formative

due to limited data specifically targeting the presence and/or severity of psychiatric symptomatology. As over 60% of reviewed cases are associated with some form of psychiatric comorbidity, a comprehensive and standardized assessment of the psychiatric profile associated with *PCDH19* variants is warranted.[31]

There were no reported psychiatric comorbidities in over 75% of individuals with normal cognitive function. Determining what factors are unique to this group might shed some light on what causes the clinical variability observed in GCE. Previous reports suggest that ID becomes apparent sometime after seizure onset[8, 9, 14], suggesting that seizure and epileptic activity may contribute to cognitive deficits. However, we observed that development prior to the onset of seizures was delayed for 15% of individuals indicating that *PCDH19* variants produce a developmental encephalopathy as well as an epileptic encephalopathy in some cases. Given that prior development was not reported, unclear, or unknown in 130 cases, and that obtaining such information retrospectively or prior to the onset of seizures is often challenging; previous reports are likely underestimating the proportion of individuals showing signs of delay prior to seizure onset. It was also noted that dysfunction specific to executive functions was reported, such as problems with planning and organization[56], abstract reasoning[52], or lack of inhibitory control.[31] Therefore, executive functions may be compromised in GCE. Moreover, definitions of ID now include deficits in adaptive behavior, with the severity of ID based on adaptive behavior impairment rather than exclusively on IQ score.[57]

Conclusion

Given the limited information in the literature concerning comorbid symptomatology, there is a need to formally characterize the neuropsychiatric profile of GCE. Neuropsychiatric disorders can be very responsive to early intervention[58-60]; therefore, a better understanding of these comorbidities may help to inform treatment and ultimately lead to better developmental outcomes for individuals affected by GCE. In addition, transmitting males may exhibit mild neuropsychiatric features. An assessment of these males may identify a clinical profile unique to this group, which may lead to carrier testing and has implications for genetic counseling. We have shown that seizure onset within the first 12 months is significantly associated with more severe ID. Therefore, knowledge of an individual's seizure onset will aid prognostic counseling, providing valuable information for clinicians managing affected individuals and their families.

Chapter 3: **A standardized patient-centered characterization of the phenotypic spectrum of PCDH19 girls clustering epilepsy**

3.1 Preamble

The aim of the second study is to use the information gleaned from the first study to systematically characterize the neuropsychiatric profile associated with *PCDH19* variants using standardized assessments. I also validate the findings from the meta-analysis using more sophisticated methods. This was possible due to the nature of the data collection and an *a priori* understanding of the variables to be tested. Additional predictive variables were also evaluated. I begin by illustrating the importance of characterizing the neuropsychiatric profile associated with *PCDH19* variants. Knowledge of comorbidities will assist families and individuals affected by GCE by expanding diagnostic criteria and tailoring treatment. Previous work in this area is limited and focused only on females. Here, I describe the neuropsychiatric profile for both females and males. Findings from this work form the basis of the detailed analysis of treatment efficacy and *PCDH19*-variant males in chapters 4 and 5, respectively.

3.2 Statement of authorship

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Principal author

Name of Principal Author (Candidate)	Kristy Kolc		
Contribution to the Paper	Major contribution to the research question. Designed study, performed data collection, analysis, and interpretation, prepared manuscript, and performed all required revisions.		
Overall percentage (%)	80%		
Certification:	This manuscript reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this manuscript.		
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Co-Author contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Professor Lynette Sadleir		
Contribution to the Paper	Assisted with development of work, data interpretation, and manuscript evaluation.		
Signature		Date	Feb 13 2020

Name of Co-Author	Dr Christel Depienne		
Contribution to the Paper	Assisted with development of work, data collection, and manuscript editing.		
Signature		Date	Feb 13 2020

Name of Co-Author	Dr Carla Marini		
Contribution to the Paper	Assisted with development of work, data collection, and manuscript editing.		
Signature		Date	Feb 13 2020

Name of Co-Author	Laureate Professor Ingrid Scheffer		
Contribution to the Paper	Assisted with development of work, data interpretation, and manuscript evaluation.		
Signature		Date	Feb 14 2020

Name of Co-Author	Dr Rikke Møller		
Contribution to the Paper	Assisted with data collection and manuscript editing.		
Signature		Date	Feb 13 2020

Name of Co-Author	Dr Marina Trivisano		
Contribution to the Paper	Assisted with data collection and manuscript editing.		
Signature		Date	Feb 13 2020

Name of Co-Author	Dr Nicola Specchio		
Contribution to the Paper	Assisted with data collection.		
Signature		Date	Feb 13 2020

Name of Co-Author	Dr Duyen Pham		
Contribution to the Paper	Assisted with data interpretation.		
Signature		Date	

Name of Co-Author	Dr Raman Kumar		
Contribution to the Paper	Supervised development of work and assisted with manuscript evaluation.		
Signature		Date	Feb 13 2020

Name of Co-Author	Associate Professor Rachel Roberts		
Contribution to the Paper	Supervised development of work, assisted with design, data interpretation, and manuscript evaluation.		
Signature		Date	Feb 15 2020

Name of Co-Author	Professor Jozef Gecz		
Contribution to the Paper	Supervised development of work, assisted with design, data interpretation, and manuscript evaluation.		
Signature		Date	Feb 13 2020

3.3 Accepted manuscript

Title: A standardized patient-centered characterization of the phenotypic spectrum of PCDH19 girls clustering epilepsy.

Running Title: CHAPTER 3

List of Authors: Kristy L Kolc[1], Lynette G Sadleir[2], Christel Depienne[3,4], Carla Marini[5], Ingrid E Scheffer[6,7,8], Rikke S Møller[9,10], Marina Trivisano[11], Nicola Specchio[11], Duyen Pham[1,12], Raman Kumar[1,12], Rachel Roberts[13], and Jozef Gecz[1,12,14]

Affiliations: [1]Adelaide Medical School, the University of Adelaide, SA, Australia, [2]Department of Paediatrics and Child Health, University of Otago, Wellington, New Zealand, [3]Institute of Human Genetics, University Hospital Essen, University Duisburg-Essen, Essen, Germany, [4]Inserm U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris, 06 UMR S 1127, Institut du Cerveau et de la Moelle épinière, ICM, F-75013, Paris, France, [5]Child Neurology and Psychiatric unit, Pediatric Hospital G. Salesi, Ospedali Riuniti Ancona, Ancona, Italy, [6]Department of Medicine, Epilepsy Research Centre, The University of Melbourne, Austin Health, Melbourne, VIC, Australia, [7]Department of Paediatrics, Royal Children's Hospital, The University of Melbourne, VIC, Australia, [8]The Florey and Murdoch Institutes, Melbourne, VIC, Australia, [9]Department of Epilepsy Genetics and Personalized Medicine, The Danish Epilepsy Centre, Dianalund, Denmark, [10]Department for Regional Health Research, University of Southern Denmark, Odense, Denmark, [11]Rare and Complex Epilepsy Unit, Department of Neuroscience, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, [12]Robinson Research Institute, the University of Adelaide, SA, Australia, [13]School of Psychology, The University of Adelaide, SA, Australia, [14]Women and Kids, South Australian Health and Medical Research Institute, SA, Australia.

Conflict of Interest

I.E.S serves on the editorial boards of Neurology® and Epileptic Disorders; may accrue future revenue on a pending patent re: Therapeutic compound; has received speaker honoraria from Athena Diagnostics, UCB, GSK, Eisai, and Transgenomics; has received scientific advisory board honoraria from Nutricia, UCB, and GSK; and receives/has received research support from the NHMRC, ARC, NIH, Health Research Council of New Zealand, March of Dimes, CURE, US Department of Defense, and the Perpetual Charitable Trustees. The remaining authors declare no conflict of interest.

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Abstract

PCDH19 pathogenic variants cause an early-onset seizure disorder called GCE. GCE is an X chromosome disorder that affects heterozygous females and mosaic males, however hemizygous "transmitting" males are spared. We aimed to define the neuropsychiatric profile associated with *PCDH19* pathogenic variants and determine if a clinical profile exists for transmitting males. We also examined genotype- and phenotype-phenotype associations. We developed an online PCDH19 survey comprising the following standardized assessments: The BRIEF; the SRS-2; the SDQ; and the DOCS. Genetic, seizure, and developmental information were also collected. The survey was completed by patients or by caregivers on behalf of patients. Of the 112 individuals represented (15 males), there were 70 unique variants. Thirty-five variants were novel and included a newly identified recurrent variant Ile781Asnfs*3. There were no significant differences in phenotypic outcomes between published and unpublished cases. Seizures occurred in clusters in 94% of individuals, with seizures resolving in 28% at an average age of 17.5 years. Developmental delay prior to seizure onset occurred in 18% of our cohort. Executive dysfunction and ASD occurred in approximately 60% of individuals. The ASD profile included features of ADHD. In addition, 21% of individuals met criteria for OCD that appeared to be distinct from ASD. There were no phenotypic differences between heterozygous females and mosaic males. We describe a mosaic male and two hemizygous males with atypical clinical profiles. Earlier seizure onset age and increased number of seizures within a cluster were associated with more severe ASD symptoms ($p = .001$), with seizure onset also predictive of executive dysfunction ($p = 4.69 \times 10^{-4}$) and prosocial behavior ($p = .040$). No clinical profile was observed for transmitting males. This is the first patient-derived standardized assessment of the neuropsychiatric profile of GCE. These phenotypic insights will inform diagnosis, management, and prognostic and genetic counseling.

Introduction

PCDH19 pathogenic variants cause an infantile onset seizure disorder called GCE. GCE is an X chromosome disorder with a unique expression pattern where heterozygous females and males with postzygotic somatic variants ("mosaic males") are affected, but hemizygous ("transmitting") males are unaffected. The hallmark of GCE is clusters of focal seizures, often coinciding with fever.[25] While the seizure semiology associated with GCE has been characterized,[3, 25, 61, 62] the neuropsychiatric profile has not been well established.

PCDH19 pathogenic variants are frequently associated with ID and psychiatric disturbances.[14, 31, 61, 63] These neuropsychiatric comorbidities are highly heterogeneous, with ID ranging from mild to profound, and combinations of autistic, attention-deficit/hyperactive, obsessive, or aggressive features. The natural history of GCE shows that seizures become less frequent with age and cognition plateaus over time.[9, 31] Psychiatric symptoms often increase with age and become the most disabling feature in some patients.[9, 61] The specific cognitive deficits, and the severity and prevalence of comorbidities remain unknown.

In our systematic review and meta-analysis, we identified that executive dysfunction and hyperactive, autistic, and obsessive-compulsive features were most frequently reported in individuals with *PCDH19* pathogenic variants. We also showed that individuals with seizure onset before 12 months of age had more severe ID than those with seizure onset after 12 months.[64] A recent retrospective study validated this finding and also showed an association between earlier seizure onset and the presence of ASD.[62] Reviewed studies were typically based on small samples, lacked systematized approaches, and focused on only one clinical outcome.

Here we aim to define the neuropsychiatric profile associated with *PCDH19* pathogenic variants using standardized assessments that specifically target executive functions and symptoms associated with ASD, ADHD, and OCD, and interrogate which factors predict the severity of these neuropsychiatric comorbidities.

Method

Study design and participants

The PCDH19 survey was developed in English, Italian, and French using Survey Monkey (www.surveymonkey.com/) and was available from April 2017 through March 2019. Invited participants (n = 186) were parents or caregivers responding on behalf of individuals aged 2 years and over with a *PCHD19* variant or individuals over 10 years of age with a *PCDH19* variant who were able to self-report. Exclusion was based on our determination that the *PCDH19* variant was likely benign based on frequency in the general population and *in silico* assessment.

Outcomes

We collected demographic and clinical information using the EQ, which we developed based on literature review and discussion with health professionals (Appendix A6). We assessed ASD using the SRS-2 (Appendix A1).[65] As a French translation of the SRS-2 was not available, we also utilized the SCQ (Appendix A2).[66] Behavioral difficulties were assessed via the SDQ (Appendix A3).[67] We used the four-band categorization cut-off scores to assess symptoms of depression and anxiety (emotional problems scale), aggression (conduct problems and prosocial scales), ADHD (hyperactivity-inattention scale), and social deficits (peer problems and prosocial scales).[68] SDQ categories were re-classified from "close to average" to "average", "slightly raised/lowered" to "mild", "high/low" to "moderate", and "very high/very low" to "severe" to align our analysis with other assessed constructs. Executive dysfunction was assessed using the BRIEF (Appendix A4).[69] BRIEF inconsistency, negativity, and infrequency scales were included to assist in detecting bias associated with rating scales. We assessed OCD using the DOCS (Appendix A5).[70] The internal consistency of all assessments was acceptable: SRS-2 (Cronbach's alpha = .97), SCQ (α = .86), SDQ scales (M = 0.73; Supplementary Table 3.1), BRIEF forms (M = 0.98), and DOCS (α = .96).

Translation of the EQ, survey scripts, and study material were performed and checked by either a professional translator or by an individual familiar with GCE and fluent in the relevant languages. Published authorized translations of the SRS-2, SCQ, SDQ, BRIEF, and DOCS were utilized. License agreements were obtained to reproduce assessments in

an online format. The project was approved by the University of Adelaide Human Research Ethics Committee (H-2016-184). Electronic informed consent was obtained from all participants.

Statistical analysis

All genetic variants were mapped to the longest isoform of the PCDH19 mRNA (NM_001184880.1) and protein (NP_001171809.1) reference sequences (https://www.ncbi.nlm.nih.gov/). Variant annotation was based on nomenclature for the description of sequence variants (http://www.hgvs.org/mutnomen/). We identified whether the *PCDH19* variant had been previously reported, then assessed the pathogenicity of all novel variants based on gnomAD (http://gnomad.broadinstitute.org/) frequency and *in silico* prediction tools through the web-based ANNOVAR (http://wannovar.wglab.org/). Data were analyzed using SPSS version 25. ID severity was coded as previously described.[64] To test associations, we used a linear regression model and set statistical significance at $p = .05$. We used a deductive category analytical approach to evaluate qualitative data.[71]

Results

Of the 122 completed surveys, 7 participants were excluded prior to, and 3 following, *in silico PCDH19* variant assessment (Supplementary Table 3.2). Seven individuals reported secondary variants in other genes, which were not predicted to be likely pathogenic (Supplementary Table 3.3), so these participants remained in the analysis. Following exclusions, 112 individuals remained; including 97 heterozygous females (90 affected, 7 unaffected) and 15 males (6 hemizygous, 9 mosaic). The mean age at time of study (n = 112) was 17.6 years (SD = 15.6, *range* = 1.5 to 70 years).

The characteristic phenotype for *PCDH19* heterozygous females and mosaic males is epilepsy. Individuals without epilepsy were classified as “non-penetrant”. Of the 106 heterozygous females and mosaic males, there were eight non-penetrant individuals (seven females). Therefore, the penetrance of GCE in our cohort was 92%. The non-penetrant mosaic male (#63) was ascertained as they were the father of an affected female (#28). Of the seven non-penetrant females (#49, #56, #58, #62, #93, #97, #99), two (#58, #93) had a single febrile seizure.[72] Interestingly, our cohort included two symptomatic

hemizygous males, one with epilepsy (#39) and one with ASD and no epilepsy (#47). The remaining four hemizygous males were classified as "transmitting", as they were the asymptomatic fathers of affected females.

PCDH19 DNA Variants

There were 70 unique *PCDH19* variants, of which 35 were novel. Variants included 25 frameshift, 14 nonsense, 60 missense, 3 in-frame duplications (Fig. 3.1), 6 whole gene deletions, and 4 splicing. Almost half (54/112) arose *de novo*, with 18 paternal, 21 maternal, or 19 of unknown origin. There were four recurrent variants: p.Arg886* (2), p.Ile781Asnfs*3 (2), p.Asn340Ser (3), and p.Tyr366Leufs*10 (6). The p.Ile781Asnfs*3 variant is newly identified as recurrent (Supplementary Table 3.4).

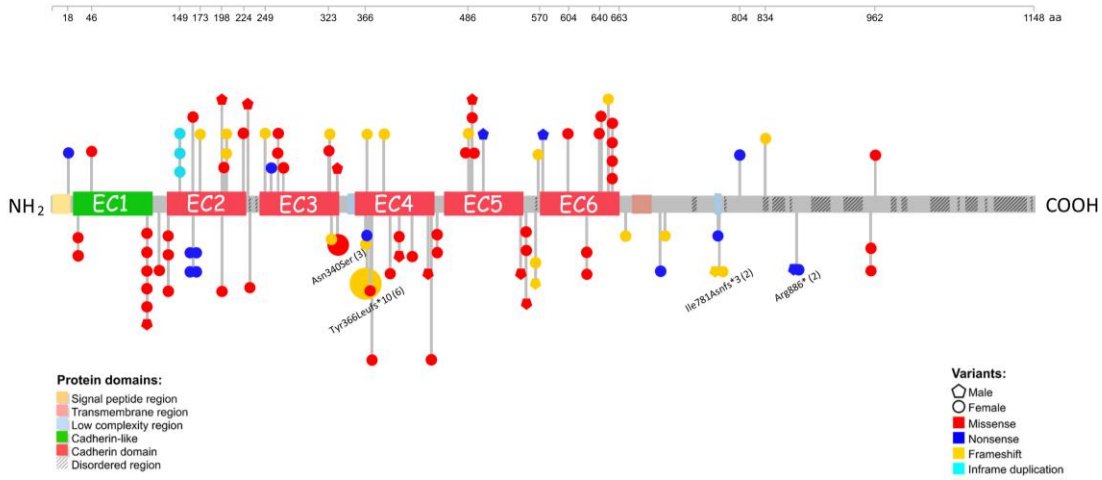

Figure 3.1 Lollipop plot illustrating all *PCDH19* variants in our cohort (*n* = 102) excluding whole gene deletions and splicing variants. Lollipop size is exponentially proportional to the number of times the variant has been observed in unrelated individuals (recurrent). At a given locus, the number of lollipops represents the number of related individuals with that variant, with the exception of Asn340Ser (2 unrelated families and one sporadic case) where this is illustrated in text. Unpublished (novel) variants (*n* = 34) are located above the protein and published variants (*n* = 32) are below the protein.

Development

Early development was ascertained only for surveys completed by a parent or caregiver (*n* = 83; Supplementary Table 3.5a). Developmental delay occurred in 50/83 (60%). The average age of first developmental concern was 12.8 months (*SD* = 11.1, *range* = 1 to 48 months). All developmental domains were affected, however, communication (24/50) and motor skills (22/50) were most affected. Delay prior to the onset of seizures occurred in 15/49 individuals (31%). For one individual (#47), developmental delay was noted at 12 months in the absence of seizures. Regression occurred in 37/83 (46%), and regression

following a seizure cluster occurred in 30/37 (81%).

The intellect of the entire cohort was based on pre-existing diagnosis. Information regarding the degree of ID was not available for one individual (#22), therefore they were excluded from this analysis. Normal intellect was reported in 62/111 (56%), borderline in 4/111 (3.5%), mild ID in 20/111 (18%), moderate ID in 9/111 (8%), severe ID in 14/111 (12.5%), and profound ID in 2/111 (2%) of our patient cohort (Supplementary Table 3.5b).

Seizures

Age at seizure onset for heterozygous females (*n* = 90) ranged from 1.5 to 60 months (*M* = 12.2, *SD* = 9.27, *median* = 10 months) and for mosaic males (*n* = 8) from 5 to 96 months (*M* = 20.6, *SD* = 30.9, *median* = 9 months). The most common age at onset was 8 months (14%, *n* = 98). Cluster duration (*n* = 93) ranged from 1 to 24 days (*M* = 4.61, *SD* = 4.29) and the average number of seizures within a cluster ranged from 2 to 100 (*M* = 15.53, *SD* = 13.99), with most clusters lasting for 2 days and averaging 10 seizures per cluster (Fig. 3.2; see also Supplementary Table 3.6).

Seizure offset for individuals aged 11 years and over (based on the youngest reported seizure offset age) had occurred in 28% of our cohort. Age at seizure offset ranged from 11 to 38 years (*M* = 17.6, *SD* = 7.43). Epilepsy is classified as resolved when an individual has been seizure free for 10 years, with the last five years free from antiepileptic medication.[72] Sixteen individuals (16%), aged 22 to 52 years (*M* = 32.8, *SD* = 8.55) were seizure-free for at least 10 years. Eleven of the 16 were taking antiepileptic medication. For the five individuals who were seizure-free without medication, age at seizure offset ranged from 11 to 38 years (*M* = 20.7, *SD* = 10.8).

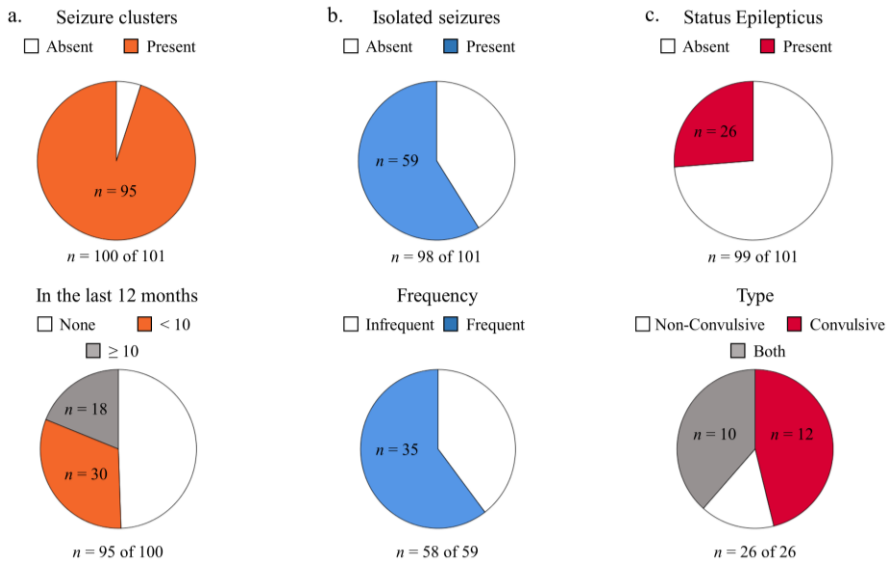

Figure 3.2 Seizure characteristics: a) proportion of individuals with seizure clusters and number of clusters in the last 12 months; b) proportion of individuals with isolated seizures and the frequency of isolated seizures (infrequent refers to less than yearly and frequent to occurrence ranging from daily to yearly); and c) proportion of individuals with episodes of status epilepticus and type of status epilepticus.

Neuropsychiatric comorbidities

Autism Spectrum Disorder. All participants completed either the SRS-2 (n = 104) or the SCQ (n = 8). Total average SRS-2 scores fell in the normal range for transmitting males and non-penetrant females and in the clinical range for all other groups (Fig. 3.3). Total SRS-2 scores were in the clinical range in 68% of females (56/82) including one non-penetrant female (#58), 75% of mosaic males (6/8) including the non-penetrant male (#63), and both hemizygous males (#39, #47). There was no sex difference in severity of ASD (p = .781; Supplementary Fig. 3.1). The SCQ average total score ranged from 8 to 32 (M = 18.5, SD = 8.1), and fell in the clinical range for five individuals (63%).

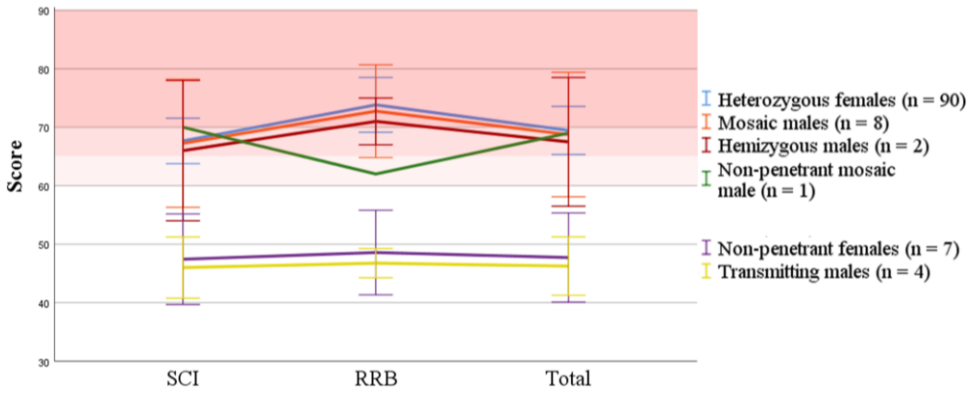

Figure 3.3 Average (±2 SEM) SRS-2 total and DSM-5 domain t scores by group. Darkening shades of red correspond to increasing degrees of severity. *SCI*, social communication and inhibition; *RRB*, restricted interests and repetitive behavior.

Behavioral assessment. The SDQ was completed by parents/caregivers of individuals aged between 2 and 17 years (n = 73). Elevated scores were observed across all SDQ domains, with the hyperactivity-inattention, peer problems, and prosocial behavior domains most severely affected (Supplementary Table 3.7). Impact scores were in the very high range for approximately 75% of participants (Supplementary Table 3.7). This was further supported by 65% of respondents endorsing behavior as the most challenging aspect each day.

Executive dysfunction. One participant (#87) was excluded due to missing data (n = 111). For individuals aged between 2-4 years (n = 17), BRIEF total global executive composite t scores (GECt) fell within the clinical range for heterozygous females (M = 68.1) and mosaic males (M = 62.5). Inhibit (M = 68.5) and working memory (M = 70.9) domains were the most affected for females, with working memory also elevated for males (M = 72.5). For individuals aged between 5-17 years (n = 57), GECt scores fell within the clinical range for heterozygous females (M = 73.2) and mosaic males (M = 67.0), and within the normal range for one hemizygous male (#39). Elevation in all domains were observed for females, with the exception of organization of materials. Shift, working memory and plan/organize were elevated for males. For individuals aged 18 and over (n = 37), GECt scores fell in the normal range for all but one hemizygous male (#47).

Average scores in the shift domain were elevated for most groups, including one hemizygous male (#47) and the non-penetrant mosaic male (#63; Supplementary Fig. 3.2). Overall, executive dysfunction was observed in 72% of heterozygous females (64/89), 50% of mosaic males (4/8), one non-penetrant female (1/7) and one hemizygous male (1/2). Again, no difference was observed between males and females (p = .482; Supplementary Fig. 3.1). Inconsistency, negativity, and infrequency scores were in the acceptable range. Figure 3.4 summarizes the neuropsychiatric profile of our cohort.

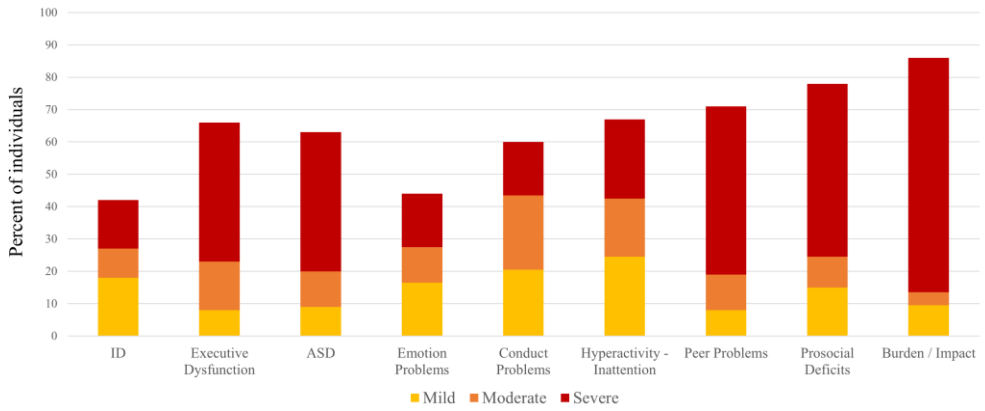

Figure 3.4 The percentage of each comorbidity associated with *PCDH19* variants. *ID,* intellectual disability; *ASD,* autism spectrum disorder (severity based on SRS-2 *t* scores only). Severe ID included two individuals with profound ID. DOCS scores omitted as no cutoff exists for severity.

Obsessive Compulsive Disorder. The DOCS was administered only to individuals who could complete the assessment themselves (n = 29). One participant (#56) was excluded due to missing responses (n = 28). Six individuals (21%), attained a total score that was consistent with a possible OCD diagnosis. All six were heterozygous females, including one non-penetrant female (#58). All transmitting males scored in the normal range (Supplementary Table 3.8).

Predicting the severity of neuropsychiatric comorbidities

Five outliers (#39, #50, #58, #64, & #112) were removed prior to statistical analyses due to seizure onsets 2 standard deviations above the mean. There was a significant negative association between age at seizure onset and ID for novel variants (p = .007) as well as for the entire cohort (p = .010). For every one month increase in seizure onset age, there was a 0.07 decrease in average ID severity, controlling for age at time of study (estimate = -0.07, 95% CI: -0.12, -0.02). There was a significant negative association between age at seizure onset and executive dysfunction for novel variants (p = .044) as well as the entire cohort ($p = 4.69 \times 10^{-4}$; Fig. 7a). On average, increasing seizure onset age by 1 month was associated with a 0.82 decrease in average GEC*t* scores, controlling for age at time of study (estimate = -0.82, 95% CI: -1.27, -0.37). For the ASD analysis, SRS-2 and SCQ total scores were converted to *z* scores and combined. Seizure onset age was significantly associated with ASD (p = .001; Fig. 7b) and prosocial behavior (p = .040). On average, increasing seizure onset age by 1 month was associated with a 0.50 decrease in SCQ/SRS-2 combined scores (estimate = -0.50, 95% CI: -0.08, -0.02) and a 0.13

increase in prosocial behavior scores (estimate = 0.13, 95% CI: 0.01, 0.25), controlling for age at time of study. No significant association was observed for the hyperactivity-inattention and peer problems scales.

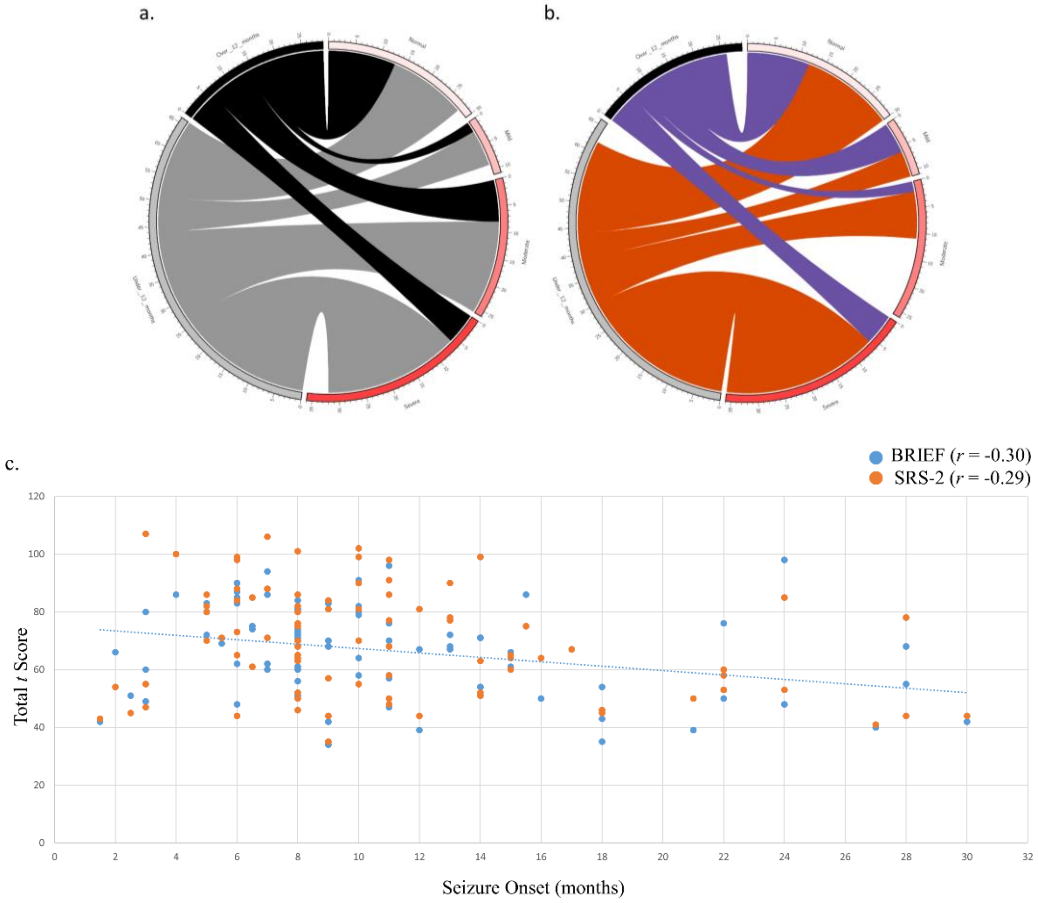

Figure 3.5 Circos and scatterplot illustrating phenotype-phenotype association: a) The variable cognitive profile of GCE (n = 95) against age at seizure onset: ≤12 months, represented by grey links (n = 66) and >12 months, represented by black links (n = 29); b) The variable ASD profile of GCE (n = 88) against age at seizure onset: ≤12 months, represented by orange links (n = 62) and >12 months, represented by purple links (n = 26). Axes show the number of individuals in each category; c) A moderate negative association between age at seizure onset and clinical outcome, as measured by the BRIEF (n = 93) and SRS-2 (n = 86).

We hypothesized that seizure activity (operationalized as the average number of seizures per day in a cluster) combined with onset would strengthen these associations. An additional outlier (#26) was removed from this analysis. For a one-way ANOVA, seizure onset was categorized as *"early"* (≤12 months) or *"late"* (>12 months)[64] and seizure activity was categorized as *"mild"* (≤15 seizures/day in a cluster) or *"severe"* (>15 seizures/day in a cluster) based on the group average. Seizure activity was associated with executive dysfunction, $F_{3,81} = 4.71$, $p = .004$, ASD, $F_{3,81} = 7.45$, $p = 1.82 \times 10^{-4}$, and prosocial behavior, $F_{3,66} = 3.10$, $p = .033$. We predicted that the greatest phenotypic

difference would be observed between individuals with late onset/mild seizure activity and individuals with early onset/severe seizure activity. Our prediction was supported for all outcomes: executive dysfunction (Supplementary Fig. 3a; t_{81} = -3.45, p = .001), ASD (Supplementary Fig. 3b; t_{81} = -4.66, p = 1.2 x 10^{-5}), and prosocial behavior (t_{66} = -2.92, p = .005), with an earlier age at seizure onset combined with severe seizure activity being associated with more severe outcomes. An examination of the means revealed that seizure onset was more strongly associated with cognitive outcomes whereas seizure activity was more strongly associated with psychiatric outcomes (Supplementary Table 3.9).

Given that 50% of our cohort had novel variants, we wanted to exclude that the clinical outcome for published cases would be more severe than that of unpublished cases due to selection or admission bias.[73] We investigated this via *t* tests and found no statistically significant difference for either executive dysfunction ($M_{\text{published}}$ = 67.5; $M_{\text{unpublished}}$ = 62.2) or standardized ASD symptom severity ($M_{\text{published}}$ = 0.14; $M_{\text{unpublished}}$ = -0.24). The SDQ and DOCS were excluded, as fewer individuals had scores on these measures. Consistent with our previous finding, there were no notable genotype-phenotype associations (Supplementary Table 3.10).

Discussion

We performed the first comprehensive patient-derived standardized assessment of *PCDH19*-variant individuals, including males (both germline and mosaic) and non-penetrant females. Females with GCE are typically described as having normal early development and regressing in infancy.[8-10] We found that delayed development prior to seizure onset occurred in 18% of individuals, replicating observations in two smaller studies.[3, 61] This may provide scope for early detection, especially for siblings of affected individuals. Seizures occurred in clusters in 94% of individuals. We believe the hallmark features of GCE to be threefold: 1) focal seizure clusters with affective semiology,[25] often triggered by fever; 2) seizure onset at 8 months of age; and 3) predominantly affecting females. The molecular diagnosis for many infantile neurodevelopmental disorders including epilepsy occurs at a mean age of 3 years, which represents delays of months to years for patients with pathogenic variants.[74] Early clinical identification of GCE will result in earlier molecular diagnosis and may impact outcome by allowing optimization of both seizure management and developmental progress.

Overall, 69/112 (62%) individuals met criteria for ASD. GCE has previously been associated with ASD,[3, 14] suggesting that this genetic etiology underlies epilepsy, ASD, and ID. The frequency of ASD in our cohort was lower than previous estimates, but may be more accurate given the much smaller sample sizes and lack of systemized approaches in these studies.[14, 31, 61, 62] We found that executive dysfunction occurs in 70/111 (63%) individuals. Of these, 62 (89%) individuals also met criteria for ASD. It has been posited that the social and non-social deficits observed in ASD stem from deficits in executive functions and might explain the co-occurrence of these disorders.[75]

The SDQ revealed that peer problems and prosocial behavior were the most affected domains. This is expected given the high proportion of individuals meeting criteria for ASD. Most individuals who met criteria for ASD, also scored high on the BRIEF inhibit and shift subscales. The inhibit domain is relatively preserved in ASD, yet impaired in ADHD whereas shift domain deficits are characteristic of ASD rather than ADHD.[76] The ASD group with elevated inhibit (ADHD-like) and shift (ASD-like) scores also scored very high on hyperactivity-inattention (see Supplementary output). This may represent an ASD profile with features of ADHD or a general deficit in executive functions that underlies these comorbidities. SDQ impact assesses chronicity, distress, social impairment, and burden for others. Most (75%) scores were in the very high range. This finding, combined with qualitative accounts, supports reports that psychiatric comorbidities become the most concerning feature in GCE.

Consistent with a recent report,[61] 21% of our cohort had obsessive-compulsive symptomatology revealed by the DOCS. This may be an accurate estimate or may reflect the similarities between OCD and other disorders, such as ASD. Only one of the six individuals meeting criteria for OCD also met criteria for ASD in conjunction with a moderately elevated shift score (#58), suggesting that OCD is distinct from ASD in GCE.

We demonstrated that the clinical profile for heterozygous females and mosaic males is the same. We describe seven non-penetrant females and one male. This non-penetrance may reflect absence of mosaicism in the brains of these individuals, consistent with the cellular interference model.[2, 4] We confirmed there is no clinical profile for transmitting males, although identified two hemizygous males with ASD in addition to executive dysfunction (#42) or seizures (#39). If their phenotypes are due to their *PCDH19* variants,

these findings will expand the phenotypic spectrum; however, they may be due to additional genetic or environmental factors.

Consistent with our previous meta-analysis, earlier seizure onset age was associated with more severe ID. We also showed that earlier seizure onset age predicted greater executive dysfunction, prosocial behavior, and ASD severity, and that increased seizure activity strengthened these associations. It could be that earlier and more frequent seizures cause more adverse outcomes,[77] or the *PCDH19* variant is enhanced by polygenic or epigenetic burden.[51]

Although the frequency of comorbid ASD in the GCE population is likely to be correct, we are limited by inherent difficulties in delineating ASD from other impairments. For example, parent reports are not entirely reliable in discriminating children with ASD from those with language impairment.[78] An assessment of language was beyond the scope of this study. As language delay may be associated with GCE,[64] future work should incorporate a formal assessment of ASD and language impairment.

Biases also exist with self-reported data.[79] Validity checks within the BRIEF addressed these biases to some extent. Retrospective accounts are inherently less reliable than direct observation of behavior or events. However, standardized assessments are particularly useful in contexts such as these, as they minimize any potential confounds that are likely to emerge as a result of subjectivity and different administrators or methodology.[80]

GCE is a distinctive epilepsy with early onset of seizure clusters, with or without ID. We show that individuals with *PCDH19* pathogenic variants may have associated executive dysfunction, ASD, ADHD, and OCD, thus characterizing the neuropsychiatric profile of GCE. Our data show that approximately 20% of individuals have developmental delay prior to seizure onset. We confirm the association between earlier seizure onset age and more severe ID and demonstrate that the association with earlier seizure onset age extends to neuropsychiatric comorbidities. We also demonstrate an association between increased seizure frequency and poorer clinical outcomes. We show that the clinical profile for heterozygous females and mosaic males is the same and describe seven non-penetrant females and one male. We also show that there is no clinical profile for transmitting males, but identify two affected hemizygous males. These phenotypic insights will lead to better diagnosis and management of GCE.

