

# NEUROBIOLOGY OF PCDH19-FEMALE EPILEPSY

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## Abstract

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PCDH19-female epilepsy (PCDH19-FE) is a female-limited epilepsy characterised by a spectrum of neurodevelopmental and behavioural problems. It is caused by predicted, loss of function mutations in an X-chromosome gene, *Protocadherin 19* (*PCDH19*). PCDH19-FE presents because of cellular mosaicism, typically in females where heterozygous mutations cause mosaicism resulting from random X-inactivation. Currently, limited cell and molecular biology data is available to explain how mutations in *PCDH19* cause this debilitating disorder. *PCDH19* shows spatially and temporally regulated expression in the developing and adult brain, with layer-specific expression in the cortex and expression in the neurogenic regions of the developing brain. Thus, PCDH19 is likely to have important functions in both the developing and adult cortex. To investigate the role of PCDH19 in cortical brain development, both mouse and human based *in vitro* models were developed and employed.

Utilising wildtype (WT) and *Pcdh19* knockout (KO) mice, neural stem and progenitor cells (NSPCs) were isolated and cultured as neurospheres to investigate NSPCs behaviours and the cellular mosaicism of the PCDH19-FE individuals. In the absence of *Pcdh19*, increased neuronal migration was observed. This was associated with increased neuronal differentiation at the expense of oligodendrocyte differentiation. The mosaic cultures showed a phenotype intermediate to the *Pcdh19* WT and *Pcdh19* KO cells in all assays, suggesting cell intrinsic properties were maintained. Genome-wide expression analysis implicated multiple genes and gene networks involved in neuronal development. In particular, genes involved in the regulation of the actin cytoskeleton via Rho GTPases were highlighted, a pathway which could underlie the cellular phenotypes observed.

Human induced pluripotent stem cells (hiPSC) provide a unique system to study neurological disorders using disease relevant cells that are otherwise unattainable from the patients. hiPSCs were generated from patient skin fibroblasts with a pathogenic *PCDH19* missense mutation. Additionally, an optimised *in vitro* protocol of human cortical development was developed and shown to be reproducible across multiple pluripotent stem cell lines. Using this protocol with WT and *PCDH19* Mutant hiPSCs, PCDH19-FE was modelled by again replicating the cellular mosaicism of the patient brain. PCDH19 was found to be important for the maintenance of NSPC polarity during cortical development, with *PCDH19* Mutants being able to form neural rosette structures, but unable to properly maintain these structures as evidenced by a decrease in lumen size and number of polarised neural rosette structures/rosette colony area. A

significant increase in the number of neurons at the edge of the rosette colonies was also observed suggesting premature neuronal differentiation. PCDH19 was also shown to regulate axonal extensions with *PCDH19* Mutant and Mosaic neurons having an increased primary neurite length.

Taken together this study has shown that PCDH19 has important functional roles during the early stages of cortical brain development. This work identifies novel roles for PCDH19 in NSPC polarity, neurogenesis, neuronal migration and neuronal morphology. This study suggests that the PCDH19-FE pathology is attributed to the presence of two differing cell populations (WT and Mutant/KO) resulting in abnormal brain development and neuronal network formation at later stages of cortical development.

## 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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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Bachelor of Science (Honours)

Student number: a1119925

Signed

**DATE** ...../...../.....

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---

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## Abbreviations

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### Standard Terms

+	Plus
-	Minus
%	Percentage
°C	Degrees Celsius
bp	Base pair
cDNA	Complementary DNA
DNA	Deoxyribonucleic Acid
E	Embryonic day
g	Gravitational force
gDNA	Genomic DNA
hrs	Hours
Kb	Kilobase
KO	Knockout
L	Litre
M	Molar
min	Minutes
mL	Millilitre
mM	Millimolar
mRNA	Message RNA
ng	Nanograms
RNA	Ribonucleic Acid
Rpm	Revolutions per minute
RQ	Relative quantity
UV	Ultra Violet
V	Volts
WT	Wildtype
µg	Microgram
µL	Microliter
µm	Micrometer
µM	Micromolar

### Materials and Methods

2D	Two dimensional
3D	Three dimensional
ANOVA	Analysis of variance
AR	Androgen receptor
ASP	Allele specific PCR
BSA	Bovine serum albumin
BSD	Blasticidin S
Ct	Cross threshold
DAPI	4',6-diamidino-2-phenylindole
DMEM	Dulbecco's modified eagle medium
DMEM/F12	Dulbecco's modified eagle medium: nutrient mixture F-12
DMSO	Dimethyl sulfoxide
dNTP	Deoxyribonucleotide
DPBS	Dulbecco's phosphate buffered saline
EB	Embryoid bodies

ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
ESC	Embryonic stem cell
eGFP	Enhanced green fluorescent protein
FACS	Fluorescence-activated cell sorting
FAM	6-Fluorescein amidite
FBS	Foetal bovine serum
FCS	Foetal calf serum
FGF	Fibroblast growth factor
FITC	Fluorescein isothiocyanate
GFP	Green fluorescent protein
HBSS	Hank's balanced salt solution
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hESC	Human embryonic stem cell
hPSC	Human pluripotent stem cell
hiPSC	Human induced pluripotent stem cell
HPA	Hypothalamic-pituitary-adrenal axis
HSD	Honest significant difference
HUMARA	Human androgen receptor clonality assay
IF	Immunofluorescence
iPSC	Induced pluripotent stem cell
IVF	In vitro fertilisation
KSR	Knockout serum replacement
L15	Leibovitz's L-15 medium
LB	Luria-broth
MEF	Embryonic mouse fibroblasts
MGB	Minor groove binder
MOI	Multiplicity of infection
MQ	Ultrapure water
NCFC	Neural colony-forming cell
NEAA	Non-essential amino acids
NFQ	Non-fluorescence quencher
NPC	Neural progenitor cell
NSC	Neural stem cell
NSPC	Neural stem and progenitor cell
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline Tween20
PCR	Polymerase chain reaction
Pen/Strep	Penicillin-Streptomycin
PFA	Paraformaldehyde
PLL	Poly-L-Lysine
PSC	Pluripotent stem cell
ROCK inhibitor	Y-27632 dihydrochloride
RT-qPCR	Quantitative real-time PCR
TAE	Tris-acetate-EDTA
X-gal	5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside

### Non-Standard Terms

AJ	Adherens junctions
AP	Apical progenitor
ASD	Autism spectrum disorders
AVE	Anterior visceral endoderm

BFIE	Benign familial infantile epilepsy
BFNE	Benign familial neonatal epilepsy
BFNS	Benign familial neonatal seizures
bHLH	Basic helix-loop-helix
BiFC	Biomolecular fluorescence complementation
bIP	Basal intermediate progenitor
BMP	Bone morphogenetic proteins
BP	Basal progenitor
CFNS	Craniofrontonasal syndrome
CGH	Comparative genomic hybridisation
CNS	Central nervous system
CNV	Copy number variation
CP	Cytoplasmic domain
DG	Dentate gyrus
DGCs	Dentate gyrus cells
DS	Dravet Syndrome
EC	Extracellular cadherin motif
ECS	Electroconvulsive shock
EEG	Electroencephalogram
EFMR	Epilepsy and Mental Retardation Limited to Females
EIEE	Early infantile epileptic encephalopathy
E-RG	Early radial glial
FXS	Fragile X syndrome
Hpf	Hours post fertilisation
ICM	Inner cell mass
ID	Intellectual disability
IGE	Idiopathic generalised epilepsies
IQ	Intelligent quotient
iSVZ	Inner SVZ
LIF	Leukaemia inhibitory factor
Lpd	Lamellipodin
MRI	Magnetic resonance imaging
MGE	Medial ganglionic eminence
mIPSCs	Miniature inhibitory post synaptic currents
M-RG	Late radial-glial
MZ	Monozygotic
NECs	Neuroepithelial cells
NMD	Nonsense mediated mRNA decay
oSVZ	Outer subventricular zone
PCDH	Protocadherins
PCDH19-FE	PCDH19-female epilepsy
PMDD	Pre-menstrual dysphoric disorder
PNS	Peripheral nervous system
PTC	Premature termination codon
PS	Pregnenolone sulphate
RGC	Radial glial cell
R-NSC	Rosette-stage neural stem cell
SAP	Subapical progenitors
shRNA	Short hairpin RNA
SNP	Single nucleotide polymorphism
SV	Synaptic vesicles
SVZ	Subventricular zone
TLE	Temporal lobe epilepsy

TM	Transmembrane domain
UTR	Untranslated region
VZ	Ventricular zone
WRC	Wave regulatory complex
XCI	X-chromosome inactivation
XCR	Inactive X-chromosome
XLID	X-linked ID

## Genes and Proteins

\*Given most of the comparisons in this thesis are between human and mouse, to facilitate identification of the species, human proteins are all in upper case and mouse proteins will have an upper case first letter with the rest of the protein in lower case. Genes follow the same nomenclature but are italicised.

