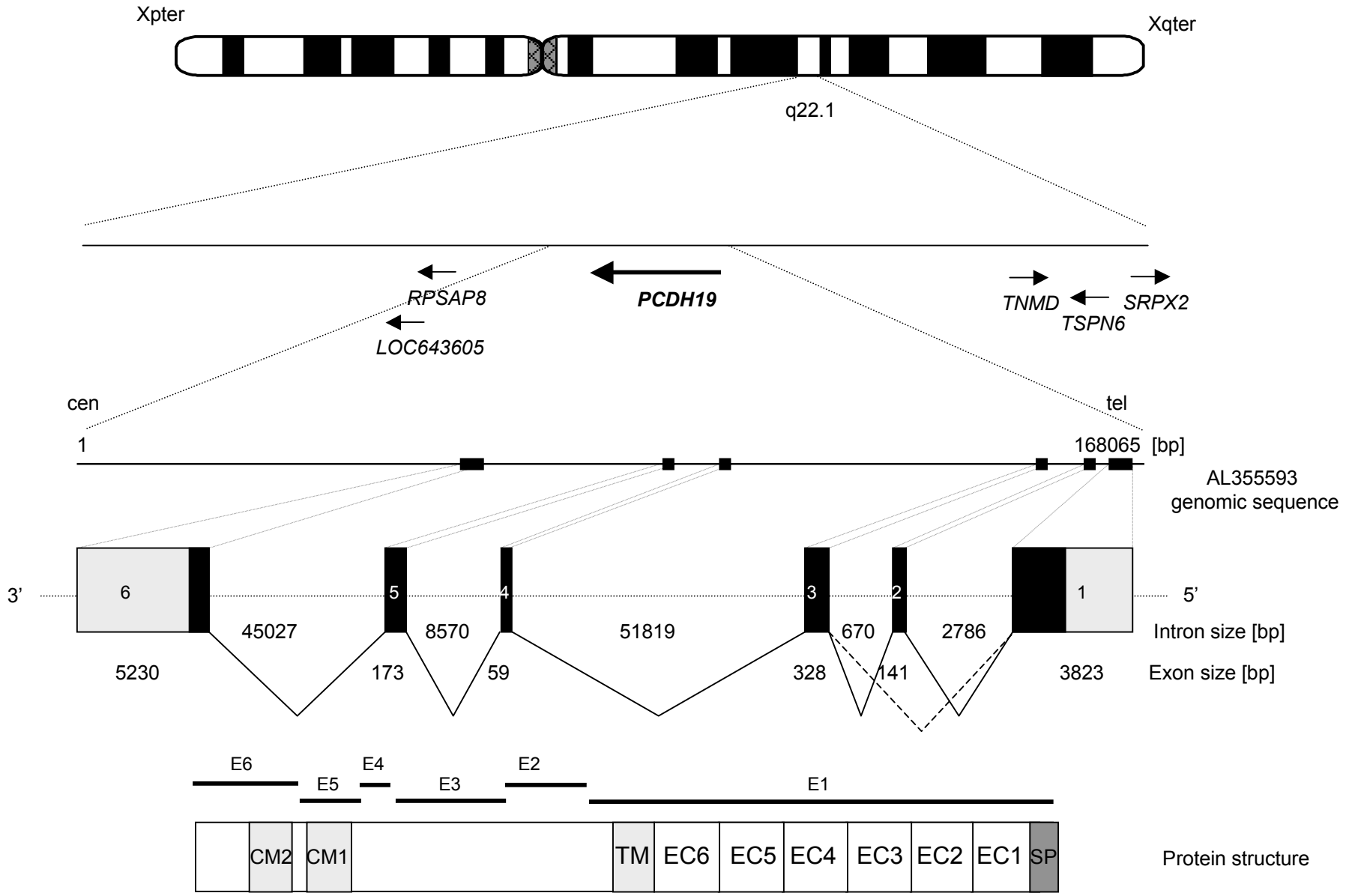
X-linked *protocadherin 19* mutations cause female-limited epilepsy and cognitive impairment.

