

**Mutations in the
protocadherin 19 gene
cause epilepsy in females
by a mechanism called
cellular interference**

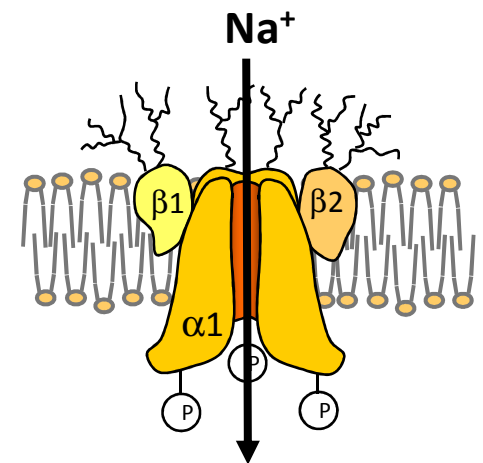
Christel Depienne

**UF de neurogénétique & CRicm
Hôpital de la Pitié-Salpêtrière, Paris**

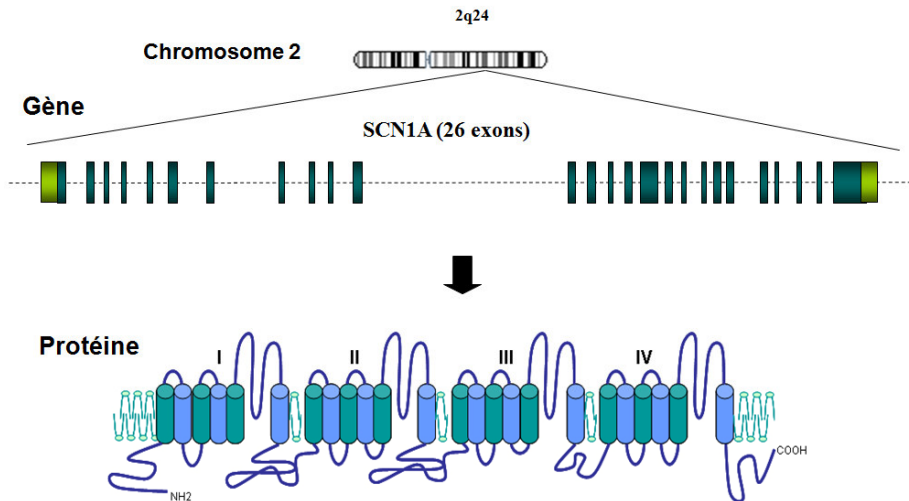
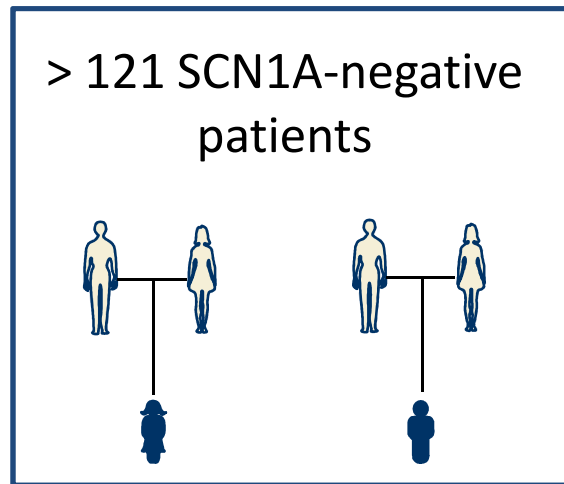
**Clinical Implications of Arrays & Next Generation Sequencing Technologies
Seminar, Paris, 10/05/2010**

Severe myoclonic epilepsy of infancy or Dravet syndrome

- Normal PMD & MRI before the onset of seizures
- Onset < 1 yr: seizures +++ triggered by fever
- Febrile and afebrile polymorphic seizures (generalized, partial seizures & absences)
- Pharmacoresistant
- Psychomotor delay, myoclonic jerks > 2 yrs
- Mental retardation, behavioral disturbances
- Sporadic cases
- **70-80% *de novo* mutations in the *SCN1A* gene** (voltage-gated sodium channel alpha 1 or Nav1.1)