Abi-1	Abl interactor 1
ACTB	Actin Beta
AF6	Afadin 6
AKRs	Aldo-Keto Reductase Families
AKR1C1	Aldo-Keto Reductase Family 1 Member C1
AKR1C2	Aldo-Keto Reductase Family 1 Member C2
AKR1C3	Aldo-Keto Reductase Family 1 Member C3
AKR1C4	Aldo-Keto Reductase Family 1 Member C4
CD133	Prominin 1
CDC42	Cell division cycle 42
CNP1/CNPase	2',3'-cyclic nucleotide 3' phosphodiesterase
CTIP2	B-cell CLL/lymphoma 11B
CUX1	Cut like homeobox 1
CYFIP1	Cytoplasmic FMR1-interacting protein 1
CYFIP2	Cytoplasmic FMR1-interacting protein 2
CYP17a1	Cytochrome P450, family 17, subfamily a, polypeptide1
DACH1	dachshund family transcription factor 1
DCX	Doublecortin
DDX3X	DEAD-box helicase 3, X-linked
DKK	Dickkopf
DLX2	Distal-less homeobox 2
EFNB1	Ephrin B1
EFNA5	Ephrin A5
EPHA2	EPH receptor A2
ER	Estrogen receptor
FAIM2	Fas apoptotic inhibitory molecule 2
FMRP	Fragile X mental retardation protein
GABA <sub>A</sub>	Gamma-aminobutyric acid A receptor, subunit gamma1
GABARG <sub>2</sub>	Gamma-aminobutyric acid receptor subunit gamma-2
GABRA2	Gamma-aminobutyric acid type A receptor alpha2 subunit
GABRA3	Gamma-aminobutyric acid type A receptor alpha3 subunit
GABRD	Gamma-aminobutyric acid receptor delta
GAD67 /GAD1	Glutamate decarboxylase 1
GFAP	Glial fibrillary acidic protein
GluR2	Glutamate receptor 2
GPRIN3	GPRIN family member 3
GPCR	G-protein coupled receptor
GRIA1	Glutamate ionotropic receptor AMPA type subunit 1
H3K4	Histone 3 Lys4

H3K27	Histone 3 Lys27
HES1	Hes family bHLH transcription factor 1
HSF1	Heat shock transcription factor 1
IGF	Insulin-like growth factor
JNK	c-Jun N-terminal kinase
KCNQ2	Potassium voltage-gated channel subfamily Q member 2
KCNQ3	Potassium voltage-gated channel subfamily Q member 3
MAPK	Mitogen-activated protein kinase
MAP2	Microtubule-associated protein 2
MECP2	Methyl-CpG binding protein 2
MEF2	Myocyte enhancer receptor 2
mGluR	Group 1 metabotropic glutamate receptor
MKI67	Marker of proliferation Ki-67
mSin3A	SIN3 transcription regulator family member A
NAP1	Nck-associated protein 1
NCAD	N-cadherin
NEUROG2	Neurogenin 2
NFκB	Nuclear factor kappa B
NMP35	Neural membrane protein 35
NONO	Non-POU-domain-containing, octamer binding protein
OCT4	Octamer-binding transcription factor 4
OXTR	Oxytocin receptor
PAR3	Par-3 family cell polarity regulator
PAX6	Paired box protein 6
PCDH10	Protocadherin 10
PCDH11X	Protocadherin 11X
PCDH19	Protocadherin 19
PCDH17	Protocadherin 17
pHH3	Phosphohistone H3
αPKC	Protein kinase C, alpha
PKCλ	Protein kinase C, lambda
PPAR	Peroxisome proliferator-activated receptor
PR	Progesterone receptor
PRRT2	Proline-rich transmembrane protein 2
PSD-95	Post-synaptic density 95
PSF	Protein associated splicing factor
PTCH1	Patched 1
Rab5	RAB5A, member RAS oncogene family
Rac1	Ras-related C3 botulinum toxin substrate 1
RBM4	RNA binding motif protein 4
RhoA	Ras homolog family member A
SATB2	SATB homeobox 2
SCN1A	Sodium voltage-gated channel alpha subunit 1
SCN1B	Sodium voltage-gated channel beta subunit 1
SCN2A	Sodium voltage-gated channel alpha subunit 2
SNAP25	Synaptosomal-associated protein 25
SOX2	SRY-box 2
SOX10	SRY-box 10
STAT3	Signal transducer and activator of transcription 3
STXBP1	Syntaxin binding protein 1
TBC1D24	TBC1 domain family, member 24
TBR2	T-box brain protein 2
TBR1	T-box, brain 1

THDOC	Allotetrahydrodeoxycorticosterone
TP53	Tumour protein p53
USP9X	Ubiquitin specific peptidase 9, X-linked
VASP	Vasodilator-stimulated phosphoprotein
VGLUT1	Solute carrier family 17 member 7
VGLUT2	Solute carrier family 17 member 6
WAVE	WASP family verprolin homologous protein
ZO-1	Zona occludens 1