Chapter 4: **Human Disease Genes website series – PCDH19 website**

4.1 Preamble

Based on the findings from the first two studies, I developed a PCDH19 website as part of the Human Disease Genes website series https://humandiseasegenes.nl/pcdh19/. The Human Disease Genes website series is an international library of websites for professional information about genes and copy number variances and their clinical consequences. Through discussions with individuals and families affected by *PCDH19* variants and GCE we discovered that having access to information was extremely important, as well as being able to connect with PCDH19 researchers, clinicians, and other families affected by this disorder. The PCDH19 website provides clinical and genetic information about patients with disease-causing changes in the *PCDH19* gene. We are dedicated to ensuring access to new information about *PCDH19* as soon as it becomes available. The PCDH19 website will provide a platform for clinicians, researchers, and families to connect, share information, or learn more about this disorder.

4.2 Statement of authorship

Title of Paper	Human Disease Genes website series – PCDH19 website.
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Kolc, K. L., Sadleir, L. G., Scheffer, I. E., Palmer, E., & Gecz, J. (2019). PCDH19 website. Human Disease Genes website series.

Principal author

Name of Principal Author (Candidate)	Kristy Kolc		
Contribution to the Paper	Major contribution to the development of the PCDH19 website, including content and all required revisions.		
Overall percentage (%)	80%		
Certification:	This website reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this website.		
Signature		Date	Feb 14 2020

Co-Author contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Professor Lynette Sadleir		
Contribution to the Paper	Assisted with development of work and website evaluation.		
Signature		Date	Feb 13 2020

Name of Co-Author	Laureate Professor Ingrid Scheffer		
Contribution to the Paper	Assisted with development of work and website evaluation.		
Signature		Date	Feb 14 2020

Name of Co-Author	Dr Emma Palmer		
Contribution to the Paper	Assisted with development of work and website evaluation.		
Signature		Date	Feb 13 2020

Name of Co-Author	Professor Jozef Gecz		
Contribution to the Paper	Assisted with development of work and website evaluation.		
Signature		Date	Feb 13 2020

4.3 Published website

Name: Miss Kristy Kolc
Function: PhD Candidate
Affiliation: Adelaide Medical School, The University of Adelaide
City, country: Adelaide, Australia
Email address: kristy.kolc@adelaide.edu.au

Name: Professor Jozef Gecz
Function: PhD
Affiliation: Adelaide Medical School, The Robinson Research Institute, The University of Adelaide
City, country: Adelaide, Australia
Email address: jozef.gecz@adelaide.edu.au

Name: Dr (Elizabeth) Emma Palmer
Function: MD
Affiliation: Genetics of Learning Disability Service, Hunter Genetics
City, country: Sydney, Australia
Email address: elizabeth.palmer1@health.nsw.gov.au

Name: Professor Ingrid Scheffer
Function: MBBS PhD FRACP
Affiliation: The University of Melbourne, Austin Health and Royal Children's Hospital
City, country: Melbourne, Australia
Email address: i.scheffer@unimelb.edu.au

Name: Professor Lynette Sadleir
Function: PhD
Affiliation: Department of Paediatrics and Child Health, University of Otago
City, country: Wellington, New Zealand
Email address: lynette.sadleir@otago.ac.nz

Gene Specific Homepage

This website provides clinical and genetic information about patients with disease-causing changes in the *PCDH19* gene.

Abnormalities in the *PCDH19* gene are usually associated with epilepsy in girls. The seizures often start at 8 months (median onset = 10 months), most girls having seizure onset by 3 years of age. Commonly, girls have clusters of many seizures over several days followed by periods where they are seizure free. This distinctive pattern has led to the disorder being often referred to as GCE to assist in earlier recognition of *PCDH19* epilepsies. Affected girls often have behavioral difficulties, learning problems, or ID that may not be apparent until after the seizures start.

The *PCDH19* gene is located on the X chromosome, and was first implicated in epilepsy in 2008. Changes that affect a gene's ability to function are referred to as pathogenic variants or mutations. Many different pathogenic variants in the *PCDH19* gene are associated with GCE. The *PCDH19* gene is important because it encodes a protein that is involved in how brain cells (neurons) communicate with other cells and move in forming the brain.

GCE typically affects females, but not all females with *PCDH19* pathogenic variants are affected. The penetrance (the extent to which the condition is present with the pathogenic variant) of GCE is estimated to be 90%. Males who inherit a *PCDH19* pathogenic variant from their mother are generally not affected; they do not have seizures or learning difficulties.

However, in rare cases, males have a *PCDH19* pathogenic variant in some, but not all, of their cells (called mosaicism). These males usually have similar features to females with GCE. Mosaicism can result from a pathogenic variant occurring in the male during early development (post fertilization of the sperm and egg).

This website was created to facilitate the collection and sharing of information about *PCDH19*-associated conditions. Our aim is to enhance the understanding of these disorders and improve treatment of individuals with *PCDH19* pathogenic variants through PCDH19 research.

Professionals – General Information

GCE is a condition caused by pathogenic variants in the X chromosome gene *PCDH19*. *PCDH19* encodes the protocadherin-19 protein. This protein is important in individual cell function, cell-cell adhesion, and cell-cell communication. Due to the normal X-chromosome inactivation in females or the development of a somatic (postzygotic) pathogenic variant in males, individuals with pathogenic variation in this gene are cellular mosaics. That is, they have some cells that express functional protocadherin-19 protein and some cells that do not express protocadherin-19 protein or express its non-functional form.

Main clinical features. *PCDH19* pathogenic variants cause a distinctive phenotype for which the name GCE has been proposed to aid in early recognition of the distinguishing phenotypic features. Although GCE can show marked inter-individual variation in severity, it is readily identifiable clinically. Typically developing girls present with clusters of frequent seizures usually in the first year of life, often triggered by fever. Seizures are typically focal, although bilateral tonic-clonic (BTCS) and tonic seizures may occur. Clusters may be difficult to abort and the epilepsy is often pharmaco-resistant. During the second decade of life (mean 18 years) the epilepsy can improve, with many girls becoming seizure free in adolescence. Developmental course is varied, with many girls showing developmental slowing during childhood. Although some girls have abnormal development from birth (approx. 20%). There may be developmental regression with clusters of seizures. Approximately half of affected individuals have ID ranging from mild to profound. Comorbid psychiatric disorders are common, most frequently ASD and behavioral problems; later onset psychosis occurs in approximately 20% of females. These features also have a significant impact on the quality of life for these individuals and their families.

Prevalence. *PCDH19* is considered one of the most clinically relevant genes in epilepsy, second only to *SCN1A*. The prevalence of GCE is estimated to be 1 in 15,000. To date, over 300 individuals with pathogenic variants in *PCDH19* are reported in the literature.

Inheritance. *PCDH19* pathogenic variants can be *de novo* or inherited. Females may present as sporadic cases or with a striking family history in which only women have seizures, ID, or ASD. The X-chromosome-linked inheritance pattern observed in families

is unique: only females with heterozygous pathogenic variants are affected, whereas males with hemizygous pathogenic variants are unaffected. A hemizygous or "transmitting" male must pass the pathogenic variant onto all his daughters who are likely to be affected. The penetrance (the extent to which the condition is present with the pathogenic variant) is estimated to be 90%.

Sporadic cases are generally the result of a *de novo* ("new") pathogenic variant and are observed in approximately half of the reported *PCDH19* cases. However, in rare instances, the unaffected father may be mosaic thus increasing the recurrence risk of having another girl with GCE in those families.

Males with a *de novo* somatic pathogenic variant are mosaic, that is, some of their cells express *PCDH19* and others do not. These males exhibit a similar clinical profile (with seizures and ID) to heterozygous affected females. No significant clinical differences have been observed among individuals with inherited or *de novo* pathogenic variants. There are two frequent recurrent variants: p.Asn340Ser and p.Tyr366Leufs*10 that have been identified in 25 (20 unrelated) and 30 (11 unrelated) cases, respectively. There are no marked similarities among individuals with the same variant.

Professionals – Clinical Characteristics

Clinical characteristics of affected females with a PCDH19 pathogenic variant

The clinical spectrum of GCE varies significantly, even amongst individuals in the same family ((including mother-daughter, sister-sister, and monozygotic (MZ) twin pairs)) and non-related individuals with the same *PCDH19* variant.

Epilepsy. The median age of seizure onset is 10 months (range 1 to 70 months), with 95% of cases beginning before 25 months of age.

Seizures are often focal and include FIAS, FMS and FBTCS but can progress quickly to BTCS. Ictal fear is an initial feature in ~80% of seizures with screaming reported in ~60%. FIAS are prominent in the first few years and can have subtle semiology with a fearful expression, behavioral arrest accompanied by an arrest, loss of muscle tone, hypopnea, cyanosis, and desaturation. The motor semiology is often tonic and more common in older girls. Seizures can arise independently from either hemisphere. TCS are common in infancy, occurring in clusters and often triggered by fever. Generalised

absence, myoclonic, and atonic seizures are rare but there are no reports of epileptic spasms. The more severe cases have a developmental and epileptic encephalopathy.

For 95% of individuals, seizures cluster and are often triggered by fever and illness, and, less commonly, by vaccination. Clusters typically comprise of many seizures per day over several days for up to a week. The severity of the clusters varies from several brief seizures per day with normal interictal state to hourly seizures, or status epilepticus requiring intensive care management. Video monitoring shows that most (71%) seizures occur during sleep. Although girls can have months of seizure freedom between clusters, the epilepsy is often pharmaco-resistant and it can be difficult to both abort a cluster of seizures and prevent clusters and sporadic seizures from occurring in the first decade of life. In adolescence, seizures often improve, with at least 30% of girls becoming seizure free. The age of seizure onset does not predict seizure outcome.

Intellectual disability. Infants are usually developmentally normal at seizure onset, although some are delayed from birth. Regression often occurs with seizure clusters. Initially, development subsequently improves with a return to normal between clusters. Over time, with subsequent clusters, return to previous levels of function may not occur. Intellectual outcome varies from normal (~30%) to severe or profound ID (~15%), with most individuals having mild to moderate cognitive difficulties (~55%) with predominant language impairment.

Early seizure onset (≤ 12 months of age) is associated with more severe ID. As delayed development prior to the onset of seizures is observed in ~15% of infants and seizure persistence is not correlated with intellectual outcome, it is likely that *PCDH19* pathogenic variants have an independent effect on seizures and cognition, which may be additive. In a set of identical twins with the same variant, the sister who had more frequent, intense, and longer clusters had a significantly worse cognitive and behavioral outcome than the sister who had fewer seizures. This is consistent with a developmental and epileptic encephalopathy. Although the association of early seizure onset with more severe ID may reflect the underlying severity of the disorder in that individual, it is likely that the early, frequent seizure activity also contributes to poorer cognitive outcomes.

Psychiatric comorbidities. Many females with *PCDH19* pathogenic variants develop significant mental health disorders. ASD is often diagnosed in childhood. Although

seizures typically improve by the second decade of life, behavioral difficulties become the main feature in adolescence and significantly impact the quality of life for both the individual and their family. These difficulties affect over half of females and males and, in addition to autistic features, include aggression, obsessions, depression, hyperactivity, panic attack, hysteria/somatoform disorder, anxiety, impulsivity, disinhibition, and dysexecutive syndrome. Autistic features and executive dysfunction are the most prominent, occurring in approximately 60% of individuals. Reports describe that 22% of individuals have multiple psychiatric comorbidities that predominantly include combinations of autistic, aggressive, hyperactive, and/or obsessive features. Psychosis occurs in about 20% of women, beginning in adolescence or adult life.

More research is required to further characterize the psychiatric aspects of the clinical profile associated with *PCDH19* pathogenic variants. Currently, research in this area is being led by Professor Jozef Gecz at the University of Adelaide, Australia. Further details can be found under “Ongoing Research” on the “Parents” page.

EEG and neuroimaging findings: EEGs are often normal between seizure clusters but may show focal slowing and interictal focal or multifocal epileptiform discharges between and, more frequently, during seizure clusters. Focal discharges are typically located in the frontal and temporal regions. Focal discharges can have a diffuse field in up to 25% of EEGs with true generalized spike wave or polyspike wave occurring infrequently and in patients who also have focal discharges.

The ictal EEG shows seizures arise from the temporal (83%), frontal (6%), parieto-occipital (6%), or central (5%) regions. Within a cluster, seizures can start independently from either hemisphere or have bilateral onset. In 20% of seizures, interhemispheric asynchrony is observed with seizures originating in one hemisphere and migrating to involve only the other hemisphere.

Neuroimaging is usually normal, although there is a report of focal cortical malformation in 5 individuals with *PCDH19* pathogenic variants.

Differential diagnosis. At presentation, the main differential diagnoses include a self-limited infantile epilepsy syndrome, focal structural epilepsy, and DS. There is some clinical overlap between GCE and DS, as both syndromes present at a similar age, with seizures often triggered by fever. However, the syndromes are quite distinguishable: a

younger age of onset in DS (mean 5 months) comparted with GCE (mean 14 months); often presenting with hemiclonic SE in DS compared to clusters of brief FIAS or TCS with ictal fear in GCE; and generalized spike wave often seen later in DS but uncommon in GCE.

It can be difficult to differentiate GCE from self-limited focal infantile epilepsy at presentation. For example, sporadic or familial proline rich transmembrane protein 2 (PRRT2) self-limited focal infantile epilepsy presents at a similar age with clusters of brief FS. With time, the features of the syndromes diverge with continuing normal development and seizure resolution in most PRRT2 epilepsies and developmental slowing and continuing seizure clusters in GCE.

Clinical characteristics of "mosaic" males with a de novo PCDH19 pathogenic variant

At least ten males have been reported with *de novo PCDH19* pathogenic variants. All had a normal complement of sex chromosomes. It was confirmed that each male was mosaic, with some cells containing the *PCDH19* pathogenic variant and some containing wild-type *PCDH19*. This mixed population of *PCDH19* variant and wild-type cells is termed cellular mosaicism and arises from a pathogenic variant that occurs after fertilisation, but during early development. The mosaic cellular expression resembles an affected heterozygous female who has two X chromosomes, one with variant and one with wild-type *PCDH19*.

These mosaic males have a similar clinical profile to heterozygous females. Nine of the ten reported mosaic males have comorbid psychiatric features including behavioral disturbances (i.e., aggression and rigidity), ADHD, anxiety, OCD, and oppositional defiant disorder.

An additional five males have recently been described, with intellectual function ranging from normal to severely impaired. Executive dysfunction, autistic features, hyperactivity, and social problems were also common.

Clinical characteristics of hemizygous males with a germline PCDH19 pathogenic variant

As transmitting males do not have epilepsy or significant cognitive difficulties, they are considered unaffected. However, it has been noted that they may exhibit behavioral

features reminiscent of a broader autism-like phenotype or obsessive-compulsive features. Five transmitting males have been described with inflexible, rigid personalities, and obsessive traits.

Professionals – Management

Management and Surveillance. Currently, there is no known cure for GCE. Epilepsy should be managed by a pediatric neurologist or a pediatrician with expertise in epilepsy. Early developmental and behavioral diagnosis and intervention with education programs can maximize cognitive and social potential.

Parents should be encouraged to promptly discuss concerns about possible seizures, learning difficulties, or behavioral problems with their child's pediatrician or pediatric neurologist so that these symptoms can be appropriately diagnosed and managed.

Table 4.1 Management and surveillance

Possible health risk	*Suggested surveillance*
Seizure control/management	Epilepsy should be managed by a pediatric neurologist or a pediatrician with expertise in epilepsy.
Learning (including speech and language)	Developmental surveillance by a pediatrician with appropriate referral for early cognitive assessment, intervention (including speech therapy), and ongoing support at school and on leaving school.
Behavioral problems	Developmental surveillance by a pediatrician with appropriate referral for early intervention and ongoing support by a psychologist or psychiatrist, if required. For more severely affected individuals, behavioral medications may be required.

Genetic counseling. Individuals and their families with a *PCDH19* pathogenic variant should be referred to a geneticist and for genetic counseling. This is especially important given the unusual X-linked dominant pattern of inheritance with male sparing.

Approximately half of the individuals with GCE have a family history of epilepsy. However, the expression pattern is unusual given only females are affected and daughters can inherit the variant from their unaffected fathers or affected or unaffected mothers (Fig. 4.1). The expression pattern in GCE is the "inverse" of typical X-linked inheritance, where males are affected, and females are unaffected carriers (Fig. 4.2).

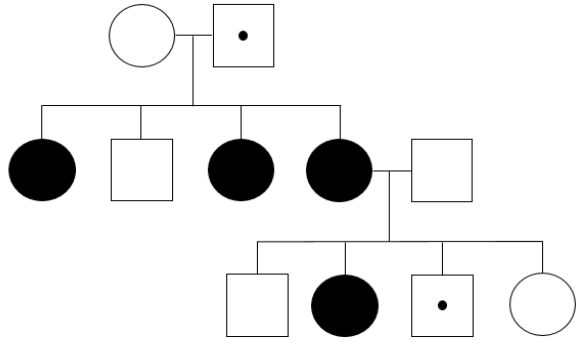

Figure 4.1 X-linked dominant inheritance with male sparing and expression pattern in GCE. Note that all daughters of an unaffected (transmitting) male inherit the *PCDH19* pathogenic variant and most are affected. A female with a *PCDH19* pathogenic variant will transmit this to 50% of her daughters.

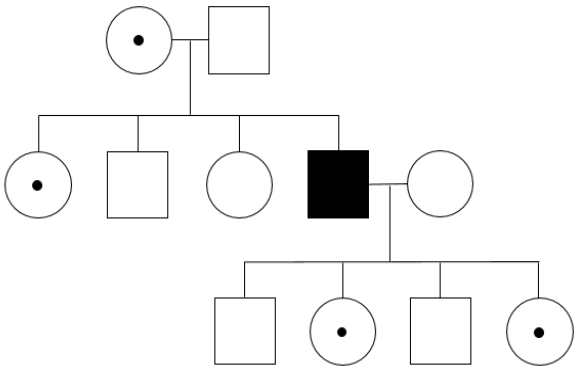

Figure 4.2 Typical X-linked recessive inheritance

Males inherit an X chromosome from their mother and a Y chromosome from their father. Males who inherit an X chromosome with an abnormal *PCDH19* gene are unaffected and are therefore considered transmitting carriers of GCE. Females have two X chromosomes; one inherited from their mother and one from their father. Each X chromosome has a *PCDH19* gene. However, while females have two X chromosomes in each of their cells, only one X chromosome is generally active. This is due to an evolutionarily old molecular process called XCI (see also below), which prevents the two X chromosomes from being expressed in one cell. Females who have a pathogenic variant in one of their *PCDH19* genes are called heterozygotes and are generally affected (90% penetrance).

Around half of the females with GCE are sporadic, with no family history of the disorder. The majority of these have a *de novo PCDH19* pathogenic variant. However, in a small percentage germline mosaicism can occur where the genetic change occurs later in the parents' early development. The pathogenic variant is not present in the mother or

father's brain, so they are unaffected. The variant is present in their germline cells. Therefore, the chance that they may have another affected child is lower than for a heterozygous female carrier (50% chance of transmission to daughters) or a transmitting male (100% chance of transmitting to daughters). However, the risk of having another affected child for individuals with gonadal mosaicism is not negligible. Due to the complex nature of GCE inheritance, it is important that families are offered comprehensive genetics counseling, especially if they are planning another pregnancy. Their children should also be offered counseling when they grow up. The sons of a mother with a *PCDH19* pathogenic variant have a 50% chance of inheriting the pathogenic variant and then a high risk of having affected daughters.

What are the chances of a female with a PCDH19 pathogenic variant having a child with GCE?

Females with a *PCDH19* pathogenic variant who are on the mild end of the disease spectrum or asymptomatic carriers may want to have children. Females with a *PCDH19* pathogenic variant, regardless of their disease penetrance, have a 50% chance of passing on the X chromosome with the working *PCDH19* gene and a 50% chance of passing on the X chromosome with the *PCDH19* pathogenic variant (see diagram).

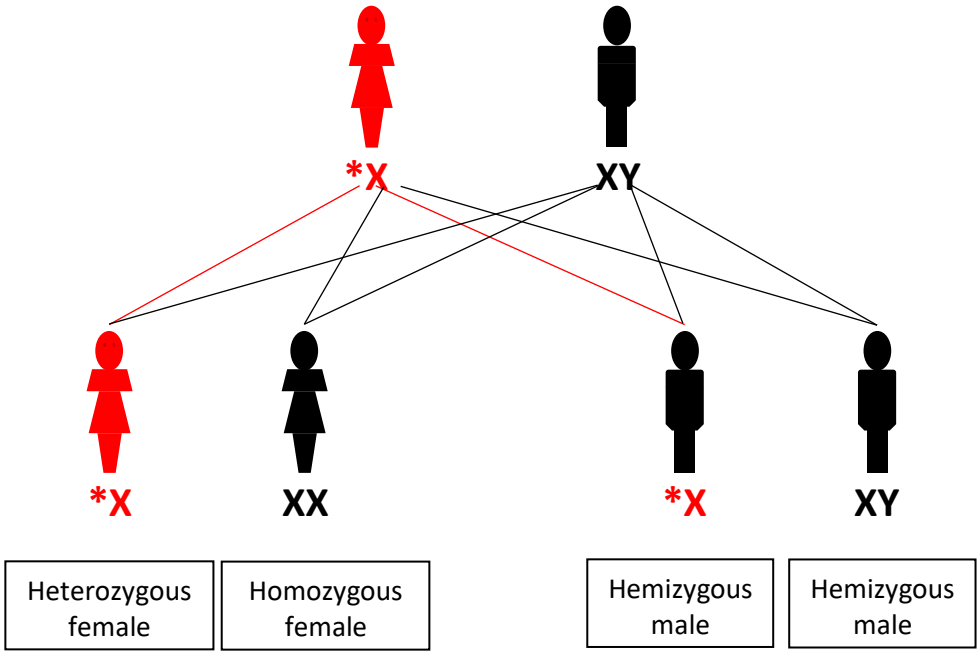

Figure 4.3 Transmission of the *PCDH19* gene from the mother.

What are the chances of a male (hemizygous or mosaic) with a PCDH19 pathogenic variant having a child with GCE?

Males pass on their X chromosome to all their daughters and a Y chromosome to all their sons. A carrier male will pass on the X chromosome with the *PCDH19* pathogenic variant to all his daughters. This means all their daughters will be at high (due to ~90% penetrance) risk of developing GCE. As a male with a *PCDH19* pathogenic variant passes on his Y chromosome to all his sons they will **not** carry their father's *PCDH19* pathogenic variant.

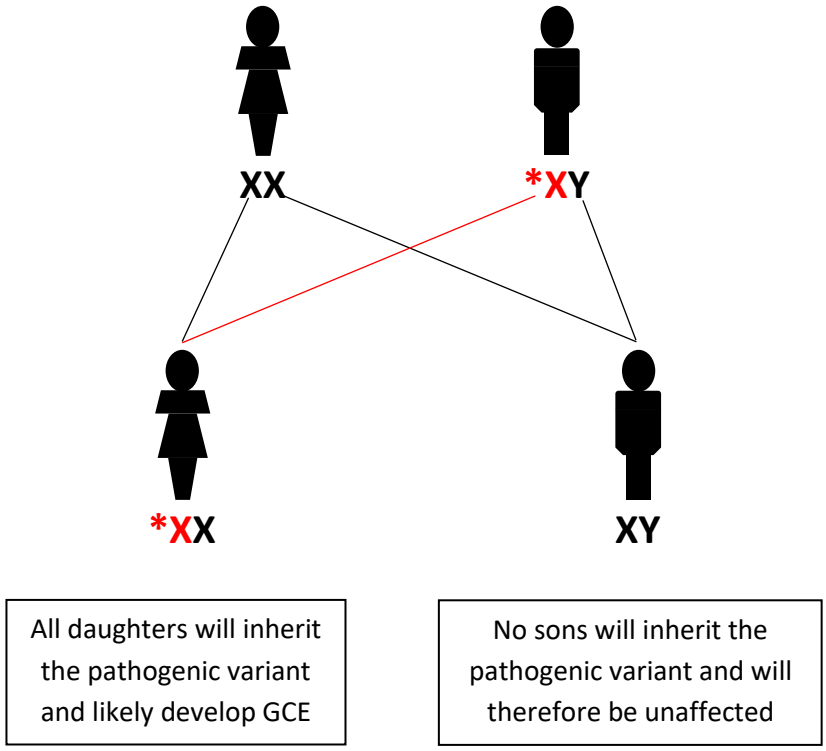

Figure 4.4 Transmission of the *PCDH19* gene from the father.

Professionals – Molecular Characteristics

The *PCDH19* gene is located at Xq22.1 and consists of six exons. The gene encodes a 1148 amino acid protein with typical features of the δ2-protocadherin sub-family, with 23 amino acid signal peptides, six conserved cadherin repeats in the EC domain, a transmembrane domain, and conserved motifs (CM1-CM2) in the C-terminal region. The first exon encodes the EC and transmembrane domains, as well as a small portion of the C-terminal region. While the rest of the C-terminal region is encoded by exons 2–6, the second, and likely the third exon are subject to alternative splicing. Exons 5 and 6 encode for CM1 and CM2 domains, respectively. More than 80% of the reported GCE

pathogenic variants are observed in the EC domain of the protein encoded by exon 1. Of the reported variants in this region, almost half are in the EC3 and EC4 domains (20% and 23%, respectively). Missense variants are most frequently reported (45%), followed by frameshift (27%), and nonsense variants (20%). In total, 145 unique germline *PCDH19* pathogenic variants have been identified in GCE, both in large families as well as singleton cases. Most *PCDH19* variants are non-recurrent (exclusive to that individual or family) with the exception of p.Asn340Ser and p.Tyr366Leufs*10, which have been reported in 25 and 30 individuals, respectively.

Several mechanisms have been suggested to account for the unusual mode of inheritance. Of these, cellular interference has received the most support. Cellular interference is a mechanism reminiscent of metabolic interference and postulates that random inactivation of one X chromosome in females with a *PCDH19* pathogenic variant generates cellular mosaicism in *PCDH19*-expressing tissue (i.e. co-existence of *PCDH19*-normal or *PCDH19*-abnormal cells). Such cellular mosaicism causes the condition by altering cell-cell interactions, function, and therefore neural networks in the brain. Cellular interference is consistent with the clinical finding that males hemizygous for a *PCDH19* pathogenic variant in all their cells are typically unaffected as they have only one population of cells albeit with the pathogenic variant, whereas males with somatic mosaicism are affected similarly to heterozygous females.

The identification of affected males who are mosaic for *PCDH19*, and therefore have a mixture of *PCDH19*-normal and *PCDH19*-abnormal cells, strongly supports the hypothesis of cellular interference as the main pathogenic mechanism associated with *PCDH19* pathogenic variants. The co-existence of normal and abnormal cells and the proportion of each population in the brain of these males cannot, however, be extrapolated from available tissues i.e., skin fibroblasts or lymphocytes. To establish that cellular interference is the pathogenic mechanism, it is necessary i) to demonstrate that neuronal cells are mosaic, but also that ii) females who are homozygous for *PCDH19* pathogenic variants or deletions are also unaffected, akin to hemizygous males. Some support for the first point lies in the findings from a *Pcdh19* knockout mouse model. Simultaneous labelling of wild-type *Pcdh19* and null *Pcdh19* cells in *Pcdh19* heterozygous mice revealed a striking pattern of alternating *Pcdh19* +ve and *Pcdh19* -ve regions. This pattern was particularly obvious in the developing cortex where it

resembled “tiger stripes”. Although pathogenesis in cells that express the abnormal allele corresponds to a loss-of-function, cellular interference would result in a gain-of-function at the tissue level, because of abnormal interactions between normal and abnormal cells. This hypothesis supposes that the loss of *PCDH19* is compensated for, but by a mechanism that is relatively independent of gender. For the latter, there is yet to be a report of a female homozygous for loss of function pathogenic variant in *PCDH19*.

Molecular diagnosis

Testing for GCE requires sequencing of the *PCDH19* gene, either by traditional Sanger sequencing or massively parallel sequencing methodology (panel testing, exome, or genome). If no pathogenic variants are identified, then microarrays for copy number testing i.e., deletions or duplications should be performed in individuals presenting with the typical phenotype or inheritance pattern.

Determining the pathogenicity of novel variants requires the use of *in silico* tools and segregation in additional family members. In some cases, molecular/functional studies may be required to build evidence that a detected variant is causal of the individual’s condition. Whether the phenotype is consistent with the disease should also be considered.

Repository of the variant and levels of evidence for causality in publicly available international databases like ClinVar https://www.ncbi.nlm.nih.gov/clinvar/ or DECIPHER https://decipher.sanger.ac.uk/ is recommended to aid interpretation of novel variants.

Professionals – Publications

Juberg RC et al. A new familial form of convulsive disorder and mental retardation limited to females. J Pediatr. 1971;79:726–32. PMID: 5116697

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Professionals – Research Collaboration

Ongoing Research

1. Understanding individual differences associated with *PCDH19* pathogenic variants (H-2016-184)

This research, led by Professor Jozef Gecz at the University of Adelaide, aims to understand the symptoms associated with *PCDH19* pathogenic variants. The project also aims to provide a clear picture of how these symptoms change over time. This information will help us to better understand *PCDH19*-related disorders.

Participants for this project are individuals with a *PCDH19* pathogenic variant or their caregivers who can report on behalf of someone with a *PCDH19* pathogenic variant. Participation involves responding to an online PCDH19 survey.

For more details and to participate, please contact Kristy Kolc
Kristy.kolc@adelaide.edu.au

2. PCDH19 Registry

With funding provided by the PCDH19 Alliance, researchers at UCSF Benioff Children's Hospital and Boston Children's Hospital have created a registry for individuals with *PCDH19*-Related Epilepsy. The registry is an important tool to help researchers gain a better understanding of *PCDH19*-Related Epilepsy and ultimately develop more effective treatment options. The registry is open to any individual who has been diagnosed with *PCDH19*-Related Epilepsy.

For more information visit https://www.pcdh19info.org/pcdh19-patient-registry

Support Organisations

There are several organisations around the world that provide support, information, and fund research related to *PCDH19*.

USA: PCDH19 Alliance

http://pcdh19info.org/

France: PCDH19 France

http://www.pcdh19france.fr/

Italy: ONLUS Insieme per la Ricerca PCDH19

www.pcdh19research.org

Caregivers – General Information

The *PCDH19* gene is an important gene contributing to brain function. Genetic changes (called pathogenic variants or mutations) in the *PCDH19* gene cause disease. This predominantly occurs in girls and the characteristic feature of this disease is seizures

occurring in clusters, hence the name GCE. Some girls (20%) with a *PCDH19* pathogenic variant have no clinical features. Affected females with pathogenic variants vary in the severity of their illness, ranging from mild epilepsy, to mild epilepsy with learning difficulties to a severe treatment-resistant epilepsy accompanied by ID and mental health disorders, such as ASD and behavioral problems. How much this condition affects an individual varies significantly, even amongst individuals in the same family with the same pathogenic variant.

The first symptoms are usually epileptic seizures that often begin in the first year of life, but may begin up to 3 years of age. The seizures come in clusters, that is, many seizures occur over several days followed by periods of time (up to months) without seizures. Girls may have slow development and behavioral problems, and sometimes lose their skills. The more severely affected will develop ID, ranging from mild to profound. Individuals with GCE are otherwise healthy, with normal functioning of the rest of their bodies.

The *PCDH19* gene lies on the X chromosome, which is one of the sex chromosomes. Males have a single X chromosome and a Y chromosome, while females have two X chromosomes. GCE can be passed down (inherited) within a family or found in an individual with no family history of the disorder. Females with a pathogenic variant have a high likelihood of being affected, but males with the pathogenic variant typically do not have seizures and have normal intelligence.

Only a small number of males have been reported who are affected by *PCDH19* pathogenic variants. This is due to a situation called “somatic mosaicism”, where the affected males have a mixture of normal and abnormal copies of the *PCDH19* gene in their brains.

Pathogenic variants in *PCDH19* are a common genetic cause of epilepsy. As the gene was only identified in 2008, we are rapidly learning about this condition as more people are being diagnosed.

It is crucial that we capture as many individuals as possible with *PCDH19* pathogenic variants (including those not reported in the medical literature). This includes females as well as males, so we can build a more comprehensive understanding of this condition.

Caregivers – Clinical Characteristics

Features of GCE. Not all individuals with a pathogenic variant in the *PCDH19* gene have clinical features. How much a pathogenic variant results in symptoms (e.g. seizures, learning difficulties) in an individual varies, even amongst affected individuals in the same family. The main clinical features of this disorder are seizures, abnormal development, and behavioral problems.

Seizures. The most common age of seizure onset is 8 months, but children can develop seizures anytime between 1.5 and 70 months of age. The first seizures often occur in the setting of a fever. Most seizures occur in clusters and girls can have many seizures in a day for several days. The types of seizures are most commonly focal seizures, but tonic-clonic convulsive seizures can also occur. During a seizure, girls appear fearful and may scream. They are not aware of what is going on around them. They may have stiffening or jerking of one limb, which can sometimes spread to involve the entire body. Most seizures are short, lasting less than a few minutes, but sometimes there are so many of them in a cluster that the child does not recover between seizures. Anti-epileptic drugs (AEDs) are used to stop the seizures and prevent further clusters. In some individuals, the AEDs do not work well and it is difficult to prevent seizures occurring. Fortunately, the epilepsy often improves over time, with seizures becoming less severe and less frequent during adolescence. Some individuals become seizure-free around this time.

Development. Most, but not all, girls have normal development prior to the onset of seizures. Approximately 40% of females with GCE have normal intelligence throughout their lives. For the remaining 60% of girls, development slows during childhood and they ultimately develop ID, which can vary in severity from mild (i.e., will likely be able to attend mainstream education) to severe or profound (will need assistance in all areas of life). It is important to treat the clusters of seizures, as frequent and severe seizures can cause slowing of development. Many affected females have specific problems with planning and organisation, abstract reasoning, and the ability to control their behaviors ("executive functions").

Mental health and behavioural outcomes. Many children develop autistic features, as well as hyperactivity, attention-deficit disorder, and/or other behavioural disturbances. In adult life, some woman have psychiatric disorders, such as depression and psychosis.

Caregivers – Management

The same text will be displayed as in **Professionals – Management**.

Caregivers – Molecular Characteristics

Many changes in the genetic code of the *PCDH19* gene have been found to cause GCE. Changes which affect the gene's ability to function (and therefore cause disease) are referred to as pathogenic variants or mutations. Every person has a unique genetic code and most changes to this code do not cause disease. These non-disease-causing changes are called benign variants. When a diagnosis of GCE is supported by the finding of a pathogenic variant by a molecular laboratory, the identified variation is recorded on the report from the testing laboratory.

Most individuals with GCE have a pathogenic variant that is unique to their family, and therefore it can be difficult to determine if a variant is a benign (non-disease-causing) change or a pathogenic (disease-causing) change. More evidence may be required to be sure that the genetic change in *PCDH19* is causing disease. Testing of other family members can be helpful and provide evidence for the variant being either benign or pathogenic. A clinical genetics service can advise on the next steps after a *PCDH19* pathogenic variant is identified.

The international scientific and diagnostic community is working hard to build tools to improve our ability to determine whether a variant is disease-causing or not.

Caregivers – Publications

The same text will be displayed as in **Professionals – Publications**.

Chapter 5: **Levetiracetam efficacy in PCDH19 girls clustering epilepsy**

5.1 Preamble

An interesting finding to emerge from the second study was the self-reported efficacy of the anti-epileptic medication levetiracetam. This independent finding was corroborated following discussions with our collaborators. Clinical evidence also suggested that levetiracetam was efficacious in ameliorating seizures and, in some cases, improving behavior. For this work, I collaborated with pediatric neurologists based in Melbourne and New Zealand to review the effect of levetiracetam as a treatment for GCE. The importance of this work is pivotal. Presently, there are no effective treatments available, as GCE is pharmaco-resistant. Our lab has been involved in a clinical trial of ganaxolone, a synthetic analogue of allopregnanolone. We had previously shown a deficiency in allopregnanolone in girls with *PCDH19* variants.[50] Data from my first two studies was utilized by Marinus Pharmaceuticals to inform the design of their phase III clinical trial.

5.2 Statement of authorship

Title of Paper	Levetiracetam efficacy in PCDH19 girls clustering epilepsy.
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	*Sadleir, L. G., *Kolc, K. L., King, C., Mefford, H. C., Dale, R. C., Gecz, J., & Scheffer, I. E. (2020). Levetiracetam efficacy in PCDH19 girls clustering epilepsy. European Journal of Paediatric Neurology. *Equal first authors

Principal author

Name of Principal Author (Candidate)	Kristy Kolc		
Contribution to the Paper	Major contribution to the research question. Designed study, performed data collection, analysis, and interpretation, assisted with manuscript preparation and evaluation.		
Overall percentage (%)	40%		
Certification:	This manuscript reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this manuscript.		
Signature		Date	Feb 14 2020

Co-Author contributions

By signing the Statement of Authorship, each author certifies that:

iv. the candidate's stated contribution to the publication is accurate (as detailed above);

v. permission is granted for the candidate in include the publication in the thesis; and

vi. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Professor Lynette Sadleir		
Contribution to the Paper	Major contribution to the research question. Designed study, performed data collection, analysis, and interpretation, assisted with manuscript preparation and evaluation.		
Signature		Date	Feb 13 2020

Name of Co-Author	Ms Chontelle King		
Contribution to the Paper	Assisted with development of work and manuscript evaluation.		
Signature		Date	Feb 17 2020

Name of Co-Author	Assistant Professor Heather Mefford		
Contribution to the Paper	Assisted with development of work and manuscript evaluation.		
Signature		Date	Feb 12 2020

Name of Co-Author	Professor Russell Dale		
Contribution to the Paper	Assisted with development of work and manuscript evaluation.		
Signature		Date	Feb 14 2020

Name of Co-Author	Professor Jozef Gecz		
Contribution to the Paper	Assisted with development of work and manuscript evaluation.		
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Name of Co-Author	Laureate Professor Ingrid Scheffer		
Contribution to the Paper	Assisted with development of work and manuscript evaluation.		
Signature		Date	Feb 14 2020

5.3 Published manuscript

Title: Levetiracetam efficacy in PCDH19 girls clustering epilepsy.

Running Title: CHAPTER 5

List of Authors: Sadleir, L. G[a,1]., Kolc, K[b,1]. L., King, C[a]., Mefford, H. C[c]., Dale, R. C[d]., Gecz, J[b,e,f]., Scheffer, I. E[g,h,i].

[1]Equal first authors

Affiliations: [a]Department of Paediatrics and Child Health, University of Otago, Wellington, New Zealand, [b]Adelaide Medical School, The University of Adelaide, SA, Australia, [c]Department of Pediatrics, Division of Genetic Medicine, University of Washington, Seattle, WA, 98195, USA, [d]Kids Neuroscience Centre. Children's Hospital at Westmead, University of Sydney, Australia, [e]Robinson Research Institute, The University of Adelaide, SA, Australia, [f]Healthy Mothers and Babies, South Australian Health and Medical Research Institute, SA, Australia, [g]Department of Medicine, Epilepsy Research Centre, The University of Melbourne, Austin Health, Melbourne, VIC, Australia, [h]Department of Paediatrics, Royal Children's Hospital, The University of Melbourne, VIC, Australia, [i]The Florey Institute and Murdoch Children's Research Institute, Melbourne, VIC, Australia.

Conflict of Interest

The authors declare no conflict of interest.

