# Molecular Genetics of Epilepsy and Mental Retardation Limited to Females (EFMR)

A thesis submitted for the degree of Doctor of Philosophy to the University of Adelaide


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*Courage does not always roar*. *Sometimes*, it is the *quiet voice at the end of the day* saying, "I will try again tomorrow".

- Maryanne Radanbacher

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## Summary

Epilepsy and mental retardation limited to females (EFMR) is an intriguing X-linked disorder, which exhibits counterintuitive sex specific phenotype presentation, with heterozygous females affected and hemizygous males spared. Affected females suffer from epilepsy, which typically begins prior to three years of age. Onset of seizures often coincides with developmental regression resulting in intellectual disability.

- Using high throughput sequencing of the X-chromosome we have identified 7 different mutations in protoccadherin-19 (*PCDH19*) in 7 families with EFMR (Dibbens et al., 2008), including the original EFMR family published in 1971 (Juberg and Hellman, 1971). The discovery of mutations in *PCDH19*, a calcium cell-cell adhesion molecule, in EFMR highlights protocadherins and cell adhesion molecules in general, as a novel class of genes involved in intellectual disability and epilepsy. Publication 1, appendix A, (Dibbens et al., 2008)
- We have conducted follow up screening in three additional cohorts and identified additional mutations in the *PCDH19* gene in smaller families and a sporadic case with EFMR. *PCDH19* was not found to be a major cause of Rett syndrome or Autism Spectrum Disorder alone. Publication 2, appendix B, (Hynes et al., 2009)
- We have also identified an additional, large, black Afro-American family with EFMR with a missense mutation in *PCDH19* that is predicted to result in loss of function of PCDH19 protein. (Publication in preparation).
- We have performed genome-wide microarray expression profiling analyses and identified altered expression of genes involved in axon guidance and cell migration in EFMR females. We also unexpectedly found that EFMR females had a “male-like” expression profile of more than 100 genes and altered expression of 10 genes which function in aspects of estrogen production, activation and degradation or that are

known downstream targets of estrogen. This suggests that response to estrogen may be disrupted in certain cells. (Publication in preparation).

- Through genome-wide microarray expression profiling analyses we also identified altered expression of 13 genes involved in the Ephrin / Eph signalling pathway in EFMR females. The altered expression of Ephrin / Eph signalling genes and the parallels identified between EFMR and CFNS (craniofrontonasal syndrome), with respect to inheritance pattern, lead us to hypothesise a potential interaction between *PCDH19* and Ephrin / Eph signalling.

In conclusion, we have identified the causative gene for EFMR and gained novel insights into additional genes and signalling pathways that are disrupted as a result of mutations in *PCDH19* in females.

# Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Kim Hynes 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.

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Publications, which work outline in this thesis, has contributed to

1) Dibbens, L.M., et al., *X-linked protocadherin 19 mutations cause female-limited epilepsy and cognitive impairment.* Nat Genet, 2008. **40**(6): p. 776-81.

2) Hynes, K., et al., *Epilepsy and mental retardation limited to females with PCDH19 mutations can present de novo or in single generation families.* J Med Genet, Published online September 14, 2009.

Signed …….  Date

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To all my friends back home, sorry! Sorry I did not kept in touch as much as I should have but I assure you I was thinking of you all and hope that I will be able to see you again soon!

# List of abbreviations

| | |
|---|---|
| A, C, T, G | nucleotides; adenine, cytosine, guanine, thymine |
| aa | amino acid |
| ANOVA | analysis of variance |
| APC | anterior polar cataracts |
| ASD | Autism Spectrum Disorder |
| bp | base pairs |
| $Ca^{2+}$ | Calcium |
| cDNA | complementary DNA |
| CFNS | Craniofrontonasal syndrome |
| cM | centimorgans |
| CNS | central nervous system |
| CNV | copy number variation |
| CYP19 | aromatase cytochrome P450 |
| DAVID | Database for Annotation, Visualisation and Integrated Discovery |
| DNA | deoxyribonucleic acid |
| DS | Dravet Syndrome |
| EC | extracellular cadherin |
| ECM | extracellular matrix |
| EFMR | Epilepsy and mental retardation limited to females |
| ER | estrogen receptor |
| ES | embryonic stem |
| EST | expressed sequence tag |
| Fra(X) | fragile(X) |
| gDNA | genomic DNA |
| hpf | hours post fertilisation |
| GEFS+ | Genetic (generalised) Epilepsy with febrile seizers plus |
| GPI | glycosylphosphatidylinositol |
| GWAS | genome wide association study |
| IC | inferior colliculus |
| ID | intellectual disability |
| IGE | idiopathic generalised epilepsy |
| IGOLD | International Genetics of Learnig Disability group |
| IQ | intelligence quotient |
| kb | kilobase |
| Mb | megabase |
| MR | mental retardation |
| mRNA | messenger RNA |
| NCBI | National Centre for Biotechnology Information |
| NMD | nonsense mediated mRNA decay |
| NS-XLID | non-syndromic XLID |
| OMIM | Online Mendelian Inheritance in Man |
| ORF | open reading frame |
| PAC | P1 artificial chromosome |
| PCA | principal component analysis |
| PCDH19-DS | PCDH19 associated Dravet Syndrome |
| PCR | polymerse chain reaction |
| PTC | premature termination codon |
| RESDX | Rolandric Epilepsy, mental retardation and Speech Dyspraxia, X-linked |
| RNA | ribonucleic acid |

| | |
|---|---|
| RS | Rett Syndrome |
| RT-PCR | reverse transcribe PCR |
| RT-qPCR | quantitative real time PCR |
| SC | superior colliculus |
| SIFT | Sorting Intolerant From Tolerant |
| SNP | single nucleotide polymorphism |
| SP | signal peptides |
| SXLID | syndromic XLID |
| TM | transmembrane |
| UCSC | UC Santa Cruz genome browser |
| UTR | untranslated region |
| XLMR | X-linked mental retardation |
| XLID | X-linked intellectual disability |

## List of gene abreviations

| | |
|---|---|
| AKR | aldo-keto reductase |
| CDKL5 | cyclin-dependent kinase-like 5 |
| CDON | Cdon homolog |
| CXCR7 | chemokine (C-X-C motif) receptor 7 |
| DCX | double cortin |
| EFNA5 | ephrin-A5 |
| EPHA2 | EPH receptor A2 |
| GAS7 | growth arrest-specific 7 |
| IGF1R | insulin-like growth factor 1 receptor |
| ITGA3 | integrin, alpha 3 |
| ITGA6 | integrin, alpha 6 |
| LAMB1 | laminin, beta 1 |
| MECP2 | methyl-CpG-binding protein 2 |
| MET | met proto-oncogene |
| NR2F2 | nuclear receptor subfamily 2, group F, member 2 |
| NR4A1 | nuclear receptor subfamily 4, group A, member 1 |
| NTF3 | neurotrophin 3 |
| PCDH11X | protocadherin 11X |
| PCDH11Y | protocadherin 11Y |
| PCDH19 | protocadherin 19 |
| PDGFC | platelet derived growth factor C |
| PPARG | peroxisome proliferator-activated receptor gamma |
| SH3BP5 | SH3-domain binding protein 5 (BTK-associated) |
| SRPX2 | sushi-repeat-containing protein X-linked |
| STAT3 | signal transducer and activator of transcription 3 |
| STAT5A | signal transducer and activator of transcription 5A |
| TGFBR1 | transforming growth factor, beta receptor I |
| ZFP36L1 | zinc finger protein 36, C3H type-like 1 |