Search for new genes responsible for Dravet syndrome

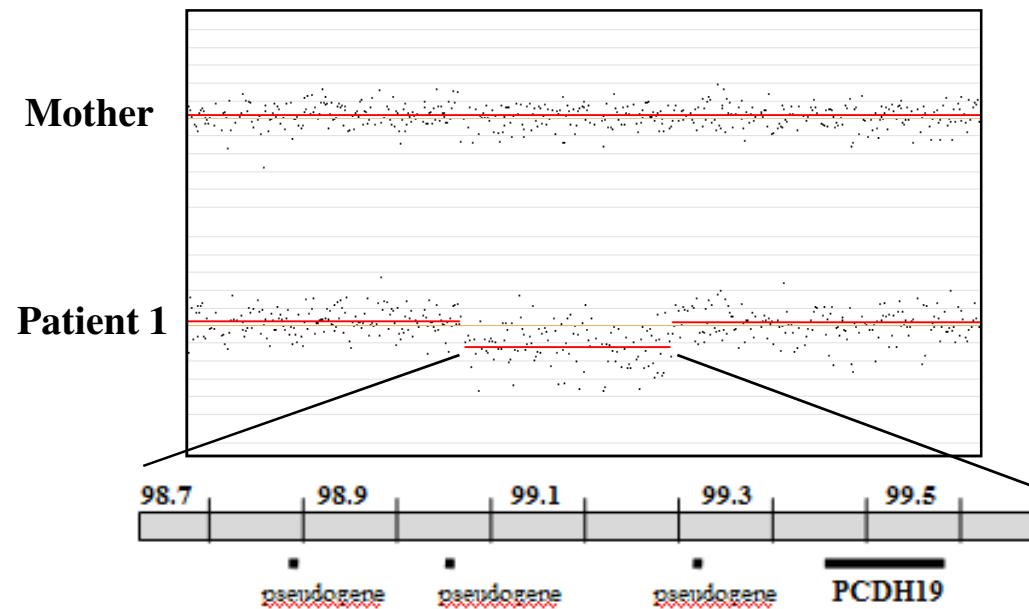
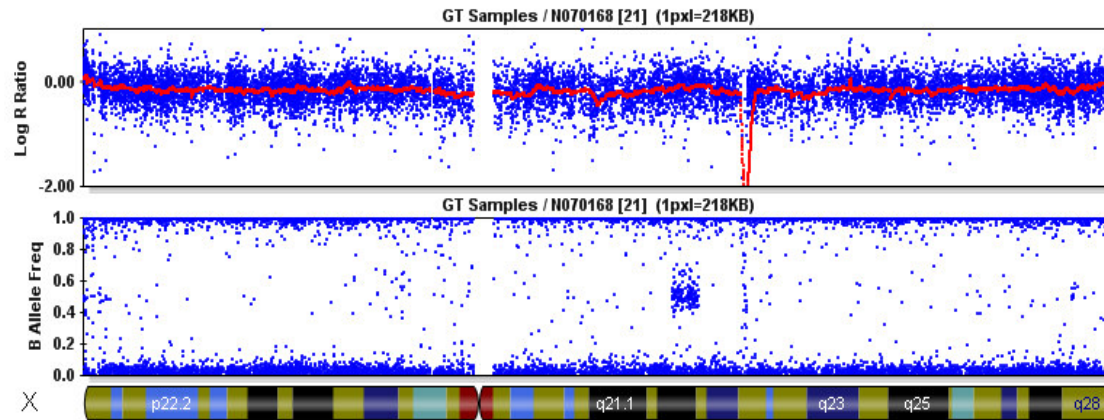
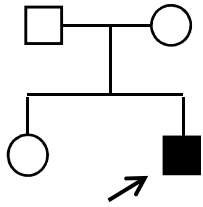


Search for micro-rearrangements

Illumina 370K arrays



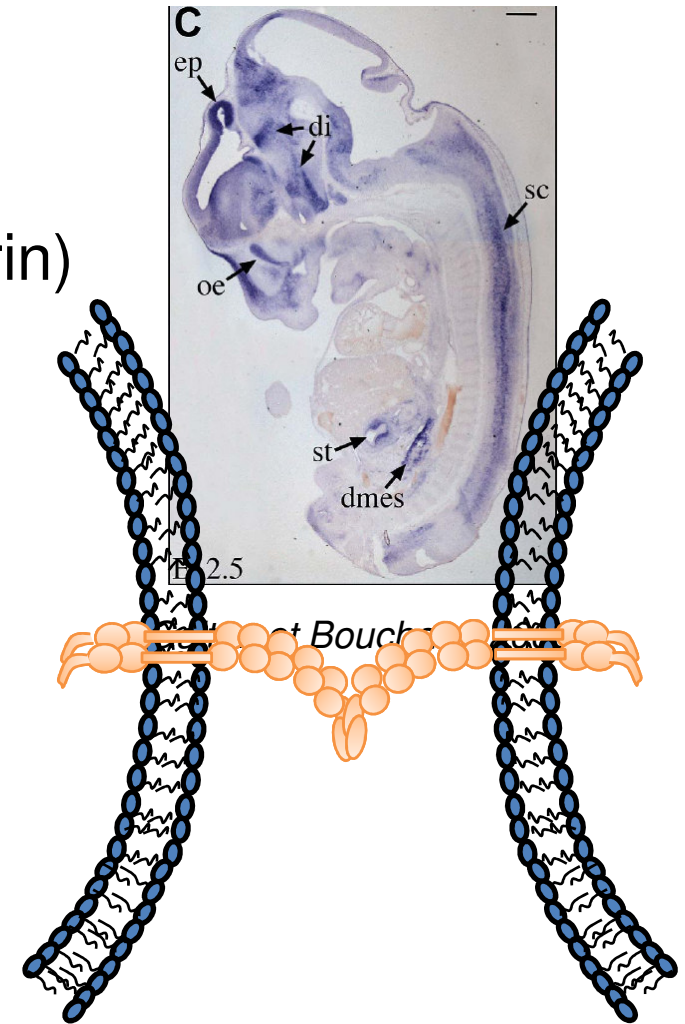
Identification of a male patient with a hemizygous Xq22.1 deletion