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Abstract

Background: GCE has a phenotypic spectrum that includes developmental and epileptic encephalopathy. GCE presents with clusters of seizures in the first years of life. Although patients typically outgrow their seizures, many are left with ID. Here we retrospectively assess the effect of levetiracetam in two independent cohorts of females with GCE. Methods: Cohort A was identified by searching our epilepsy genetics research database for girls with GCE who had trialled levetiracetam. Cohort B consisted of girls aged 2 years or older, including women, participating in an international online questionnaire. Information regarding seizure frequency and levetiracetam use was obtained by in-person patient interview and review of clinical records for cohort A, and by patient report for cohort B. Results: Cohort A consisted of 17 females, aged 3-37 years, who had a trial of levetiracetam at an average age of 10.7 years. 13/17 females became seizure free for >12 months; while 10/17 remained seizure free for >24 months. Cohort B comprised 62 females, aged 1.5-41 years. 26/62 became seizure free for >12months, and 19/62 for >24 months on levetiracetam therapy. Discussion: Levetiracetam was effective in two cohorts of females with GCE where 42% and 76% of females became seizure free for >12 months, respectively. Levetiracetam is an effective therapy for females with GCE and should be considered early in the management of the highly refractory clusters of seizures that characterize this genetic disease.

Introduction

PCDH19 causes a distinctive epilepsy syndrome known as GCE.[2, 3] This syndrome has a broad range of phenotypes ranging from a mild self-limited disorder to a highly refractory developmental and epileptic encephalopathy. A recent Scottish epidemiology study found an incidence of *PCDH19* pathogenic variants of 1 in 20,500 live female births; it was the second most common cause of developmental and epileptic encephalopathy in girls.[81] *PCDH19* is an X chromosome gene. It encodes a member of the delta-2 non-clustered protocadherins (d2-PCDHs), which belong to the cadherin super-family of cell-cell adhesion molecules with diverse roles in neuronal migration, neuronal cell specification, or synaptic function.[82] In addition to its canonical role in brain wiring,[51] PCDH19 protein has also been proposed to co-regulate gene expression with estrogen receptor alpha (ERa).[50] While the fine detail of the underlying mechanism(s) of GCE is not yet fully understood, there is mounting evidence suggesting that cellular mosaicism, that is the presence of cells with and cells without functioning PCDH19 protein, is the fundamental disease driver.[51]

Girls with GCE present at a mean age of 11.8 months (range 4-72) with clusters of FIAS and FBTCS.[3, 8, 25, 47, 56, 61] Often triggered by fever, clusters can consist of up to 30 seizures and last 1-14 days.[8, 11, 22, 25, 28, 56, 83] Inter-cluster intervals last weeks to months.[3, 8, 11, 22, 25, 28, 56, 83] Typically, development is normal at onset of seizures and may regress with clusters.[3, 8, 11, 25] Initially skills can be regained during the inter-cluster interval but eventually ID may be evident.[8, 11, 25, 64] Seizures often cease in the second to third decade.[3, 8, 11, 25, 28, 56, 63, 64] Two-thirds of women have ID, which ranges from mild to severe. A significant number have psychiatric comorbidities such as ASD and psychosis.[8, 11, 25, 28, 63, 64] Seizure onset before 12 months of age is associated with a poorer cognitive outcome.[56, 64]

Preventing and aborting clusters of seizures is difficult and, despite trials of multiple AEDs, girls often require recurrent hospital admissions.[56] Through clinical practice, we noted remarkable seizure control with levetiracetam for several girls with drug-resistant GCE. Here, we report the impact of levetiracetam on girls with GCE in two cohorts: a research cohort in which detailed historical clinical information was available and an international cohort of individuals with *PCDH19* variants who were surveyed via an

online questionnaire

Method

We evaluated response to levetiracetam in two cohorts of patients with GCE.

Cohort A

We searched our Australian and New Zealand Epilepsy Genetics research database for individuals with epilepsy and pathogenic variants in *PCDH19* who had received a trial of levetiracetam. The parents of each girl were interviewed using a standardized epilepsy questionnaire[84] and seizure videos were reviewed where available. All medical records, EEGs, and neuroimaging were obtained. Seizures and epilepsy syndromes were diagnosed according to the 2017 International League Against Epilepsy classification.[85, 86] Clinical records were analyzed for seizure frequency and AED history.

Cohort B

We conducted a large online international survey of patients with *PCDH19* pathogenic variants; only patients who had trialed levetiracetam were included in this study. We invited families with girls aged 2 years or older to participate. Patients were recruited via our international collaborators and PCDH19 family associations. The survey was carried out between April 2017 and March 2019 and was available in English, Italian, or French. Participants were proficient in one of these languages. The survey was completed by affected women of normal intellect, or a parent or carer in the case of minors or women with ID. Families provided their specific *PCDH19* variant and results of any other genetic testing. Patients were excluded if they had any of the following:

- an additional pathogenic variant(s) in another gene(s)
- their *PCDH19* variant was likely benign based on: frequency of their variant in gnomAD (http://gnomad.broadinstitute.org/) and *in silico* prediction tools CADDv1.3 (including Polyphen2[87], GPP[88], MutPred[89], Mutation Assessor[90], PROVEAN[91], and SIFT[92]) through the web-based ANNOVAR (http://wannovar.wglab.org/).

For cohort B, participants were asked questions relating to seizure frequency and AED

history. Individuals were asked to list all the AEDs ever trialled, and to specify if any had resulted in seizure freedom and for how long.

Written or online informed consent was obtained from all patients or their parents or legal guardians in the case of minors or those with ID. The study was approved by the New Zealand, Austin Health, and University of Adelaide Ethics committees.

Results

Cohort A

We identified 40 females with epilepsy due to *PCDH19* pathogenic variants from 19 Australian and New Zealand families. 17/40 (43%) females had trialed levetiracetam. Adequate efficacy data were available for 14 girls (Table 5.1), and for 3, behavioral exacerbation meant that levetiracetam was discontinued before seizure efficacy could be assessed. The 14 girls who had an adequate trial of levetiracetam were previously drug-resistant; they had an average of 5 AEDs prior to the introduction of levetiracetam. The average age at trial of levetiracetam was 10.7 years. Of the 17 girls that trialed levetiracetam, 13/17 (76%) became seizure free for 12 months or more; 10 (59%) were seizure free for at least 24 months. For nine girls, a detailed yearly analysis of seizure burden and AED history was available from the medical history and revealed a striking effect of levetiracetam (Fig. 5.1). For the remaining five girls, detailed yearly seizure burden information was not available.

Table 5.1 Clinical information for cases who had adequate trial of levetiracetam

Name	*Family*	*Case*	*Age at study*	*Age of seizure onset*	*Initial seizure type*	*Other seizure types*	*Age delay first noted*	*ID*	*ASD*	*Psychosis*	*Prior AEDs*	*Age LEV started*	*Duration of seizure freedom on LEV*	*Age of seizure offset*	*Subsequent AEDs*	*PCDH19 variant*
CP	A	A1	13y	10m	FIAS	Nil	3.3y	Severe	Y	N	CLB, CBZ, LTG, VPA, TPM	5.1y	7.9y	5.2y	None	c.497_498insA p.Tyr166X
JP	A	A2	13y	10m	FIAS	Nil	3.4y	Mild	N	N	CLB, CBZ, LTG, TPM	5.3y	7.7y	5.1y	None	c.497_498insA p.Tyr166X
JR	B	B3	17.5y	16m	FIAS	FBTCS	3.7y	Borderline	N	N	CLB, CBZ, TPM, VPA, LTG	4.8y	5.6y	11.8y	None	c.1919T>G p.Leu640Arg
AB	C	C4	22y	19m	TS	FIAS, FBTCS	1.5y	Mild	Y	Y	PRD, CLB, CBZ, VPA, ACTH, PB, PT, AZD	20y	1y	21y	None	Exon 6 deletion
SB	C	C5	31y	14m	FBTCS	FIAS	1.1y	Mild	Y	Y	PB, PHT, CLB, AZD, CBZ, LTG, VPA, TPM, CZP	22y	9y	22y	None	Exon 6 deletion
CJ	D	D6	8.9y	11m	GTCS	FIAS, FMS, FBTCS	2.9y	Normal	N	N	VPA, CZP	2.9y	3.1y	7.5y	None	c.1031G>A p.Pro344Leu
MK	E	E7	27y	8m	FIAS	GTCS	5y	Moderate	N	Y	CBZ, PHT, CZP, PHT, DZP, VPA, CLB	Unknown	8y	19y	None	c.2534C>T p.Ser845Asn
HK	F	F8	35y	9m	FMS	FIAS	9m	Mild	N	Y	VPA, NZP, VGB, LTG, GP, PHT, PB, TPM, TGB, CZP, CBZ	28y	4y	31y	None	c.2123_2124del p.Lys708ArgfsX9
KK	F	F9	3.3y	7m	TS	FIAS, FBTC	7m	DD	N	N	None	7m	1y	Ongoing	TPM	c.2123_2124del p.Lys708ArgfsX9
RV	G	G10	17.6y	8m	TS	FAS, GTCS	8m	Moderate	Y	Y	OX, CZP	12y	4.7y	13y	None	c.74T>C p.Leu25Pro
AK	H	H11	32y	24m	FMS	FIAS	Unknown	Mild	N	N	CBZ, LTG, VPA, TPM	16y	16y	16y	None	c.1671C>G p.Asn557Lys
GS	I	H12	9.5y	9m	GTCS	TS, FBTCS, FIAS	2.4y	Mild	Y	N	CLB, LTG, VPA, PRD	1.8y	1.2y	Ongoing	None	c.1019A>G (het) p.Asn340Ser
PC	J	J13	4.3y	18m	FBTCS	FIAS, TS, FMS	1.5y	DD	Y	N	None	1.5y	NA	Ongoing	CZP, OX, VPA, PHT, TPR, CLB, CBD	c.496_498delinsAA p.Tyr166Lys
TK	K	K14	17y	17m	FIAS	FIAS, FMS, FBTCS	3y	Borderline	N	Y	VPA, CBZ, CLB	13y	2y	Ongoing		c.498C>A p.Tyr166X

In the Family column, the superscript number denotes the reference number of the paper in which this individual has been previously published. The ID denotes the specific patient identification number in that publication. *ASD*, autism spectrum disorder; *AEDs*, antiepileptic drugs; *y*, years; *m*, months; *Y*: yes; *N*, no; *NA*, not affective; *PCDH19*, protocadherin-19, *DD*, developmental delay; *ID*, intellectual disability; *FIAS*, focal impaired awareness seizure; *FBTCS*, focal to bilateral tonic-clonic seizure; *TS*, tonic seizure; *GTCS*, generalised tonic clonic seizure; *FMS*, focal motor seizure; *LEV*, Levetiracetam; *CLB*, clobazam; *CBZ*, carbamazepine; *LTG*, lamotrigine; *VPA*, sodium valproate; *TPM*, Topiramate; *PRD*, pyridoxine; *ACTH*, adrenocorticotropic hormone; *PB*, phenobarbital; *AZD*, acetazolamide; *PHT*, phenytoin; *CZP*, clonazepam; *DZP*, diazepam; *VGB*, vigabatrin; *GP*, gabapentin; *NZP*, nitrazepam; *TGB*, tiagabine; *OX*, oxcarbazepine; *CBD*; cannabidiol; *ETX*, ethosuximide.

Case example

Patient 3 is an 18-year-old adolescent who presented at age 14 months with a cluster of 11 focal seizures over 2 days. In retrospect she had had clusters of brief FIAS from age 9 months that were thought to be day dreaming. She continued to have clusters of 11-60 seizures over 2-11 days every 3 months. Her early development was normal. She walked at 14 months and her first words were at 9 months. During clusters her language and development would regress however, in her first 4 years, development would return to normal within 2 weeks of a cluster. By her fifth year, cognitive assessment showed that her verbal abilities were in the "low average" range and non-verbal abilities in the "mildly impaired" range (1st percentile). She was drug-resistant to clobazam, carbamazepine, topiramate, valproate, and lamotrigine, and her longest seizure-free period was 4 months. In the four years prior to levetiracetam, she had 245, 129, 202 and 259 seizures per year, respectively resulting in 12, 38, 34 and 27 days in hospital over 15 admissions. Levetiracetam was started just before her 5th birthday and controlled her seizures. She has not required any hospital admission subsequently. She had 4 seizures in her 6th year but after the levetiracetam dose reached 60 mg/kg/day she only had rare break-through seizures with growth at age 8 and 11 years. She has been seizure free since.

Cohort B

Eighty-nine females or their carers completed the online questionnaire. Of these, 82 had epilepsy and 62 had trialed levetiracetam. The average age when the survey was completed was 11.5 years (1.5 - 41 years). The average age of seizure onset was 10.3 months (1.5 - 28 months). The majority (59/62, 95%) had been admitted to hospital at least twice with 68% (42/62) having at least 5 admissions. Nineteen cases (31%) had at least one episode of SE. 26/62 (42%) reported becoming seizure free for at least 12 months with levetiracetam. This included 19 (31%) who were seizure free for 2-16 years. An additional 4 girls (6%) reported 4-12 months seizure freedom with levetiracetam. Duration of seizure freedom was associated with age at survey completion, with older girls more likely to report seizure freedom on levetiracetam ($t_{(43.7)} = 2.47$, $p = .018$).

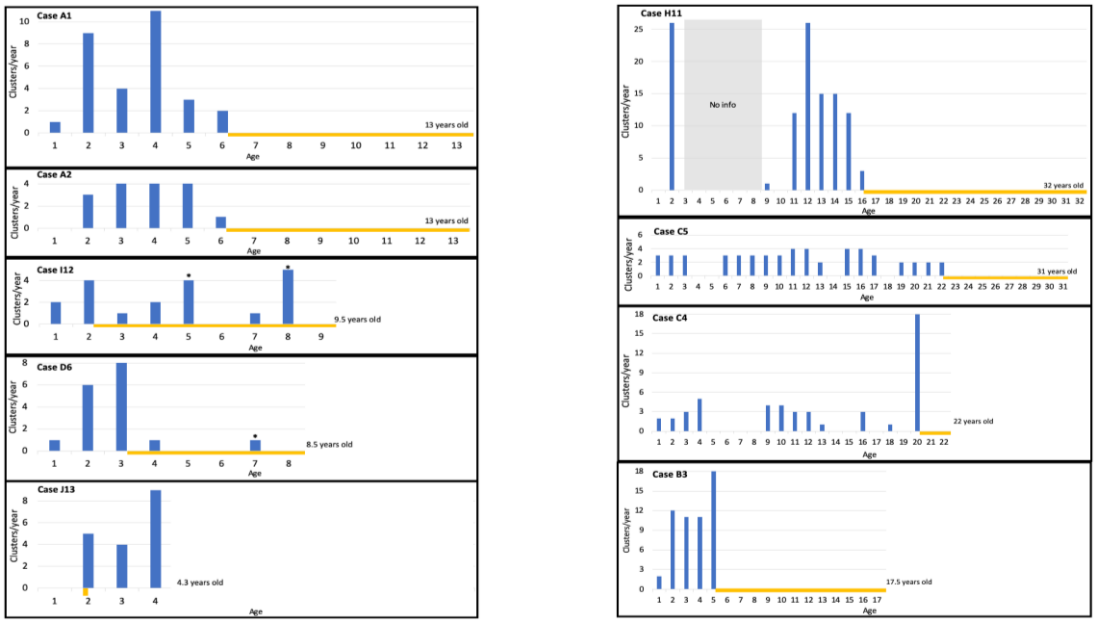

Figure 5.1 Seizure clusters (blue bars) for nine patients showing a marked reduction in cluster frequency with the introduction of levetiracetam (yellow bar). A) Girls younger than 14 years of age. B) Adolescent and adult patients. Length of X axis denotes the current age of the patient. Grey box indicates that no hospital records were available for this period.

Discussion

GCE has a characteristic clinical presentation where typically developing infant girls present with clusters of seizures, which often prove highly refractory.[11, 25] Earlier age of seizure onset is associated with poorer cognitive outcome.[56, 64] Although there are no studies to show that the seizure burden per se impacts negatively on their development, the regression of development associated with more severe clusters suggests that seizure load contributes independently to poor cognitive outcome. Initiating effective AEDs early is therefore likely to have a positive effect on developmental outcome. There are no published clinical trials of AEDs in females with GCE. A small series of 5 children with GCE reported a reduction in the duration of a cluster with the use of IV corticosteroid therapy however ongoing steroids did not prevent future clusters.[83] A retrospective multicentre review of AED therapy in 58 females with GCE from 12 countries reported that the most effective AEDs were clobazam and bromide.[93] Clinicians completed a questionnaire based on parental reports of seizure frequency. The age at trial of AEDs and the order of AED use was not reported. Here we report the effect of levetiracetam in two cohorts of females with GCE. Following independent recognition by two authors (LGS and IES) in clinical practice of the remarkable effect of levetiracetam in girls with GCE, we searched our epilepsy research database for all girls treated with levetiracetam (cohort

A) to ascertain if this was coincidental. Levetiracetam resulted in at least 12 months' seizure freedom in 76% of cohort A. In an independent cohort (cohort B), 42% of females reported seizure freedom for 12 months or more with levetiracetam. Although the data from both cohorts is retrospective, the seizure freedom rates are considerably higher than those reported in ten randomised placebo-controlled trials of levetiracetam in focal epilepsies where only 2-18% of individuals became seizure free for 3-6 months.[50, 94-104] In previous clinical series of GCE, a positive effect of levetiracetam has been reported in individual cases but was not systematically assessed.[4, 28, 56] Delving into the effect of levetiracetam in the report by Lotte and colleagues, 38 cases had at least a 3 month trial of levetiracetam of which 13 (34%) had a 50% or more reduction in seizures and 5 (15%) became seizure free.[93] Fourteen cases trialed levetiracetam for 12 months with 8 (57%) obtaining a 50% reduction in seizures and 3 (21%) being seizure free. Our cohort A, which was ascertained in a similar manner, demonstrated a much greater effect of levetiracetam (76% seizure free for12 months) than reported in the Lotte et al. study.[93] A greater positive effect (42% seizure free for 12 months) was also seen independently in cohort B. Identifying AEDs that are effective in GCE is complicated as the natural history of this distinctive syndrome is that most girls become seizure free in adolescence.[3, 4, 11, 56] Therefore, retrospective reports of which AED controlled seizures are confounded by the age at which the AED was introduced, as it may have coincided with the age at which the girls would naturally outgrow their seizures. Seizure cessation may then be inappropriately attributed to the AED in use at that time. Age of specific AED introduction was not available for cohort B nor was it provided in the Lotte study.[93] To definitively ascertain if an AED is effective, randomised, double-blind, trials of either placebo or proven alternative treatments are required. However, in our research cohort A, the average age at which levetiracetam was introduced was 10.7 years, which is younger than studies show most girls become seizure free.[3, 8, 11, 25, 28, 56, 63, 64] Of the 7 cases in cohort A who trialed levetiracetam prior to their 6th birthday, six became seizure free for at least 1 year, and four only had break through seizures with iatrogenic reduction of the levetiracetam dose or when the girl grew (Fig. 5.1). This provides support to the effect being real and not due to natural seizure remission.

Why Levetiracetam may be beneficial in GCE can only be speculated. Evidence from iron-chloride induced epilepsy in rats suggests that levetiracetam leads to suppression of glutamate overflow and enhancement of GABAergic inhibition.[105] While the suppression of the glutamate overflow effect could be explained through levetiracetam modulation of the synaptic vesicle glycoprotein 2A (SV2A) function, the modulation of GABAergic inhibition is less clear. The fact that the PCDH19 protein has recently been shown to physically interact with gamma aminobutyric acid alpha receptor ($GABA_AR$)[106] may lead to testable hypotheses in this regard.

In conclusion, the data from our two independent cohorts suggest that levetiracetam is an effective AED for GCE, rendering some girls seizure free. A randomized, double-blind, placebo-controled trial is required to prove the efficacy of levetiracetam in GCE but, given our promising data, levetiracetam should be considered early in the management of this severe disorder.

Chapter 6: **PCDH19 variants in males – Expanding the phenotypic spectrum**

6.1 Preamble

When GCE (EFMR, at the time) was first described in 1971 by Juberg and Helman, a phenotype was only evident among females in the original family. Subsequent reports describe a “female-limited” disorder with “male-sparing”. It was not until 2009, that the first male was identified by Depienne and colleagues. Since then, only 12 males have been described with *PCDH19* pathogenic variants and a GCE-like phenotype. In most cases, a single case is reported and no large cohorts have been presented. As such, males represent an under-recognized group. Using the information gleaned from the second study, I conduct a precise and comprehensive analysis of phenotypic abnormalities in the largest cohort of *PCDH19*-variant males to date. This work will facilitate the identification of males with GCE, useful to both families and clinicians. GCE can no longer be considered a disorder limited to females and has been re-named X-linked clustering epilepsy (XCE) to facilitate the growing number of affected male cases. Therefore, these findings expand the phenotypic spectrum of this disorder.

6.2 Statement of authorship

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Principal author

Name of Principal Author (Candidate)	Kristy Kolc		
Contribution to the Paper	Major contribution to the research question. Designed study, performed data collection, analysis, and interpretation, prepared manuscript, and performed all required revisions.		
Overall percentage (%)	80%		
Certification:	This manuscript reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this manuscript.		
Signature		Date	Feb 14 2020

Co-Author contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Dr Raman Kumar		
Contribution to the Paper	Supervised development of work and manuscript evaluation.		
Signature		Date	Feb 13 2020

Name of Co-Author	Dr Rikke Møller		
Contribution to the Paper	Provided patient samples and manuscript editing.		
Signatur		Date	Feb 13 2020

Name of Co-Author	Professor Lynette Sadleir		
Contribution to the Paper	Assisted with development of work and manuscript evaluation.		
Signature		Date	Feb 13 2020

Name of Co-Author	Laurette Professor Ingrid Scheffer		
Contribution to the Paper	Assisted with development of work and manuscript evaluation.		
Signature		Date	Feb 14 2020

Name of Co-Author	Professor Jozef Gecz		
Contribution to the Paper	Supervised development of work, assisted with design, data interpretation, and manuscript evaluation.		
Signature		Date	Feb 13 2020

6.3 Submitted manuscript

Title: PCDH19 pathogenic variants in males: Expanding the phenotypic spectrum.

Running Title: CHAPTER 6

List of Authors: Kristy L Kolc[1,2], Rikke S Møller[3,4], Lynette G Sadleir[5], Ingrid E Scheffer[6,7,8], Raman Kumar[1,2], and Jozef Gecz[1,2,9]

Affiliations: [1]Adelaide Medical School, the University of Adelaide, SA, Australia, [2]Robinson Research Institute, the University of Adelaide, SA, Australia, [3]Department of Epilepsy Genetics and Personalized Medicine, The Danish Epilepsy Centre, Dianalund, Denmark, [4]Department for Regional Health Research, University of Southern Denmark, Odense, Denmark, [5]Department of Paediatrics and Child Health, University of Otago, Wellington, New Zealand, [6]Department of Medicine, Epilepsy Research Centre, The University of Melbourne, Austin Health, Melbourne, VIC, Australia, [7]Department of Paediatrics, Royal Children's Hospital, The University of Melbourne, VIC, Australia, [8]The Florey and Murdoch Institutes, Melbourne, VIC, Australia, [9]Healthy Mothers and Babies, South Australian Health and Medical Research Institute, South Australia, Australia.

Conflict of Interest

Ingrid Scheffer serves/has served on the editorial boards of the Annals of Neurology, Neurology and Epileptic Disorders; may accrue future revenue on pending patent WO61/010176 (filed: 2008): Therapeutic Compound; has a patent for SCN1A testing held by Bionomics Inc and licensed to various diagnostic companies; she has a patent molecular diagnostic/theranostic target for benign familial infantile epilepsy (BFIE) [PRRT2] 2011904493 & 2012900190 and PCT/AU2012/001321 (TECH ID:2012-009) with royalties paid. She has served on scientific advisory boards for UCB, Eisai, GlaxoSmithKline, BioMarin, Nutricia, Rogcon and Xenon Pharmaceuticals; has received speaker honoraria from GlaxoSmithKline, Athena Diagnostics, UCB, BioMarin, Biocodex and Eisai; has received funding for travel from Athena Diagnostics, UCB, Biocodex, GlaxoSmithKline, Biomarin and Eisai. She receives/has received research support from the National Health and Medical Research Council of Australia, Health Research Council of New Zealand, CURE, March of Dimes and NIH/NINDS.

The remaining authors declare no conflict of interest.

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Abstract

PCDH19 pathogenic variants cause an infantile onset epilepsy syndrome called GCE due to the vast majority of affected individuals being female. This syndromic name was developed to foster early recognition and diagnosis in infancy. It has, however, sparked debate, as, there are rare males with postzygotic somatic, and therefore, mosaic, *PCDH19* pathogenic variants with similar clinical features to females. Conversely, "transmitting" males with germline inherited *PCDH19* variants are considered asymptomatic. To date, there has been no standardized neuropsychiatric assessment of males with *PCDH19* pathogenic variants. Here, we studied 15 males with *PCDH19* pathogenic variants (nine mosaic and six transmitting) aged 2 to 70 years. Our families completed a survey including standardized clinical assessments: SRS-2, SDQ, BRIEF, and DOCS. We identified neuropsychiatric abnormalities in two males with germline *PCDH19* possibly pathogenic variants. One had a prior history of a severe encephalopathic illness, which may have been unelated. We also describe a non-penetrant somatic mosaic male with mosaicism confirmed in blood, but not identified in skin fibroblasts. Our data suggest that transmitting hemizygous males are generally unaffected, in contrast to males with postzygotic somatic mosaic variants who show a similar neuropsychiatric profile to females who are naturally mosaic, due to XCI. The penetrance of *PCDH19* pathogenic variants has been estimated to be 80%. Like females, not all mosaic males are affected. From our small sample, we estimate that males with mosaic *PCHD19* pathogenic variants have a penetrance of 85%. With these insights into the male phenotypic spectrum of *PCDH19* epilepsy, we propose the new term X-linked Clustering Epilepsy (XCE) to highlight the striking X-linked pattern of affected females with males being spared, unless they are mosaic. Both affected females and males typically present with infantile onset of clusters of seizures.

Introduction

Pathogenic variants in *PCDH19*, encoding protocadherin-19, cause an infantile onset epilepsy syndrome called GCE, as it typically presents in girls with clusters of seizures. Affected females often have ID, executive dysfunction, and a range of psychiatric disorders including ASD, OCD, and ADHD.[1, 3, 64] Mosaicism describes an individual with two or more populations of cells and can develop at any point following fertilization.[107] With regard to *PCDH19* epilepsy, all females are effectively mosaic due to inactivation of one of their two X chromosomes, resulting in some cells containing wild-type *PCDH19* and some containing variant *PCDH19.* Males are typically hemizygous having only one X chromosome, however, when they have a postzygotic variant of *PCDH19* they also become mosaic and are phenotypically similar to affected females. Twelve males with postzygotic somatic *PCDH19* variants[4-6, 17, 34, 108] and one male with Klinefelter syndrome and a heterozygous *PCDH19* pathogenic variant[109] have been identified with a similar phenotype to females. Although the reported clinical features of mosaic males are similar to affected females,[64] there has been no standardized neuropsychiatric assessment of males with *PCDH19* pathogenic variants.

Males who inherit a germline loss-of-function *PCDH19* variant are generally asymptomatic and referred to as "transmitting" males.[1, 29, 30] However, there have been four transmitting males reported with neuropsychiatric abnormalities.[16, 35, 36] One male with High Functioning ASD (previously known as Asperger's syndrome), inherited his probably damaging missense variant from his asymptomatic mother,[16] another male with ASD had unknown inheritance of his probably damaging missense variant,[35] and two cases with ID.[36] With such a small number of reported males, it is unclear if these clinical features are caused by the germline *PCDH19* variant. Here, we report the phenotypic profile of 15 mosaic and transmitting males with *PCDH19* pathogenic variants using standardized assessments conducted in conjunction with our PCDH19 survey.[64]

Method

Participants

Our 15 male participants were ascertained through the international PCDH19 survey.[110] This cohort comprised 9 mosaic males (#1, #2, #3, #4, #5, #6, #7, #8, #9), with an age

range of 2 to 41 years (*M* = 9.67, *SD* = 12.2) and 6 transmitting males (#10, #11, #12, #13, #14, #15), with an age range of 16 to 70 years (*M* = 44.0, *SD* = 21.5). English speaking participants were from Ireland (1), Argentina (1), Republic of Moldova (1), United States (3), United Kingdom (1), Israel (1), Denmark (2), Netherlands (1), and Australia (1). Three participants from Italy spoke Italian and no English. Thirteen were Caucasian, with the remaining two Hispanic or Africana. Variant pathogenicity was determined by gnomAD (http://gnomad.broadinstitute.org/) frequency and *in silico* assessment through the web-based ANNOVAR (http://wannovar.wglab.org/).

PCDH19 Survey

The PCDH19 survey was developed and scored as previously described[110] and incorporated the following: SRS-2[65] for the assessment of ASD-related symptoms, SDQ[67] for the assessment of ADHD-related symptoms, BRIEF[111] for the assessment of executive dysfunction, and DOCS[70] for the assessment of OCD-related symptoms. Demographic, development, variant, medication, and seizure information was captured using the EQ.[110] Participants or parents responding on behalf of their child were contacted to clarify or obtain additional information. Where possible, the treating clinician was also consulted to verify reported information. Italian translation of the EQ, survey scripts, and study material were performed and checked by either a professional translator or by an individual familiar with GCE and fluent in the relevant languages. Published authorized translations of the SRS-2, BRIEF, and DOCS were utilized. License agreements were obtained to reproduce assessments in an online format. The project was approved by the University of Adelaide Human Research Ethics Committee (H-2016-184). Electronic informed consent was obtained from all participants or their parents or legal guardians in the case of minors or those with ID.

Results

Males with mosaic PCDH19 variants

Five of the nine mosaic males represented in the survey harbored novel *PCDH19* variants (#2, #3, #5, #6, #8). These included two nonsense (p.Glu574*; p.Tyr516*) and three missense (p.Arg198Pro, p.Asp230His, p.Asn340Lys) variants affecting the EC domain, which is a highly conserved region of the PCDH19 protein.[10] All five variants were

predicted to be likely pathogenic by *in silico* assessment.[110] The remaining were previously published and included: nonsense (1), missense (2), and splice-site (1) *PCDH19* pathogenic variants. Inheritance information showed that the *PCDH19* variant arose *de novo* in eight males and was not available for one individual (Table 6.1).

Seizures presented in clusters for eight of the nine mosaic males. For the eight mosaic males with seizures, the median age at seizure onset was 10 months. One male (#8) experienced seizure onset at 8 years of age. This is the latest seizure onset recorded for an individual with XCE. His seizures occurred in clusters for an average of 1 day, with approximately 5 seizures per day in a cluster, according to parent report. His seizures remain ongoing at 12 years of age. The mosaic nature of this variant was confirmed in saliva and predicted to be deleterious.

One mosaic male (#9) had never had a seizure and no prior neuropsychiatric diagnoses. This male harbors a missense variant (c.1240G>A; p.Glu414Lys) that was transmitted to his daughter, who is affected (#28 in [110]). We tested the blood DNA of this male and detected the *PCDH19* variant allele in approximately the same ratio as the wild-type allele, that is 50:50. The daughter of #9 displayed the typical features associated with *PCDH19* pathogenic variants, with seizure onset at 8 months of age, normal early development, autistic features, and mild ID. This variant is recurrent and has been reported in a female with seizures between 14 months and 5 years of age, aggression, and ID.[24] The variant was inherited from an asymptomatic mother and was predicted to be deleterious by three independent *in silico* tools.[24]

Transmitting males with a germline PCDH19 variant

Six males with inherited *PCDH19* variants were included. Four scored in the normal range on all neuropsychiatric measures (Table 6.1). Two males had neuropsychiatric abnormalities. The first male (#11) was a 23-year-old with mild ID, ASD, and impulsive behavior. His parent reported first behavioural concerns at 12 months. He had no seizures. His parent and physician report that, despite his severe ASD, he has well-developed language skills. Our assessment revealed a global executive composite score that was greater than 99% of scores from the standardization sample of 18-29 year-olds, indicative of an executive functions deficit. Although all domains were affected, the shift domain was especially elevated. Such inflexibility and rigidity is common in ASD and would

therefore be expected given his severe ASD. Scores on the SRS-2 supported the presence of ASD, with a total score in the moderate range. Elevation was also observed in the two DSM-5 equivalent domains; social communication and interaction and restricted interests and repetitive behavior. This male was reported to have a *de novo* frameshift variant (c.2341dup; p.Ile781Asnfs*3). This recurrent variant has been previously reported in a female with refractory seizure clusters and attention-deficit features.[25, 31]

The second male was 16 years old. The following medical information was gained from parental report. He was a healthy boy with normal development until 9.5 years of age when he developed febrile infection-related epilepsy syndrome (FIRES).[112] Following a prodromal illness he presented with rapidly escalating tonic-clonic seizures requiring a phenobarbital induced coma for a month. Serial MRI scans showed progressive atrophy. Investigations for infectious and autoimmune etiologies were negative. Following the acute illness, he was left with motor and cognitive neurological sequelae, as well as pharmaco-resistant focal epilepsy. It took more than a year for him to regain walking and he remains ataxic with foot drop. He has almost monthly focal seizures consisting of a visual aura which can progress to a bilateral tonic-clonic seizure despite felbamate and phenytoin. EEGs showed multifocal epileptiform discharges. Our neuropsychiatric assessment found normal executive functions, mild ASD symptoms, and no other behavioral disturbances. This profile is consistent with a diagnosis of FIRES and may not relate to the *PCDH19* variant. This male was reported to have a missense *PCDH19* variant (c.1672 G>C; p.Asp558His) inherited from his unaffected mother. The variant is located in the calcium-binding domain (important for protein structure/folding). There is a previous report of this variant as likely pathogenic in a commercial gene company series (GeneDx) so this may be the same individual.[74]

Table 6.1 Clinical characteristics of *PCDH19* males

General			Seizures					Neuropsychiatric comorbidities									*PCDH19* Variant				
Pt.	*Age*	*Zygosity*	*Onset*	*SE*	*Clusters*	*Isolated*	*Hosp.*	*ICU*	*ID*	*ED*	*EP*	*CoP*	*ADHD*	*PP*	*SD*	*ASD*	*OCD*	*cDNA; Protein*	*Type (loc)*	*Inheritance*	*Novel*	*In silico Prediction*
1	2.5y	Mosaic	22m	-	+	-	Once	Yes	No	No	Av	Mild	Mild	Sev	Sev	No	NC	c.2656C>T; p.Arg886*	Non (CP)	*De novo*	-	Deleterious[10, 16]
2	2y	Mosaic	8m	-	+	-	Twice	No	No	No	Av	Sev	Mild	Av	Mild	No	NC	c.1720G>T; p.Glu574*	Non (EC6)	*De novo*	+	Likely pathogenic
3	5y	Mosaic	5m	-	+	+	5+	Yes	Sev	Sev	Sev	Mod	Mod	Sev	Sev	Sev	NC	c.593G>C; p.Arg198Pro	Miss (EC2)	*De novo*	+	Likely pathogenic
4	10y	Mosaic	11m	-	+	-	5+	No	Sev	Sev	Av	Mild	Mod	Sev	Sev	Sev	NC	c.2147+2T>C; p.?	(Ex/Int1)	*De novo*	-	Deleterious[34]
5	5y	Mosaic	8m	-	+	-	5+	Yes	No	No	Av	Av	Mild	Mod	Sev	Mild	NC	c.688G>C; p.Asp230His	Miss (EC2)	*De novo*	+	Likely pathogenic
6	2.5y	Mosaic	5m	-	+	-	5+	No	Mild	Mod	Av	Av	Sev	Mod	Sev	Sev	NC	c.1020T>A; p.Asn340Lys	Miss (EC3)	*De novo*	+	Likely pathogenic
7	7y	Mosaic	10m	-	+	-	5+	Yes	No	No	Av	Av	Av	Av	Mild	No	NC	c.1352C>T; p.Pro451Leu	Miss (EC4)	*De novo*	-	Deleterious[5]
8	12y	Mosaic	96m	-	+	+	5+	No	Bord	Mod	Mod	Av	Av	Sev	Sev	Mod	NC	c.1548C>A; p.Tyr516*	Non (EC5)	Unknown	+	Likely pathogenic
9	41y	Mosaic	NA	NA	NA	NA	NA	NA	No	No	NC	NC	NC	NC	NC	Mod	No	c.1240G>A; p.Glu414Lys	Miss (EC4)	*De novo*	-	Deleterious[24]
10	16y	Hemi	114m	-	+	+	Once	Yes	No	No	Mod	Av	Av	Mod	Av	Mild	NC	c.1672G>C; p.Asp558His	Miss (EC5)	Maternal	-	Deleterious[74]
11	23y	Hemi	NA	NA	NA	NA	NA	NA	Mild	Sev	NC	NC	NC	NC	NC	Mod	NC	c.2341dup; p.Ile781Asnfs*3	F/S (CP)	Maternal	-	Deleterious[25, 31]
12	63y	Hemi	NA	NA	NA	NA	NA	NA	No	No	NC	NC	NC	NC	NC	No	No	c.1671C>G; p.Asn557Lys	Miss (EC5)	Unknown	-	Deleterious[2, 56]
13	70y	Hemi	NA	NA	NA	NA	NA	NA	No	No	NC	NC	NC	NC	NC	No	*No	c.370G>A; p.Asp124Asn	Miss (EC1)	Maternal	-	Deleterious[24]
14	41y	Hemi	NA	NA	NA	NA	NA	NA	No	No	NC	NC	NC	NC	NC	No	No	c.1683_1696del; p.Val562Thrfs*4	F/S (EC5)	Unknown	-	Deleterious[74]
15	51y	Hemi	NA	NA	NA	NA	NA	NA	No	No	NC	NC	NC	NC	NC	No	No	c.1463T>A; p.Val488Asp	Miss (EC5)	Unknown	+	Likely pathogenic

Abbreviations: *ADHD*, attention-deficit hyperactivity disorder; *ASD;* autism spectrum disorder; *Av*, average; *Bord*, borderline; *CoP*, conduct problems; *CP*, cytoplasmic domain; *EC*, extracellular cadherin domain; *ED*, executive dysfunction; *EP*, emotional problems; *Ex*, exon; *F/S*, frameshift; *Hemi*, hemizygous; *Hosp.*, hospitalizations; *ICU*, Intensive Care Unit admissions; *ID*, intellectual disability; *Int*, intron; *isolated*, presence of non-clustering seizures; *loc*, location; *m*, months; *Miss*, missense; *Mod*, moderate; *NA*, not applicable; *NC*, not covered; *Non*, nonsense; *NP*, not provided; *OCD*, obsessive compulsive disorder; *PP*, peer problems; *Pt*, participant; *SD*, social deficits; *SE*, status epilepticus; +, present; -, absent; *Sev*, severe

*Score just below the clinical threshold (18 or higher)

Neuropsychiatric profile

Executive dysfunction occurred in 44% (4/9) mosaic males and one transmitting male (#11). All five individuals had a diagnosis of ID. As the SDQ is only available to individuals 18 years and younger, results from this measure were obtained for 8/9 mosaic and 1/6 transmitting males. This one transmitting male scored in the moderate range for emotional and peer problems and in the average range for all other measures. Emotional problems were recorded for 25% (2/8), conduct problems for 50% (4/8), inattention/hyperactivity for 75% (6/8), peer problems for 75% (6/8), and social deficits were recorded for all eight mosaic males. Sixty seven percent of mosaic males (6/9) including the non-penetrant male (#9) and two transmitting males (#10, #11) scored in the ASD clinical range. DOCS scores were all in the normal range, however, one male (#13) attained a total score just below the clinical threshold for OCD. Elevation in the harm, thoughts, and order domains were reported by this individual. The remaining transmitting males (#12, #14, and #15) scored within the normal range on all relevant measures. The neuropsychiatric profile of our mosaic males was similar to affected females (Fig. 6.1).[110]

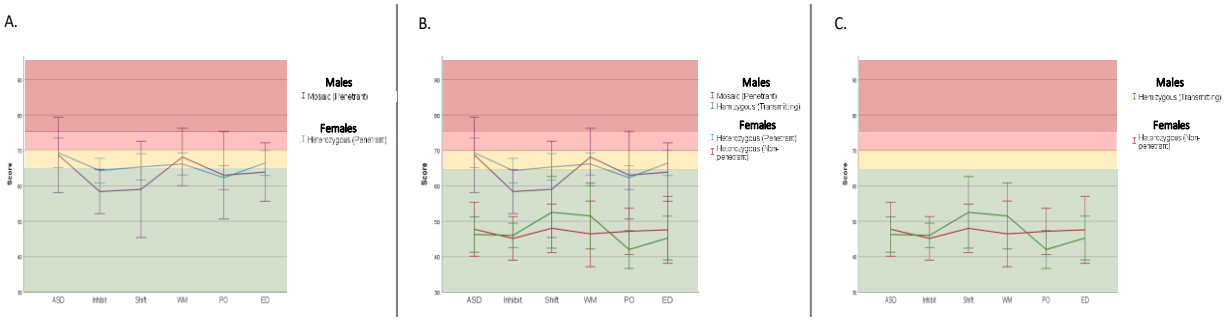

Figure 6.1 Neuropsychiatric profile of males with *PCDH19* pathogenic variants based on average (±2 SEM) SRS-2 total and BRIEF total and subscale *t* scores. A) mosaic males B) males compared to the females from our previous publication,[110] and C) hemizygous males. Green shaded region represents the normal range; yellow, mildly elevated; orange, moderately elevated; and red represents severely elevated *t* scores. *ASD*; autism spectrum disorder; *ED*, executive dysfunction; *PO*, plan/organize; *WM*, working memory.