*Leanne M Dibbens[1,2], *Patrick S Tarpey[3], Kim Hynes[1,4], Marta A Bayly[1], Ingrid E Scheffer[5,6], Raffaella Smith[3], Jamee Bomar[7], Edwina Sutton[4], Lucianne Vandeleur[1], Cheryl Shoubridge[1], Sarah Edkins[3], Samantha J Turner[5], Claire Stevens[3], Sarah O'Meara[3], Calli Tofts[3], Syd Barthorpe[3], Gemma Buck[3], Jennifer Cole[3], Kelly Halliday[3], David Jones[3], Rebecca Lee[3], Mark Madison[3], Tatiana Mironenko[3], Jennifer Varian[3], Sofie West[3], Sara Widaa[3], Paul Wray[3], John Teague[3], Ed Dicks[3], Adam Butler[3], Andrew Menzies[3], Andrew Jenkinson[3], Rebecca Shepherd[3], James F Gusella[8], Zaid Afawi[9], Aziz Mazarib[9], Miriam Y Neufeld[9], Sara Kivity[10], Dorit Lev[11], Tally Lerman-Sagie[11], Amos D Korczyn[8], Christopher P Derry[5], Grant R Sutherland[1,2,4], Kathryn Friend[1], Marie Shaw[1], Mark Corbett[1], Hyung-Goo Kim[8], Daniel H Geschwind[7], Paul Thomas[4], Eric Haan[1,4], Stephen Ryan[12], Shane McKee[13], Samuel F Berkovic[5], P Andrew Futreal[3], Michael R Stratton[3], John C Mulley[1,2,4], Jozef Gécz[1,2,4] .

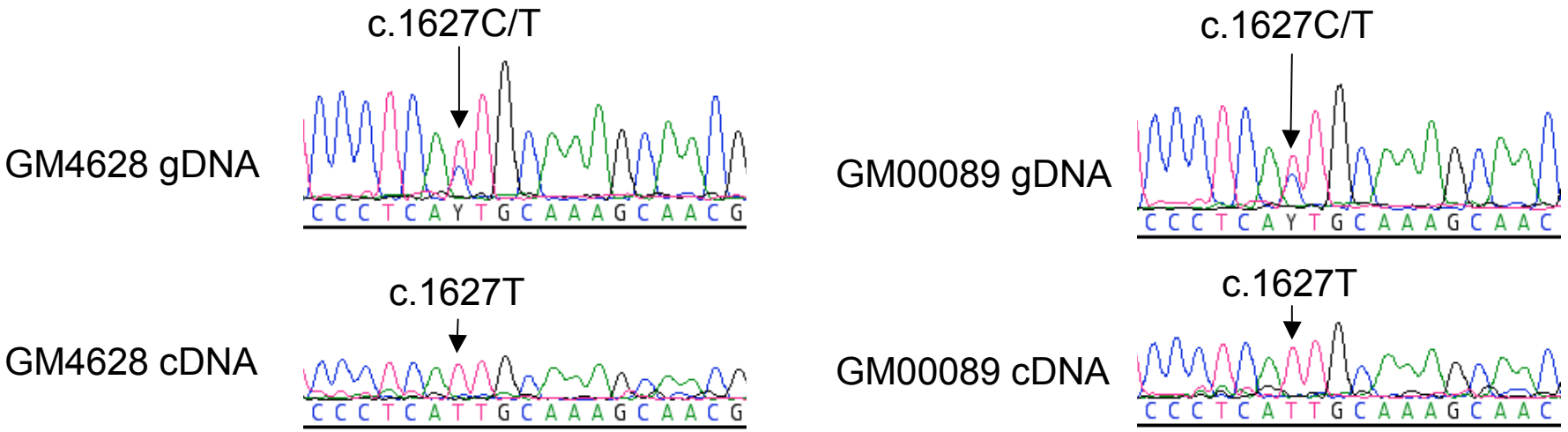
1 Department of Genetic Medicine, Women's and Children's Hospital, North Adelaide, SA 5006, Australia
2 School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, Australia
3 Wellcome Trust Sanger Institute, Hinxton UK
4 School of Molecular & Biomedical Science, University of Adelaide, Adelaide, Australia
5 Epilepsy Research Centre and Department of Medicine, University of Melbourne, Austin Health, Heidelberg, Victoria, Australia.
6 Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Melbourne, Australia
7Program in Neurogenetics and Neurobehavioral Genetics, Neurology Department and Semel Institute for Neuroscience and Behavior, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, California 90095-1769, USA.
8 Molecular Neurogenetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, Massachusetts, 02114 USA..
9 Department of Neurology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel.
10 Department of Neurology, Schneider Children's Medical Centre, Petaq Tikvah, Israel.
11 Metabolic Neurogenetic Clinic, Wolfson Medical Centre, Holon, Israel.
12 AstraZeneca, Wilmington, Delaware, USA.
13 Northern Ireland Regional Genetics Service, Belfast City Hospital, Belfast, Northern Ireland, UK.