Del ChrX: 98.731.380-99.618.794

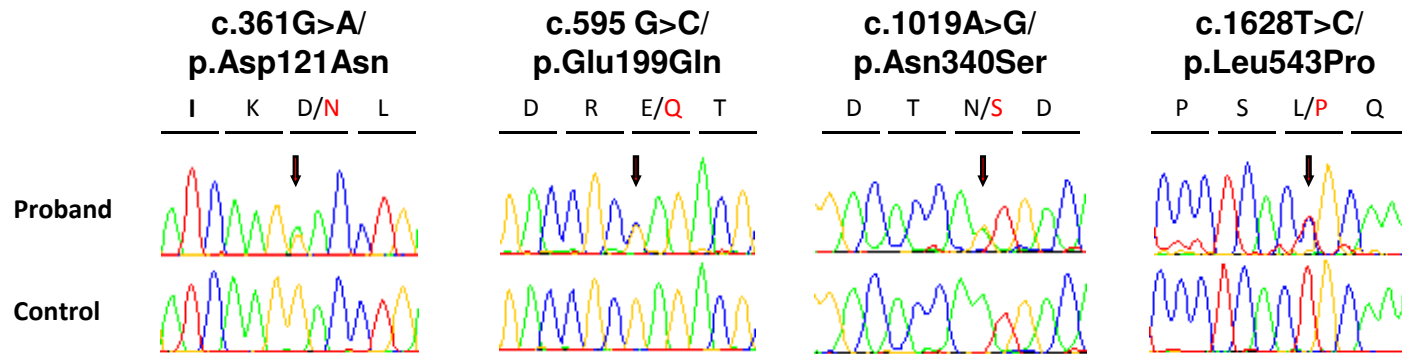
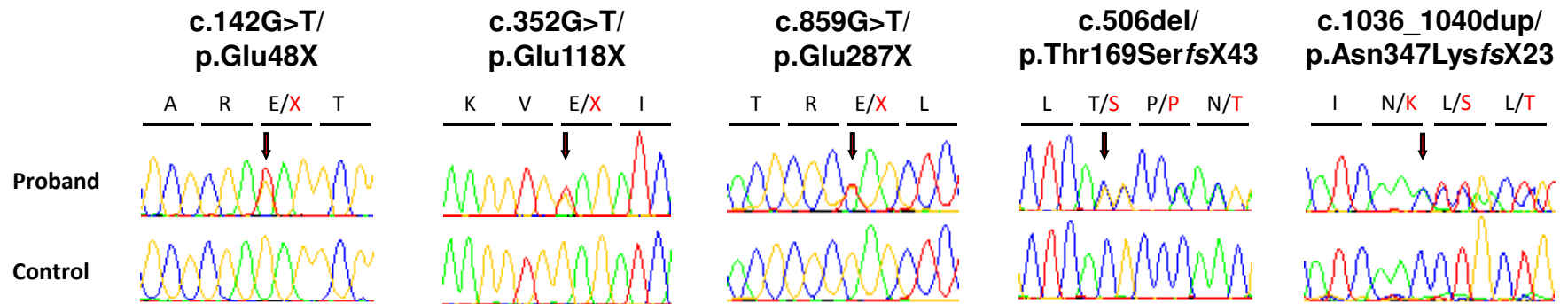
Function of protocadherins in the CNS

- Highly expressed in CNS
- Cadherin superfamily ($\delta 2$ protocadherin)
- Membrane protein (6 extracellular EC domains, 2 intracellular CM domains)
- Ca^{2+} -dependent homophilic/heterophilic Interactions
- Potential role in neuronal survival & migration during development



➔ Screening of *PCDH19* in 73 *SCN1A*-negative DS patients

Identification of 9 different point mutations in 11 unrelated female patients

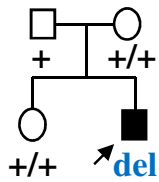


Human	PCDH19	ICVIRKVEI	D	LNDNAPSFP		LVVEKSLD	E	TQSHYSFRI		KVTVSVLDT	N	NDNPPVINLL		LAKDGG	LPS	-	LQSNATV	VRVI
Rat	PCDH19	ICVIRKVEI	D	LNDNAPSFP		LVVEKSLD	E	TQSHYSFRI		KVTVSVLDT	N	NDNPPVINLL		LAKDGG	MPS	-	LQSNATV	VRVI
Mouse	PCDH19	ICVIRKVEI	D	LNDNAPSFP		LVVEKSLD	E	TQSHYSFRI		KVTVSVLDT	N	NDNPPVINLL		LAKDGG	LPS	-	LQSNATV	VRVI
Dog	PCDH19	ICVIRKVEI	D	LNDNAPSFP		LVVEKSLD	E	TQSHYSFHI		KVTVSVLDT	N	NDNPPVINLL		LAKDGG	LPS	-	LQSNATV	VRVI
Tetraodon	PCDH19	ICVIRVEI	D	LNDNAPRFF		LVVQKSLD	E	TQSHYTYEI		KVTVNVD	N	NDNPPVISLL		LAKDGG	GPS	-	LQSNATV	VRVI
Human	PCDH17	ICMIRKVEI	Q	DINDNAPSFS		LVIQKALD	R	EQNHHTLVL		KVTVKLID	N	NDNAPSIGFV		LAKDSG	APL	-	LQSNATV	VRVT
Human	PCDH10	LFQVEVEI	V	DINDNPPSFP		KVLVRLVD	A	NDNAPESFS		KVLVRLVD	A	NDNAPESFS		EARDAG	SPQ	-	LAGNATV	NL
Human	PCDH18	LFHIEVEI	V	DINDNSPQFS		KI I I KVV	D	NDNKPEININ		KI I I KVV	D	NDNKPEININ		EARDGG	SPR	-	LVSN	TVVLT
Human	PCDH8	LHVHEVEI	D	NDHAPRFP		KVIVRIRD	N	NDNAPDIAIT		KVIVRIRD	N	NDNAPDIAIT		QASDGG	SPQ	-	LQSSALV	QVR
Human	PCDH11X	LVKIRFLI	H	DINDNAPLFP		MVLVNVTD	N	NDNVP SIDIR		MVLVNVTD	N	NDNVP SIDIR		LAKDNG	VPP	-	LVSNVT	VFVS
Human	PCDH11Y	LVKIRFLI	H	DINDNAPLFP		LIVQKELD	R	EKKDTYVMKV		MVLVNVTD	N	NDNVP SIDIR		LAKDNG	VPP	-	LVSNVT	VFVS
Human	PCDH9	LIKIKIIV	H	DINDNAPMFP		LIVQQLD	R	EKKDTYVMKI		TVTINVT	D	NDNPPNIDLR		TARDNG	TPP	-	LQSQAAV	I
Human	PCDH1	LLEGQIEV	Q	DINDNTPNFA		LIVMGNLD	R	EWDSYDLTI		QVVVTVK	D	NDNAPTIEIR		NAKDKG	IPV	-	LQGSTT	VIVQ
Human	PCDH7	LEFGQVI	V	DINDNTPTFP						TVV L N I K	D	NDNVP SIEIR		VAADR	GSPS	-	LQGTATV	LVN