Our mosaic male (#9) without seizures had an SRS-2 score in the moderate range. Scores in this range indicate deficiencies in reciprocal social behavior that are clinically significant and lead to substantial interference with everyday social interactions. This finding was supported by an elevated score on the BRIEF shift domain and a slightly elevated score on the DOCS order domain, which indicates concerns in the area of mental flexibility and a need for order/symmetry, respectively. Sanger sequencing of blood-derived DNA showed somatic mosaicism of his *PCDH19* variant (c.1240G>A), however, *PCDH19* mosaicism could not be detected in fibroblast-derived DNA (Fig. 6.1).

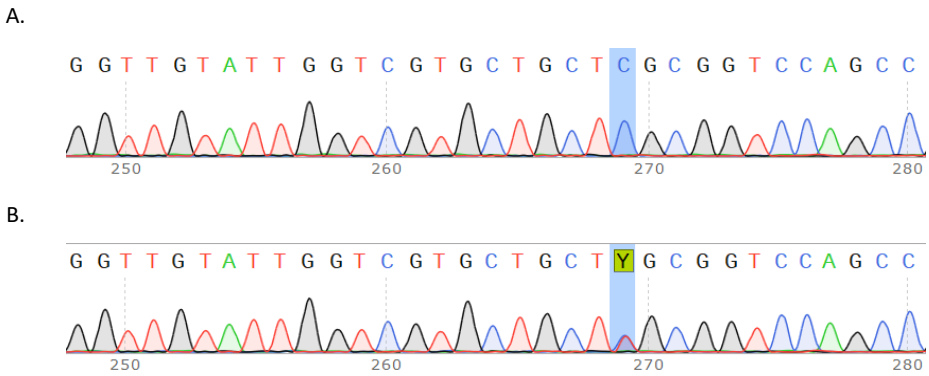

Figure 6.2 gDNA Sanger sequence illustrating the A) absence of mosaicism in skin fibroblasts and B) presence of mosaicism in blood for the non-penetrant mosaic male (#9). Sequences were generated using a reverse primer (CGTATATGTCGCCTGAGTT).

Discussion

We compared males with somatic and germline *PCDH19* pathogenic variants and systematically describe their phenotypic spectrum. The clinical features of the mosaic males in our cohort concur with previous case reports.[4-6] We describe five new cases, expanding the number of reported *PCDH19* mosaic male cases to 18. We show that mosaic males have a neuropsychiatric profile that includes executive dysfunction, ADHD, and ASD; a clinical profile comparable to that reported for females who are naturally mosaic.[64, 110] We also systematically assessed males with germline *PCDH19* variants. Previous reports are limited, however, germline males are generally asymptomatic apart from four reported with ASD [16, 35] and ID.[36] Here we describe two males with germline *PCDH19* variants and neuropsychiatric abnormalities. However, one had FIRES at 9.5 years, an acute encephalopathy with status epilepticus and fever which presents in previously normal children.[112] Most patients have cognitive, motor, and behavioral sequelae and epilepsy. Based on molecular grounds alone, there is compelling evidence for his *PCDH19* variant. However, when his clinical profile and family history are considered, it is more likely that FIRES explains the neuropsychiatric abnormalities we detected, and they are unrelated to his *PCDH19* variant.

We also describe a *PCDH19* mosaic male with moderate ASD but no seizures. It is possible that his autistic features are due to other genetic or environmental factors; however, it may also be due to neuronal *PCDH19* mosaicism. To our knowledge, there have only been two non-penetrant mosaic males reported.[108] *PCDH19* somatic mosaicism was confirmed in multiple tissues for both individuals. Interestingly, their skin fibroblasts

were not tested. Based on cellular interference, it was proposed that asymptomatic mosaic males have skewed neuronal mosaicism in favour of either wild-type or variant allele. Given that we were unable to identify mosaicism in the skin fibroblasts of our mosaic male, it is possible that a low level of mosaicism was present that was not detectable via Sanger Sequencing. Future studies should utilize more sensitive techniques (i.e., microdroplet PCR) for detection of *PCDH19* mosaicism. The penetrance of *PCDH19* variants in females has been estimated to be 80%.[64] There has been no estimate with respect to mosaic males. With the addition of our mosaic male, there have been three non-penetrant males of the twenty reported mosaic males. Hence, we estimate the penetrance of *PCDH19* variants in mosaic males to be 85%.

Conclusion

While *PCDH19* variants predominantly affect females, it is important to recognize that *PCDH19* variants also result in a spectrum of phenotypes in males, albeit less frequently. The phenotypic profile for somatic mosaic males resembles that of heterozygous affected females. We identified neuropsychiatric abnormalities in two males with germline *PCDH19* variants, however, the *PCDH19* variant in each case is likely serendipitous and our data suggest that transmitting males are generally unaffected. We also describe a non-penetrant somatic mosaic male with mosaicism confirmed in blood, but not identified in skin fibroblasts. We propose microdroplet PCR for more sensitive detection of *PCDH19* somatic mosaicism.

Chapter 7: **Investigating cellular and gene expression determinants of variable penetrance in PCDH19 X-linked clustering epilepsy**

7.1 Preamble

A unique feature of XCE is the phenotypic expression pattern of inherited *PCDH19* variants. Typically, X-linked inheritance leads to the condition in males, with females being spared. This is because females have two X chromosomes, the second of which conveys a degree of protection. The unique expression in XCE is such that heterozygous females are affected while hemizygous males are spared, contradicting classical X-chromosome linked genetics. The identification of the first symptomatic "mosaic" male spurred the cellular interference hypothesis to explain this unique expression pattern. Cellular interference posits that co-existence of variant and wild-type *PCDH19* cells (in the brain) leads to a disruption in cell-cell communications. The premise of this hypothesis is grounded in the idea that symptomatic females are cellular mosaics due to XCI of one copy of *PCDH19* in each cell. This is supported by the existence of asymptomatic hemizygous males and symptomatic postzygotic somatic mosaic males. Differences in the degree of mosaicism (in females or in males) are speculated to explain variable penetrance, yet this has not yet been demonstrated. Here we test select biological factors for their association with the penetrance in XCE. These findings will form the foundation for future work in this area.

7.2 Statement of authorship

Title of Paper	Investigating cellular and gene expression determinants of variable penetrance in PCDH19 X-linked clustering epilepsy.
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Kolc, K. L., Kumar, R., & Gecz, J. (Prepared for submission). Investigating cellular and gene expression determinants of variable penetrance in PCDH19 X-linked clustering epilepsy.

Principal author

Name of Principal Author (Candidate)	Kristy Kolc		
Contribution to the Paper	Major contribution to the research question. Designed study, performed data collection, analysis, and interpretation, prepared manuscript, and performed all required revisions.		
Overall percentage (%)	80%		
Certification:	This manuscript reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this manuscript.		
Signature		Date	Feb 14 2020

Co-Author contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Dr Raman Kumar		
Contribution to the Paper	Supervised development of work, assisted with design, experimental work, data interpretation, and manuscript evaluation.		
Signature		Date	Feb 13 2020

Name of Co-Author	Professor Jozef Gecz		
Contribution to the Paper	Supervised development of work, assisted with design, data interpretation, and manuscript evaluation.		
Signature		Date	Feb 13 2020

7.3 Prepared manuscript

Title: Investigating cellular and gene expression determinants of variable penetrance in PCDH19 X-linked clustering epilepsy.

Running Title: CHAPTER 7

List of Authors: Kristy Kolc[1,2], Raman Kumar[1,2], and Jozef Gecz[1,2,3]

Affiliations: [1]Adelaide Medical School, the University of Adelaide, South Australia, Australia, [2]Robinson Research Institute, the University of Adelaide, South Australia, Australia, [3]Healthy Mothers and Babies, South Australian Health and Medical Research Institute, South Australia, Australia.

Abstract

The clinical presentation of XCE is postulated to be underpinned by cellular mosaicism due to some cells expressing the wild-type and others loss-of-function or no PCDH19 protein. XCE is an infantile onset epilepsy syndrome with unusual genetics whereby heterozygous females and postzygotic somatic mosaic males are affected, but hemizygous males are unaffected. The cellular mosaicism underpinning the XCE phenotype is driven by XCI in females and the developmental timing of the *de novo* variant in mosaic males. We have collected primary skin fibroblasts from clinically penetrant and non-penetrant XCE females and tested their XCI patterns, and relative levels of their wild-type and variant PCDH19 mRNA and protein expression. We investigated 13 females heterozygous for *PCDH19* variants, including three discordant MZ twin pairs. Three out of four tested non-penetrant females showed completely wild-type *PCDH19* expression. We show that the pattern of XCI in these cell lines does not correlate with the mRNA expression of the respective wild-type and variant *PCDH19*. This suggests that XCI does not predict the ratio of wild-type to variant *PCDH19* mRNA expression. We suggest that the relative ratio of wild-type to variant *PCDH19* expression, but not XCI, may provide a better molecular tool to assess the penetrance and the clinical severity of XCE. This may be beneficial for future diagnosis and prognosis of XCE.

Introduction

PCDH19 pathogenic variants cause XCE; an infantile onset epilepsy syndrome with unusual genetics whereby heterozygous females and mosaic males are affected, but hemizygous males are unaffected. This is hypothesized to be a consequence of the presence of two neuronal populations; one expressing the wild-type and the other the variant PCDH19 protein. PCDH19 is a cell-cell adhesion molecule and, as such, this mix of neuronal cell populations results in a loss of or abnormal cell-cell interactions, referred to as cellular interference.[4] Cellular mosaicism, either due to XCI in females or early somatic variant in males, is a unifying feature of XCE and the best hypothesis that can explain why *PCDH19* variants are pathogenic in individuals with two different populations of neurons.

The penetrance of XCE is predicted to be greater than 90%. However, our recent systematic review estimates this to be closer to 80% due to increased reports of asymptomatic carrier females.[64] The first such report described two families with *PCDH19* variants inherited from asymptomatic mothers (c.1700C>T and c.1852G>A, respectively).[10] Following this, a mother with a novel *PCDH19* variant (c.2705dupA) was reported to have an IQ of 98, no behavioral problems, and never manifested seizures.[12] Another report described an affected female with the recurrent *PCDH19* variant (c.1019A>G) inherited from an asymptomatic mother.[23] Importantly, this *PCDH19* variant has been identified in approximately 20 unrelated XCE cases, demonstrating that other modifying factors are affecting the variable penetrance of XCE.

In mammals, XCI is a process of transcriptional silencing of one of the two female X chromosomes early in embryonic development.[113] In X-linked disorders, XCI may act as a protective mechanism if skewed in favor of the chromosome with the wild-type allele.[114] As *PCDH19* hemizygous males are asymptomatic, skewing of XCI in females, whether in favor of the wild-type or the variant allele is speculated be protective in XCE. Several studies have examined XCI in XCE.[2, 8, 9, 115-117] However, only two compared penetrant and non-penetrant individuals. In the first study examining XCI in a family with incomplete penetrance, a random XCI was observed in the proband and her asymptomatic sisters.[28] In another study, the proband showed the same ratio of variant and wild-type alleles, however, her asymptomatic mother showed variability in the tested tissues (blood, saliva, hair), with complete wild-type expression detected in urinary cells.[23] Accessible

ectodermal tissue would be expected to provide the best phenotypic correlate, as they share the same developmental origin as neuronal tissue. However, in the Terracciano et al. study, hair roots showed no genotype-phenotype correlation. As neuronal mosaicism has been demonstrated in a *Pcdh19*$^{+/-}$ mouse,[51] it would be important to identify the most appropriate correlate for brain tissue in XCE, if at all possible.

Here we analyzed penetrant and non-penetrant XCE females to determine XCI patterns, wild-type to variant *PCDH19* mRNA expression, and PCDH19 protein levels. Specifically, we hypothesized that skewing in favor of one *PCDH19* allele would be associated with non-penetrance or a milder phenotype and that XCI patterns observed in fibroblasts but not whole blood would provide a better correlate with the XCE phenotype.

Method

Patients

Our cohort of 13 females with *PDH19* variants, included a previously unpublished Danish sister pair (c.1469A>G, p.Tyr490Cys), an unpublished Danish family (c.370G>A, p.Asp124Asn), an Australian sister pair (c.1671C>G, p.Asn557Lys),[2] a pair of MZ twins from Italy (c.1300_1301delCA, p.Gln434GlufsX11),[9, 25] a MZ twin pair from Japan (c.1019A>G, p.Asn340Ser),[26] and previously unpublished MZ twins from New Zealand (c.497_498insA, p.Tyr166X). All related individuals demonstrated a degree of phenotypic discordance, with the Danish sisters, family with the p.Asp124Asn variant, and the Italian MZ twins representing cases of complete discordance. The study was approved by the Women's and Children's Health Network Human Research Ethics Committee and written informed consent was obtained from the participating families.

Clinical assessment

Clinical information was previously collected via our PCDH19 survey.[110] The PCDH19 survey incorporated the following: SRS-2[65] for the assessment of ASD symptoms, the SDQ[67] for the assessment of ADHD symptoms, the BRIEF[109] for the assessment of executive dysfunction, the DOCS[70] for the assessment of OCD symptoms, and the EQ[110] for demographic, development, variant, medication, and seizure information.

Skin fibroblast cell culture

Established cultures were grown in RPMI-1640 medium (Sigma-Aldrich) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Waltham, MA, USA), and 2 mM L-glutaMAX (Sigma-Aldrich).

Genomic DNA isolation

gDNA was isolated from frozen fibroblast cell pellets as per the manufacturer's instructions (DNeasy Blood & Tissue Kit; Qiagen, Hilden, Germany). We also had access to gDNA from blood cells, buccal mucosa, and saliva for the Japanese MZ twins.

Expression Analysis

X-chromosome inactivation assay. Five hundred nanograms of gDNA was digested overnight at 37°C in 25 µl with 1.5 µl *Hpa*II enzyme (10 U/µl) (New England Biolabs, Beverly, MA, U.S.A.). Double digestion was performed with 1 µl *Dde*I enzyme (10 U/µl).[118] After digestion, the enzyme was inactivated at 80°C for 20 min, and PCR was performed on both digested and undigested DNAs (200 ng) with primers specific for the androgen receptor (AR) gene (5' - TCC AGA ATC TGT TCC AGA GCG TGC - 3' and 5' – FAM-GCT GTG AAG GTT GCT GTT CCT CAT - 3'). The PCR mixture contained 5× GC-Rich Phusion PCR Buffer, 10mM dNTPs, primers (10 µM), 5% DMSO, and Phusion HiFi DNA Polymerase in 25 µl. Amplification was performed at 98°C for 3 min, followed by 98°C for 10 s, 60°C for 10 s, 72°C for 30 s for 27 cycles, and a final extension at 72°C for 10 min. Analysis of fluorescent samples was performed using ABI 3100 Genetic Analyzer (Applied Biosystems). XCI was classified as random if the ratio between the two chromosomes was between 50:50 and 80:20 or skewed if the ratio was greater than 80:20.

PCDH19 Relative Allelic Expression

RNA isolation and cDNA synthesis. RNA was isolated from frozen cell pellets according to the manufacturer's instructions (QIAshredder; RNAeasy Mini Kit; Qiagen, Hilden, Germany). cDNA synthesis was performed using SUPERSCRIPT IV Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions.

cDNA PCR. *PCDH19* was amplified from patient fibroblast cDNA with primers 5′- CTG CTG GTC ACC AAG CAG AAG ATT GA -3′ and 5′- AAC AGC CGA GGA GAC AAG TGA TGG -3′ or 5′- CTG CTG GTC ACC AAG CAG AAG ATT GA -3′ and 5′-

CCG AGA TGC AAT GCA GAC ACT TGC TG -3′. Amplified products were resolved on a 1% agarose gel, specific cDNA bands purified by gel extraction (QIAquick Gel Extraction Kit, Qiagen), and sequenced by Sanger sequencing.

Sanger sequencing. Confirmation of variants and cDNA analysis was performed by Sanger sequencing using standard methods. Primer sequences (gDNA) included: 5′- ACC ACG AGT TCG GCA AA -3′ (#3, #4, #5, #12, and #13); 5′- CGT AGA TGT CGC CTG AGT T -3′ (#8, #9, #10, and #11); 5′- TGG GCA ATG TGC CCT TT -3′ (#1, #2, #6, and #7). Primer sequences (cDNA) included: 5′- TCG GAA GCT GTA GTG CGA CTG CGT C -3′ (#3, #4, #5, #12, and #13); 5′- AAC GGC ACT GCA CAC GTC CAT TGA -3′ (#10); 5′- CCA CCA GAA TAG TGG AGA AG -3′ (#11); 5′- GGA TCG CAG CGC GTA GAT GTC GCC T -3′ (#1 and #2); 5′- GAA CGC CTT GGT CTG CTC GTG GTT A -3′ (#8 and #9); and 5′- GTC ACC AGG TAG CCT ATG CCA GAG TT -3′ (#6 and #7).

Cycloheximide assay. Approximately 3×10^6 early passage fibroblasts from two discordant MZ female twins (#8 and #9; #12 and #13) were cultured in presence of either 100 μg/ml cycloheximide (Sigma-Aldrich) or DMSO (Sigma-Aldrich) at 37°C with 5% CO_2 for 6 hours. Cells were harvested, washed once with 1×PBS (Sigma-Aldrich), and snap frozen until RNA isolation using QIAshredder and RNeasy Mini Kit (Qiagen) and generation of cDNA. An additional pellet of untreated cells from each individual was simultaneously harvested and gDNA isolated using DNeasy Blood and Tissue kit (Qiagen) for XCI analysis.

PCDH19 Protein Analysis

siRNA PCHD19 knockdown. MCF-7 cells were transfected with either PCDH19 ON-TARGETplus SMARTpool (5′- GUU CCU AGC UUU ACG CAU U -3′, 5′- CAA UCA AGU GCA AGC GAG A -3′, 5′- UGG AGC UGA UAG CGA GAA A -3′, and 5′- AAU GGA AAU CUG CGU GAU A -3′) or scrambled (5′- UAG CGA CUA AAC ACA UCA A -3′) siRNAs (Dharmacon) using Lipofectamine RNAiMAX reagent according to the manufacturer's specifications (Life Technologies). MCF-7 cells were harvested 48 h after 6 h of siRNA transfection at 37ºC, washed once in 1×PBS (Sigma-Aldrich), and snap frozen until protein isolation. PCDH19 protein expression was determined by western blotting.

Western blot analysis. Protein lysates were prepared in lysis buffer (50 mM Tris-HCl, pH 7.5, 0.2% Triton X-100, 2 mM EDTA, 150 mM NaCl, 0.01% SDS, 50 mM NaF, 0.1 mM Na3VO4, and 1× protease inhibitor/No EDTA, Roche Applied Sciences) followed by sonication and centrifugation. Protein concentration was determined by Pierce BCA Protein Assay Kit (Thermo Scientific). Lysates were resolved by 6% SDS-PAGE and transferred onto nitrocellulose membrane (Amersham Biosciences). Western blot was probed with rabbit anti-PCDH19 antibody (0.2 μg/ml; Bethyl Laboratories), detected with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (Dako) and visualized by enhanced chemiluminescence (GE Healthcare). β-Tubulin was employed as a loading control using rabbit anti-β-tubulin antibody (Abcam, Cambridge, UK).

PCDH19 reference sequences

All PCDH19 cDNAs and proteins were based on the following reference sequences, which represent the longest isoform of PCDH19 mRNA and protein: NM_001184880.2 and NP_001171809.1 (https://www.ncbi.nlm.nih.gov/).

Results

Demographic and clinical characteristics of the 13 females are summarized in Table 7.1.

Table 7.1 Characteristics of *PCDH19* females

ID	Country	Epilepsy (yes/no)	Onset age (seizure type)	Intellectual disability	Psychiatric comorbidities	Variant (inheritance)	Reference
1	Denmark	No	N/A	No	No	c.1469A>G (paternal)	This study
2	Denmark	Yes	15 months (GTCS)	Mild	ASD, CP, PP, SD	c.1469A>G (paternal)	This study
3	Denmark	No	N/A	No	No	c.370G>A (paternal)	This study
4	Denmark	Yes	7 months (TS)	Severe	ASD	c.370G>A (paternal)	This study
5	Denmark	Yes	6.5 months (GTCS)	Severe	ASD, CP, SD	c.370G>A (maternal)	This study
6	Australia	Yes	18 months (FeS)	No	No	c.1671C>G (paternal)	2
7	Australia	Yes	24 months (HCS)	Mild	No	c.1671C>G (paternal)	2
8	Italy	No	N/A	No	No	c.1300_1301delCA (*de novo*)	9
9	Italy	Yes	14 months (GTCS)	Moderate	ASD	c.1300_1301delCA (*de novo*)	9
10	Japan	No	25 months (GTCS[#])	No	No	c.1019A>G (*de novo*)	26
11	Japan	Yes	13 months (FS)	No	No	c.1019A>G (*de novo*)	26
12	New Zealand	Yes	8 months (FIAS)	Mild	ASD, EP, CP, SD	c.497_498insA (*de novo*)	This study
13	New Zealand	Yes	8 months (FIAS)	Severe	ASD, EP, CP, SD	c.497_498insA (*de novo*)	This study

Abbreviations: *ADHD*, attention-deficit hyperactivity disorder; *ASD*, autism spectrum disorder; *CP*, conduct problems; *EP*, emotional problems; *FeS*, febrile seizure; *FIAS*, focal impaired awareness seizure; *FS*, focal seizure; *GTCS*, generalized tonic-clonic seizures; *HCS*, hemiclonic seizure; *N/A*, not applicable; *OCD*, obsessive compulsive disorder; *PP*, peer problems; *SD*, social deficits; [#], isolated seizure

Molecular analysis

***PCDH19* variants.** All variants were located within exon 1, which corresponds to the EC repeat domain of the PCDH19 protein (Fig. 7.1).

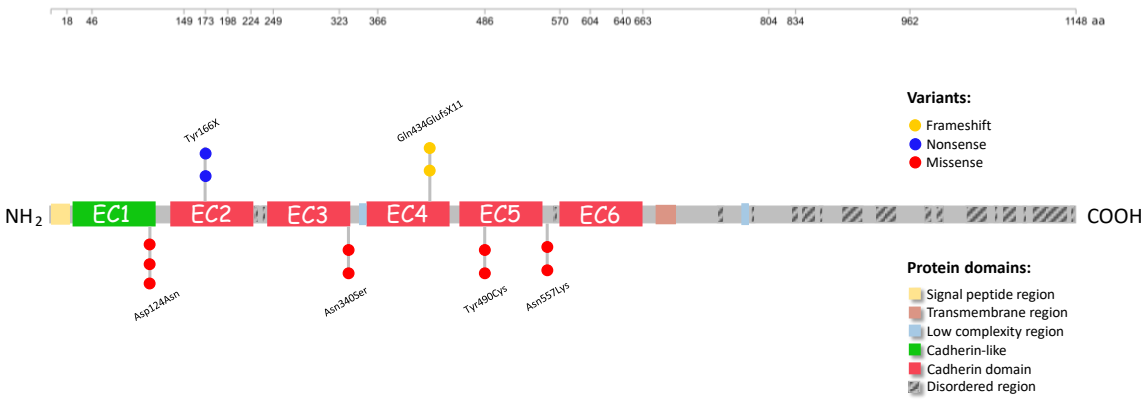

Figure 7.1 Lollipop plot illustrating all *PCDH19* variants represented in the analysis (n = 13).

Sanger sequence analysis confirmed the presence of a *PCDH19* variant in the gDNA extracted from the relevant skin fibroblast cell line of all 13 females (Fig. 7.2). All

sequences demonstrated the presence of both the wild-type and the variant *PCDH19* sequence, as expected.

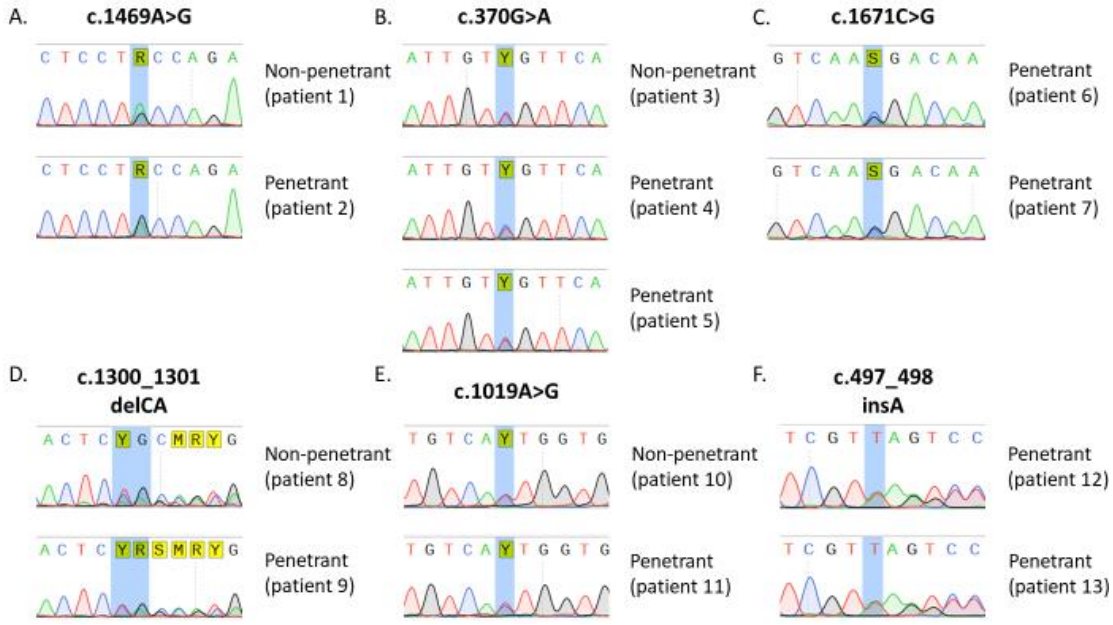

Figure 7.2 gDNA sequences for all individuals grouped by family relationship included in the present study. A) Danish siblings (#1 and #2); B) Danish family (#3, #4, and #5); C) Australian siblings (#6 and #7); D) Italian MZ twins (#8 and #9); E) Japanese MZ twins (#10 and #11); and F) New Zealand MZ twins (#12 and #13). The variants are highlighted in blue.

X-chromosome inactivation. XCI analysis was performed on fibroblast gDNAs from all 13 patients. For six patients (#1, #2, #3, #4, #6, and #7) parental alleles could not be distinguished at the AR locus and were therefore not informative. Six of the seven patients who were informative at the AR locus had a skewed XCI (Table 7.2). For the Japanese MZ twins, we had additional tissue available for analysis. Whereas saliva and skin fibroblasts of the non-penetrant twin (#10) showed a completely skewed XCI pattern, a random XCI pattern was detected in her blood (27:73). A random XCI pattern was observed in blood (25:75) and saliva (28:72), and a skewed pattern in the skin fibroblasts (12:88) of the penetrant twin (#11). The buccal mucosa did not give a conclusive result for either twin, likely due to low DNA content.

Relative *PCDH19* cDNA expression. In an attempt to provide an independent and direct measure of XCI, we have measured relative *PCDH19* mRNA expression in the skin fibroblasts of all these 13 females (Fig. 7.3).

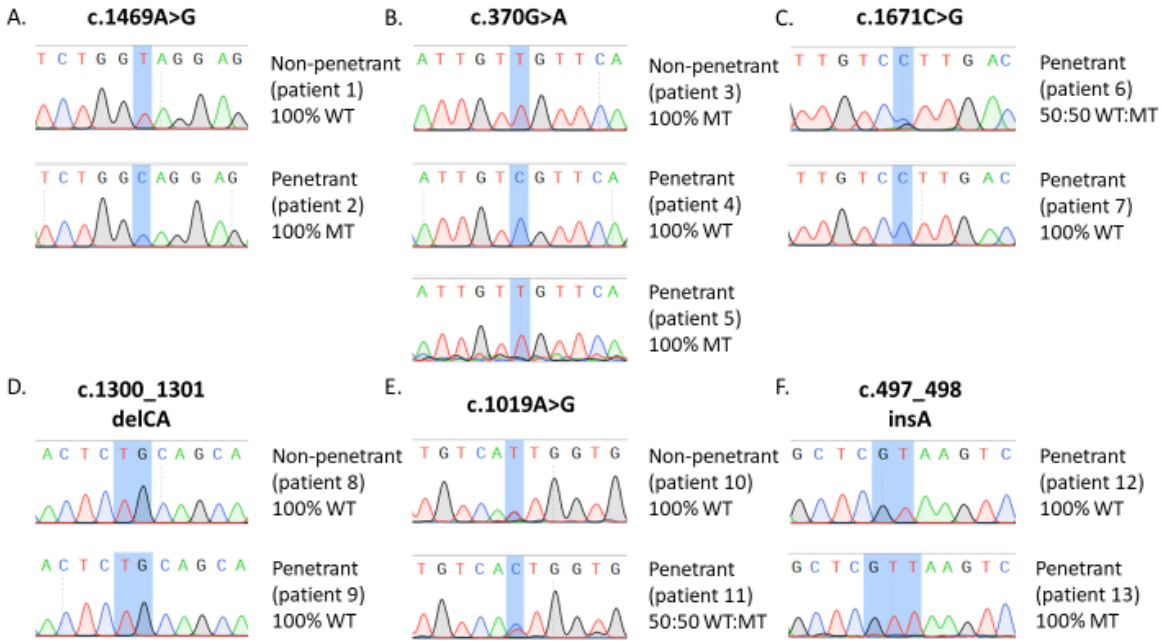

Figure 7.3 cDNA sequences for all families. A) Danish siblings; B) Danish family; C) Australian siblings; D) Italian MZ twins; E) Japanese MZ twins; and F) NZ MZ twins. All sequences generated using reverse primers (see Methods for primer sequences). The variants are highlighted in blue.

Between individual discordance in relative wild-type to variant *PCDH19* mRNA expression was observed for all related individuals, except the Italian MZ twins (#8 and #9). Interestingly, three out of the four non-penetrant females (#1, #8, and #10) showed 100% wild-type *PCDH19* mRNA expression. However, the non-penetrant female from the Danish family (#3) exhibited 100% variant allele expression. Completely wild-type expression was also observed for mildly affected females #7 and #12. All remaining affected females showed either completely variant (#2, #4, #5, and #13) or an approximate 50:50 wild-type and variant mRNA expression (#6, #9, and #11). Taken together, these data suggest that: i) there is no correlation between XCI and penetrance in these individuals, ii) the wild-type and variant *PCDH19* allele expression does not explain the phenotypic differences either, however, iii) there also appears to be no correlation between XCI and relative wild-type to variant *PCDH19* mRNA expression.

Of the six *PCDH19* variants in the present study, two involve the introduction of a premature termination codon (PTC) into the *PCDH19* mRNA (#8, #9, #12, & #13). PTC-containing mRNA transcripts are typically eliminated by the nonsense mediated decay (NMD) pathway.[119, 120] Of these individuals, only the Italian MZ twins (#8 and #9) provided a case of complete phenotypic discordance. Consequently, we treated the skin fibroblasts of these individuals with cycloheximide to inhibit NMD[121] and were able to

rescue the PTC-containing mRNA transcript for one of the twins, that is the penetrant twin (Fig. 7.4).

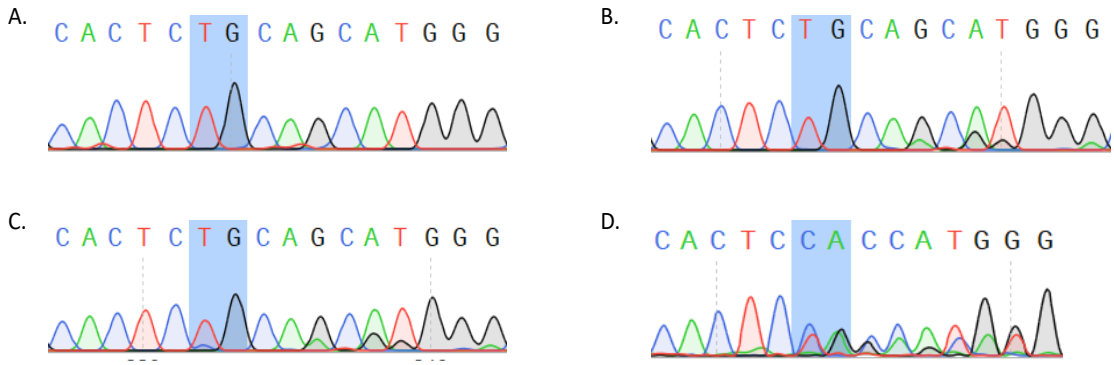

Figure 7.4 NMD of PCDH19 transcript in skin fibroblasts. Sequence chromatogram from A) non-penetrant female (patient 8) and B) penetrant female (patient 9) showing the absence of the mutant sequence in untreated cDNA; and C) minimal rescue of the variant transcript for the non-penetrant female and D) near complete rescue of the variant transcript for the penetrant female following cycloheximide treatment, which inhibits the pioneer round of translation and thus NMD. The position of the variant is highlighted in blue.

Protein expression. PCDH19 has a molecular mass of 126kD[122] and is weakly expressed in human fibroblasts. To confirm our ability to detect and specify the PCDH19 protein in fibroblasts via western blot, we used lysates from MCF-7 cells treated with PCDH19 or scrambled siRNA. PCDH19 was detectable in all patient and MCF-7 cells (Supplementary Fig. 7.1), confirming a very low PCDH19 expression in fibroblasts, therefore rendering subtle differences in expression levels between related individuals difficult to detect. Conversely, PCDH19 was easily detectable in MCF-7 cells.

Genotype-phenotype associations. We obtained clinical information for eight patients (#2, #3, #4, #5, #6, #7, #12, and #13) via the PCDH19 survey.[110] There was no consensus between the pattern of XCI and clinical outcomes when comparing related individuals or the group as a whole. However, relative *PCDH19* cDNA expression demonstrated some agreement with phenotype (Table 7.2), such that complete variant expression or an approximate even expression of variant and wild-type was associated with more seizures (#6 and #13) and more severe psychiatric comorbidities (#2, #5, and #13). Conversely, wild-type expression was associated with no seizures (#1, #8, and #10), fewer seizures (#7) and no psychiatric comorbidities (#1, #7, #8, and #10).

Table 7.2 Genotype-phenotype associations

General	Molecular		Seizures		Neuropsychiatric comorbidities							
Patient	*XCI*	*cDNA*	*Penetrance*	*# per cluster (average/day)*	*Executive Dysfunction*	*Emotional Problems*	*Conduct Problems*	*ADHD*	*Peer Problems*	*Social deficits*	*ASD*	*OCD*
1	NI	100% WT	-	N/A	No	No	No	No	No	No	No	No
2	NI	100% MT	+	12	Mild	Average	Moderate	Average	Moderate	Severe	Mild	Not tested
3	NI	100% MT	-	N/A	No	No	No	No	No	No	No	No
4	NI	100% WT	+	20	Mild	Not tested	Not tested	Not tested	Not tested	Not tested	Moderate	Not tested
5	99:1	100% MT	+	20	Moderate	Average	Mild	Average	Average	Mild	Severe	Not tested
6	NI	50:50	+	20	No	Not tested	Not tested	Not tested	Not tested	Not tested	No	No
7	NI	100% WT	+	8	No	Not tested	Not tested	Not tested	Not tested	Not tested	No	No
8	84:16	100% WT	-	N/A	No	No	No	No	No	No	No	No
9	25:75	100% MT	+	Unknown	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested
10	1:99	100% WT	-	N/A	No	No	No	No	No	No	No	No
11	12:88	50:50	+	Unknown	No	No	No	No	No	No	No	No
12	100:0	100% WT	+	30	Moderate	Mild	Mild	Average	Average	Mild	Moderate	Not tested
13	0:100	50:50	+	30	Moderate	Average	Mild	Average	Average	Mild	Moderate	Not tested

Abbreviations: *ADHD*, attention-deficit hyperactivity disorder; *ASD;* autism spectrum disorder; *MT*, mutant; *NI*, non-informative; *OCD*, obsessive compulsive disorder; *WT*, wild-type; *XCI*, X-chromosome inactivation; - non-penetrant, + penetrant

NB: “No” refers to information obtained from publications or clinicians indicating that the individual is unaffected/has no comorbidities whereas “average” refers to the results of the assessment in the PCDH19 survey (indicating no presence of the given comorbidity).

Discussion

In this study, we show that the pattern of XCI in the skin fibroblasts of 13 females does not correlate with their *PCDH19* allelic expression. As XCI analysis relies on the ability to distinguish between the alleles inherited from each parent at a given locus, there are limitations to this method. For the AR assay, parental alleles are distinguished by variation in the length of a CAG short tandem repeat located within the first exon of the AR gene.[123] It is speculated that around 90% of the female population is heterozygous at this locus, however, this percentage is often lower in clinical settings.[124, 125] For these reasons, we suggest using the ratio of wild-type to variant *PCDH19* mRNA expression estimated/measured from sequence chromatograms of specific regions of cDNAs amplified by PCR.

Three of four non-penetrant females represented in this analysis showed 100% wild-type *PCDH19* mRNA expression however, there was no unanimous association between mRNA expression and penetrance. The Italian MZ twins with the p.1300_1301delCA variant had complete wild-type *PCDH19* mRNA expression. When NMD was inhibited in their skin fibroblasts, we were only able to rescue the variant transcript for the penetrant twin. So, in one twin we were able to see the silenced transcript yet in the other we did not. This suggests that the mechanisms responsible for *PCDH19* mRNA expression can differ between related individuals and that there is something else which is suppressing transcription of the variant allele in the tissue of the non-penetrant twin. Why we were unable to rescue the variant *PCDH19* transcript in the skin fibroblasts for the non-penetrant twin remains unknown. Given the XCI pattern is approximately similar for both twins (so we assume there is expression of the variant allele), we speculate that the variant transcript is not subjected to NMD for the non-penetrant twin and that other transcriptional silencing factors are involved. Furthermore, the observed difference in relative wild-type to variant mRNA expression between related individuals may be due to transcriptional mechanisms inhibiting one of the two transcripts.

Several hypotheses have been proposed to explain the unusual clinical phenotype in XCE.[2, 4] One that has received considerable attention is that of cellular interference.[4] This draws on the idea that neuronal populations expressing both wild-type and variant *PCDH19* results in abnormal neuronal interactions and communication. A recent *Pcdh19* knockout mouse model showed that heterozygous *Pcdh19* mice had elevated cortical

activity that was not present in mice completely lacking *Pcdh19*, reflecting the human condition.[51] Furthermore, deletion of the wild-type *Pcdh19* allele in heterozygous mice restored normal network activity.[51] Here, we were unable to provide support for the mechanism of cellular interference through our finding clinical phenotypes in individuals with markedly skewed XCI. Conversely, the mouse model did not display an overt seizure or behavioral phenotype in heterozygous females.