Supplementary Fig. 1



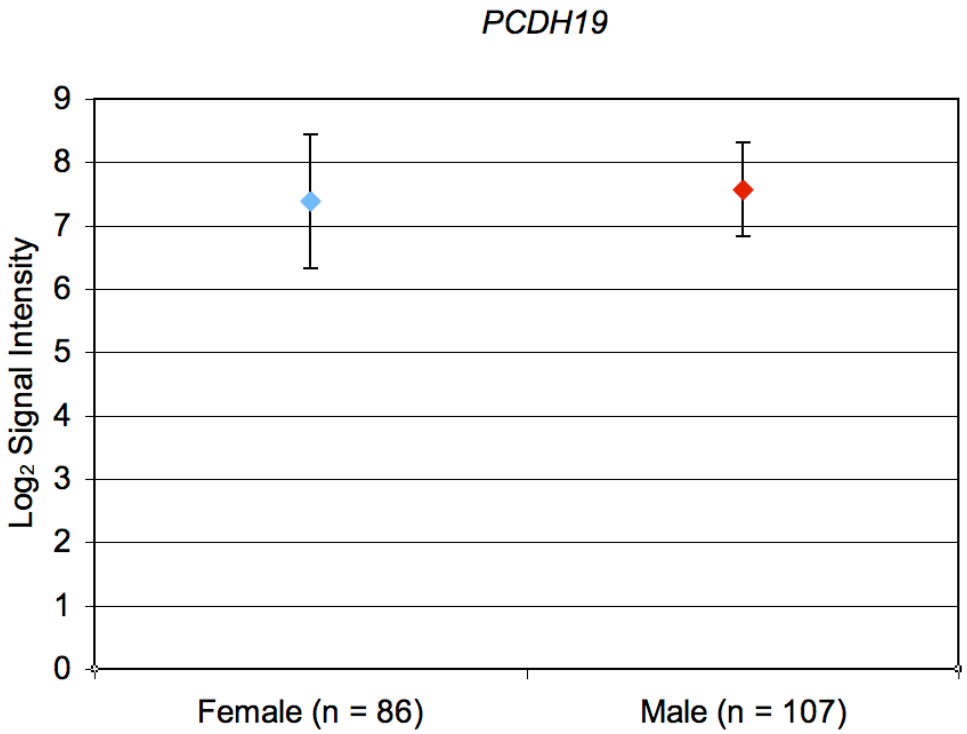
Supplementary Fig. 1 Genomic, gene and PCDH19 protein structure. Below the ideogram of the human X chromosome a genomic region of the *PCDH19* gene is shown. The *PCDH19* gene is located within the Xq22.1 region and not in Xq13.3 as indicated in the Entrez Gene database. Only the closest flanking genes and pseudogenes (together with their orientation indicated by arrows) are shown. Exon/intron structure of *PCDH19* is shown on the background of its genomic sequence (GenBank accession AL355593). The position of individual *PCDH19* exons is not drawn to scale. Individual *PCDH19* exons (n=6) are labeled and their sizes shown in nucleotide base pairs [bp]. The individual intron sizes are also indicated. The *PCDH19* mRNA is subject to alternative splicing, which involves exon 2. This is indicated with dotted line below the exon structure together with *PCDH19* mRNA transcription orientation (from Xq telomere to centromere). The lower panel shows protein structure of PCDH19 with signal peptide (SP), six extracellular cadherin repeats (EC1-6), transmembrane domain (TM) and known cytoplasmic domains (CM1 and CM2).