Pedigrees & segregation of the mutations in the families

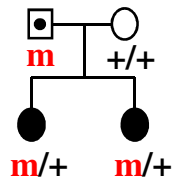
Family 1

del = whole gene deletion



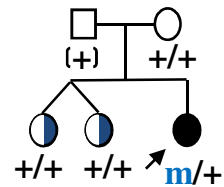
Family 2

m = p.Glu48X



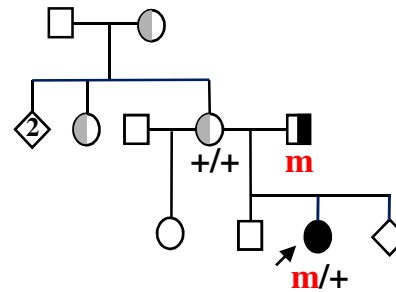
Family 3

m = p.Glu118X



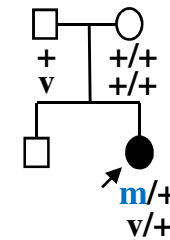
Family 4

m = p.Glu287X



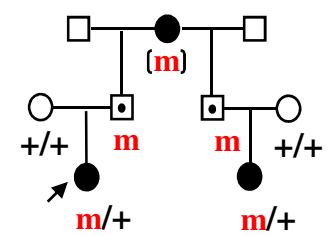
Family 5

m = p.Glu287X
v = p.Arg1107Gly



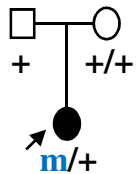
Family 6

m = c.1036_1040dup5



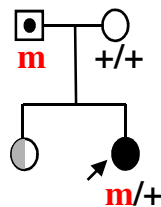
Family 7

m = c.506delC



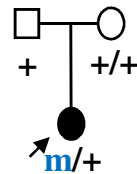
Family 8

m = p.Asp121Asn



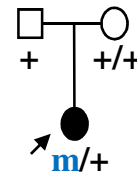
Family 9

m = p.Glu199Gln



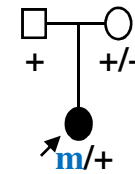
Family 10

m = p.Asn340Ser



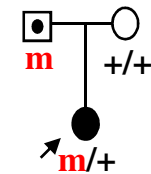
Family 11

m = p.Asn340Ser



Family 12

m = p.Leu543Pro



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

Leanne M Dibbens^{1,2,14}, Patrick S Tarpey^{3,14}, Kim Hynes^{1,4}, Marta A Bayly¹, Ingrid E Scheffer^{5,6}, Raffaella Smith³, Jamee Bomar⁷, Edwina Sutton⁴, Lucianne Vandeleur¹, Cheryl Shoubbridge¹, Sarah Edkins³, Samantha J Turner⁵, Claire Stevens³, Sarah O'Meara³, Calli Tofts³, Syd Barthorpe³, Gemma Buck³, Jennifer Cole³, Kelly Halliday³, David Jones³, Rebecca Lee³, Mark Madison³, Tatiana Mironenko³, Jennifer Varian³, Sofie West³, Sara Widaa³, Paul Wray³, John Teague³, Ed Dicks³, Adam Butler³, Andrew Menzies³, Andrew Jenkinson³, Rebecca Shepherd³, James F Gusella⁸, Zaid Afawi⁹, Aziz Mazarib⁹, Miriam Y Neufeld⁹, Sara Kivity¹⁰, Dorit Lev¹¹, Tally Lerman-Sagie¹¹, Amos D Korczyn⁸, Christopher P Derry⁵, Grant R Sutherland^{1,2,4}, Kathryn Friend¹, Marie Shaw¹, Mark Corbett¹, Hyung-Goo Kim⁸, Daniel H Geschwind⁷, Paul Thomas⁴, Eric Haan^{1,4}, Stephen Ryan¹², Shane McKee¹³, Samuel F Berkovic⁵, P Andrew Futreal³, Michael R Stratton³, John C Mulley^{1,2,4} & Jozef Gécz^{1,2,4}