There are several limitations associated with our approach. First, we were unable to directly measure the ratio of wild-type to variant *PCDH19* expression from the brain tissue, although post-mortem brain tissue as a source of mRNA for gene expression profiling remains a possibility. We were also unable to demonstrate a correlation between fibroblasts, blood, and saliva mRNA expression for individuals where additional tissue types were available. Secondly, later passage (>10) fibroblast cultures have been associated with XCI skewing in favor of the dominant X [126]. To address this concern, we utilized early stage passage fibroblasts, however, the effect of passaging on skewing cannot be disregarded. Additional experiments comparing XCI in a primary biopsy with cultured cells at different passages from the same individual are required to determine if there are any effects of culture on XCI. Lastly, analysis on a small number of non-penetrant females (n = 4) limited our ability to perform statistical analyses and draw firm conclusions. Given the limited number of non-penetrant females in XCE (penetrance ~90%), this number may be representative, however, accessing additional non-penetrant female samples would increase the validity of these findings.

Conclusion

We have shown that XCI does not correlate with the ratio of the wild-type to variant *PCDH19* mRNA expression, invalidating the use of this assay to predict relative *PCDH19* mRNA expression. Without access to brain tissue, the ratio of the wild-type to variant *PCDH19* mRNA expression may provide a better molecular tool to assess the penetrance and the clinical severity of XCE than XCI. This may benefit future diagnosis and prognosis of XCE.

Chapter 8: **Discussion**

This discussion begins with a brief overview of the thesis, and then discusses the key findings in a series of four outcomes. The first outcome is the statistical association between earlier seizure onset age and more severe ID. This association was first identified in Chapter 2 and later validated in Chapter 3. In Chapter 3, we also identified an association between age at seizure onset and the severity of ASD symptoms, as well as an association between the frequency of seizures within a cluster and the severity of ASD symptoms. The second outcome involves the characterization of the neuropsychiatric profile associated with *PCDH19* variants. These findings are discussed in terms of the entire cohort (Chapter 3) as well as a detailed analysis of *PCDH9*-variant males (Chapter 6). This outcome formed the basis of the PCDH19 website as part of the Human Disease Genes Website Series (Chapter 4). The third outcome explores the efficacy of the AED Levetiracetam in the treatment of XCE (Chapter 5). The fourth outcome is the correlation between *PCDH19* cDNA relative expression and clinical outcome (Chapter 7). Following is a discussion of the clinical implications. Finally, the strengths and limitations of the project are considered, as well as recommendations for future research.

8.1 Overview

This thesis examined the variable clinical and molecular expressivity associated with *PCDH19* variants. Data used were obtained via a systematic review and meta-analysis of the *PCDH19* literature, the PCDH19 survey, and patient gDNA, RNA, and protein derived from a variety of tissue.

Five separate studies were conducted, with key results summarised below:

Study 1 (**Chapter 2)** identified the comorbidities frequently associated with XCE in the literature and potential associations of clinical and molecular factors with neuropsychiatric outcomes. *PCDH19* variants were associated with psychiatric comorbidities in approximately 60% of females, 80% of affected mosaic males, and reported in nine hemizygous males. Hyperactive, autistic, and obsessive-compulsive features were most frequently reported. We found that seizure onset ≤ 12 months was significantly associated ($p = 4.127 \times 10^{-7}$) with more severe ID compared with onset > 12 months. We identified two recurrent variants p.Asn340Ser and p.Tyr366Leufs*10, occurring in 25 (20 unrelated) and 30 (11 unrelated) cases, respectively.

Study 2 **(Chapters 3 and 6)** validated the prevalence of the comorbidities identified in Study 1 using standardized neuropsychiatric assessments that were administered as part of an online international survey (the PCDH19 survey). Executive dysfunction and ASD occurred in approximately 60% of individuals. The ASD profile included features of ADHD. In addition, 21% of individuals met criteria for OCD that appeared to be distinct from ASD. There were no phenotypic differences between heterozygous females and mosaic males. We describe a mosaic male and two hemizygous males with atypical clinical profiles. We also validated the association identified in Study 1 and identified additional associations. Earlier seizure onset age and increased number of seizures within a cluster were associated with more severe ASD symptoms, with seizure onset also predictive of executive dysfunction and prosocial behavior. We identified 35 novel variants, which included a newly identified recurrent variant Ile781Asnfs*3.

In study 3 **(Chapter 4)** data collected from Study 1 and 2, as well as information gleaned directly from health professionals formed the basis of the *PCDH19* gene website as part of the Human Disease Genes website series. This website connects families with clinicians and researchers and provides a platform where information can be shared or obtained regarding *PCDH19* and XCE. As new information becomes available, it is uploaded to the site either by clinicians sharing data concerning a new patient or by the PCDH19 page moderators when new research findings are obtained. In this way, the website provides the latest data, statistics, information, and research opportunities for individuals wishing to learn more about this disorder.

Study 4 **(Chapter 5)** evaluated the effectiveness of the AED levetiracetam in two independent cohorts of females with XCE. Cohort A consisted of 17 females, aged 3-37 years, who had a trial of levetiracetam at an average age of 10.7 years. 13/17 females became seizure free for >12 months; while 10/17 remained seizure free for >24 months. Cohort B comprised 62 females, aged 1.5-41 years. 26/62 became seizure free for >12 months, and 19/62 for >24 months on levetiracetam therapy.

Study 5 **(Chapter 7)** explored biological factors associated with penetrance in XCE. We included 13 females with *PCDH19* variants, including three discordant MZ twin pairs. We showed that XCE penetrance is not associated with the degree of relative wild-type to variant *PCDH19* cDNA expression in skin fibroblasts. Three of four non-penetrant females showed completely wild-type *PCDH19* cDNA expression. We showed that the pattern of

XCI does not correlate with relative *PCDH19* cDNA expression, invalidating the use of this assay to infer *PCDH19* wild-type to variant expression.

8.2 Outcome 1: Predicting clinical outcomes

Results across two studies, utilizing two independent cohorts support an association between the age an individual is when they have their first seizure and the severity of their clinical outcome. In addition, we showed that increased frequency of seizures within a cluster was associated with ASD symptom severity. This section discusses these associations and implications for clinical practice.

8.2.1 Earlier seizure onset age is significantly associated with more severe cognitive impairment.

The first study found that seizure onset ≤ 12 months was significantly associated with more severe ID compared with onset > 12 months. This finding derived from a meta-analysis of studies that included information about age at seizure onset and degree of ID. ID was then re-classified as a numeric variable to aid the analysis. This approach was limited due to the nature of the data collected. ID was reported categorically (borderline, mild, moderate, severe, or profound) and therefore differences that exist within categories are lost. Restrictions also exist with respect to the analyses that can be performed and the conclusions that can be drawn. This limitation was addressed in the second study through the use of standardized assessments.

In our systematic review we identified that executive dysfunction was frequently reported in individuals with *PCDH19* pathogenic variants. We also identified that EEG reports of abnormal activity in frontotemporal brain regions were relatively common in affected girls. The frontotemporal region of the brain is involved in executive functions such as decision making, inhibition, emotional regulation, as well as speech production.[127] We also found that many girls appeared to have difficulties in these areas, such as inattention, aggression and other emotion regulation problems, and difficulties with speech, especially expressive language.

To be better understand the specific cognitive deficits in XCE and validate the association identified in the first study, we examined the association between seizure onset and executive dysfunction in addition to ID. We validated the association between earlier

seizure onset age and more severe ID and expanded this to also include executive dysfunction (Study 2).

8.2.2 Earlier seizure onset age and more frequent seizures within a cluster are significantly associated with more severe ASD symptoms.

The second study also found that earlier seizure onset age and increased number of seizures within a cluster were associated with more severe ASD symptoms, with seizure onset also predictive of prosocial behavior. We found that executive dysfunction occurred in 63% of our cohort. Of these, 89% also met criteria for ASD. It has been posited that the social and non-social deficits observed in ASD stem from deficits in executive functions and might explain the co-occurrence of these disorders and why seizure onset and activity might be predictive of both outcomes.[75] The prosocial behavior scale within the SDQ is a measure of social deficits. Social deficits are a core feature of ASD and therefore the association between seizure onset and prosocial behavior provides further validation for the impact of earlier seizures on behavior.

8.2.3 Clinical implications.

Developmental delay prior to seizure onset occurred in 18% of our cohort. The molecular diagnosis for many infantile neurodevelopmental disorders including epilepsy occurs at a mean age of 3 years, which represents delays of months to years for patients with pathogenic variants. Early clinical identification of XCE will result in earlier molecular diagnosis and may impact outcome by allowing optimization of both seizure management and developmental progress.

We are the first to show that earlier seizure onset age predicts greater executive dysfunction, prosocial behavior, and ASD severity, and that increased seizure activity strengthens these associations. Clinicians are already using this information to inform prognosis. Prior to this, we had no information as to what an individual clinical outcome might entail. Through our work, we can now provide guidance and targeted intervention for patients and their families.

8.3 Outcome 2: The neuropsychiatric profile of *PCDH19* variants

This section discusses the neuropsychiatric profile that was characterized for XCE and provides a brief description of the neuropsychiatric assessments that were utilized (Study

2). This section finishes with a discussion of the implications these findings have for families, as well as in clinical practice.

8.3.1 The Social Responsiveness Scale, second edition and the Social Communication Questionnaire.

The SRS-2 original parent form demonstrates very good test-retest reliability[128, 129] and a moderate to strong positive correlation with the Autism Diagnostic Interview-Revised (ADI-R)[129], which is the accepted gold-standard parent-report interview for the clinical diagnosis of ASD.[130] The SRS-2 is based on DSM criteria with two subscales (Social Communication and Restricted Interests and Repetitive Behavior) that are combined to produce a total score. The SRS-2 total score is the most reliable measure for social deficits related to ASD.

The SCQ is based on the ADI-R, which is very time intensive, taking upwards of two hours to complete.[131] Conversely, the SCQ takes 10 minutes to complete.[132] A total cutoff score of 15 is used for differentiating different pervasive developmental disorders from other diagnoses, with a sensitivity of 0.96 and a specificity of 0.80 (intellectual impairment excluded) and a sensitivity of 0.96 and a specificity of 0.67 (intellectual impairment included).[133]

8.3.2 Autism spectrum disorder.

For a diagnosis of ASD, there must be evidence of restricted, repetitive patterns of behavior, interests, or activity as well as deficits in social communication and interaction.[57] Current prevalence estimates of ASD are around 2% of school-age children in the world,[134] with this figure continuing to rise.[57] Comparatively, we found an occurrence of ASD-like symptoms in approximately 60% of our cohort. This figure is well above the rates observed in the general population and suggests a possible common underlying mechanism in XCE and ASD. ASD also demonstrates significant overlap with other psychiatric disorders.[57] The ASD profile observed in our cohort included features of ADHD, and may represent a general deficit in executive functions that underlies these comorbidities. A targeted clinical assessment will help delineate these features and determine a differential diagnosis.

8.3.3 The Strengths and Difficulties Questionnaire.

The SDQ has been used successfully in the screening and diagnosis of ADHD in community[135] and clinical[136] samples, respectively. All versions of the SDQ demonstrate very good psychometric properties, such as discriminant validity,[137, 138] external validity,[139] internal consistency,[140] and inter-rater reliability.[141]

8.3.4 Attention-deficit hyperactivity disorder.

ADHD is a neurodevelopmental disorder characterized by an inability to pay attention and control behavior.[57] For a diagnosis of ADHD, symptoms must manifest before the age of 12, be age-inappropriate, remain for more than 6 months, and be apparent in more than one setting (i.e., home and school).[57] ADHD is particularly difficult for the family; as troublesome behavior often involves anger and aggressive outbursts,[142] in addition to inattention, hyperactivity, and disruptive behavior.[57] According to the World Health Organization, ADHD is estimated to affect almost 40 million people,[143] with a prevalence rate of around 5% in most cultures.[57]

Neurological disorders (i.e., epilepsy) can lead to ADHD-like symptoms, such as deficits in attention and may explain the higher prevalence of ADHD observed in seizure disorders.[144, 145] Therefore, the pathophysiology of ADHD likely involves a genetic predisposition. This is further supported by reports detailing the concordance for symptoms of ADHD among MZ and dizygotic (DZ) twins. There is greater concordance among MZ compared with DZ twins,[146, 147] with some reports finding either complete[148] or near complete[149] concordance among MZ twins. Similar findings have been observed across the lifespan, with higher concordance among MZ twins.[150] Collectively, these findings suggest that ADHD is highly heritable and likely shares genetic vulnerabilities with other neurodevelopmental disorders and might explain the co-occurrence of ADHD features with ASD in our XCE cohort.

8.3.5 The Dimensional Obsessive-Compulsive Scale.

The DOCS demonstrates a stable factor structure, high total score (α = .93) and subscale (α = .83-.96) internal consistency, adequate test-retest reliability over a 12 week interval (r = .55-.66), good discriminant and convergent validity,[70] and has been validated for internet administration.[151]

8.3.6 Obsessive-compulsive disorder.

Obsessions and compulsions are the hallmark features of OCD and, for a clinical diagnosis, obsessive and compulsive behaviors must interfere with an individual's life and cause them some degree of discomfort.[57] OCD affects around 1.5% of the population, with females being affected at a slightly higher rate than males in adulthood.[57] OCD is generally diagnosed in individuals during adolescence, though symptom onset is earlier in males, with 25% of males being diagnosed by the age of 10.[57] For this reason, males are more commonly affected than females in childhood. OCD symptomology was observed in 21% of our cohort. This may be an accurate estimate or may reflect the similarities between OCD and other disorders, such as ASD. Only one of the six individuals meeting criteria for OCD also met criteria for ASD, suggesting that OCD is distinct from ASD and that later onset psychiatric comorbidities should be considered with a diagnosis of XCE.

8.3.7 The Behavior Rating Inventory of Executive Function.

All versions of the BRIEF demonstrate very good psychometric properties. Based on normative samples, test-retest reliabilities have been reported for the GEC of the parent (r = .86)[111] and self-report (r = .89)[152] forms, and, in general, the correlations between the parent and self-report ratings are moderate to high. The clinical utility of the BRIEF has also been established through convergent and ecological validity.[153] The BRIEF is a particularly appropriate because it is written to capture the qualitative aspects of a child's executive functions in a real world-setting, and has been used successfully in studies involving children with developmental and psychiatric disorders (for review, see[154]).

8.3.8 Executive functions.

Executive functions are a set of cognitive processes that include attention, working memory, inhibition, mental flexibility, problem-solving, planning, and reasoning.[155] These processes are necessary for the cognitive control of behavior, that is, successfully monitoring and regulating behaviors in a goal-oriented manner.[156] Cognitive control deficits underlie many psychiatric disorders, such as ADHD, ASD, and OCD. These disorders were shown to be associated with XCE in this thesis. The considerable overlap between executive dysfunction and ASD/ADHD symptomatology in our cohort, supports the notion that deficits in executive functions underlie the observed psychiatric features in XCE.

8.3.9 Clinical implications.

SDQ impact assesses chronicity, distress, social impairment, and burden for others. Impact scores were in the very high range for approximately 75% of our cohort. This was further supported by 65% of respondents endorsing behavior as the most challenging aspect each day. To our knowledge, this is the first study to specifically incorporate a direct measure of burden on the individual and their family in the context of XCE. Impact scores have been shown to better discriminate community from clinical groups compared to symptom scores and may be a useful tool for determining caseness.[157] Our findings afford a more comprehensive understanding of the caregiving context and its outcomes, and may lead to a practical application in devising effective support strategies for families, which still seem to be lacking.

8.4 Outcome 3: Treatment efficacy in X-linked clustering epilepsy

This section discusses levetiracetam and the implications our findings have for clinical practice.

8.4.1 Levetiracetam.

Levetiracetam (Keppra®, E Keppra®) is a second-generation AED. It is chemically unrelated to, and has low potential for clinically relevant pharmacokinetic interactions with, other currently available AEDs.[158] The mechanism of action for levetiracetam is postulated to involve binding to SV2A, leading to inhibition of presynaptic neurotransmitter release;[159] reducing calcium release;[160] and activating GABA current by opposing the action of zinc and β-carbolines on GABA- and glycin-gated currents.[161]

Levetiracetam has demonstrated therapeutic efficacy across a broad spectrum of epilepsies as an adjunctive therapy and as a monotherapy (see[162] for a review). Interestingly, levetiracetam has been associated with an improvement in health-related quality of life.[163, 164] This is important to note with respect to XCE, as the burden experienced by individuals and families affected by this disorder is high (refer Fig. 3.4). To date, the therapeutic efficacy of levetiracetam in XCE has not been systematically assessed. Several isolated cases have reported efficacy, and, in the Lotte study,[93] seizure freedom was recorded for approximately a quarter of those trialling levetiracetam for 12 months. Given the present pharmaco-resistant nature of XCE, levetiracetam should be considered early in the management seizure clusters.

There have been several isolated cases of adverse events following increased levetiracetam dosage,[165, 166] however, levetiracetam is generally regarded as comparable to placebo.[167] Cognition appears to be unaffected by levetiracetam administration, though an association with psychiatric and behavioral adverse events has been established.[168] This should be considered when administering levetiracetam for the treatment of seizures in XCE, given the high prevalence of late-onset psychosis.[63]

8.4.2 Clinical implications.

The mainstay of treatment of XCE is pharmacological therapy with AEDs. Identifying AEDs that are effective in XCE is complicated as the natural history of this disorder is that many females become seizure free in adolescence. Therefore, retrospective reports of which AED controlled seizures are confounded by the age at which the AED was introduced, as it may have coincided with the age at which patients would naturally outgrow their seizures. Seizure cessation may then be inappropriately attributed to the AED in use at that time. To definitively ascertain if an AED is effective randomized, double-blind, trials of either placebo or proven alternative treatments are required.

8.5 Outcome 4: Penetrance of *PCDH19* pathogenic variants

In this section I discuss the major theory that has been proposed to explain penetrance in XCE, the association between cDNA expression and penetrance identified in Study 4, and how this can be interpreted with respect to theory.

8.5.1 The cellular interference model.

The current perspective accounting for the pathogenesis of XCE is the cellular interference model, which posits an alteration of cell-cell interactions. Here, *PCDH19* loss-of-function at the cellular level would result in a gain of (or altered) function at the tissue level due to altered interactions between wild-type and variant cells.[22] The co-existence of both cell types in the brain may result in a skewing of cell-cell communication due to the formation of separate cellular networks (e.g. wild-type and wild-type) or deregulation of the networks from altered cell-cell signalling (Fig. 8.1).[4] Normal individuals, hemizygous males with *PCDH19* variants or highly skewed females, whom are essentially homogeneous for either wild-type cells or variant cells will be asymptomatic or “non-penetrant”.

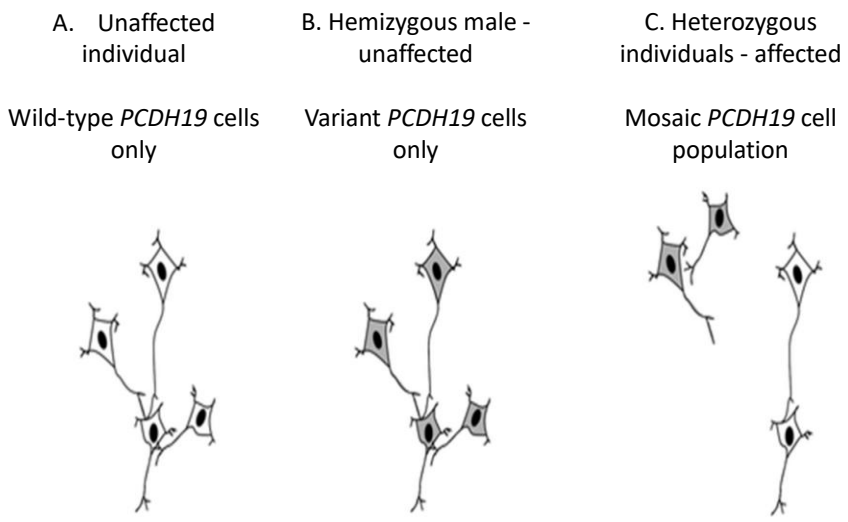

Figure 8.1 Cellular interference model as an explanation of pathogenesis in XCE: A) Interaction of wild-type cells or B) variant cells both have a normal phenotype; (C) A mosaic population of wild-type and variant cells are unable to communicate correctly resulting in the disease expression. Modified from[4].

8.5.2 Association between PCDH19 cDNA expression and clinical outcomes.

No clear association was observed between penetrance in XCE and the degree of variant and wild-type *PCDH19* mRNA expression in skin fibroblasts. The precise mechanism by which penetrance is determined is yet to be elucidated; however, is likely to involve a complex interplay of post-transcriptional regulatory factors. Cellular interference posits that skewing in favour of complete, or near-complete, wild-type or variant expression would be associated with a milder phenotype or absence of symptoms. Although we were able to identify three non-penetrant females with 100% wild-type *PCDH19* expression, we were unable to provide support for the mechanism of cellular interference through our finding clinical phenotypes in individuals with markedly skewed XCI. Our cohort consisted of six individuals with 100% wild-type expression, of whom three had seizures with varying neuropsychiatric profiles and three were non-penetrant. There were four with 100% variant expression, of whom one was non-penetrant and three had seizures, two with neuropsychiatric comorbidities and one with no information in this regard. There were three individuals with 50:50 expression, all with seizures but two with no neuropsychiatric comorbidities. These unexpected findings suggest that a new model of pathogenesis in this condition may be needed. While males with mosaic *PCDH19* pathogenic variants provide support for cellular interference, the identification of tissue mosaicism in the brain and asymptomatic females with homozygous *PCDH19* variants would lend further support to this model.

8.6 Strengths and limitations

A limitation that was identified in this research thesis was a lack of consensus with respect to how comorbidities are identified and assessed. As an initial step, standardized clinical assessments will address this discrepancy and is a strength of this research thesis. Standardized measures are particularly useful in contexts such as these, as they minimize any potential confounds that are likely to emerge as a result of different time points of administration, different administrators, and differences in the assessments themselves. Such differences render any comparisons across studies less meaningful and can lead to variation in results.

Moreover, research settings are often constrained by available resources and time, therefore highlighting the need for tools that can be administered quickly and easily.[169] The self-report is a type of assessment whereby respondents respond to questions about their personal circumstances (i.e., psychiatric symptoms) without researcher involvement. A written questionnaire is an example of a highly structured self-report that can be systematically administered by different researchers in different contexts, thus reducing the influence of potential confounds. They are also able to be administered online, which makes them a very quick and easy way to obtain information, particularly if respondents are spread geographically, as is the case in XCE. Variations to the self-report include the "parent" or "other" report, which are completed by someone on behalf of the individual being assessed. These reports are particularly useful if the individual demonstrates poor insight into their circumstances or is unable to comprehend instructions, such is the case among young children[170, 171] or individuals with ID.[172]

While the use of standardized assessments was a major strength of this study, limitations also exist with relation to this methodology. Surveys such as the PCDH19 survey, involve the collection of data at a given time point thus limiting the ability to demonstrate causal associations. Biases also exist with self-reported data.[73, 173] Validity checks within the BRIEF addressed these biases to some extent. Retrospective accounts are inherently less reliable than direct observation of behavior or events.[174] The overwhelming gap in the literature is empirical evaluation and data on neuropsychiatric comorbidities in XCE. Longitudinal studies of the comorbidities in XCE are required to assess which early symptoms are markers for increased severity and chronicity, as well as long-term impact on functioning and wellbeing. Understanding the progression of neuropsychiatric

comorbidities and the role they play in future physiological and psychological health will determine if early intervention of neuropsychiatric comorbidities can prevent disease progression.

Participation bias is a concern in all survey studies. Non-response bias may limit the ability to generalize the study's findings. The response rate for the PCDH19 survey of 65% is very high.[175] Additionally, responses were obtained primarily from the primary caregivers of affected individuals. Contact with many survey respondents was ongoing and clarification or additional information could be sought around survey responses. A notable strength of this thesis is the large representative sample of the population of interest. Having access to an international cohort comprising the parents of affected individuals (male and female), as well as asymptomatic carriers of a *PCDH19* variant provided the most comprehensive overview of the phenotypic spectrum associated with *PCDH19* variants to date.

This thesis has made several novel contributions to the field. The first is the characterization of the neuropsychiatric profile associated with *PCDH19* variants. This is the first study to provide a comprehensive, standardized assessment of neuropsychiatric comorbidities, of which families identify as an area of major concern. This information provides a foundation for future longitudinal studies and will lead to the development of targeted intervention and guidance for patients and their families. The second contribution is the identified statistical associations between seizure onset age, frequency, and clinical outcome. This information is already being used by clinicians to inform prognosis and sheds light on possible mechanisms that underlie these clinical features. Lastly, we identify levetiracetam as an effective pharmaco-therapy in the management of the highly refractory clusters of seizures that characterize this genetic disease.

8.7 Recommendations for further research

Our comprehensive assessment of the neuropsychiatric comorbidities may provide an understanding of the pathological mechanisms involved. We identified significant overlap with relation to features of executive dysfunction and ASD, suggesting a common pathway underlying the neuropsychiatric comorbidities in this disorder. Of importance is an understanding of the neurobiological mechanisms of both epileptic seizures and neuropsychiatric comorbidities and to identify the contribution of these features to disease pathophysiology. The identification of blood biomarkers (in addition to skin biopsies) will

lead to more accurate and earlier diagnosis, which in turn has implications for early intervention.

ID is formally diagnosed based on the following criteria: deficits in intellectual and adaptive functioning, and an onset of intellectual and adaptive deficits during the developmental period.[57] Almost 75% of the reviewed cases reported individual FSIQ scores as a way of classifying ID. While FSIQ is an effective and accurate way to measure global intelligence, it does not adequately explain where specific deficits in cognition occur. It also fails to take an individual's adaptive functioning into account. Adaptive functioning is a measure of how well an individual is able to meet acceptable standards of personal independence and social responsibility when compared to others of similar age and sociocultural background.[57] Adaptive functioning is assessed via a combination of clinical and standardized assessments, the latter of which are generally administered to a knowledgeable informant (e.g., parents). Therefore, FSIQ assessments that also incorporate adaptive functioning measures will more accurately reflect the level of dysfunction that exists for the individual.

The development of a framework for the assessment of neuropsychiatric comorbidities in XCE (including those identified in this thesis) could improve research, management, and outcomes. To validate the patient's experience and that experienced by their families, as well as explain symptoms to patients, such frameworks should incorporate all dimensions of distress suffered by individuals. This can be achieved through more comprehensive assessments of impact and distress (similar to that employed by the impact supplement in the SDQ). In doing so, perhaps we can tailor interventions to meet the needs of patients and their families, thereby improving treatment adherence and outcomes.

Besides the mechanism of cellular interference, sex-specific differences likely contribute to the heterogeneity and variable expressivity associated with *PCDH19* pathogenic variants. Sex hormones, in particular those associated with the menstrual cycle and menopause, can trigger gender specific epilepsy.[176, 177] Genes regulated by steroid hormone receptors, such as aldo-keto reductase family 1 member C3 (*AKR1C3*), are dysregulated in individuals with *PCDH19* pathogenic variants.[50] *AKR1C3* converts androstenedione to testosterone, which is involved in the epigenetic modulation of gene expression. Taken together, these data raise the possibility that, in addition to or in conjunction with alterations

in cell-cell interactions, epigenetic dysregulation – specifically of genes regulated by steroid hormone receptors – underlies the pathogenesis in XCE.

Treatment options for both seizures and neuropsychiatric comorbidities in XCE require further research, particularly the exploration of neuropsychiatric treatment options. Presently, Marinus Pharmaceuticals are running a phase III clinical trial for the treatment of epileptic seizures in XCE using ganaxolone. In phase II of this clinical trial, it was identified that treatment outcomes included an improvement in behaviour. This effect on behavior has been incorporated into the phase III clinical trial, which has been informed by this thesis.

8.8 Conclusions

We have shown that XCI patterns do not correlate with relative *PCDH19* cDNA expression in fibroblasts, thus invalidating use of this assay to infer *PCDH19* expression. We were also unable to provide support for cellular interference through our finding clinical phenotypes in individuals with markedly skewed XCI, suggesting that a new model of pathogenesis in this condition may be required.

We identified three recurrent variants p.Asn340Ser, p.Tyr366Leufs*10, and Ile781Asnfs*3, as well as 35 novel *PCDH19* pathogenic variants, thus significantly expanding the number of cases in the *PCDH19* literature. This represents the largest cohort reported and allowed for the characterization of the clinical profile associated with *PCDH19* variants. Executive dysfunction and ASD occurred in approximately 60% of individuals. The ASD profile included features of ADHD. OCD symptomology was observed in 21% individuals. There were no phenotypic differences between heterozygous females and mosaic males. Neuropsychiatric disorders can be very responsive to early intervention;[58-60] therefore, a better understanding of these comorbidities may help to inform treatment and ultimately lead to better developmental outcomes for individuals affected by XCE.

We show that both seizure onset age and activity are associated with clinical outcomes. Clinicians can use this information to inform prognosis and provide targeted intervention and guidance for patients and their families.

Appendix A: Survey Assessments

A1 Social Responsiveness Scale, second edition (SRS-2)

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https://www.parinc.com/Frequently-Asked-Questions?fbclid=IwAR08LMaEm-tbtHewmKd48oyZJcnUXd0F0m-7v-vJkKuQonhIgz1uFBe29pQ#9029-copyright-trademark-and-permissions

A2 Social Communication Questionnaire (SCQ)

Lifetime Form **SCQ**™

Response Sheet

unlocking potential

Social Communication Questionnaire

Michael Rutter, MD, FRS, Anthony Bailey, MD, Sibel Kazak Berument, PhD,
Catherine Lord, PhD, and Andrew Pickles, PhD

Name of Subject		Gender
		Male Female
Date of Interview	Date of Birth	Chronological Age
Name of Respondent	Relation to Subject	
Clinician Name	School/Clinic	

Directions

Thank you for taking the time to complete this questionnaire. Please answer each question by circling *yes* or *no*. A few questions ask about several related types of behavior; please circle *yes* if *any* of these behaviors have ever been present. Although you may be uncertain about whether some behaviors were ever present or not, please answer *yes* or *no* to every question on the basis of what you think.

1. Is she/he now able to talk using short phrases or sentences? If *no*, skip to question 8.	Yes	No
2. Can you have a to and fro "conversation" with her/him that involves taking turns or building on what you have said?	Yes	No
3. Has she/he ever used odd phrases or said the same thing over and over in almost exactly the same way (either phrases that she/he has heard other people use or ones that she/he has made up)?	Yes	No
4. Has she/he ever used socially inappropriate questions or statements? For example, has she/he ever regularly asked personal questions or made personal comments at awkward times?	Yes	No
5. Has she/he ever got her/his pronouns mixed up (e.g., saying *you* or *she/he* for *I*)?	Yes	No

6. Has she/he ever used words that she/he seemed to have invented or made up her/himself; put things in odd, indirect ways; or used metaphorical ways of saying things (e.g., saying *hot rain* for *steam*)?	Yes	No
7. Has she/he ever said the same thing over and over in exactly the same way or insisted that you say the same thing over and over again?	Yes	No

Continued on next page

8. Has she/he ever had things that she/he seemed to have to do in a very particular way or order or rituals that she/he insisted that you go through?	Yes	No
9. Has her/his facial expression usually seemed appropriate to the particular situation, as far as you could tell?	Yes	No
10. Has she/he ever used your hand like a tool or as if it were part of her/his own body (e.g., pointing with your finger, putting your hand on a doorknob to get you to open the door)?	Yes	No
11. Has she/he ever had any interests that preoccupy her/him and might seem odd to other people (e.g., traffic lights, drainpipes, or timetables)?	Yes	No
12. Has she/he ever seemed to be more interested in parts of a toy or an object (e.g., spinning the wheels of a car), rather than using the object as it was intended?	Yes	No
13. Has she/he ever had any special interests that were *unusual* in their intensity but otherwise appropriate for her/his age and peer group (e.g., trains, dinosaurs)?	Yes	No
14. Has she/he ever seemed to be *unusually* interested in the sight, feel, sound, taste, or smell of things or people?	Yes	No
15. Has she/he ever had any mannerisms or odd ways of moving her/his hands or fingers, such as flapping or moving her/his fingers in front of her/his eyes?	Yes	No
16. Has she/he ever had any complicated movements of her/his whole body, such as spinning or repeatedly bouncing up and down?	Yes	No
17. Has she/he ever injured her/himself deliberately, such as by biting her/his arm or banging her/his head?	Yes	No
18. Has she/he ever had any objects (*other* than a soft toy or comfort blanket) that she/he *had* to carry around?	Yes	No
19. Does she/he have any particular friends or a best friend?	Yes	No

For the following behaviors, please focus on the time period between the child's fourth and fifth birthdays. You may find it easier to remember how things were at that time by focusing on key events, such as starting school, moving house, Christmastime, or other specific events that are particularly memorable for you as a family. If your child is not yet 4 years old, please consider her or his behavior in the past 12 months.

20. When she/he was 4 to 5, did she/he ever talk with you just to be friendly (rather than to get something)?	Yes	No
21. When she/he was 4 to 5, did she/he ever *spontaneously* copy you (or other people) or what you were doing (such as vacuuming, gardening, or mending things)?	Yes	No

22. When she/he was 4 to 5, did she/he ever spontaneously point at things around her/him just to show you things (not because she/he wanted them)?	Yes	No
23. When she/he was 4 to 5, did she/he ever use gestures, other than pointing or pulling your hand, to let you know what she/he wanted?	Yes	No
24. When she/he was 4 to 5, did she/he nod her/his head to mean *yes*?	Yes	No
25. When she/he was 4 to 5, did she/he shake her/his head to mean *no*?	Yes	No
26. When she/he was 4 to 5, did she/he usually look at you directly in the face when doing things with you or talking with you?	Yes	No
27. When she/he was 4 to 5, did she/he smile back if someone smiled at her/him?	Yes	No
28. When she/he was 4 to 5, did she/he ever show you things that interested her/him to engage your attention?	Yes	No
29. When she/he was 4 to 5, did she/he ever offer to share things other than food with you?	Yes	No
30. When she/he was 4 to 5, did she/he ever seem to want you to join in her/his enjoyment of something?	Yes	No
31. When she/he was 4 to 5, did she/he ever try to comfort you if you were sad or hurt?	Yes	No
32. When she/he was 4 to 5, when she/he wanted something or wanted help, did she/he look at you and use gestures with sounds or words to get your attention?	Yes	No
33. When she/he was 4 to 5, did she/he show a normal range of facial expressions?	Yes	No
34. When she/he was 4 to 5, did she/he ever spontaneously join in and try to copy the actions in social games, such as *The Mulberry Bush* or *London Bridge Is Falling Down*?	Yes	No
35. When she/he was 4 to 5, did she/he play any pretend or make-believe games?	Yes	No
36. When she/he was 4 to 5, did she/he seem interested in other children of approximately the same age whom she/he did not know?	Yes	No
37. When she/he was 4 to 5, did she/he respond positively when another child approached her/him?	Yes	No
38. When she/he was 4 to 5, if you came into a room and started talking to her/him without calling her/his name, did she/he usually look up and pay attention to you?	Yes	No
39. When she/he was 4 to 5, did she/he ever play imaginative games with another child in such a way that you could tell that they each understood what the other was pretending?	Yes	No
40. When she/he was 4 to 5, did she/he play cooperatively in games that required joining in with a group of other children, such as hide-and-seek or ball games?	Yes	No

A3 Strengths and Difficulties Questionnaire (SDQ)

A3.1 Parent Form (2-4)

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain. Please give your answers on the basis of your child's behaviour over the last six months.

Your child's name ..... Male/Female

Date of birth.....

	Not True	Somewhat True	Certainly True
Considerate of other people's feelings	☐	☐	☐
Restless, overactive, cannot stay still for long	☐	☐	☐
Often complains of headaches, stomach-aches or sickness	☐	☐	☐
Shares readily with other children, for example toys, treats, pencils	☐	☐	☐
Often loses temper	☐	☐	☐
Rather solitary, prefers to play alone	☐	☐	☐
Generally well behaved, usually does what adults request	☐	☐	☐
Many worries or often seems worried	☐	☐	☐
Helpful if someone is hurt, upset or feeling ill	☐	☐	☐
Constantly fidgeting or squirming	☐	☐	☐
Has at least one good friend	☐	☐	☐
Often fights with other children or bullies them	☐	☐	☐
Often unhappy, depressed or tearful	☐	☐	☐
Generally liked by other children	☐	☐	☐
Easily distracted, concentration wanders	☐	☐	☐
Nervous or clingy in new situations, easily loses confidence	☐	☐	☐
Kind to younger children	☐	☐	☐
Often argumentative with adults	☐	☐	☐
Picked on or bullied by other children	☐	☐	☐
Often volunteers to help others (parents, teachers, other children)	☐	☐	☐
Can stop and think things out before acting	☐	☐	☐
Can be spiteful to others	☐	☐	☐
Gets along better with adults than with other children	☐	☐	☐
Many fears, easily scared	☐	☐	☐
Good attention span, sees chores or homework through to the end	☐	☐	☐

Do you have any other comments or concerns?

Overall, do you think that your child has difficulties in one or more of the following areas: emotions, concentration, behaviour or being able to get on with other people?

No	Yes-minor difficulties	Yes-definite difficulties	Yes-severe difficulties
☐	☐	☐	☐

If you have answered "Yes", please answer the following questions about these difficulties:

• How long have these difficulties been present?

Less than a month	1-5 months	6-12 months	Over a year
☐	☐	☐	☐

• Do the difficulties upset or distress your child?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

• Do the difficulties interfere with your child's everyday life in the following areas?

	Not at all	Only a little	Quite a lot	A great deal
HOME LIFE	☐	☐	☐	☐
FRIENDSHIPS	☐	☐	☐	☐
LEARNING	☐	☐	☐	☐
LEISURE ACTIVITIES	☐	☐	☐	☐

• Do the difficulties put a burden on you or the family as a whole?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

Signature ..... Date .....

Mother/Father/Other (please specify:)

A3.2 Parent Form (5-10)

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain. Please give your answers on the basis of your child's behaviour over the last six months.

Your child's name ..... Male/Female

Date of birth.....

	Not True	Somewhat True	Certainly True
Considerate of other people's feelings	☐	☐	☐
Restless, overactive, cannot stay still for long	☐	☐	☐
Often complains of headaches, stomach-aches or sickness	☐	☐	☐
Shares readily with other children, for example toys, treats, pencils	☐	☐	☐
Often loses temper	☐	☐	☐
Rather solitary, prefers to play alone	☐	☐	☐
Generally well behaved, usually does what adults request	☐	☐	☐
Many worries or often seems worried	☐	☐	☐
Helpful if someone is hurt, upset or feeling ill	☐	☐	☐
Constantly fidgeting or squirming	☐	☐	☐
Has at least one good friend	☐	☐	☐
Often fights with other children or bullies them	☐	☐	☐
Often unhappy, depressed or tearful	☐	☐	☐
Generally liked by other children	☐	☐	☐
Easily distracted, concentration wanders	☐	☐	☐
Nervous or clingy in new situations, easily loses confidence	☐	☐	☐
Kind to younger children	☐	☐	☐
Often lies or cheats	☐	☐	☐
Picked on or bullied by other children	☐	☐	☐
Often volunteers to help others (parents, teachers, other children)	☐	☐	☐
Thinks things out before acting	☐	☐	☐
Steals from home, school or elsewhere	☐	☐	☐
Gets along better with adults than with other children	☐	☐	☐
Many fears, easily scared	☐	☐	☐
Good attention span, sees chores or homework through to the end	☐	☐	☐

Do you have any other comments or concerns?