Supplementary Fig. 2



Supplementary Fig. 2 *PCDH19* is subject to X-inactivation. To test whether the *PCDH19* gene is subject to X-inactivation we isolated DNA and RNA from multiple (n = 7) fibroblast (n = 5) and lymphoblast (n = 2) cell lines of patients with balanced X;autosome translocations. For most of these patients (n = 5) there was published evidence that the normal X-chromosome was late replicating and thus preferentially inactivated (*Carrel L, Willard HF, Proc Natl Acad Sci U S A. 96(13):7364-7369, 1999; Gecz et al., Genomics 62(3):356-368*, 1999; *Kalscheuer et al., Am J Hum Genet 72(6):1401-1411, 2003*). Only two of these cell lines, GM4628 (case #68) and GM00089 (case #51; *Carrel L, Willard HF, Proc Natl Acad Sci U S A. 96(13):7364-7369, 1999*) were informative for *PCDH19* cSNP c.1627C/T. The two panels above show the results of genomic DNA (gDNA) and cDNA re-sequencing of the corresponding region of the *PCDH19* gene. While in genomic DNA both alleles are present, in cDNA there is only one. Together with the fact that these females have only one of their X-chromosome active these results strongly suggest that *PCDH19* is subject to X-inactivation. The position of the cSNP c.1627C/T is indicated on each sequence chromatogram with arrows.

Supplementary Fig. 3



Supplementary Fig. 3 The levels of gene expression of *PCDH19* did not vary significantly between males and females in human cortex. Average expression values and standard deviations corresponding to the *PCDH19* gene (probeset GI_37546220-S) in human female (n = 86) and male (n = 107) cortex were determined from the dataset of *Myers et al., Nature Genet 39(12):1494-1499, 2007;* GEO accession GSE8919.

Supplementary note:

Mouse in situ hybridization studies. To investigate the expression of *Pcdh19* in the developing murine CNS, we performed *in situ* hybridization analysis of embryonic (15.5 days post coitum (dpc)) and postnatal day 2 tissue. *Pcdch19* was expressed in a widespread, symmetrical pattern in the embryonic forebrain and frequently localized to discrete cell clusters within the cortex, thalamus and hypothalamus (arrowheads **Fig. 3a**, **b**). In the cortex, expression was restricted to the cortical plate (**Fig. 3c**) and extended medially into the intercerebral fissure (**Fig. 3d**). Robust expression was also detected in the ganglionic eminence that abuts the dorsolateral wall of the lateral ventricles (asterisk, **Fig. 3e**). At this stage hippocampal expression was not observed on the medial edge of the lateral ventricle in the presumptive hippocampus (**Fig. 3b, e**). Analysis of anterior forebrain sections revealed *Pcdh19* expression in the epithelial lining of the nasal cavity (consistent with a previous report24) and in the olfactory bulbs (**Fig. 3f**). At postnatal day 2, *Pcdh19* expression was maintained in discrete regions of the cortex and the thalamus however, unlike the embryonic brain, expression was also observed in the hippocampus (**Fig. 3g-i**). In the cortex, expression was restricted to a band of cells that spanned layers II-IV (arrows, **Fig. 3j, k**) whilst the most prominent *Pcdh19* signal was observed in the CA1 and CA3 regions of the hippocampus (**Fig. 3h, l**). *Pcdh19* transcripts were not detected in white matter tracts including the corpus callosum (**Fig. 3h**). Together these data indicate that *Pcdh19* has widespread expression in both the embryonic and adult brain including the developing cortex and hippocampus and are consistent with our finding that mutation of this gene in humans is associated with cognitive impairment.

Supplementary Table 1

Primer	Product size (bp)	Direction	Primer Sequence (5‘ to 3‘)	Nucleotides	Genbank Acc.
PCDH19 northern probe	374	F	CCGGATTCTTGGCCACTCTGAC	2884-3257	NM_921478
		R	CAATGGTGTAAGACACGGAAG		
PCDH11X/Y probe 1	489	F	AGATCCTGGTTGCAGCTGTT	3280-3768	NM_032969.2
Human *in situs*		R	AGGCCACAAAGGTGTTATCG		
PCDH11X/Y probe 2	492	F	AACGTCCTGATAGGCAACTTGT	954-1445	NM_032969.2
Human *in situs*		R	GGCATCTTGTCTCCTTCTGG		
PCDH19 probe 1	500	F	TACTTCCCAATGCCGCTACT	2374-2873	NM_020166.1
Human *in situs*		R	TGATTGGAGAGAGGGAATTTCT		
PCDH19 probe 2	600	F	TGATTGGAAAGCATTGTAGGT	5780-6379	NM_020166.1
Human *in situs*		R	TAACACCATTTGAAACATAACTGTAA		

Supplementary Table 1 Primer sequences used to generate the probe used in Northern Blot analysis of human *PCDH19* and the probes used for *in situ* hybridization analyses of *PCDH11X/Y* and *PCDH19* transcript localization in human tissues.