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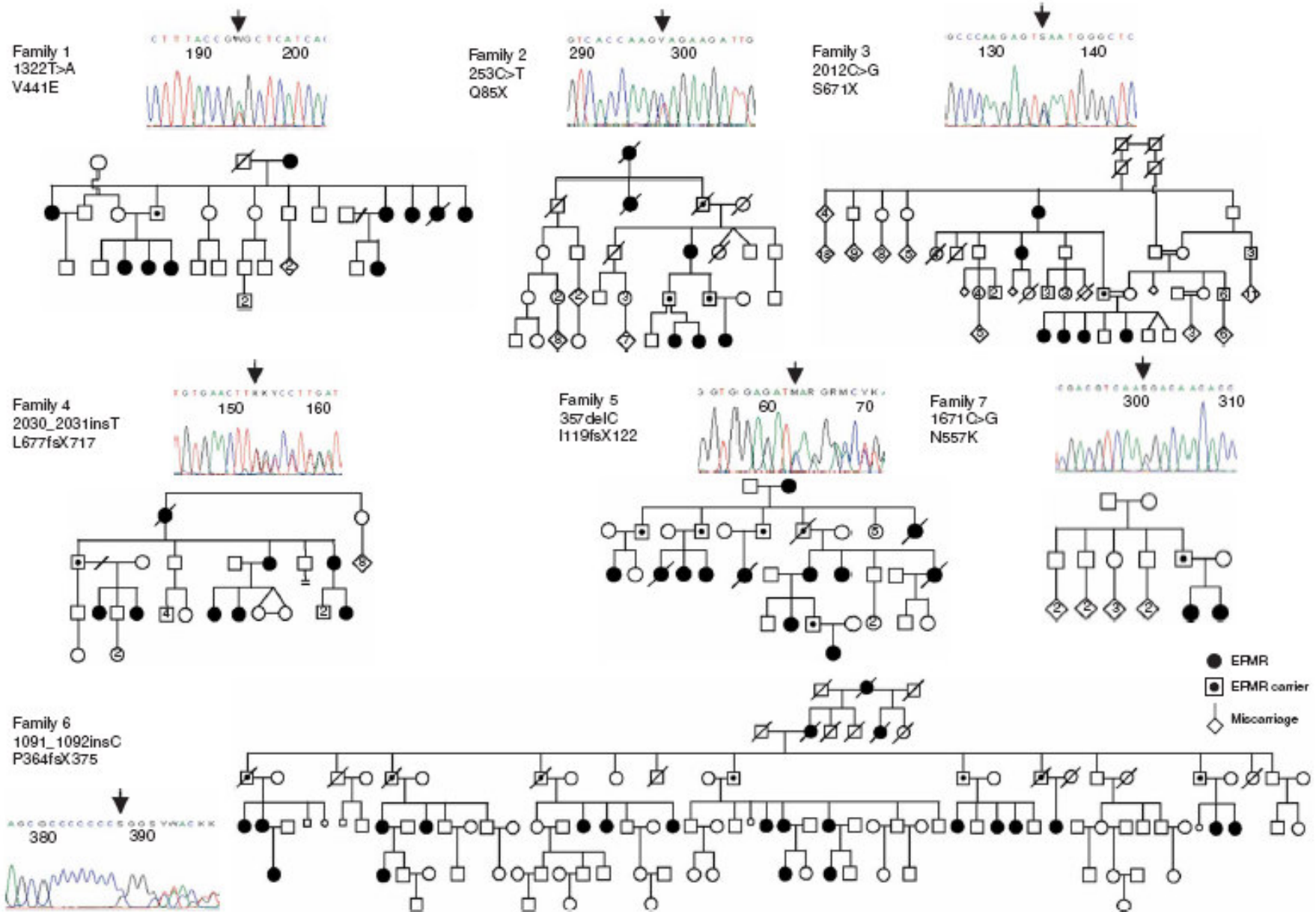
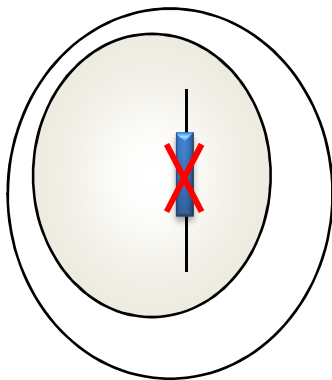
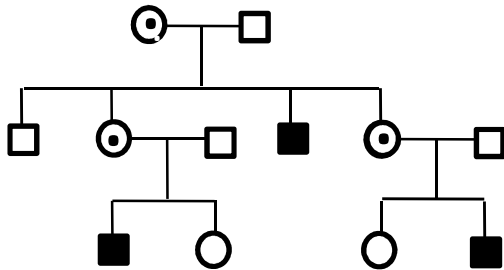


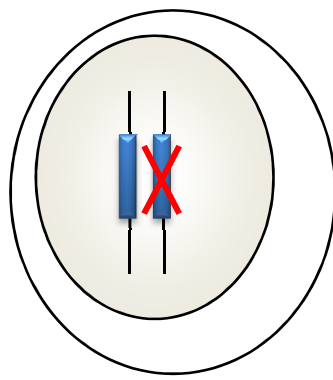
Figure 1 Mutations in *PCDH19* cause EFMR. Pedigrees of the seven EFMR families showing the characteristic inheritance pattern of affected females and transmitting males. Each of the X chromosome–encoded *PCDH19* mutations segregated with the EFMR clinical phenotype. An example of a sequence chromatogram of a *PCDH19* mutation as detected in an affected female is shown for each family.