Overall, do you think that your child has difficulties in one or more of the following areas: emotions, concentration, behaviour or being able to get on with other people?

No	Yes-minor difficulties	Yes-definite difficulties	Yes-severe difficulties
☐	☐	☐	☐

If you have answered "Yes", please answer the following questions about these difficulties:

• How long have these difficulties been present?

Less than a month	1-5 months	6-12 months	Over a year
☐	☐	☐	☐

• Do the difficulties upset or distress your child?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

• Do the difficulties interfere with your child's everyday life in the following areas?

	Not at all	Only a little	Quite a lot	A great deal
HOME LIFE	☐	☐	☐	☐
FRIENDSHIPS	☐	☐	☐	☐
CLASSROOM LEARNING	☐	☐	☐	☐
LEISURE ACTIVITIES	☐	☐	☐	☐

• Do the difficulties put a burden on you or the family as a whole?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

Signature ..... Date .....

Mother/Father/Other (please specify:)

A3.3 Parent Form (11-17)

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain. Please give your answers on the basis of your child's behaviour over the last six months.

Your child's name ..... Male/Female

Date of birth.....

	Not True	Somewhat True	Certainly True
Considerate of other people's feelings	☐	☐	☐
Restless, overactive, cannot stay still for long	☐	☐	☐
Often complains of headaches, stomach-aches or sickness	☐	☐	☐
Shares readily with other youth, for example CD's, games, food	☐	☐	☐
Often loses temper	☐	☐	☐
Would rather be alone than with other young people	☐	☐	☐
Generally well behaved, usually does what adults request	☐	☐	☐
Many worries or often seems worried	☐	☐	☐
Helpful if someone is hurt, upset or feeling ill	☐	☐	☐
Constantly fidgeting or squirming	☐	☐	☐
Has at least one good friend	☐	☐	☐
Often fights with other young people or bullies them	☐	☐	☐
Often unhappy, depressed or tearful	☐	☐	☐
Generally liked by other young people	☐	☐	☐
Easily distracted, concentration wanders	☐	☐	☐
Nervous in new situations, easily loses confidence	☐	☐	☐
Kind to younger children	☐	☐	☐
Often lies or cheats	☐	☐	☐
Picked on or bullied by other young people	☐	☐	☐
Often volunteers to help others (parents, teachers, children)	☐	☐	☐
Thinks things out before acting	☐	☐	☐
Steals from home, school or elsewhere	☐	☐	☐
Gets along better with adults than with other young people	☐	☐	☐
Many fears, easily scared	☐	☐	☐
Good attention span, sees chores or homework through to the end	☐	☐	☐

Do you have any other comments or concerns?

Overall, do you think that your child has difficulties in one or more of the following areas: emotions, concentration, behavior or being able to get on with other people?

No	Yes-minor difficulties	Yes-definite difficulties	Yes-severe difficulties
☐	☐	☐	☐

If you have answered "Yes", please answer the following questions about these difficulties:

• How long have these difficulties been present?

Less than a month	1-5 months	6-12 months	Over a year
☐	☐	☐	☐

• Do the difficulties upset or distress your child?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

• Do the difficulties interfere with your child's everyday life in the following areas?

	Not at all	Only a little	Quite a lot	A great deal
HOME LIFE	☐	☐	☐	☐
FRIENDSHIPS	☐	☐	☐	☐
CLASSROOM LEARNING	☐	☐	☐	☐
LEISURE ACTIVITIES	☐	☐	☐	☐

• Do the difficulties put a burden on you or the family as a whole?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

Signature ..... Date .....

Mother/Father/Other (please specify:)

A3.4 Self-Report Form (11-17)

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain . Please give your answers on the basis of how things have been for you over the last six months.

Your name..... Male/Female

Date of birth.....

	Not True	Somewhat True	Certainly True
I try to be nice to other people. I care about their feelings	☐	☐	☐
I am restless, I cannot stay still for long	☐	☐	☐
I get a lot of headaches, stomach-aches or sickness	☐	☐	☐
I usually share with others, for example CD's, games, food	☐	☐	☐
I get very angry and often lose my temper	☐	☐	☐
I would rather be alone than with people of my age	☐	☐	☐
I usually do as I am told	☐	☐	☐
I worry a lot	☐	☐	☐
I am helpful if someone is hurt, upset or feeling ill	☐	☐	☐
I am constantly fidgeting or squirming	☐	☐	☐
I have one good friend or more	☐	☐	☐
I fight a lot. I can make other people do what I want	☐	☐	☐
I am often unhappy, depressed or tearful	☐	☐	☐
Other people my age generally like me	☐	☐	☐
I am easily distracted, I find it difficult to concentrate	☐	☐	☐
I am nervous in new situations. I easily lose confidence	☐	☐	☐
I am kind to younger children	☐	☐	☐
I am often accused of lying or cheating	☐	☐	☐
Other children or young people pick on me or bully me	☐	☐	☐
I often volunteer to help others (parents, teachers, children)	☐	☐	☐
I think before I do things	☐	☐	☐
I take things that are not mine from home, school or elsewhere	☐	☐	☐
I get along better with adults than with people my own age	☐	☐	☐
I have many fears, I am easily scared	☐	☐	☐
I finish the work I'm doing. My attention is good	☐	☐	☐

Do you have any other comments or concerns?

Overall, do you think that you have difficulties in one or more of the following areas: emotions, concentration, behavior or being able to get on with other people?

No	Yes-minor difficulties	Yes-definite difficulties	Yes-severe difficulties
☐	☐	☐	☐

If you have answered "Yes", please answer the following questions about these difficulties:

- How long have these difficulties been present?

Less than a month	1-5 months	6-12 months	Over a year
☐	☐	☐	☐

- Do the difficulties upset or distress you?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

- Do the difficulties interfere with your everyday life in the following areas?

	Not at all	Only a little	Quite a lot	A great deal
HOME LIFE	☐	☐	☐	☐
FRIENDSHIPS	☐	☐	☐	☐
CLASSROOM LEARNING	☐	☐	☐	☐
LEISURE ACTIVITIES	☐	☐	☐	☐

- Do the difficulties make it harder for those around you (family, friends, teachers, etc.)?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

Your Signature ..... Today's Date .....

A4 Dimensional Obsessive-Compulsive Scale (DOCS)

This questionnaire asks you about 4 different types of concerns that you might or might not experience. For each type there is a description of the kinds of thoughts (sometimes called *obsessions*) and behaviors (sometimes called *rituals* or *compulsions*) that are typical of that particular concern, followed by 5 questions about your experiences with these thoughts and behaviors. Please read each description carefully and answer the questions for each category based on your experiences in the last month.

Category 1: Concerns about Germs and Contamination

Examples…

-Thoughts or feelings that you are contaminated because you came into contact with (or were nearby) a certain object or person.
-The feeling of being contaminated because you were in a certain place (such as a bathroom).
-Thoughts about germs, sickness, or the possibility of spreading contamination.
-Washing your hands, using hand sanitizer gels, showering, changing your clothes, or cleaning objects because of concerns about contamination.
-Following a certain routine (e.g., in the bathroom, getting dressed) because of contamination
-Avoiding certain people, objects, or places because of contamination.

The next questions ask about your experiences with thoughts and behaviors related to contamination over the last month. Keep in mind that your experiences might be different than the examples listed above. Please circle the number next to your answer:

1. About how much time have you spent each day thinking about contamination and engaging in washing or cleaning behaviors because of contamination?
   0 None at all
   1 Less than 1 hour each day
   2 Between 1 and 3 hours each day
   3 Between 3 and 8 hours each day
   4 8 hours or more each day

2. To what extent have you avoided situations in order to prevent concerns with contamination or having to spend time washing, cleaning, or showering?
   0 None at all
   1 A little avoidance
   2 A moderate amount of avoidance
   3 A great deal of avoidance
   4 Extreme avoidance of nearly all things

3. If you had thoughts about contamination but could not wash, clean, or shower (or otherwise remove the contamination), how distressed or anxious did you become?
   0 Not at all distressed/anxious
   1 Mildly distressed/anxious
   2 Moderately distressed/anxious
   3 Severely distressed/anxious
   4 Extremely distressed/anxious

4. To what extent has your daily routine (work, school, self-care, social life) been disrupted by contamination concerns and excessive washing, showering, cleaning, or avoidance behaviors?
   0 No disruption at all.
   1 A little disruption, but I mostly function well.
   2 Many things are disrupted, but I can still manage.
   3 My life is disrupted in many ways and I have trouble managing.
   4 My life is completely disrupted and I cannot function at all.

5. How difficult is it for you to disregard thoughts about contamination and refrain from behaviors such as washing, showering, cleaning, and other decontamination routines when you try to do so?
   0 Not at all difficult
   1 A little difficult
   2 Moderately difficult
   3 Very difficult
   4 Extremely difficult

Category 2: Concerns about being Responsible for Harm, Injury, or Bad Luck

Examples…

-A doubt that you might have made a mistake that could cause something awful or harmful to happen.
-The thought that a terrible accident, disaster, injury, or other bad luck might have occurred and you weren't careful enough to prevent it.
-The thought that you could prevent harm or bad luck by doing things in a certain way, counting to certain numbers, or by avoiding certain "bad" numbers or words.
-Thought of losing something important that you are unlikely to lose (e.g., wallet, identify theft, papers).
-Checking things such as locks, switches, your wallet, etc. more often than is necessary.
-Repeatedly asking or checking for reassurance that something bad did not (or will not) happen.
-Mentally reviewing past events to make sure you didn't do anything wrong.
-The need to follow a special routine because it will prevent harm or disasters from occurring.
-The need to count to certain numbers, or avoid certain bad numbers, due to the fear of harm.

The next questions ask about your experiences with thoughts and behaviors related to harm and disasters over the last month. Keep in mind that your experiences might be slightly different than the examples listed above. Please circle the number next to your answer:

1. About how much time have you spent each day thinking about the possibility of harm or disasters and engaging in checking or efforts to get reassurance that such things do not (or did not) occur?

0 None at all
1 Less than 1 hour each day
2 Between 1 and 3 hours each day
3 Between 3 and 8 hours each day
4 8 hours or more each day

2. To what extent have you avoided situations so that you did not have to check for danger or worry about possible harm or disasters?

0 None at all
1 A little avoidance
2 A moderate amount of avoidance
3 A great deal of avoidance
4 Extreme avoidance of nearly all things

3. When you think about the possibility of harm or disasters, or if you cannot check or get reassurance about these things, how distressed or anxious did you become?

0 Not at all distressed/anxious
1 Mildly distressed/anxious
2 Moderately distressed/anxious
3 Severely distressed/anxious
4 Extremely distressed/anxious

4. To what extent has your daily routine (work, school, self-care, social life) been disrupted by thoughts about harm or disasters and excessive checking or asking for reassurance?

0 No disruption at all.
1 A little disruption, but I mostly function well.
2 Many things are disrupted, but I can still manage.
3 My life is disrupted in many ways and I have trouble managing.
4 My life is completely disrupted and I cannot function at all.

5. How difficult is it for you to disregard thoughts about possible harm or disasters and refrain from checking or reassurance-seeking behaviors when you try to do so?

0 Not at all difficult
1 A little difficult
2 Moderately difficult
3 Very difficult
4 Extremely difficult

Category 3: Unacceptable Thoughts

Examples…

-Unpleasant thoughts about sex, immorality, or violence that come to mind against your will.
-Thoughts about doing awful, improper, or embarrassing things that you don't really want to do.
-Repeating an action or following a special routine because of a bad thought.
-Mentally performing an action or saying prayers to get rid of an unwanted or unpleasant thought.
-Avoidance of certain people, places, situations or other triggers of unwanted or unpleasant thoughts

The next questions ask about your experiences with unwanted thoughts that come to mind against your will and behaviors designed to deal with these kinds of thoughts over the last month. Keep in mind that your experiences might be slightly different than the examples listed above. Please circle the number next to your answer:

1. About how much time have you spent each day with unwanted unpleasant thoughts and with behavioral or mental actions to deal with them?

0 None at all
1 Less than 1 hour each day
2 Between 1 and 3 hours each day
3 Between 3 and 8 hours each day
4 8 hours or more each day

2. To what extent have you been avoiding situations, places, objects and other reminders (e.g., numbers, people) that trigger unwanted or unpleasant thoughts?

0 None at all
1 A little avoidance
2 A moderate amount of avoidance
3 A great deal of avoidance
4 Extreme avoidance of nearly all things

3. When unwanted or unpleasant thoughts come to mind against your will how distressed or anxious did you become?

0 Not at all distressed/anxious
1 Mildly distressed/anxious
2 Moderately distressed/anxious
3 Severely distressed/anxious
4 Extremely distressed/anxious

4. To what extent has your daily routine (work, school, self-care, social life) been disrupted by unwanted and unpleasant thoughts and efforts to avoid or deal with such thoughts?

0 No disruption at all.
1 A little disruption, but I mostly function well.
2 Many things are disrupted, but I can still manage.
3 My life is disrupted in many ways and I have trouble managing.
4 My life is completely disrupted and I cannot function at all.

5. How difficult is it for you to disregard unwanted or unpleasant thoughts and refrain from using behavioral or mental acts to deal with them when you try to do so?

0 Not at all difficult
1 A little difficult
2 Moderately difficult
3 Very difficult
4 Extremely difficult

Category 4: Concerns about Symmetry, Completeness, and the Need for Things to be "Just Right"

Examples…

-The need for symmetry, evenness, balance, or exactness.
-Feelings that something isn't "just right."
-Repeating a routine action until it feels "just right" or "balanced."
-Counting senseless things (e.g., ceiling tiles, words in a sentence).
-Unnecessarily arranging things in "order."
-Having to say something over and over in the same way until it feels "just right."

The next questions ask about your experiences with feelings that something is not "just right" and behaviors designed to achieve order, symmetry, or balance over the last month. Keep in mind that your experiences might be slightly different than the examples listed above. Please circle the number next to your answer:

1. About how much time have you spent each day with unwanted thoughts about symmetry, order, or balance and with behaviors intended to achieve symmetry, order or balance?

   0 None at all
   1 Less than 1 hour each day
   2 Between 1 and 3 hours each day
   3 Between 3 and 8 hours each day
   4 8 hours or more each day

2. To what extent have you been avoiding situations, places or objects associated with feelings that something is not symmetrical or "just right?"

   0 None at all
   1 A little avoidance
   2 A moderate amount of avoidance
   3 A great deal of avoidance
   4 Extreme avoidance of nearly all things

3. When you have the feeling of something being "not just right," how distressed or anxious did you become?

   0 Not at all distressed/anxious
   1 Mildly distressed/anxious
   2 Moderately distressed/anxious
   3 Severely distressed/anxious
   4 Extremely distressed/anxious

4. To what extent has your daily routine (work, school, self-care, social life) been disrupted by the feeling of things being "not just right," and efforts to put things in order or make them feel right?

   0 No disruption at all.
   1 A little disruption, but I mostly function well.
   2 Many things are disrupted, but I can still manage.
   3 My life is disrupted in many ways and I have trouble managing.
   4 My life is completely disrupted and I cannot function at all.

5. How difficult is it for you to disregard thoughts about the lack of symmetry and order, and refrain from urges to arrange things in order or repeat certain behaviors when you try to do so?

   0 Not at all difficult
   1 A little difficult
   2 Moderately difficult
   3 Very difficult
   4 Extremely difficult

A5 Behavior Rating Inventory of Executive Function (BRIEF)

A6 Epilepsy Questionnaire (EQ)

DEMOGRAPHICS

YOUR DETAILS: Please enter your details, as outlined below.
YOUR CHILD'S DETAILS: Please enter your child's details, as outlined below.
THE INDIVIDUAL'S DETAILS: Please enter the details of the individual you are responding on behalf of, as outlined below.

***First name:**

***Surname:**

***Date of birth: dd/mm/yyyy**

***Sex:**
☐ Male
☐ Female

Race:
☐ African
☐ Asian
☐ Caucasian
☐ Indigenous
☐ Other (please specify)

Country of birth:

Mother's age (in years):

Father's age (in years):

Age of other parent (in years):

Relationship to individual:

Age (in years) of mother (if known/applicable):
Age (in years) of father (if known/applicable):

DEVELOPMENTAL DETAILS

The following questions relate to your development.
The following questions relate to your child's development.
The following questions relate to the development of the individual you are responding on behalf of.

*Have you had a neurocognitive assessment?
*Has your child had a neurocognitive assessment?
*Has the individual had a neurocognitive assessment?
☐ YES
☐ NO
☐ UNSURE

If you are happy to share your neurocognitive assessment, please attach it here.
If you are happy to share their neurocognitive assessment, please attach it here.
Choose File

PREVIOUS DEVELOPMENT

The following questions relate to your child's previous development.

Is your child developmentally normal?
☐ YES
☐ NO
☐ UNSURE

At what age (in months) did you first notice they were not developing as they should?

What aspects of their development were affected?

Did your child lose any developmental skills that they previously had?
☐ YES
☐ NO
☐ UNSURE

How many times did this happen?
☐ Once
☐ Twice
☐ Three times
☐ Four times
☐ Five times or more

Was this associated with a cluster of seizures?
☐ YES
☐ NO
☐ UNSURE
☐ N/A

Was this associated with seizures lasting longer than 30 minutes?
☐ YES
☐ NO
☐ UNSURE
☐ N/A

CURRENT DEVELOPMENT

The following questions relate to your development at the present time.
The following questions relate to your child's development at the present time.
The following questions relate to the individual's development at the present time.

What year/grade are you in at school?
What year/grade is your child in school?
☐ Preschool/Nursery school/Day care
☐ Kindergarten/Reception
☐ Year 1
☐ Year 2
☐ Year 3
☐ Year 4
☐ Year 5
☐ Year 6
☐ Year 7
☐ Year 8
☐ Year 9
☐ Year 10
☐ Year 11
☐ Year 12
☐ Not currently attending school
☐ Never attended school

What is the highest level of education you have attained?
What is the highest level of education they attained?
☐ Attended some school
☐ Graduated high/secondary school
☐ Diploma
☐ Undergraduate degree
☐ Postgraduate degree
☐ Have never attended school

Are they able to do age appropriate work?
Are they able to do age-appropriate tasks?
☐ YES
☐ NO
☐ UNSURE

Do they require assistance/help with completing age-appropriate work?
Do they require assistance/help with completing age-appropriate tasks?
☐ YES
☐ NO
☐ UNSURE

*Have you been diagnosed with an intellectual disability?
*Has your child been diagnosed with an intellectual disability?
*Have they been diagnosed with an intellectual disability?
☐ YES
☐ NO
☐ UNSURE

*What level of intellectual disability have you been diagnosed with?
*What level of intellectual disability has your child been diagnosed with?
*What level of intellectual disability have they been diagnosed with?
☐ Borderline
☐ Mild
☐ Moderate
☐ Severe
☐ Profound
☐ Unsure

Which of these most accurately describes your child's language proficiency?
Which of these most accurately describes the individual's language proficiency?
☐ Pre-verbal (e.g., coos and makes pleasure sounds)
☐ Distinct consonant/vowel sounds (e.g., babbles in a speech-like way)
☐ Single word utterances (e.g., "Hi", "dog", "Dada", or "Mama")
☐ Two word utterances (e.g., "Where kitty?" or "more cookie")
☐ Two or three word phrases (i.e., speaks in a way that is understood by family members and friends)
☐ Four or more word sentences (i.e., speaks easily without having to repeat syllables or words)
☐ Fluency in native language (i.e., communicates easily with other children and adults)

MUTATION DETAILS

The next questions relate to your diagnosed mutation.
The next questions relate to your child's diagnosed mutation.
The next questions relate to the individual's diagnosed mutation.

*What is your diagnosed mutation? *E.g., c.1019A>G or p.D340N*
*What is your child's diagnosed mutation? *E.g., c.1019A>G or p.D340N*
*What is their diagnosed mutation? *E.g., c.1019A>G or p.D340N*
If you are unsure, just type "unsure."

*Please select the option that reflects the inheritance of your mutation.
☐ Only I have the mutation
☐ I have the mutation but neither parent has been tested
☐ Both my mother and I have the mutation
☐ Both my father and I have the mutation
☐ Unsure

*Please select the option that reflects the inheritance of your child's mutation.
☐ Only my child has the mutation
☐ My child has the mutation but neither parent has been tested
☐ My child and I have the mutation
☐ My child and his/her other parent have the mutation
☐ Unsure

*Please select the option that reflects the inheritance of their mutation.
☐ Only the individual has the mutation
☐ The individual has the mutation but neither parent has been tested
☐ The individual and their mother have the mutation
☐ The individual and their father have the mutation
☐ Unsure

*Have you been diagnosed with any additional mutations?
*Has your child been diagnosed with any additional mutations?
*Has the individual been diagnosed with any additional mutations?
☐ YES
☐ NO
☐ UNSURE

Please provide details of any additional mutations that you have been diagnosed with. *E.g., SCN1A, c.2837A>G or p.R466H*
Please provide details of any additional mutations that your child has been diagnosed with. *E.g., SCN1A, c.2837A>G or p.R466H*
Please provide details of any additional mutations the individual has been diagnosed with. *E.g., SCN1A, c.2837A>G or p.R466H*

SCREENING

Has your child ever had a seizure?
Has the individual ever had a seizure?
Have you ever had a seizure?
☐ YES
☐ NO

MEDICATION DETAILS

The next questions relate to your seizure medication.
The next questions relate to your child's seizure medication.
The next questions relate to the individual's seizure medication.

Please select all the medication that you have **previously taken** for your seizures.
Please select all the medication that your child has **previously taken** for their seizures.
Please select all the medication that the individual has **previously taken** for their seizures.

Please select all the medication that you are **currently taking** for your seizures.
Please select all the medication that your child is **currently taking** for their seizures.
Please select all the medication that the individual is **currently taking** for their seizures.

☐ Acetazolamide (e.g., Diamox)
☐ Carbamazepine (e.g., Tegretol)
☐ Cannabidiol (e.g., Sativex)
☐ Clobazam (Onfi, Frisium)
☐ Clonazepam (e.g., Klonotin)
☐ Clorazepate (Tranxene)
☐ Ethosuximide (Zarontin)
☐ Gabapentin (e.g., Neurontin)
☐ Lacosamide (Vimpat)
☐ Lamotrigine (e.g., Lamictal)
☐ Levetiracetam (Keppra)
☐ Nitrazepam (e.g., Alodorm)
☐ Oxcarbazepine (Oxtellar, Trileptal)
☐ Perampanel (Fycompa)
☐ Phenytoin (e.g., Dilantin)
☐ Piracetam (e.g., Nootropil)
☐ Potassium Bromide (Dibro-Be mono)
☐ Primidone (Mysoline)
☐ Pyridoxine/Vitamin B6 (e.g., Neuro-K)
☐ Retigabine/Ezogabine (Potiga, Trobalt)
☐ Rufinamide (Banzel, Inovelon)
☐ Stiripentol (Diacomit)
☐ Sultiame/Sulthiame (Ospolot)
☐ Tiagabine (Gabitril)
☐ Topiramate (Topamax)
☐ Valproate/Valproic Acid (e.g., Depakote)
☐ Vigabatrin/Gamma Vinyl GABA (Sabril)
☐ Zonisamide (Zonegran)
☐ Other (please specify)

*Have your seizures been controlled with medication for a period of time?
*Have your child's seizures been controlled with medication for a period of time?
*Have the individual's seizures been controlled with medication for a period of time?
☐ YES
☐ NO

Are you currently seizure-free?
Is your child currently seizure-free?
Is the individual currently seizure-free?
☐ YES
☐ NO

Please provide details of the medication or medications that have been successful in controlling your seizures. Please provide the duration (in months) that you have remained seizure free in brackets following each medication listed. *E.g., Valproate (13 months), Lamotrigine (5 months)*

Please provide details of the medication or medications that have been successful in controlling your child's seizures. Please provide the duration (in months) that your child remained seizure free in brackets following each medication listed. *E.g., Valproate (13 months), Lamotrigine (5 months)*

Please provide details of the medication or medications that have been successful in controlling the individual's seizures. Please provide the duration (in months) that they remained seizure free in brackets following each medication listed. *E.g., Valproate (13 months), Lamotrigine (5 months)*

SEIZURE DETAILS

The next questions relate to your seizures.
The next questions relate to your child's seizures.
The next questions relate to the individual's seizures.

*At what age (in months) did you begin having seizures?
*At what age (in months) did your child begin having seizures?
*At what age (in months) did they begin having seizures?
If you are unsure, just type "unsure."

How many hospital admissions for seizures have you had?
How many hospital admissions for seizures has your child had?
How many hospital admissions for seizures have they had?
☐ 0
☐ 1
☐ 2
☐ 3
☐ 4
☐ 5+
☐ Unsure

Have you been admitted to ICU as a result of seizures?
Has your child been admitted to ICU as a result of seizures?
Have they been admitted to ICU as a result of seizures?
☐ YES
☐ NO

*Have you ever had a seizure lasting longer than 30 minutes?
*Has your child ever had a seizure lasting longer than 30 minutes?
*Have they ever had a seizure lasting longer than 30 minutes?
☐ YES
☐ NO
☐ UNSURE

How often do you have seizures that last longer than 30 minutes?
How often does your child have seizures that last longer than 30 minutes?
How often do they have seizures that last longer than 30 minutes?
☐ Daily
☐ Weekly
☐ Monthly
☐ Yearly
☐ Other (please specify)

How long do these seizures last? *Multiple options may be selected.*
☐ 30-40 minutes
☐ 40-50 minutes
☐ 50-60 minutes
☐ 60+ minutes

Are these long seizures convulsive (limbs shaking)?
☐ YES
☐ NO
☐ UNSURE

Are these long seizures non-convulsive?
☐ YES
☐ NO
☐ UNSURE

*Do your seizures cluster (many seizures occur together then there are long periods of time with no seizures)?
*Do your child's seizures cluster (many seizures occur together then there are long periods of time with no seizures)?
*Do their seizures cluster (many seizures occur together then there are long periods of time with no seizures)?
☐ YES
☐ NO
☐ UNSURE

What is the average number of days a cluster lasts?

What is the average number of seizures per day in a cluster?

How many seizure clusters have you had in the last 12 months?
How many seizure clusters has your child had in the last 12 months?
How many seizure clusters have they had in the last 12 months?

* Do you ever experience isolated seizures (not in a cluster)?
*Does your child ever experience isolated seizures (not in a cluster)?
*Do they ever experience isolated seizures (not in a cluster)?
☐ YES
☐ NO
☐ UNSURE

When you have experienced isolated seizures, how often did they occur?
When they have experienced isolated seizures, how often did they occur?
☐ Daily
☐ Weekly
☐ Monthly
☐ Yearly
☐ Other (please specify)

When was your last seizure?
When was your child's last seizure?
When was the individual's last seizure?

FEEDBACK

What do you find to be most challenging each day?
As a parent, what do you find to be most challenging each day?
As someone who is close to this individual, what do you find to be most challenging each day?

What do you find to be the most helpful in responding to these challenges?

Is there any other information that you wish to share (such as, seizures are commonly associated with temperature instability)?

Appendix B: Scripts

B1 Survey

UNDERSTANDING INDIVIDUAL DIFFERENCES ASSOCIATED WITH PROTOCADHERIN-19 (PCDH19) MUTATIONS

Page 1: WELCOME TO THE SURVEY

Thank you for taking the time to access this survey for a Doctor of Philosophy student research project.

It should take no more than 45 minutes to complete.

Your responses will not be individually identifiable.

Your honesty in providing your responses would be greatly appreciated.

The survey will be open until March 2019.

NEXT

Page 2: Human Research Ethics Committee (HREC) consent form

(This project has been approved by HREC: H-2016-184). Any concerns or queries can be directed to the HREC Secretariat - hrec@adelaide.edu.au).

1. I have read the Participant Information Sheet provided via email and agree to take part in the following research project:

Understanding individual differences associated with PCDH19 mutations.

2. I am aged 18 years or older and am able to give consent freely.

3. I have had the project, so far as it affects me, fully explained to my satisfaction by the researcher via the Participant Information Sheet (attached to the original email invite). My consent is given freely.

4. I understand the purpose of the research project and understand that my involvement will not be of any benefit to me directly.

5. I understand that information gained during the study may be published, and I will not be identified, nor will my personal results be shared.

6. I understand that I am free to withdraw from the project without consequence.

7. I am aware that I should keep a copy of this consent form (can take a screen shot) and the Participant Information Sheet provided previously.

8. I understand that by submitting my survey response I consent to my data being used for this project.

*I have read the informed consent and freely provide my consent to participate.

☐ YES
☐ NO

Please provide your email address below.

PREV/NEXT

Page 3: Screening

*Are you completing this survey on behalf of yourself or someone else?

☐ Yourself
☐ Someone else

Page 4: Screening

*How old are you?

☐ Younger than 11 years old
☐ 11 – 14 years old
☐ 15 – 17 years old
☐ Older than 17 years old

Page 5: Assent

We are from the University of Adelaide in Australia and we are asking you to be in our research. We do research to learn more about how the world works and why people act the way they do. In this study, we want to learn more about epilepsy.

What we are asking you to do:
We would like to ask you to take a 30-minute online survey about your feelings, thoughts, and the things you do.

Do I have to be in this study?
You do not have to participate in this study. It is up to you. You can say no now or you can even change your mind later. No one will be upset with you if you decide not to be in this study.

Will being in this study hurt or help me in any way?
Being in this study will bring you no harm. There are no direct benefits to you for participating in this study. It will hopefully help us learn more about epilepsy.

What will you do with information about me?
We will be very careful to keep your answers to the survey questions private. Before and after the study we will keep all information we collect about you locked up and password protected and no individual will be identified in any report of the findings.

If you have questions about the study, contact: kristy.kolc@adelaide.edu.au

If you have questions about your rights in the study, contact: Human Research Ethics Committee, hrec@adelaide.edu.au

If you become upset during the survey or would like to talk to someone about how you are feeling, contact: rachel.roberts@adelaide.edu.au

*I agree to be in the study described above

☐ YES
☐ NO

Page 6: Screening

*Is the person you are responding on behalf of your child?

☐ YES

☐ NO

Page 7: Screening

*How old is your child?

☐ Between 2 and 4 years old

☐ Between 5 and 10 years old

☐ Between 11 and 17 years old

☐ 18 years old or older

Page 8: Screening

*How old is the individual you are responding on behalf of?

☐ Under 18 years of age

☐ 18 years or over

Page 9: Thank you

We're sorry. You do not meet the requirements for this survey. We sincerely thank you and appreciate your time and effort.

If you have or know a parent willing to complete this survey, please kindly forward them the survey link.

Again, thank you and have a nice day.

Page 267: THIRD PARTY CONSENT

*If you are unable to provide mutation and/or seizure details, and/or (where applicable) neurocognitive/developmental details, are you happy for the researchers in this study to contact the person (or persons) who can provide this information?

☐ YES
☐ NO
☐ UNSURE

Page 268: THIRD PARTY CONSENT

I consent to information being obtained from *(please select appropriate)*

☐ Clinician

☐ Physician

☐ GP

☐ Other (please specify)

Contact name:

Contact email:

*Is there another person who can also provide this information?

☐ YES

☐ NO

Page 271: COMPLETION: THANK YOU

This completes the survey

Thank you for your participation

Information collected in this research project may be used to answer similar questions in the future, such as to investigate the potential benefits of a given treatment. Please indicate whether or not you consent for the information you have provided being used in the future by selecting one of the boxes below:

*Consent for data to be used in future research:

☐ YES

☐ NO

Please note that no individual will be personally identifiable in any reporting of future results

This information may also be compared to existing medical data that we have if, for instance, you or the individual you are responding on behalf of, has provided a skin or saliva sample. Please indicate whether or not you consent for the information you have provided being compared to existing data by selecting one of the boxes below:

*Consent for data to be compared to existing information:

☐ YES

☐ NO

Once you have indicated your response above, please click on the DONE button located at the bottom of this page to submit your survey.

(This project has been approved by HREC: H-2016-184. Any concerns or queries can be directed to the HREC Secretariat - hrec@adelaide.edu.au)

*Indicates a required field

PREV/DONE

B2 Neuropsychiatric Assessment

Page 10: Questionnaire 1

SRS-2 AutoScore Form (Preschool)

John N. Constantino, MD

Instructions

For each question, please select the response that best describes your child's behavior **over the past 6 months**.

1 = NOT TRUE 2 = SOMETIMES TRUE 3 = OFTEN TRUE 4 = ALMOST ALWAYS TRUE

Strengths and Difficulties Questionnaire (P 2-4; © Robert Goodman, 2005)

For each item, please select the option for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain. Please give your answers on the basis of your child's behaviour over the last six months.

Please give your answers on the basis of your child's behaviour over the last six months.

BRIEF-P: Behavior Rating Inventory of Executive Function- Preschool Version

RATING FORM

Gerard A. Gioia, PhD, Kimberly Andrews Espy, PhD, and Peter K. Isquith, PhD

Instructions to Parents

Below is a list of statements that describe young children. We would like to know if your child has had *problems* with these behaviors *during the past 6 months*. Please answer *all the items* the best that you can. Please do not skip any items. Think about your child as you read these statements and select:

N if the behavior is **Never** a problem
S if the behavior is **Sometimes** a problem
O if the behavior is **Often** a problem

For example, if having tantrums when told "No" is **never** a problem, you would select **N** for this item:

Has tantrums when told "No" **N** S O

During the past 6 months, how often has each of the following behaviors been a *problem*?

N = NEVER
S = SOMETIMES
O = OFTEN

(School-Age)
(P 5-10; © Robert Goodman, 2005)

BRIEF: Behavior Rating Inventory of Executive Function PARENT FORM

Gerard A. Gioia, PhD, Peter K. Isquith, PhD, Steven C. Guy, PhD, and Lauren Kenworthy, PhD

Below is a list of statements that describe children. We would like to know if your child has had problems with these behaviors over the past 6 months. Please answer all the items the best that you can. Please DO NOT SKIP ANY ITEMS. Think about your child as you read each statement and select your response:

For example, if your child **never** has trouble completing homework on time, you would select **N** for this item:

Has trouble completing homework on time **N** S O

Over the past 6 months, how often has each of the following behaviors been a *problem*?

(P 11-17; © Robert Goodman, 2005)

(Adult Relative/Other Report)

For each question, please select the response that best describes this individual's behavior **over the past 6 months.**

BRIEF-A: Behavior Rating Inventory of Executive Function-Adult Version
INFORMANT REPORT FORM

Robert M. Roth, PhD, Peter K. Isquith, PhD, and Gerard A. Gioia, PhD

Below is a list of statements that may describe your child/parent/spouse/sibling or another person with whom you are familiar. We would like to know if he/she has had problems with these behaviors over the past month. Please answer all the items the best that you can. Please DO NOT SKIP ANY ITEMS. Think about him/her as you read each statement and then indicate your response by selecting:

For example, if he/she **never** has trouble making decisions, you would select **N** for this item:

Has trouble making decisions **N** S O

During the past month, how often has each of the following behaviors been a *problem*?

(S 11-17; © Robert Goodman, 2005)

For each item, please select the response for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain. Please give your answers on the basis of how things have been for you over the last six months.

Please give your answers on the basis of how things have been for you over the last six months.

BRIEF-SR: Behavior Rating Inventory of Executive Function- Self-Report Version RATING FORM

Steven C. Guy, PhD, Peter K. Isquith, PhD, and Gerard A. Gioia, PhD

Below is a list of statements that describe young people. We would like to know if you have had any problems with these behaviors *over the past 6 months*. Please answer *all of the items* the best that you can, even if they don't seem to apply to you. Please think about yourself as you read these statements and respond by selecting:

For example, if you **never** have trouble completing homework on time, you would select **N** for this item:

I have trouble completing homework on time **N** S O

Dimensional Obsessive-Compulsive Scale (© Jonathan S. Abramowitz, 2009)

This questionnaire asks you about 4 different types of concerns that you might or might not experience. For each type there is a description of the kinds of thoughts (sometimes called *obsessions*) and behaviors (sometimes called *rituals* or *compulsions*) that are typical of that particular concern, followed by 5 questions about your experiences with these thoughts and behaviors. Please read each description carefully and answer the questions for each category based on your experiences in the last month.

(Adult Self Report)

For each question, please select the response that best describes your behavior over the past 6 months.

BRIEF-A: Behavior Rating Inventory of Executive Function- Adult Version SELF-REPORT FORM

Robert M. Roth, PhD, Peter K. Isquith, PhD, and Gerard A. Gioia, PhD

Below is a list of statements. We would like to know if you have had problems with these behaviors over the past month. Please answer all the items the best that you can. Please DO NOT SKIP ANY ITEMS. Indicate your response by selecting:

For example, if you **never** have trouble making decisions, you would select **N** for this item:

I have trouble making decisions **N** S O

Appendix C: Survey Materials

C1 Advertisement

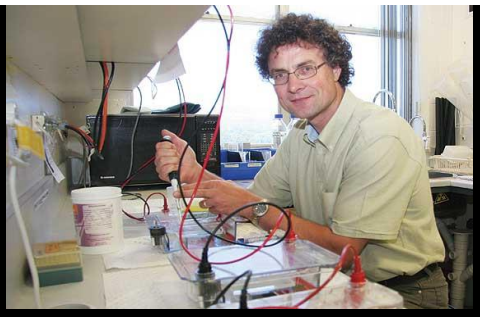
Protocadherin-19 (PCDH19) Research

(H-2016-184)

The research team at the University of Adelaide, led by Professor Jozef Gecz, are looking for people who have a protocadherin-19 (PCDH19) mutation or are able to report on behalf of someone with a PCDH19 mutation to participate in their research project. Their project aims to understand the types of symptoms associated with PCDH19 mutations, as well as the challenges faced by affected individuals and their families.

For more information, please email kristy.kolc@adelaide.edu.au

C2 Flyer



Professor Jozef Gecz is an Australian NH&MRC Senior Principal Research Fellow and Professor of Human Genetics at the University of Adelaide. He is the founding head of the Neurogenetics Research Program located at the Adelaide Women's and Children's Hospital

Protocadherin-19 (PCDH19) Research

(H-2016-184)

The research team at the University of Adelaide in Australia, led by Professor Jozef Gecz, are looking for people who have a PCDH19 mutation or are able to report on behalf of someone with a PCDH19 mutation to participate in their research project. Their project aims to understand the types of symptoms associated with PCDH19 mutations, as well as the challenges faced by affected individuals and their families.

For more information, please email kristy.kolc@adelaide.edu.au

THE UNIVERSITY of ADELAIDE

seekLIGHT

C3 Invitation

Dear [insert name],

I am contacting you on behalf of Professor Jozef Gecz, who is the head of Neurogenetics Research at the University of Adelaide, Australia and a leading researcher in Protocadherin-19 (PCDH19) epilepsy. Professor Gecz and his team are looking for people who have a PCDH19 mutation or are able to report on behalf of someone with a PCDH19 mutation to participate in their research project (H-2016-184).

Their research project aims to improve our understanding of the types of symptoms associated with PCDH19 mutations, as well as the challenges faced by affected individuals and their families. Their project also aims to provide a clear picture of how these symptoms change over time by looking at different age groups. This information will help us to better understand PCDH19 mutations, which will help to build upon existing treatment and support programs for affected individuals and their families.

Participation would involve responding to a number of online questionnaires, which should take no more than 45 minutes of your time. The survey can be completed by parents or knowledgeable informants of individuals affected by PCDH19 mutations or, where applicable, the individuals themselves (this also includes unaffected carriers of the mutation).

If you would like to know more about this research or are interested in participating, please contact kristy.kolc@adelaide.edu.au

Your decision will not impact the services you currently or may receive in any way, and you are under no obligation to participate in this research.

Should you have any questions about this project, you are encouraged to contact Kristy Kolc.

Sincerely,

[Insert name]

C4 Contact

Dear Parent/Participant,

Thank you for your interest in our study. I would like to introduce myself. My name is Kristy Kolc and I am a PhD student working under the supervision of Professor Jozef Gecz, who is the head of Neurogenetics Research at the University of Adelaide and a leading researcher in Protocadherin-19 (PCDH19) epilepsy.