An unusual X-linked inheritance

Recessive X-linked

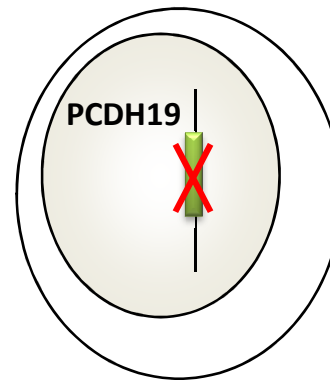
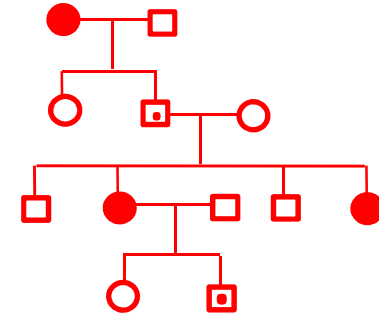


Affected

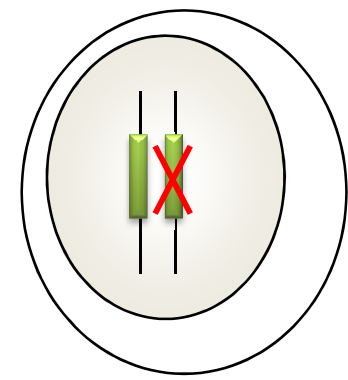


Unaffected

X-linked with male sparing



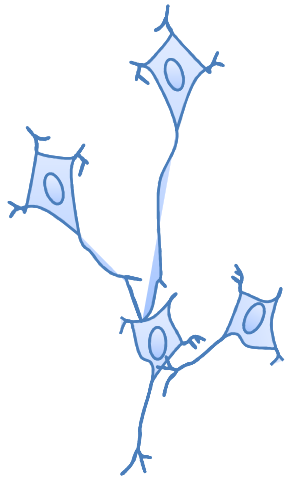
Unaffected



Affected

Principle of cellular interference

Normal individuals
(male/ female)

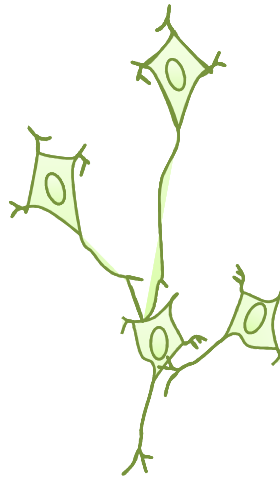


**WT protocadherin 19
is expressed in all
neurons**



Asymptomatic

Mutated males
(hemizygous)



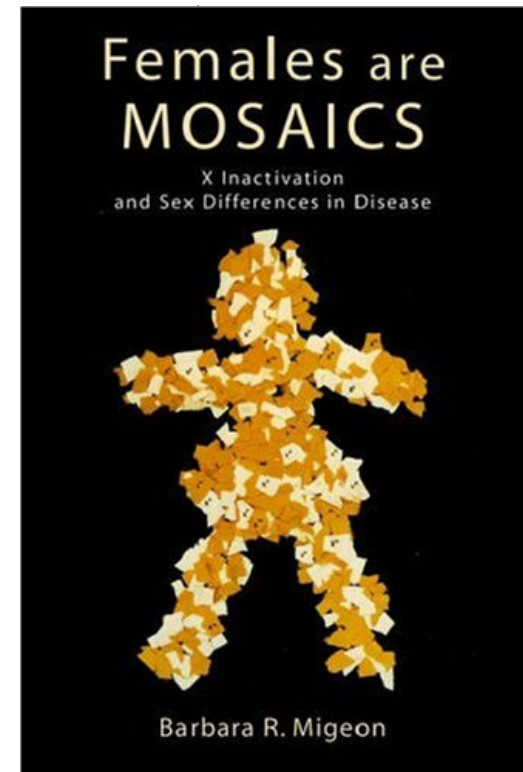
**Mutated protocadherin
19 is expressed in all
neurons**