Also attached to this email you will find a participant information sheet, which outlines all the details related to this research project and what your participation or your child's participation will entail. Please kindly read this information sheet and if you are interested in participating/your child participating please click on the link at the end of the information sheet, which will take you to the consent page and the start of the survey.

Please note that progress may be saved and the survey returned to at any time. Should you decide not to participate/for your child not to participate, we thank you for your time and wish you all the best.

Sincerely,

Kristy Kolc and the PCDH19 Research Team

C5 Reminder Email

Dear [insert name],

This is just a friendly reminder regarding your participation in our research project that aims to improve our understanding of the types of symptoms associated with PCDH19 mutations, as well as the challenges faced by affected individuals and their families. If you have decided not to participate in this research, please disregard this message.

We realize that it is often hard to find time in our busy lives, which is why we have made it possible for you to save your survey progress and come back to it later. We have also tried to minimize the amount of time needed to complete the questionnaires by only asking those questions that we believe to be directly relevant. We expect that it should take no more than 45 minutes of your time.

We have attached the participant information sheet. Once you have read this and decide that you wish to participate, please click on the link at the end of the information sheet to take you to the consent page and the start of the survey.

Please be advised that the survey closing date is **[insert date].** Following this, you will no longer be able to access the survey.

Thank you again and we wish you all the best,

Kristy Kolc and the PCDH19 Research Team

Appendix D: Consent Forms

D1 Participant Information Sheet (Parent)

PROJECT TITLE: Understanding individual differences associated with protocadherin-19 (PCDH19) mutations
HUMAN RESEARCH ETHICS COMMITTEE APPROVAL NUMBER: H-2016-184
PRINCIPAL INVESTIGATOR: Professor Jozef Gecz
STUDENT RESEARCHER: Kristy Kolc

Dear Parent,

You are invited to participate in the research project described below.

What is the project about?
The research project aims to understand the symptoms associated with protocadherin-19 (PCDH19) mutations. The project also aims to provide a clear picture of how these symptoms change over time by looking at different age groups. This information will help us to better understand PCDH19 mutations, which will help to build upon existing treatment and support programs for affected individuals and their families.

Who is undertaking the project?
This project is being conducted by Kristy Kolc. This research will form the basis for the degree of Doctor of Philosophy at the University of Adelaide, Australia under the supervision of Prof Jozef Gecz, Dr Duyen Pham, and Dr Rachel Roberts. This project is supported by an NHMRC grant and international philanthropic funding.

Why am I being invited to participate?
This research is to be undertaken amongst males and females who have a PCDH19 mutation and those who are able to report on behalf of someone with a PCDH19 mutation.

What will I be asked to do?
Participants will be asked to complete three questionnaires on behalf of their child that measure autism spectrum symptoms (*e.g., "has an unusually narrow range of interests"*), attention-deficit/hyperactivity symptoms (*e.g., "I think before I do things"*), and executive function (*e.g., problem-solving skills*). They will also be asked to provide general (*e.g., age, sex, country of residence*) and (if applicable) clinical (*e.g., medication, mutation, and seizure details*) information.

How much time will the project take?
Your participation in the project will take between 30-45 minutes.

Are there any risks associated with participating in this project?
The questionnaires are not intended to cause distress in any way, although responding to questions about personal experiences may cause distress for some participants. Please note that if you experience distress at any point, you do not need to continue your participation. Some people may feel they could benefit from speaking to someone about how they are feeling. There are a number of people you can contact in this event, such as a doctor, counsellor, or the PCDH19 Alliance (http://pcdh19info.org/). Additionally, Dr Rachel Roberts is a clinical psychologist and is available to be contacted at rachel.roberts@adelaide.edu.au.

What are the benefits of the research project?
This research will allow participants to share information about their personal experiences, which will potentially improve our understanding of the types of symptoms associated with PCDH19 mutations, as well as the challenges faced by affected individuals and their families.

Can I withdraw from the project?
Participation in this project is completely voluntary. If you agree to participate, you can withdraw from the study up until the submission of the PhD thesis. Findings from this research will be shared through publications and presentations. If material you have contributed has already been published, it will not be possible to withdraw it from that publication. However, you may withdraw your contribution from the research data and, in that case it will not be used in any further publications.

What will happen to my information?
The information collected from this project will be confidentially stored on password protected university computers, and will only be accessible to the researchers listed on this form. This information will be kept for a minimum of five years and all responses are confidential and only group responses will be reported so that no individual will be identified in any report of the findings. With your additional consent, the information collected may be used in future research addressing similar questions or linked to existing molecular data (through provision of saliva or skin samples, for example). Again, no individual will be identified in any future use of the results. The disposal of any data or personal records will be carried out in accordance with the requirements of the *State Records Act 1997* and the University Records Management Policy.

Who do I contact if I have questions about the project?
Any questions regarding this project should be addressed to:

Professor Jozef Gecz
Email: jozef.gecz@adelaide.edu.au
Phone: +618 8313 2453

or

Dr Duyen Pham
Email: duyen.pham@adelaide.edu.au
Phone: +618 8313 7955

Dr Rachel Roberts
Email: rachel.roberts@adelaide.edu.au
Phone: +618 8313 5228

or

Ms Kristy Kolc
Email: kristy.kolc@adelaide.edu.au
Phone: +618 8313 7984

What if I have a complaint or any concerns?
The study has been approved by the Human Research Ethics Committee (HREC) at the University of Adelaide (approval number H-2016-184). If you have questions or problems associated with the practical aspects of your participation in the project, or wish to raise a concern or complaint about the project, then you should consult the Principal Investigator. If you wish to speak with an independent person regarding a concern or complaint, the University's policy on research involving human participants, or your rights as a participant, please contact the HREC Secretariat on:

Phone: +61 8 8313 6028
Email: hrec@adelaide.edu.au
Post: c/- Research Services, The University of Adelaide, SA 5005 AUSTRALIA

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

If I want to participate, what do I do?
If you wish to participate, please click on the link https://www.research.net/r/PCDH19- and enter password PCDH19- (for your first child) and https://www.research.net/r/PCDH19- and enter password PCDH19- (for your second child, if applicable) to take you to the consent page and the start of the survey. Completion and submission of the survey indicates your consent to be involved in the research project.

Yours sincerely,

Prof Jozef Gecz	Dr Duyen Pham	Dr Rachel Roberts	Ms Kristy Kolc
Neurogenetics Head	Postdoctoral Fellow	Senior Lecturer	PhD Candidate

D2 Participant Information Sheet (Informant)

PROJECT TITLE: Understanding individual differences associated with protocadherin-19 (PCDH19) mutations
HUMAN RESEARCH ETHICS COMMITTEE APPROVAL NUMBER: H-2016-184
PRINCIPAL INVESTIGATOR: Professor Jozef Gecz
STUDENT RESEARCHER: Kristy Kolc

Dear Participant,

You are invited to participate in the research project described below.

What is the project about?
The research project aims to understand the symptoms associated with protocadherin-19 (PCDH19) mutations. The project also aims to provide a clear picture of how these symptoms change over time by looking at different age groups. This information will help us to better understand PCDH19 mutations, which will help to build upon existing treatment and support programs for affected individuals and their families.

Who is undertaking the project?
This project is being conducted by Kristy Kolc. This research will form the basis for the degree of Doctor of Philosophy at the University of Adelaide, Australia under the supervision of Prof Jozef Gecz, Dr Duyen Pham, and Dr Rachel Roberts. This project is supported by a NHMRC grant and international philanthropic funding.

Why am I being invited to participate?
This research is to be undertaken amongst males and females who have a PCDH19 mutation and those who are able to report on behalf of someone with a PCDH19 mutation.

What will I be asked to do?
Participants will be asked to complete two questionnaires on behalf of the individual with a PCDH19 mutation that measure autism spectrum symptoms (*e.g., "has an unusually narrow range of interests"*) and executive function (*e.g., problem-solving skills*). They will also be asked to provide general *(e.g., age, sex, country of residence)* and (if applicable) clinical (*e.g., medication, mutation, and seizure details*) information.

How much time will the project take?
Your participation in the project will take between 25-40 minutes.

Are there any risks associated with participating in this project?
The questionnaires are not intended to cause distress in any way, although responding to questions about personal experiences may cause distress for some participants. Please note that if you experience distress at any point, you do not need to continue your participation. Some people may feel they could benefit from speaking to someone about how they are feeling. There are a number of people you can contact in this event, such as a doctor, counsellor, or the PCDH19 Alliance (http://pcdh19info.org/). Additionally, Dr Rachel Roberts is a clinical psychologist and is available to be contacted at rachel.roberts@adelaide.edu.au.

What are the benefits of the research project?
This research will allow participants to share information about their personal experiences, which will potentially improve our understanding of the types of symptoms associated with PCDH19 mutations, as well as the challenges faced by affected individuals and their families.

Can I withdraw from the project?
Participation in this project is completely voluntary. If you agree to participate, you can withdraw from the study up until the submission of the PhD thesis. Findings from this research will be shared through publications and presentations. If material you have contributed has already been published, it will not be possible to withdraw it from that publication. However, you may withdraw your contribution from the research data and, in that case it will not be used in any further publications.

What will happen to my information?
The information collected from this project will be confidentially stored on password protected university computers, and will only be accessible to the researchers listed on this form. This information will be kept for a minimum of five years and all responses are confidential and only group responses will be reported so that no individual will be identified in any report of the findings. With your additional consent, the information collected may be used in future research addressing similar questions or linked to existing molecular data (through provision of saliva or skin samples, for example). Again, no individual will be identified in any future use of the results. The disposal of any data or personal records will be carried out in accordance with the requirements of the *State Records Act 1997* and the University Records Management Policy.

Who do I contact if I have questions about the project?
Any questions regarding this project should be addressed to:

Professor Jozef Gecz or Dr Duyen Pham
Email: jozef.gecz@adelaide.edu.au Email: duyen.pham@adelaide.edu.au
Phone: +618 8313 2453 Phone: +618 8313 7955

Dr Rachel Roberts or Ms Kristy Kolc
Email: rachel.roberts@adelaide.edu.au Email: kristy.kolc@adelaide.edu.au
Phone: +618 8313 5228 Phone: +618 8313 7984

What if I have a complaint or any concerns?
The study has been approved by the Human Research Ethics Committee (HREC) at the University of Adelaide (approval number H-2016-184). If you have questions or problems associated with the practical aspects of your participation in the project, or wish to raise a concern or complaint about the project, then you should consult the Principal Investigator. If you wish to speak with an independent person regarding a concern or complaint, the University's policy on research involving human participants, or your rights as a participant, please contact the HREC Secretariat on:

Phone: +61 8 8313 6028 Email: hrec@adelaide.edu.au
Post: c/- Research Services, The University of Adelaide, SA 5005 AUSTRALIA

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

If I want to participate, what do I do?
If you wish to participate, please click on the link https://www.research.net/r/PCDH19- and enter password PCDH19- to take you to the consent page and the start of the survey. Completion and submission of the survey indicates your consent to be involved in the research project.

Yours sincerely,

Prof Jozef Gecz	Dr Duyen Pham	Dr Rachel Roberts	Ms Kristy Kolc
Neurogenetics Head	Postdoctoral Fellow	Senior Lecturer	PhD Candidate

D3 Participant Information Sheet (Adult Self-Report)

PROJECT TITLE: Understanding individual differences associated with protocadherin-19 (PCDH19) mutations
HUMAN RESEARCH ETHICS COMMITTEE APPROVAL NUMBER: H-2016-184
PRINCIPAL INVESTIGATOR: Professor Jozef Gecz
STUDENT RESEARCHER: Kristy Kolc

Dear Participant,

You are invited to participate in the research project described below.

What is the project about?
The research project aims to understand the symptoms associated with protocadherin-19 (PCDH19) mutations. The project also aims to provide a clear picture of how these symptoms change over time by looking at different age groups. This information will help us to better understand PCDH19 mutations, which will help to build upon existing treatment and support programs for affected individuals and their families.

Who is undertaking the project?
This project is being conducted by Kristy Kolc. This research will form the basis for the degree of Doctor of Philosophy at the University of Adelaide, Australia under the supervision of Prof Jozef Gecz, Dr Duyen Pham, and Dr Rachel Roberts. This project is supported by an NHMRC grant and international philanthropic funding.

Why am I being invited to participate?
This research is to be undertaken amongst males and females who have a PCDH19 mutation and those who are able to report on behalf of someone with a PCDH19 mutation.

What will I be asked to do?
Participants will be asked to complete three questionnaires that measure autism spectrum symptoms (*e.g., "people think I am interested in too few topics, or that I get too carried away with those topics"*), obsessive-compulsive symptoms (*e.g., about how much time have you spent each day with unwanted unpleasant thoughts and with behavioral or mental actions to deal with them?*), and executive function (*e.g., problem-solving skills*). They will also be asked to provide general (*e.g., age, sex, country of residence*) and (if applicable) clinical (*e.g., medication, mutation, and seizure details*) information.

How much time will the project take?
Your participation in the project will take between 30-45 minutes.

Are there any risks associated with participating in this project?
The questionnaires are not intended to cause distress in any way, although responding to questions about personal experiences may cause distress for some participants. Please note that if you experience distress at any point, you do not need to continue your participation. Some people may feel they could benefit from speaking to someone about how they are feeling. There are a number of people you can contact in this event, such as a doctor, counsellor, or the PCDH19 Alliance (http://pcdh19info.org/). Additionally, Dr Rachel Roberts is a clinical psychologist and is available to be contacted at rachel.roberts@adelaide.edu.au.

What are the benefits of the research project?
This research will allow participants to share information about their personal experiences, which will potentially improve our understanding of the types of symptoms associated with PCDH19 mutations, as well as the challenges faced by affected individuals and their families.

Can I withdraw from the project?
Participation in this project is completely voluntary. If you agree to participate, you can withdraw from the study up until the submission of the PhD thesis. Findings from this research will be shared through publications and presentations. If material you have contributed has already been published, it will not be possible to withdraw it from that publication. However, you may withdraw your contribution from the research data and, in that case it will not be used in any further publications.

What will happen to my information?
The information collected from this project will be confidentially stored on password protected university computers, and will only be accessible to the researchers listed on this form. This information will be kept for a minimum of five years and all responses are confidential and only group responses will be reported so that no individual will be identified in any report of the findings. With your additional consent, the information collected may be used in future research addressing similar questions or linked to existing molecular data (through provision of saliva or skin samples, for example). Again, no individual will be identified in any future use of the results. The disposal of any data or personal records will be carried out in accordance with the requirements of the *State Records Act 1997* and the University Records Management Policy.

Who do I contact if I have questions about the project?
Any questions regarding this project should be addressed to:

Professor Jozef Gecz or Dr Duyen Pham
Email: jozef.gecz@adelaide.edu.au Email: duyen.pham@adelaide.edu.au
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Dr Rachel Roberts or Ms Kristy Kolc
Email: rachel.roberts@adelaide.edu.au Email: kristy.kolc@adelaide.edu.au
Phone: +618 8313 5228 Phone: +618 8313 7984

What if I have a complaint or any concerns?
The study has been approved by the Human Research Ethics Committee (HREC) at the University of Adelaide (approval number H-2016-184). If you have questions or problems associated with the practical aspects of your participation in the project, or wish to raise a concern or complaint about the project, then you should consult the Principal Investigator. If you wish to speak with an independent person regarding a concern or complaint, the University's policy on research involving human participants, or your rights as a participant, please contact the HREC Secretariat on:

Phone: +61 8 8313 6028 Email: hrec@adelaide.edu.au
Post: c/- Research Services, The University of Adelaide, SA 5005 AUSTRALIA

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

If I want to participate, what do I do?
If you wish to participate, please click on the link https://www.research.net/r/PCDH19- and enter password PCDH19- to take you to the consent page and the start of the survey. Completion and submission of the survey indicates your consent to be involved in the research project.

Yours sincerely,

Prof Jozef Gecz	Dr Duyen Pham	Dr Rachel Roberts	Ms Kristy Kolc
Neurogenetics Head	Postdoctoral Fellow	Senior Lecturer	PhD Candidate

D4 Participant Information Sheet (Youth Self-Report)

PROJECT TITLE: Understanding individual differences associated with protocadherin-19 (PCDH19) mutations
HUMAN RESEARCH ETHICS COMMITTEE APPROVAL NUMBER: H-2016-184
PRINCIPAL INVESTIGATOR: Professor Jozef Gecz
STUDENT RESEARCHER: Kristy Kolc

Dear Parent,

Your child is invited to participate in the research project described below.

What is the project about?
The research project aims to understand the symptoms associated with protocadherin-19 (PCDH19) mutations. The project also aims to provide a clear picture of how these symptoms change over time by looking at different age groups. This information will help us to better understand PCDH19 mutations, which will help to build upon existing treatment and support programs for affected individuals and their families.

Who is undertaking the project?
This project is being conducted by Kristy Kolc. This research will form the basis for the degree of Doctor of Philosophy at the University of Adelaide, Australia under the supervision of Prof Jozef Gecz, Dr Duyen Pham, and Dr Rachel Roberts. This project is supported by an NHMRC grant and international philanthropic funding.

Why am I being invited to participate?
This research is to be undertaken amongst males and females who have a PCDH19 mutation and those who are able to report on behalf of someone with a PCDH19 mutation.

What will I be asked to do?
Participants will be asked to complete three questionnaires that measure obsessive-compulsive symptoms (*e.g., about how much time have you spent each day with unwanted unpleasant thoughts and with behavioral or mental actions to deal with them?*), attention-deficit/hyperactivity symptoms (*e.g., "I think before I do things"*), and executive function (*e.g., problem-solving skills*). They will also be asked to provide general *(e.g., age, sex, country of residence)* and (if applicable) clinical (*e.g., medication, mutation, and seizure details*) information.

How much time will the project take?
Your child's participation in the project will take between 20-30 minutes.

Are there any risks associated with participating in this project?
The questionnaires are not intended to cause distress in any way, although responding to questions about personal experiences may cause distress for some participants. Please note that if your child experiences distress at any point, they do not need to continue their participation. Some people may feel they could benefit from speaking to someone about how they are feeling. There are a number of people you can contact in this event, such as a doctor, counsellor, or the PCDH19 Alliance (http://pcdh19info.org/). Additionally, Dr Rachel Roberts is a clinical psychologist and is available to be contacted at rachel.roberts@adelaide.edu.au.

What are the benefits of the research project?
This research will allow participants to share information about their personal experiences, which will potentially improve our understanding of the types of symptoms associated with PCDH19 mutations, as well as the challenges faced by affected individuals and their families.

Can I withdraw from the project?
Participation in this project is completely voluntary. If you agree for your child to participate, they can withdraw from the study up until the submission of the PhD thesis. Findings from this research will be shared through publications and presentations. If material they have contributed has already been published, it will not be possible to withdraw it from that publication. However, you may withdraw their contribution from the research data and, in that case it will not be used in any further publications.

What will happen to my information?
The information collected from this project will be confidentially stored on password protected university computers, and will only be accessible to the researchers listed on this form. This information will be kept for a minimum of five years and all responses are confidential and only group responses will be reported so that no individual will be identified in any report of the findings. With your additional consent, the information collected may be used in future research addressing similar questions or linked to existing molecular data (through provision of saliva or skin samples, for example). Again, no individual will be identified in any future use of the results. The disposal of any data or personal records will be carried out in accordance with the requirements of the *State Records Act 1997* and the University Records Management Policy.

Who do I contact if I have questions about the project?
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Email: jozef.gecz@adelaide.edu.au Email: duyen.pham@adelaide.edu.au
Phone: +618 8313 2453 Phone: +618 8313 7955

Dr Rachel Roberts or Ms Kristy Kolc
Email: rachel.roberts@adelaide.edu.au Email: kristy.kolc@adelaide.edu.au
Phone: +618 8313 5228 Phone: +618 8313 7984

What if I have a complaint or any concerns?
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Phone: +61 8 8313 6028 Email: hrec@adelaide.edu.au
Post: c/- Research Services, The University of Adelaide, SA 5005 AUSTRALIA

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

If I want to participate, what do I do?
If you wish to participate, please click on the link https://www.research.net/r/PCDH19- and enter password PCDH19- to take you to the consent page and the start of the survey. Completion and submission of the survey indicates yours and your child's consent to be involved in the research project.

Yours sincerely,

Prof Jozef Gecz	Dr Duyen Pham	Dr Rachel Roberts	Ms Kristy Kolc
Neurogenetics Head	Postdoctoral Fellow	Senior Lecturer	PhD Candidate

Appendix E: Further Information

If you would like to learn more about *PCDH19*, you can do so via the:

1. Human Disease Genes Website Series: https://humandiseasegenes.nl/pcdh19
2. PCDH19 Registry: https://www.pcdh19info.org/pcdh19-patient-registry

Additionally, there are several community groups dedicated to sharing information and connecting families affected by *PCDH19* pathogenic variants:

1. **USA (PCDH19 Alliance):** https://www.pcdh19info.org/
2. **Italy (Insieme per la Ricerca PCDH19):** http://www.pcdh19research.org/
3. **France (Association PCDH19 France):** https://pcdh19france.fr/

Lastly, you can read about the Marinus Pharmaceuticals clinical trial of ganaxolone on their website: https://www.marinuspharma.com/investors/news/news-2019/272-Marinus_Pharmaceuticals_Initiates_Phase_3_Study_in_Children_with_PCDH19-Related_Epilepsy

Supplementary Tables

S2.1 Seizure onset precipitate

		Frequency	Percent
Valid	Not applicable	3	1.1
	Afebrile	16	5.9
	Fever	86	31.7
	Not reported	49	18.1
	Unclear	111	41.0
	Unknown	2	.7
	Vaccination	4	1.5
	Total	271	100.0
Missing		0	
Total		271	

S2.2 Variant location

		Frequency	Percent
Valid	EC1	32	11.7
	EC2	38	13.9
	EC3	55	20.1
	EC4	63	24.8
	EC5	17	6.2
	EC5-EC6	1	.4
	EC6	29	10.6
	Cytoplasmic	20	7.3
	Not applicable	16	6.2
	Total	271	100.0
Missing		0	
Total		271	

S2.3 Type of variant

		Frequency	Percent
Valid	Frameshift	74	27.3
	In-frame deletion	1	.4
	In-frame duplication	3	1.1
	In-frame insertion	1	.4
	Missense substitution	122	45.0
	Nonsense substitution	52	19.2
	Nonsense; Missense	1	.4
	Silent substitution	1	.4
	Splice-site	5	1.8
	Whole/partial gene deletion	11	4.0
	Total	271	100.0
Missing		0	
Total		271	

S2.4 *PCDH19* cDNA

cDNA	Protein	Correct Annotation	Frequency	cDNA	Protein	Correct Annotation	Frequency
1017delC	N340Mfs*28		1	1522_1528delATCAATC	I508Pfs*59		1
1019A>G (17 recurrent)	N340S		25	152dupT	A51Rfs*37		1
1022A>G	D341G		1	1537G>C	G513R		1
1023C>G	D341E		1	1615G>C	G539R		1
1026_1027delinsAA	N342_P343delinsKT		1	1628T>C	L543P		1
1031C>G (unrel.)	P344R		2	1649G>A	R550P	R550Q	1
1031C>T	P344L		1	1671C>G (sibs)	N557K		2
1036_1040dup	N347Kfs*23		1	1681G>A	P561S	1681C>T	1
1048C>G	S350*	1049C>G	1	1682C>G (sibs+unrel.)	P561R		3
1091_1092insC (recurrent*)	Y366Lfs*10		2	1700C>T (unrel.)	P567L		2
1091delC (DZ twins+unrel.)	P364fs	P364Rfs*4	3	1765_1766delTG	V589Cfs*8	1765_1766delGT	1
1091dupC (9 recurrent*)	Y366Lfs*10		28	1780G>C	D594H		1
1098C>G	Y366*		1	1786G>C	D596H		1
1123G>T	D375Y		1	1787A>T	D596V		1
1129G>A	D377N		1	1802G>A	G601D		1
1129G>C	D377H		1	1804C>T (unrel.)	R602*		2
1131C>A	D377E		1	1825G>T	E609*		1
1143dupT	G381Wfs*19		1	1852G>A	D618N		1
1178C>T	P393L		1	1863dupT	G622Wfs*18		1
1183C>T	R395*		1	1864G>C	G622R		1
1184G>C	R395P		1	1924G>A	V642M		1
1192G>T	E398*		1	1955T>C	L652P		1
1211C>T	T404I		1	1956_1959delCTCT	S653Pfs*6		1
1240G>C	E414Q		1	2012C>G (rel.+1 unrel.)	S671*		7
1240G>A	E414K		1	2019delC	S674Lfs*2		1
1298T>C	L433P		1	2030_2031insT (rel.)	L677fs*717	L677Ffs*41	7
1300_1301delCA (MZ twins)	Q434Efs*11		2	2147+2T>C	p.? (exon/intron 1)		1
1322T>A (rel.)	V441E		10	2156T>G	L719*		1
134_135ACdel (sibs)	D45Gfs*43		2	215T>G	V72G		1
1347_1348insAAC	N449_H450insN		1	2341dupA	I781Nfs*3		1
1352C>T	P451L		1	2359C>T	R787C		1
1375C>T	Q459*		1	241dupC	L81Pfs*8		1
1408_1417delGCCTATCTGC	A470Sfs*96		1	242T>G (recurrent)	L81R		4
142G>T (sibs)	E48*		2	253C>T (rel. + 1 unrel.)	Q85*		5
1456G>C	G486R		1	2567delCGGCACT	Not provided	2567delAGGGGCC, Q856Pfs*6	1
1464_1466delCTC	S489delS		1	2568C>T[#]	S856S	2563C>T, S855S	1
1521dupC	I508Hfs*15		1	2617-1G>A	p.? (intron 3/exon 4)		1

NB: *del* = deletion, *dup* = duplication, *DZ* = dizygotic, *ins* = insertion, *sibs* = siblings, *unrel/rel.* = unrelated/related individuals, *same mutation, ~ possible repeated case, [#]unlikely to be clinically relevant

cDNA	Protein	Correct Annotation	Frequency	cDNA	Protein	Correct Annotation	Frequency
2631_2634delTTTT	F878Tfs*5		1	617T>A	F206Y		1
2656C>T (5 recurrent)	R886*		8	695A>G (recurrent)	N232S		4
2675-6A>G	p.? (intron 4/exon 5)		1	697_700delinsTAAC (sibs)	D233*		2
2675+1G>C (unrel.)	p.? (exon/intron 4)		2	701A>G	N234S		1
2697dupA	E900Rfs*8		1	706C>T	P236S		1
269A>T	D90V		1	718G>T	E240*		1
2705dupA (rel.)	D902Kfs*6	N902Kfs*6	2	729C>A	Y243*		1
2873C>T	R958Q	2873G>A	1	730dupG	A244Gfs*76		1
2903dupA	D968Efs*18		1	746A>G	E249G		1
2926G>A[#]	D976N	3070G>A, D1024N	1	747A>T	E249D		1
339C>A	C113*		1	74T>C (sibs)	L25P		2
352G>T	E118*		2~	772_773delAT	I258Pfs*61		1
357delC (unrel.)	K120Rfs*3		2	785C>A	A262D		1
361G>A	D121N		1	78delG	K26Nfs*4		1
370G>A (sibs)	D124N		2	790G>C (unrel.)	D264H		2
415_423dup (rel.)	S139_A141dup		3	799G>T	E267*		1
416C>A	S139*		1	823T>A	Y275N		1
416C>T	S139L		1	824A>C	Y275S		1
424delG	A142Pfs*70		1	826T>C	S276P		1
437C>G (rel.)	T146R		3	83C>A	S28*		1
445C>T	P149S		1	83C>A & 90A>G	S28* & E90E		1
457G>A	A153T		1	840C>G	Y280*		1
462C>A	Y154*		1	859G>T	E287*		1
462C>G	Y154*		1	859G>T & 3319C>G	E287* & R1107G		1
469G>A	D157N		1	918C>G	Y306*		1
471C>A	D157E		1	919G>A	E313K	E307K	1
473C>G	S158*		1	91G>A	E31K		1
488T>G	V163G		1	937G>A	E307K	E313K	1
497_498insA	Y166*		1	949C>T	Q317*		1
506delC (unrel.)	T169Sfs*43		2	958dupG	D320Gfs*22		1
514dupG	E172Gfs*54		1	964G>C	G322R		1
569T>G	L190R		1	C>G	H146Q	1935C>G, H645Q	1
571G>C	V191L		1	T>C	V91A	1770T>C, V590A	1
593G>T	R198L		1	**Total**			**145**
595G>C	E199Q		1				
605C>A	S202*		1	Partial gene deletion			1
608A>C & 617T>G	H203P & F206C		1	Whole gene deletion			10

NB: *del* = deletion, *dup* = duplication, *DZ* = dizygotic, *ins* = insertion, *sibs* = siblings, *unrel/rel.* = unrelated/related individuals, *same mutation, ~ possible repeated case, [#]unlikely to be clinically relevant
All corrections based on NM_001184880.1

S2.5 Inheritance

		Frequency	Percent
Valid	#Maternal	9	3.3
	De novo	110	40.6
	De novo; Paternal	1	.4
	Familial	3	1.1
	Maternal	32	11.8
	Not the father	1	.4
	Not the mother	2	.7
	Not reported	10	3.7
	Paternal	64	23.6
	Unknown	39	14.4
	Total	271	100.0
Missing		0	
Total		271	

S2.6 Cognitive function

		Frequency	Percent
Valid	Normal	55	28.2
	Borderline	10	5.1
	Mild ID	53	27.2
	Moderate ID	43	22.1
	Severe/Profound ID	34	17.4
	Total	195	100.0
Missing		76	
Total		271	

S3.1 Reliability coefficients for SDQ Parent-Report scores

		Cronbach α
SDQ scale	Total difficulties	.71
	Emotional symptoms	.77
	Conduct problems	.63
	Hyperactivity-inattention	.67
	Peer problems	.66
	Prosocial behavior	.83
	Impact	.86

SDQ Strengths and Difficulties Questionnaire

S3.2 *In silico* assessment of all novel *PCDH19* variants in our cohort

	#	Country	Position	NM_001184880.1	Mode of inheritance	CADD (P≥25)	gnomAD frequency	GPP (P ≥0.1)	Mut Pred rank (P≥0.68)	MutationAssessor rank (P≥0.59)	PROVEAN (P≤-2.5)	Polyphen2	SIFT	Prediction
Missense	7	Denmark	99661786	c.1810A>C; p.Thr604Pro	*De novo*	24.2	Absent	0.1560795	0.722	0.895	-3.68	D	Del	Likely Pathogenic
	8	Australia	99663098-99663100	c.496_498AAA; p.Tyr166Lys	*De novo*	NC	Absent	NC	0.896	NC	-8.199	D	NC	Likely Pathogenic
	16	Moldova	99663003	c.593G>C; p.Arg198Pro (mosaic)	*De novo*	31	Absent	0.4938962	0.927	0.987	-6.44	D	Del	Likely Pathogenic
	23	USA	99662127	c.1469A>C; p.Tyr490Ser	*De novo*	25.6	Absent	0.9081805	0.919	0.991	-8.43	D	Del	Likely Pathogenic
	26,56	Denmark	99662797	c.799G>A; p.Glu267Lys	Maternal, Paternal	33	Absent	0.1402426	0.887	0.771	-3.59	D	Del	Likely Pathogenic
	27	Denmark	99662127	c.1469A>G; p.Tyr490Cys	Paternal	24.5	Absent	0.8659184	0.908	0.978	-8.43	D	Del	Likely Pathogenic
	30	UK	99662908	c.688G>C; p.Asp230His (mosaic)	*De novo*	27.5	Absent	0.9995749	0.888	0.999	-6.19	D	Del	Likely Pathogenic
	40	New Zealand	99661677	c.1919T>G; p.Leu640Arg	*De novo*	25.1	Absent	0.0750358	0.87	0.972	-5.26	D	Del	Likely Pathogenic
	44	USA	99662994	c.602A>C; p.Gln201Pro	*De novo*	24.4	Absent	0.1126678	0.686	0.897	-4.9	D	Del	Likely Pathogenic
	67	Italy	99662576	c.1020T>A; p.Asn340Lys (mosaic)	*De novo*	23.9	Absent	0.5263762	0.915	0.998	-5.51	D	Del	Likely Pathogenic
	69	Italy	99661654	c.1942G>C; p.Gly648Arg	*De novo*	28.6	Absent	0.9850428	0.866	0.913	-7.06	D	Del	Likely Pathogenic
	73	Italy	99662925	c.671T>A; p.Leu224His	*De novo*	24.5	Absent	0.9962677	0.81	0.94	-5.3	D	Del	Likely Pathogenic
	78	Italy	99551837	c.2885G>A; p.Arg962Gln	Unknown	32	0.0000056	0.0001695	0.657	0.758	-1.92	D	Del	Likely Pathogenic
	85	Italy	99662817	c.779T>G; p.Leu260Arg	*De novo*	25.8	Absent	0.9746687	0.959	0.959	-5.53	D	Del	Likely Pathogenic
	89,97, 98,99	Italy	99661623	c.1973T>G; p.Val658Gly	Maternal (3) Unknown	23.4	Absent	0.8591691	0.587	0.819	-4.08	P	Del	Likely Pathogenic
	90,96	Italy	99662133	c.1463T>A; p.Val488Asp	Paternal, Unknown	26	Absent	0.3091389	0.912	0.987	-5.88	D	Del	Likely Pathogenic
	91	Italy	99663460	c.136G>C; p.Ala46Pro	Paternal	25.3	Absent	5.81E-05	0.884	0.355	-1.5	D	Tol	Likely Pathogenic
	111	France	99662628	c.968C>T; p.Pro323Leu	Paternal	26.1	Absent	0.5317213	0.803	0.132	-8.44	D	Del	Likely Pathogenic
	Ex	USA	99658583	c.2227T>A; p.Ser743Thr	Maternal (2), Unknown	6.798	0.0000115	8.40E-10	0.157	0.065	0.14	B	Tol	Likely Benign
Nonsense	11	Argentina	99661876	c.1720G>T; p.Glu574* (mosaic)	*De novo*	37	Absent	NC	NC	NC	NC	NC	NC	Likely Pathogenic
	59	Netherlands	99657726	c.2412C>A; p.Cys804*	Unknown	38	Absent	NC	NC	NC	NC	NC	NC	Likely Pathogenic
	70	Italy	99662844	c.752C>A; p.Ser251*	*De novo*	41	Absent	NC	NC	NC	NC	NC	NC	Likely Pathogenic
	107	France	99663544	c.52C>T; p.Gln18*	*De novo*	36	Absent	NC	NC	NC	NC	NC	NC	Likely Pathogenic
	112	USA	99662048	c.1548C>A; p.Tyr516*	Unknown	32	Absent	NC	NC	NC	NC	NC	NC	Likely pathogenic
Frameshift	3	Russia	99662500	c.1095_1096insG; .Tyr366Valfs*10	*De novo*	NC	Absent	NC	NC	NC	NC	NC	NC	Possibly Damaging
	4,52	Russia	99662982	c.614del; p.Ser205Thrfs*7	Maternal, *De novo*	NC	Absent	NC	NC	NC	NC	NC	NC	Possibly Damaging
	35	USA	99663061-99663088	c.518_525del; p.Leu173Profs*50	*De novo*	NC	Absent	NC	NC	NC	NC	NC	NC	Possibly Damaging
	66	Italy	99662139	c.1457del; p.Gly486Alafs*83	*De novo*	NC	Absent	NC	NC	NC	NC	NC	NC	Possibly Damaging
	71	Italy	99661880-99661886	c.1710_1716del; p.Asn570Lysfs*12	*De novo*	NC	Absent	NC	NC	NC	NC	NC	NC	Possibly Damaging
	87	Italy	99662437	c.1159delC; p.Arg387Valfs*135	Unknown	NC	Absent	NC	NC	NC	NC	NC	NC	Possibly Damaging
	100	France	99662625	c.971del; p.Asn324Ilefs*44	Maternal	NC	Absent	NC	NC	NC	NC	NC	NC	Possibly Damaging
	101	France	99661637-99661638	c.1958_1959del; p.Ser653Cysfs*64	*De novo*	NC	Absent	NC	NC	NC	NC	NC	NC	Possibly Damaging
	104	Canada	99657637	c.2501dup; p.Asn834Lysfs*13	Unknown	NC	Absent	NC	NC	NC	NC	NC	NC	Possibly Damaging
	110	France	99662851	c.745del; p.Glu249Lysfs*56	*De novo*	NC	Absent	NC	NC	NC	NC	NC	NC	Possibly Damaging
Dup	5,10, 64	USA	99663146-99663151	c.445_450dup; p.Pro149_Leu150dup	Maternal (2), Unknown	NC	Absent	NC	NC	NC	-9.85	NC	NC	Possibly Damaging
Splic	22	UK	99551874	c.2849-1G>C; p.?	Unknown	33	Absent	NC	NC	NC	NC	NC	NC	Likely Pathogenic

B, benign; *D*, probably damaging; *Del*, deleterious; *Dup*, duplication; *H*, high; *L*, low; *M*, medium; *N*, neutral; *NC*, not covered; *P*, possibly damaging; *Tol*, tolerated
NB: red text highlights benign scores, *Ex* refers to the excluded variant

S3.3 *In silico* assessment of all non-*PCDH19* variants in our cohort

	Participant	PCDH19	Secondary findings	Position	Mode of inheritance	CADD (P≥25)	gnomAD frequency	Mut Pred rank (P≥0.68)	MutationAssessor rank (P≥0.59)	PROVEAN (P≤-2.5)	Polyphen2	SIFT	Prediction
Non-PCDH19	1	c.1091dup; p.Tyr366Leufs*10	STRADA: c.1144-1G>A; p.?	63703741	Unknown	NC	Absent	NC	NC	NC	NC	NC	Possibly Damaging
			TPK1: c.337G>A:p.Glu113Lys	144320276	Unknown	17.53	Absent	0.2	0.094	-0.04	B	Tol	Likely Benign
	2	c.1335C>G; p.Asp445Glu	*KANSL1*: c.53T>C; p.Ile8Thr	44249457	Unknown	24.8	Absent	0.362	0.225	-0.117647059	D	Del	Possibly Damaging
	5#	c.445_450dup; p.Pro149_Leu150dup	*CHRNA4*: c.1183G>A:p.Val395Ile	61981052	Maternal	15.1	0.0002254	0.15	0.646	-0.8	D	Tol	Likely Benign
	6	c.2656C>T; p.Arg886* (mosaic)	WWOX: c.1057C>A:p.Gln353Lys	79245505	*De novo*	23.3	0.00001206	0.71	NC	-1.72	P	Tol	Likely Benign
			ZEB2: c.225C>T:p.Ser75=	145187442	*De novo*	NC	0.00003186	NC	NC	NC	NC	NC	Likely Benign
	10#	c.445_450dup; p.Pro149_Leu150dup	*CHRNA4*: c.1183G>A:p.Val395Ile	61981052	Maternal	15.1	0.0002254	0.15	0.646	-0.8	D	Tol	Likely Benign
	30	c.688G>C; p.Asp230His (mosaic)	*SCN9A*: c.2215A>G:p.Ile739Val	167136962	*De novo*	18.02	0.00247	0.654	0.8	-0.89	P	Del	Likely Benign
	64#	c.445_450dup; p.Pro149_Leu150dup	*CHRNA4*: c.1183G>A:p.Val395Ile	61981052	Unknown	15.1	0.0002254	0.15	0.646	-0.8	D	Tol	Likely Benign

B, benign; *CADD*, Combined Annotation Dependent Depletion; *D*, probably damaging; *Del*, deleterious; *N*, neutral; *NC*, not covered; *P*, possibly damaging; *Polyphen2*, Polymorphism Phenotyping v2; *PROVEAN*, Protein Variation Effect Analyzer; *SIFT*, Sorting Intolerance from Tolerance; *Tol*, tolerated
NB: red text highlights benign scores and # represent a mother and her two daughters

S3.4 *PCDH19* variant list

#	Group	Variant	Inheritance	Novel	Relatives
1	Heterozygous female	c.1091dup; p.Tyr366Leufs*10	Unknown		
2	Heterozygous female	c.1335C>G; p.Asp445Glu	Unknown		
3	Heterozygous female	c.1095_1096insG; p.Tyr366Valfs*10	De novo	Yes	
4	Heterozygous female	c.614del; p.Ser205Thrfs*7	Maternal	Yes	Yes (52)
5	Heterozygous female	c.445_450dup; p.Pro149_Leu150dup	Maternal	Yes	Yes (10,64)
6	Mosaic male	c.2656C>T; p.Arg886*	De novo		
7	Heterozygous female	c.1810A>C; p.Thr604Pro	De novo	Yes	
8	Heterozygous female	c.496_498AAA; p.Tyr166Lys	De novo	Yes	
9	Heterozygous female	c.370G>A; p.Asp124Asn	Maternal		Yes (42,45,53,61,62)
10	Heterozygous female	c.445_450dup; p.Pro149_Leu150dup	Maternal	Yes	Yes (5,64)
11	Mosaic male	c.1720G>T; p.Glu574*	De novo	Yes	
12	Heterozygous female	c.1019A>G; p.Asn340Ser	Maternal		Yes (54)
13	Heterozygous female	c.1335C>A; p.Asp445Glu	Maternal		Yes (49)
14	Heterozygous female	c.1091dup; p.Tyr366Leufs*10	Paternal		
15	Heterozygous female	c.1873A>G; p.Arg625Gly	Maternal		Yes (51)
16	Mosaic male	c.593G>C; p.Arg198Pro	De novo	Yes	
17	Heterozygous female	WGD	De novo		
18	Mosaic male	c.2147+2T>C; p.?	De novo		
19	Heterozygous female	c.2113C>T; p.Arg705*	De novo		
20	Heterozygous female	c.2146dup; p.Ser716Lysfs*2	Paternal		
21	Heterozygous female	c.1114C>T; p.Arg372Trp	De novo		
22	Heterozygous female	c.2849-1G>C; p.?	Unknown	Yes	
23	Heterozygous female	c.1469A>C; p.Tyr490Ser	De novo	Yes	
24	Heterozygous female	c.498C>G; p.Tyr166*	Maternal		Yes (58)
25	Heterozygous female	c.2341dup; p.Ile781Asnfs*3	De novo		
26	Heterozygous female	c.799G>A; p.Glu267Lys	Maternal	Yes	Yes (56)
27	Heterozygous female	c.1469A>G; p.Tyr490Cys	Paternal	Yes	
28	Heterozygous female	c.1240G>A; p.Glu414Lys	Paternal		Yes (63)
29	Heterozygous female	c.1091dup; p.Tyr366Leufs*10	De novo		
30	Mosaic male	c.688G>C; p.Asp230His	De novo	Yes	
31	Heterozygous female	c.1683_1696del; p.Val562Thrfs*4	Paternal		Yes (65)
32	Heterozygous female	WGD	De novo		
33	Heterozygous female	WGD	De novo		
34	Heterozygous female	WGD	Unknown		
35	Heterozygous female	c.518_525del; p.Leu173Profs*50	De novo	Yes	
36	Heterozygous female	c.497dup; p.Tyr166*	De novo		Yes (37)
37	Heterozygous female	c.497dup; p.Tyr166*	De novo		Yes (36)
38	Heterozygous female	c.593G>T; p.Arg198Leu	De novo		
39	Hemizygous male (with epilepsy)	c.1672G>C; p.Asp558His	Maternal		
40	Heterozygous female	c.1919T>G; p.Leu640Arg	De novo	Yes	
41	Heterozygous female	c.1091dup; p.Tyr366Leufs*10	Paternal		
42	Heterozygous female	c.370G>A; p.Asp124Asn	Maternal		Yes (9,45,53,61,62)
43	Heterozygous female	c.361G>C; p.Asp121His	De novo		
44	Heterozygous female	c.602A>C; p.Gln201Pro	De novo	Yes	
45	Heterozygous female	c.370G>A; p.Asp124Asn	Paternal		Yes (9,42,53,61,62)
46	Heterozygous female	c.74T>C; p.Leu25Pro	Maternal		Yes (48)
47	Hemizygous male (no epilepsy)	c.2341dup; p.Ile781Asnfs*3	Maternal		
48	Heterozygous female	c.74T>C; p.Leu25Pro	Maternal		Yes (46)
49	Non-penetrant female	c.1335C>A; p.Asp445Glu	Unknown		Yes (13)
50	Heterozygous female	c.437C>G; p.Thr146Arg	Paternal		Yes (108,109)
51	Heterozygous female	c.1873A>G; p.Arg625Gly	Unknown		Yes (15)
52	Heterozygous female	c.614del; p.Ser205Thrfs*7	De novo	Yes	Yes (4)
53	Heterozygous female	c.370G>A; p.Asp124Asn	Paternal		Yes (9,42,45,61,62)
54	Heterozygous female	c.1019A>G; p.Asn340Ser	Unknown		Yes (12)
55	Heterozygous female	c.1671C>G; p.Asn557Lys	Paternal		Yes (57,60)
56	Non-penetrant female	c.799G>A; p.Glu267Lys	Paternal	Yes	Yes (26)
57	Heterozygous female	c.1671C>G; p.Asn557Lys	Paternal		Yes (55,60)
58	Non-penetrant female	c.498C>G; p.Tyr166*	Unknown		Yes (24)
59	Heterozygous female	c.2412C>A; p.Cys804*	Unknown	Yes	
60	Transmitting male	c.1671C>G; p.Asn557Lys	Unknown		Yes (55,57)
61	Transmitting male	c.370G>A; p.Asp124Asn	Maternal		Yes (9,42,45,53,62)

62	Non-penetrant female	c.370G>A; p.Asp124Asn	Paternal		Yes (9,42,45,53,61)
63	Non-penetrant mosaic male	c.1240G>A; p.Glu414Lys	De novo		Yes (28)
64	Heterozygous female	c.445_450dup; p.Pro149_Leu150dup	Unknown	Yes	Yes (5,10)
65	Transmitting male	c.1683_1696del; p.Val562Thrfs*4	Unknown		Yes (31)
66	Heterozygous female	c.1457del; p.Gly486Alafs*83	De novo	Yes	
67	Mosaic male	c.1020T>A; p.Asn340Lys	De novo	Yes	
68	Heterozygous female	c.2338A>T; p.Lys780*	De novo		
69	Heterozygous female	c.1942G>C; p.Gly648Arg	De novo	Yes	
70	Heterozygous female	c.752C>A; p.Ser251*	De novo	Yes	
71	Heterozygous female	c.1710_1716del; p.Asn570Lysfs*12	De novo	Yes	
72	Mosaic male	c.1352C>T; p.Pro451Leu	De novo		
73	Heterozygous female	c.671T>A; p.Leu224His	De novo	Yes	
74	Heterozygous female	c.1098C>G; p.Tyr366*	De novo		
75	Heterozygous female	c.2873G>A; p.Arg958Gln	Maternal		Yes (93)
76	Heterozygous female	c2617-1G>A; p.?	De novo		
77	Heterozygous female	c.1178C>T; p.Pro393Leu	De novo		
78	Heterozygous female	c.2885G>A; p.Arg962Gln	Unknown	Yes	
79	Heterozygous female	WGD	De novo		
80	Heterozygous female	c.1091dup; p.Tyr366Leufs*10	De novo		
81	Heterozygous female	c.1019A>G; p.Asn340Ser	De novo		
82	Heterozygous female	c.1129G>C; p.Asp377His	De novo		
83	Heterozygous female	c.2675-6A>G; p.?	De novo		
84	Heterozygous female	WGD	De novo		
85	Heterozygous female	c.779T>G; p.Leu260Arg	De novo	Yes	
86	Heterozygous female	c.958dup; p.Asp320Glyfs*22	De novo		
87	Heterozygous female	c.1159delC; p.Arg387Valfs*135	Unknown	Yes	
88	Heterozygous female	c.706C>T; p.Pro236Ser	De novo		
89	Heterozygous female	c.1973T>G; p.Val658Gly	Maternal	Yes	Yes (97,98,99)
90	Heterozygous female	c.1463T>A; p.Val488Asp	Paternal	Yes	Yes (96)
91	Heterozygous female	c.136G>C; p.Ala46Pro	Paternal	Yes	
92	Heterozygous female	c.1298T>C; p.Leu433Pro	De novo		
93	Non-penetrant female	c.2873G>A; p.Arg958Gln	Unknown		Yes (75)
94	Heterozygous female	c.1019A>G; p.Asn340Ser	De novo		Yes (95)
95	Heterozygous female	c.1019A>G; p.Asn340Ser	Maternal		Yes (94)
96	Transmitting male	c.1463T>A; p.Val488Asp	Unknown	Yes	Yes (90)
97	Non-penetrant female	c.1973T>G; p.Val658Gly	Maternal	Yes	Yes (89,98,99)
98	Heterozygous female	c.1973T>G; p.Val658Gly	Maternal	Yes	Yes (89,97,99)
99	Non-penetrant female	c.1973T>G; p.Val658Gly	Unknown	Yes	Yes (89,97,98)
100	Heterozygous female	c.971del; p.Asn324Ilefs*44	Maternal	Yes	
101	Heterozygous female	c.1958_1959del; p.Ser653Cysfs*64	De novo	Yes	
102	Heterozygous female	c.1091del; p.Pro364Argfs*4	De novo		
103	Heterozygous female	c.2656C>T; p.Arg886*	De novo		
104	Heterozygous female	c.2501dup; p.Asn834Lysfs*13	Unknown	Yes	
105	Heterozygous female	c.1091dup; p.Tyr366Leufs*10	De novo		
106	Heterozygous female	c.2019del; p.Ser674Leufs*2	De novo		
107	Heterozygous female	c.52C>T; p.Gln18*	De novo	Yes	
108	Heterozygous female	c.437C>G; p.Thr146Arg	Paternal		Yes (50,109)
109	Heterozygous female	c.437C>G; p.Thr146Arg	Paternal		Yes (50,108)
110	Heterozygous female	c.745del; p.Glu249Lysfs*56	De novo	Yes	
111	Heterozygous female	c.968C>T; p.Pro323Leu	Paternal	Yes	
112	Mosaic male	c.1548C>A; p.Tyr516*	Unknown	Yes	

S3.5 Development frequencies (and percentages)

a. Early development based on parent/caregiver-report (*n* = 83)

	Heterozygous females (%) *n* = 73	**Mosaic males (%) *n* = 8**	**Hemizygous males (%) *n* = 2**
Developmental delay	43/73 (59)	6/8 (75)	1/2 (50)
-Prior to seizure onset	13/43 (30)	2/6 (33)	N/A
Regression	31/73 (42)	4/8 (50)	2/2 (100)
- ≥5 episodes of regression	17/31 (55)	3/4 (75)	1/2 (50)
-Following seizure cluster	27/31 (87)	2/4 (50)	1/2 (50)
-Following status epilepticus	5/31 (16)	0/4 (0)	0/2 (0)

b. Intellect based on self- or patient/caregiver-report (*n* = 111)

	Heterozygous females		**Mosaic males**		**Hemizygous males**	
	Affected (*n* = 89)	**Non-penetrant (*n* = 7)**	**Affected (*n* = 8)**	**Non-penetrant (*n* = 1)**	**Affected (*n* = 2)**	**Transmitting (*n* = 4)**
Normal intelligence	45	7	4	1	1	4
Borderline intelligence	3	0	1	0	0	0
Mild ID	18	0	1	0	1	0
Moderate ID	9	0	0	0	0	0
Severe ID	12	0	2	0	0	0
Profound ID	2	0	0	0	0	0

ID intellectual disability

S3.6 Seizure details

Participant			Seizures				Status Epilepticus					Clustered Seizures				Isolated seizures	
ID	Group	Age	Presence	Onset (m)	Hosp. adm.	ICU admission	Presence	Freq.	Dur.	Conv.	NC	Presence	Dur. (days)	Seiz/day (av.)	Last 12m	Last seiz	Presence	Freq.
1	AF	1.5	YES	8	3	YES	NO	N/A	N/A	N/A	N/A	YES	5	24	3	4/07/2018	NO	N/A
2	AF	3	YES	15.5	2	YES	NO	N/A	N/A	N/A	N/A	YES	4	50	3	14/08/2018	YES	Yearly
3	AF	2.5	YES	5.5	5	YES	NO	N/A	N/A	N/A	N/A	YES	1	42	14	09/07/2018	NO	N/A
4	AF	2	YES	9	3	YES	NO	N/A	N/A	N/A	N/A	UNSURE	N/A	N/A	N/A	1/10/2018	NO	N/A
5	AF	2	YES	22	0	NO	NO	N/A	N/A	N/A	N/A	YES	2	4	2	25/02/2018	YES	Weekly
6	MM	2.5	YES	22	1	YES	NO	N/A	N/A	N/A	N/A	YES	13	7	1	17/06/2017	NO	N/A
7	AF	4	YES	6	5	YES	YES	Mon	50-60min	YES	YES	YES	3	15	6	8/06/2018	NO	N/A
8	AF	2.5	YES	17	4	YES	NO	N/A	N/A	N/A	N/A	YES	10	24	4	Ongoing	YES	Twice
9	AF	4	YES	10	5	YES	NO	N/A	N/A	N/A	N/A	YES	3	17	15	24/05/2018	NO	N/A
10	AF	3	YES	14	5	YES	YES	Yearly	30-50min	NO	YES	YES	5	15	3	17/07/2017	YES	Yearly
11	MM	2	YES	8	2	NO	NO	N/A	N/A	N/A	N/A	YES	UNSURE	UNSURE	0	1/06/2016	NO	N/A
12	AF	2.5	YES	28	3	NO	NO	N/A	N/A	N/A	N/A	YES	3	8	1	10/01/2018	YES	Yearly
13	AF	8	YES	6	5	YES	YES	Yly	40-50min	YES	NO	YES	4	25	12	1/01/2019	YES	Infrequent
14	AF	9	YES	4	5	NO	YES	Rarely	30-40min	YES	NO	YES	3	40	30	6/10/2018	YES	Yearly
15	AF	7	YES	6	5	NO	YES	Yearly	30-40min	YES	NO	YES	3	40	15	30/07/2018	YES	Yearly
16	MM	5	YES	5	5	YES	NO	N/A	N/A	N/A	N/A	YES	2	2	5	26/08/2018	YES	Monthly
17	AF	10	YES	15	5	YES	NO	N/A	N/A	N/A	N/A	YES	2	15	0	1/09/2014	NO	N/A
18	MM	10	YES	11	5	NO	NO	N/A	N/A	N/A	N/A	YES	7	30	2	1/12/2017	NO	N/A
19	AF	8	YES	12	4	YES	NO	N/A	N/A	N/A	N/A	YES	1	3	0	1/05/2015	YES	Infrequent
20	AF	6	YES	11	4	YES	NO	N/A	N/A	N/A	N/A	YES	2	10	0	1/09/2017	YES	Infrequent
21	AF	5	YES	6.5	4	YES	NO	N/A	N/A	N/A	N/A	YES	16	14	0	19/02/2017	YES	Monthly
22	AF	7	YES	11	5	YES	YES	Rarely	30-40min	YES	NO	YES	10	45	0	1/08/2017	YES	Yearly
23	AF	10	YES	12	5	YES	YES	Bi-mon	30-60+min	NO	YES	YES	5	5	6	17/07/2017	NO	N/A
24	AF	6	YES	10	3	YES	NO	N/A	N/A	N/A	N/A	YES	6	8	26	30/12/2017	YES	Infrequent
25	AF	8	YES	14	3	YES	NO	N/A	N/A	N/A	N/A	YES	2	13	0	14/12/2015	YES	Monthly
26	AF	10	YES	13	5	YES	YES	Mon-yearly	30-40min	YES	NO	YES	2	100	8	21/06/2018	NO	N/A
27	AF	7	YES	15	5	NO	YES	Yearly	50-60min	YES	NO	YES	3	12	0	1/02/2016	YES	Yearly
28	AF	9	YES	8	5	YES	NO	N/A	N/A	N/A	N/A	YES	7	10	0	1/04/2017	NO	N/A
29	AF	8	YES	3	5	YES	YES	Weekly	30-60+min	YES	YES	YES	4	30	1000	Ongoing	YES	Monthly
30	MM	5	YES	8	5	YES	NO	N/A	N/A	N/A	N/A	YES	3	10	24	1/03/2018	NO	N/A
31	AF	6	YES	11	2	NO	NO	N/A	N/A	N/A	N/A	YES	3	5	0	6/10/2015	NO	N/A
32	AF	6	YES	28	1	YES	NO	N/A	N/A	N/A	N/A	YES	14	5	0	1/01/2013	NO	N/A
33	AF	8	YES	10	5	YES	YES	Daily	30-40min	NO	YES	YES	3	7	5	15/06/2006	NO	N/A
34	AF	13	YES	8	3	YES	NO	N/A	N/A	N/A	N/A	YES	14	20	0	1/10/2008	NO	N/A
35	AF	11	YES	9	5	YES	YES	No pattern	30-50min	NO	YES	YES	5	12	0	19/08/2016	NO	N/A
36	AF	11	YES	8	5	NO	YES	Rarely	30-40min	YES	YES	YES	4	30	0	1/09/2011	YES	Monthly
37	AF	11	YES	8	5	NO	YES	Rarely	30-40min	YES	YES	YES	4	30	0	1/11/2011	YES	Monthly

38	AF	11	YES	6	5	YES	UNSURE	N/A	N/A	N/A	N/A	YES	7	8	6	18/02/2018	YES	Monthly
39	AM(S)	16	YES	114	1	YES	NO	N/A	N/A	N/A	N/A	YES	5	2	12	1/12/2018	YES	Infrequent
40	AF	15	YES	14	5	YES	NO	N/A	N/A	N/A	N/A	YES	12	28	0	1/10/2008	YES	Infrequent
41	AF	12	YES	10	5	YES	YES	Once	30-40min	YES	NO	YES	5	16	1	1/08/2015	YES	Yearly
42	AF	15	YES	6.5	5	YES	NO	N/A	N/A	N/A	N/A	YES	2	20	11	13/01/2018	NO	N/A
43	AF	12	YES	13	5	YES	NO	N/A	N/A	N/A	N/A	YES	1	10	100	4/04/2018	YES	Yearly
44	AF	16	YES	8	5	YES	YES	Daily	30-40min	YES	YES	YES	4	19	12	12/08/2017	YES	Unsure
45	AF	41	YES	7	2	NO	NO	N/A	N/A	N/A	N/A	YES	2	20	0	1/01/2002	NO	N/A
46	AF	22	YES	8	5	NO	YES	Yearly	40-50min	YES	YES	YES	5	8	0	1/01/2007	YES	Yearly
47	AM(NS)	23	NO	N/A	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
48	AF	28	YES	7	5	YES	YES	Mon	30-60+min	YES	YES	YES	7	10	0	1/01/2009	YES	Yearly
49	NP	35	NO	N/A	7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
50	AF	30	YES	60	5	YES	NO	N/A	N/A	N/A	N/A	YES	3	10	0	1/01/1997	NO	N/A
51	AF	36	YES	6	6	NO	NO	N/A	N/A	N/A	N/A	YES	1	10	0	1/01/1989	YES	Yearly
52	AF	38	YES	21	6	NO	NO	N/A	N/A	N/A	N/A	YES	2	3	0	In childhood	YES	Infrequent
53	AF	35	YES	2	5	YES	YES	Once	30-40min	YES	NO	NO	N/A	N/A	N/A	1/01/1988	YES	Yearly
54	AF	36	YES	3	2	NO	NO	N/A	N/A	N/A	N/A	YES	2	3	8	13/03/2018	YES	Yearly
55	AF	35	YES	18	2	NO	NO	N/A	N/A	N/A	N/A	YES	3	20	0	1/01/1994	YES	Twice
56	NP	46	NO	N/A	7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
57	AF	32	YES	24	2	YES	YES	3 times	60+ min	YES	YES	YES	2	8	0	1/01/2002	YES	Infrequent
58	NP	33	YES	60	1	YES	NO	N/A	N/A	N/A	N/A	NO	N/A	N/A	N/A	1/01/1989	YES	Once
59	AF	41	YES	10	5	NO	NO	N/A	N/A	N/A	N/A	YES	2	5	0	1/01/2002	NO	N/A
60	TM	63	NO	N/A	7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
61	TM	70	NO	N/A	7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
62	NP	47	NO	N/A	7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
63	MM(U)	41	NO	N/A	7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
64	AF	31	YES	55	1	NO	NO	N/A	N/A	N/A	N/A	YES	1	7	0	1/08/1998	YES	Yearly
65	TM	41	NO	N/A	7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
66	AF	2	YES	11	4	NO	NO	N/A	N/A	N/A	N/A	YES	15	5	1	8/06/2018	YES	Weekly
67	MM	2.5	YES	5	5	NO	NO	N/A	N/A	N/A	N/A	YES	1	7	20	8/07/2018	NO	N/A
68	AF	3	YES	24	5	NO	NO	N/A	N/A	N/A	N/A	YES	7	10	30	1/06/2018	NO	N/A
69	AF	3	YES	8	4	NO	NO	N/A	N/A	N/A	N/A	YES	2	20	5	12/01/2018	YES	Infrequent
70	AF	2.5	YES	10	1	YES	NO	N/A	N/A	N/A	N/A	YES	6	14	6	1/02/2018	NO	N/A
71	AF	6	YES	27	1	NO	NO	N/A	N/A	N/A	N/A	YES	1	10	0	1/02/2015	NO	N/A
72	MM	7	YES	10	5	YES	NO	N/A	N/A	N/A	N/A	YES	3	15	1	1/02/2018	NO	N/A
73	AF	6	YES	14	4	YES	NO	N/A	N/A	N/A	N/A	YES	3	6	0	24/06/2015	YES	Once
74	AF	7	YES	11	3	NO	NO	N/A	N/A	N/A	N/A	YES	4	8	0	1/02/2015	YES	Infrequent
75	AF	6	YES	9	1	NO	NO	N/A	N/A	N/A	N/A	YES	3	4	0	1/08/2017	YES	Monthly
76	AF	10	YES	11	2	YES	NO	N/A	N/A	N/A	N/A	YES	2	14	0	1/08/2014	YES	Twice
77	AF	8	YES	13	5	NO	NO	N/A	N/A	N/A	N/A	YES	2	10	2	24/05/2016	YES	Once
78	AF	8	YES	8	5	YES	NO	N/A	N/A	N/A	N/A	YES	15	20	40	10/03/2018	NO	N/A
79	AF	9	YES	8	5	NO	YES	3-4m	60+ min	YES	YES	YES	4	20	4	1/01/2018	NO	N/A
80	AF	8	YES	6	5	NO	NO	N/A	N/A	N/A	N/A	YES	24	10	3	1/03/2018	YES	Yearly

81	AF	17	YES	5	5	NO	NO	N/A	N/A	N/A	N/A	YES	2	8	0	1/02/2016	YES	Infrequent
82	AF	17	YES	7	5	YES	YES	Once	40-50min	YES	NO	YES	5	30	0	1/04/2015	YES	Yearly
83	AF	16	YES	6	5	YES	YES	Twice	30-40min	YES	NO	YES	6	30	2	1/10/2017	YES	Yearly
84	AF	17	YES	15	5	YES	NO	N/A	N/A	N/A	N/A	YES	2	4	21	1/10/2018	YES	Infrequent
85	AF	13	YES	7	4	NO	NO	N/A	N/A	N/A	N/A	YES	1	25	6	21/03/2018	YES	Monthly
86	AF	15	YES	2.5	5	NO	NO	N/A	N/A	N/A	N/A	YES	14	8	0	1/01/2012	YES	Yearly
87	AF	27	YES	7	5	NO	NO	N/A	N/A	N/A	N/A	NO	N/A	N/A	N/A	30/09/2018	YES	Weekly
88	AF	43	YES	9	5	NO	NO	N/A	N/A	N/A	N/A	YES	1	5	0	1/01/2013	YES	Monthly
89	AF	21	YES	5	5	YES	NO	N/A	N/A	N/A	N/A	YES	1	7	2	1/10/2017	NO	N/A
90	AF	21	YES	16	5	YES	NO	N/A	N/A	N/A	N/A	YES	2	10	0	24/07/2003	NO	N/A
91	AF	18	YES	11	5	NO	NO	N/A	N/A	N/A	N/A	YES	3	30	0	19/02/2008	NO	N/A
92	AF	25	YES	9	5	YES	NO	N/A	N/A	N/A	N/A	NO	N/A	N/A	N/A	1/03/2008	YES	Infrequent
93	NP	45	YES	3	0	NO	NO	N/A	N/A	N/A	N/A	NO	N/A	N/A	N/A	1/08/1972	YES	Once
94	AF	52	YES	8	6	NO	UNSURE	N/A	N/A	N/A	N/A	YES			0	UNSURE	UNSURE	N/A
95	AF	23	YES	8	6	YES	YES	Yearly	60+ min	YES	YES	YES	4		0	9/05/2016	YES	Once
96	TM	51	NO	N/A	7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
97	NP	54	NO	N/A	7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
98	AF	25	YES	30	5	NO	NO	N/A	N/A	N/A	N/A	YES	15	20	0	1/01/2004	NO	N/A
99	NP	57	NO	N/A	7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
100	AF	10	YES	11	5	YES	NO	N/A	N/A	N/A	N/A	YES	1	3	0	1/01/2016	YES	Daily
101	AF	6	YES	16	5	NO	NO	N/A	N/A	N/A	N/A	YES	5	30	0	1/10/2017	NO	N/A
102	AF	6	YES	7	5	YES	NO	N/A	N/A	N/A	N/A	YES	2	7	5	26/08/2018	NO	N/A
103	AF	7	YES	3.5	5	YES	NO	N/A	N/A	N/A	N/A	YES	3	15	10	1/08/2018	YES	Yearly
104	AF	5	YES	14	5	YES	YES	Twice	50-60min	YES	NO	YES	2	10	5	1/03/2018	NO	N/A
105	AF	7	YES	10	5	YES	NO	N/A	N/A	N/A	N/A	YES	5	10	3	16/07/2018	YES	Infrequent
106	AF	14	YES	4	5	YES	NO	N/A	N/A	N/A	N/A	YES	2	6	2	1/05/2018	NO	N/A
107	AF	12	YES	22	5	YES	NO	N/A	N/A	N/A	N/A	YES	7	5	0	1/01/2010	NO	N/A
108	AF	32	YES	1.5	0	NO	NO	N/A	N/A	N/A	N/A	YES	4	20	0	1/02/2010	NO	N/A
109	AF	23	YES	22	5	YES	NO	N/A	N/A	N/A	N/A	YES	3		0	19/12/2008	YES	Twice
110	AF	23	YES	18	5	YES	NO	N/A	N/A	N/A	N/A	YES	1	Many	0	1/01/2006	NO	N/A
111	AF	33	YES	18	5	NO	YES	Yearly	30-40min	YES	UNSURE	YES	1	3	0	1/01/2002	NO	N/A
112	MM	12	YES	96	5	NO	NO	N/A	N/A	N/A	N/A	YES	1	5	50		YES	Yearly

S3.7 Frequencies (and percentage) of SDQ domain and impact scores for affected individuals

a. Heterozygous females (*n* = 65)

	SDQ Domains				
SDQ score classification	**Emotional problems**	**Conduct problems**	**Hyperactive-Inattention**	**Peer problems**	**Prosocial behavior**
Average	35 (54)	25 (38.5)	22 (34)	19 (29.5)	17 (26)
Mild	12 (18)	13 (20)	15 (23)	6 (9)	9 (14)
Moderate	7 (11)	16 (24.5)	11 (17)	6 (9)	7 (11)
Severe	11 (17)	11 (17)	17 (26)	34 (52.5)	32 (49)

b. Mosaic males (*n* = 8)

	SDQ Domains				
SDQ score classification	**Emotional problems**	**Conduct problems**	**Hyperactive-Inattention**	**Peer problems**	**Prosocial behavior**
Average	6 (75)	4 (50)	2 (25)	2 (25)	0 (0)
Mild	0 (0)	2 (25)	3 (37.5)	0 (0)	2 (25)
Moderate	1 (12.5)	1 (12.5)	2 (25)	2 (25)	0 (0)
Severe	1 (12.5)	1 (12.5)	1 (12.5)	4 (50)	6 (75)

c. Impact scores (*n* = 73)

SDQ score classification	**Heterozygous females (*n* = 65)**	**Mosaic males (*n* = 8)**
Average	9 (14)	1 (12.5)
Mild	6 (9)	1 (12.5)
Moderate	3 (5)	0 (0)
Severe	47 (72)	6 (75)

S3.8 Descriptive statistics for all neuropsychiatric measures

Group	**Measure**	**N**	**Min**	**Max**	**Mean**	**SD**
Heterozygous females	SRS-2	82	35	107	69.5*	18.6
	SCQ	8	8	32	18.5*	8.12
	SDQ	65	3	31	17.6*	6.68
	BRIEF	89	34	98	67.8*	15.8
	DOCS	17	0	33	11.4	10.7
Mosaic males	SRS-2	8	46	86	68.8*	15.1
	SDQ	8	8	29	16.5*	6.28
	BRIEF	8	51	80	63.9*	11.7
Non-penetrant females	SRS-2	7	41	70	47.7	10.1
	BRIEF	7	39	75	47.6	12.5
	DOCS	7	0	46	11.1	16.3
Transmitting males	SRS-2	4	41	51	46.3	4.99
	BRIEF	4	38	53	45.3	6.19
	DOCS	4	0	15	5.25	6.70

*Represents group averages above the clinical threshold

S3.9 Average scores on measures of executive dysfunction, ASD, and prosocial behavior based on seizure onset and activity

Group	*N*	BRIEF GEC *t* score	SRS-2/SCQ combined *z* score	*N*	SDQ Prosocial behavior
Late / Mild	22	61.0	-0.35	16	6.31
Late / Severe	6	63.7	0.01	5	4.60
Early / Mild	33	71.8	0.17	28	5.32
Early / Severe	24	75.3	0.85	21	3.52

S3.10 Genotype-phenotype associations

Dependent variable	Independent variable	N	Mean	Std. deviation	Significance
BRIEF *t* score	Non-truncating	59	61.8	16.1	$p = .067$
	Truncating	38	68.1	17.0	
ASD *z* score (SRS-2 & SCQ)	Non-truncating	59	-0.12	0.98	$p = .738$
	Truncating	38	-0.05	1.04	
SCQ emotional problems	Non-truncating	29	3.62	2.77	$p = .128$
	Truncating	32	2.63	2.27	
SCQ conduct problems	Non-truncating	29	3.41	2.01	$p = .941$
	Truncating	32	3.38	2.03	
SCQ hyperactivity-inattention	Non-truncating	29	6.52	2.23	$p = .491$
	Truncating	32	6.06	2.83	
SCQ peer problems	Non-truncating	29	4.48	2.17	$p = .190$
	Truncating	32	3.66	2.70	
SCQ prosocial behavior	Non-truncating	29	5.21	2.87	$p = .545$
	Truncating	32	4.75	2.98	
Age at seizure onset	Non-truncating	48	10.3	6.35	$p = .506$
	Truncating	34	11.2	6.20	

Supplementary Figures

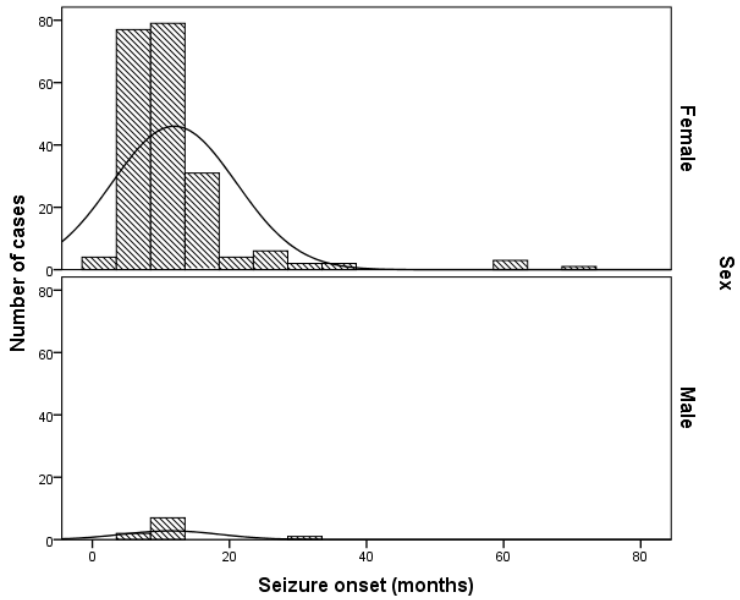

Figure S2.1 Distribution of age at seizure onset for males and females

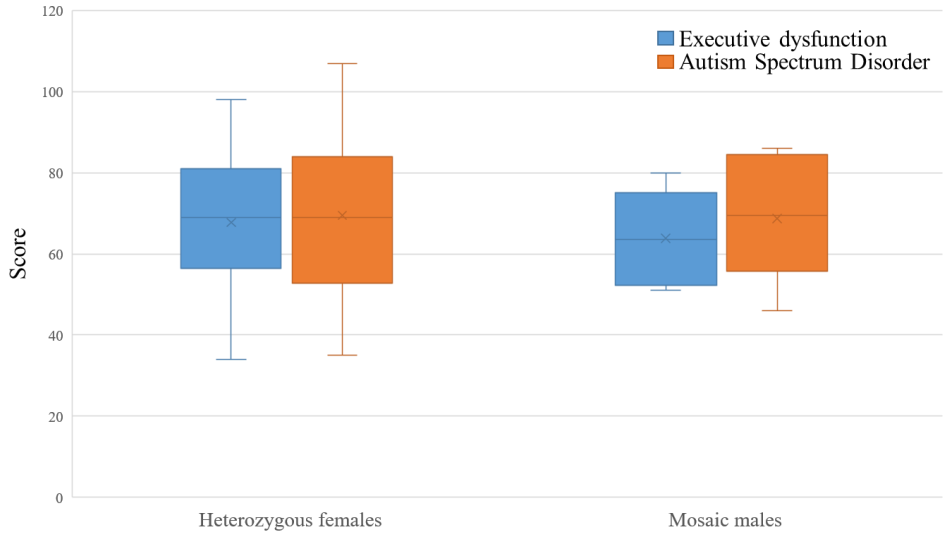

Figure S3.1 Boxplots illustrating average total BRIEF *t* scores (blue) for females (*n* = 89) and males (*n* = 8) and average total SRS-2 *t* scores (orange) for females (*n* = 82) and males (*n* = 8).

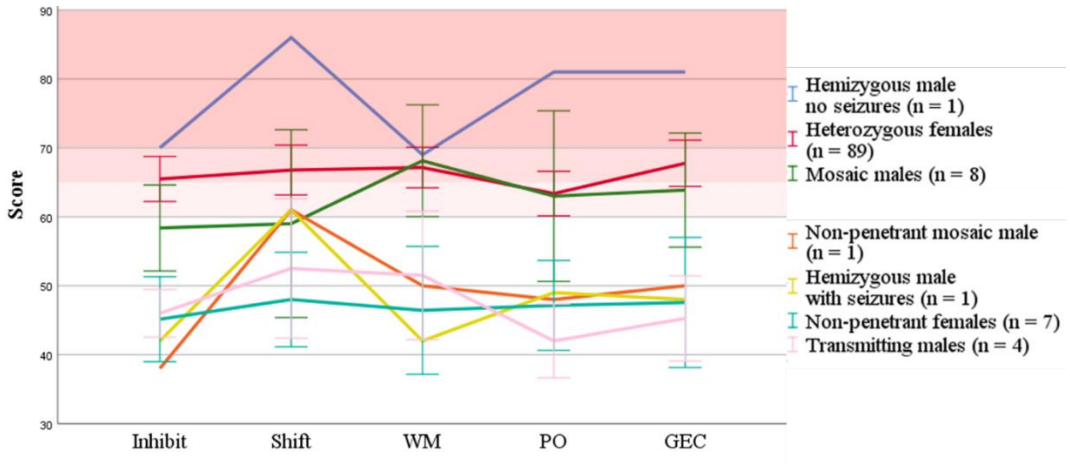

Figure S3.2 Average (±2 SEM) BRIEF total GEC and subscale *t* scores. Darkening shades of red correspond to increasing degrees of severity. *WM*, working memory; *PO*, plan/organize; *GEC*, global executive composite.

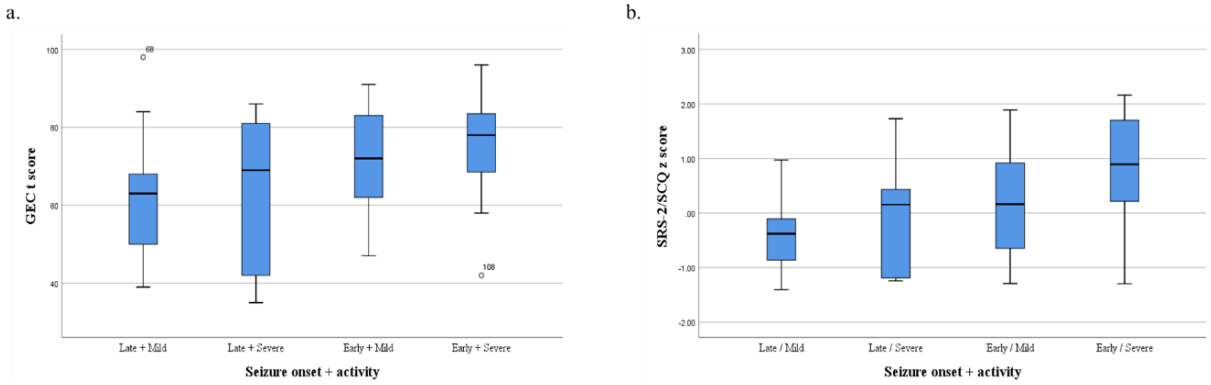

Figure S3.3 Association between seizure onset, seizure activity, and clinical outcome: a) executive dysfunction measured by the BRIEF and b) ASD outcome measured by the SRS-2 or SCQ (scores converted to *z* scores for the analysis). *Early* = ≤12 months age at onset, *Late* = >12 months seizure onset, *Mild* = ≤15 average seizures/day, *Severe* = >15 average seizures/day.

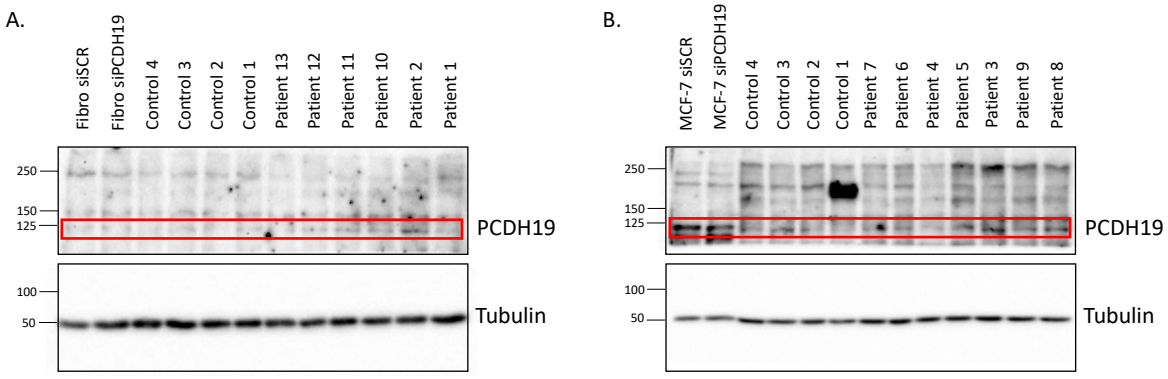

Figure S7.1 PCDH19 protein expression for all patients represented in the analysis (*n* = 13). All protein levels compared to age-matched controls (*n* = 4). PCDH19 is weakly expressed in skin fibroblasts and easily detectable in MCF-7 cells (dilution factor 1:3).

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