Asymptomatic

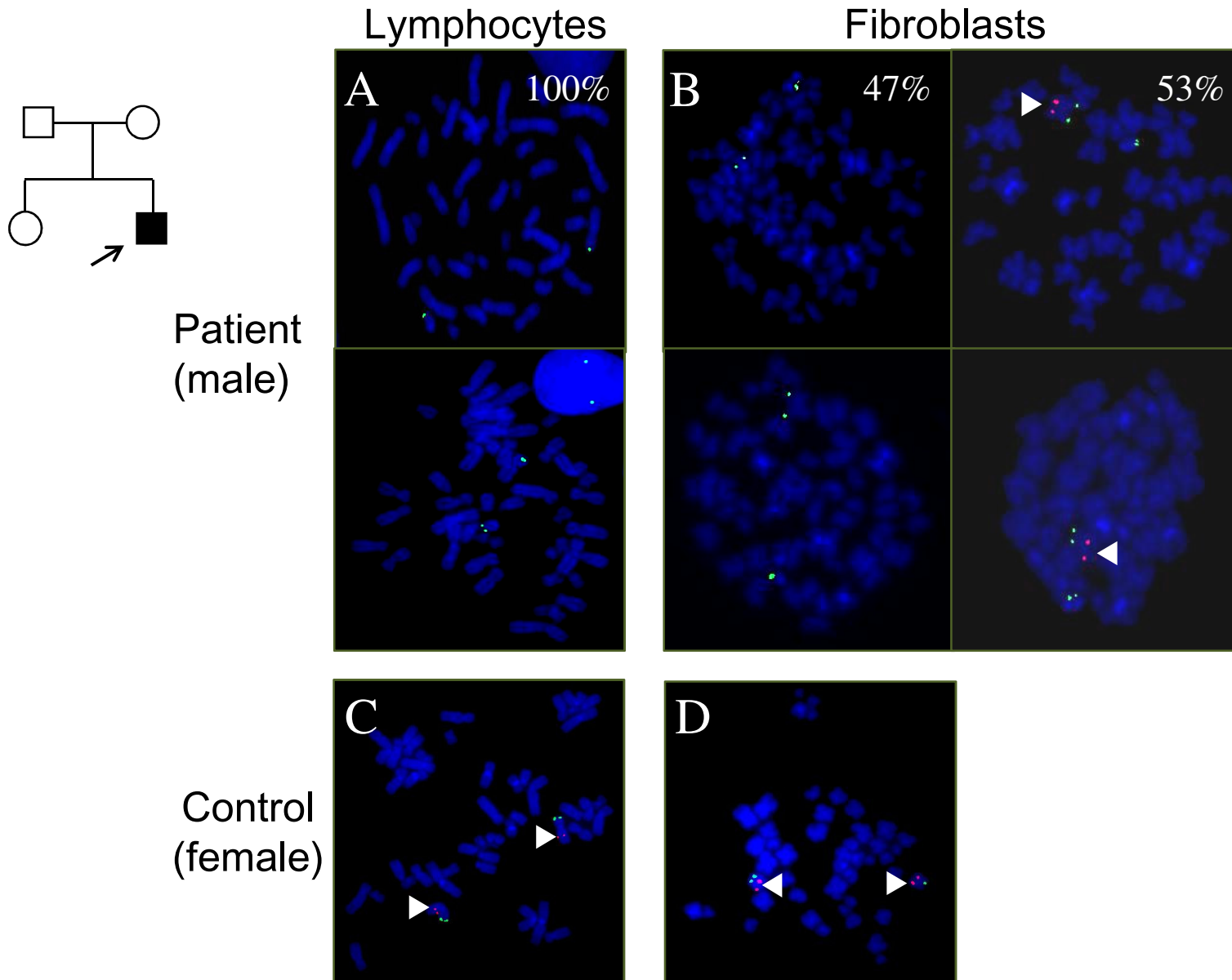
Mutated heterozygous
females

Mosaic mutated males



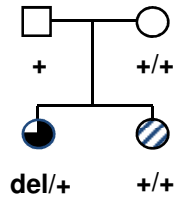
Epilepsy and MR

Mosaicism in the patient with the *PCDH19* deletion

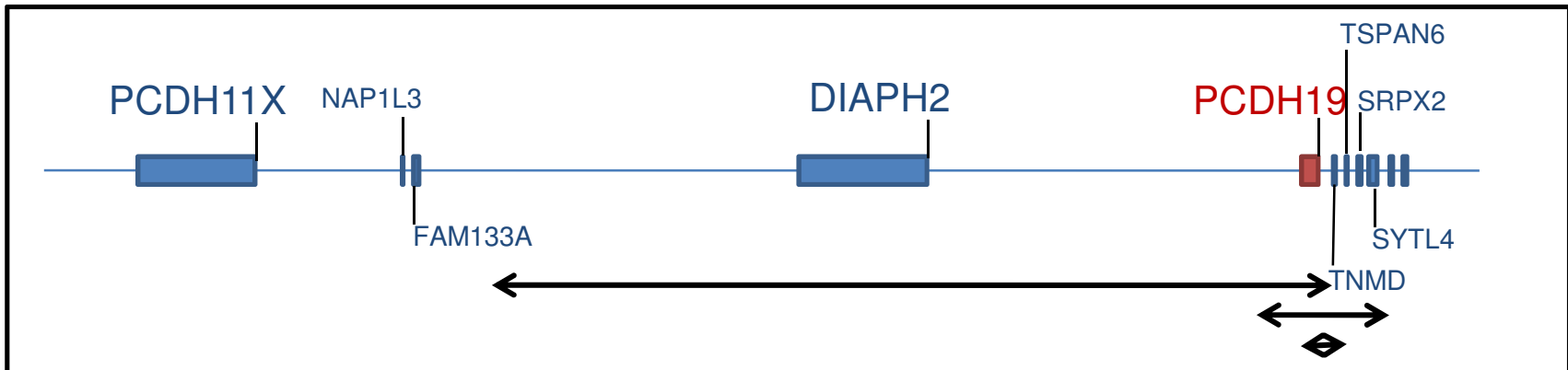
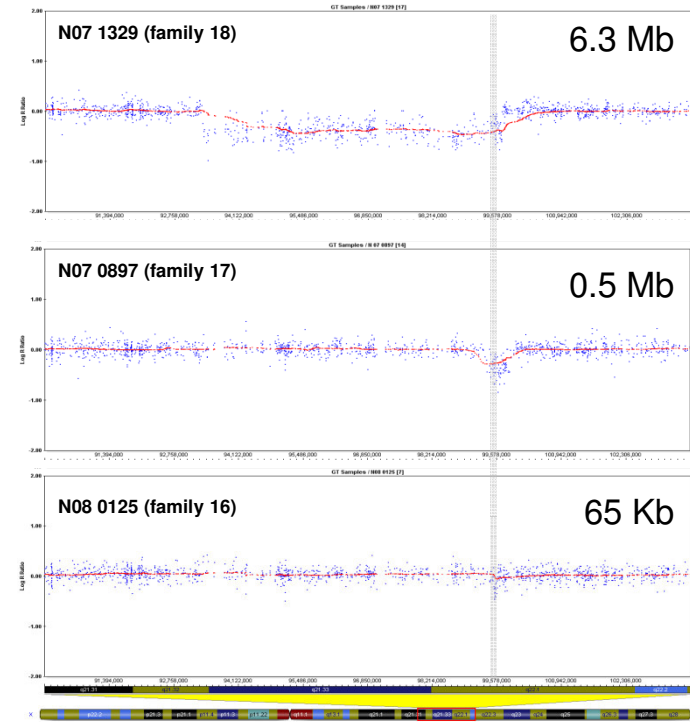
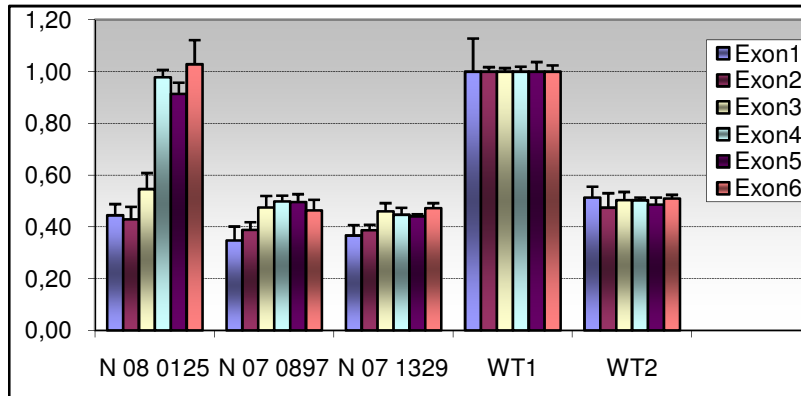
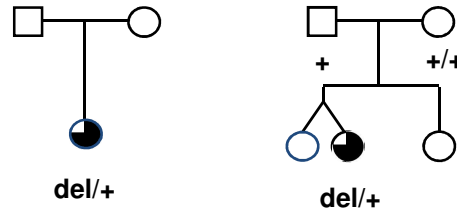


Micro-rearrangements of PCDH19 in females

Deletion of exons 1-3

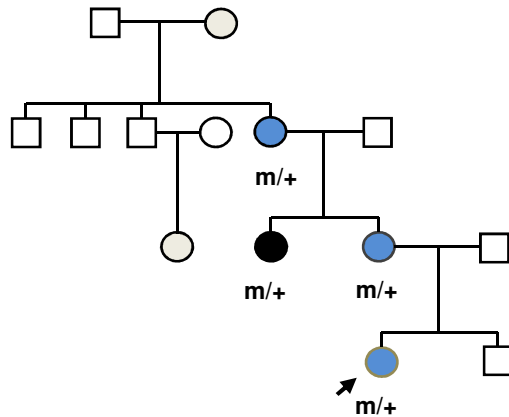


Whole gene deletions



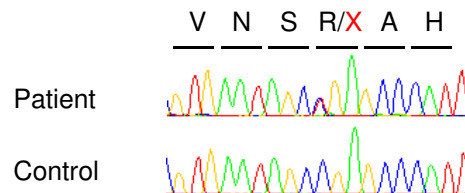
Mutations of PCDH19 in female patients with epilepsy but without cognitive impairment

Generalized epilepsy with febrile seizures

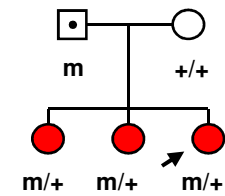


c.2656 C>T / p.Arg886X

Exon 4

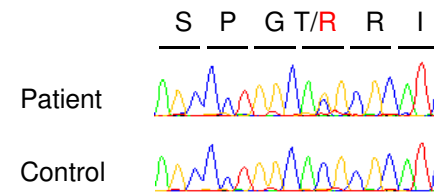


Cryptogenic partial epilepsy



c.437 C>G / p.Thr146Arg

Exon 1



Conclusions

- High-density SNP arrays are a good tool to identify micro-rearrangements and new genes
- PCDH19: new gene responsible for epilepsy +/- mental delay
- Frequent mutations: 15% of female patients with epilepsy and FS
- Unusual X-linked inheritance affecting mainly females
- Familial / sporadic cases (inherited / *de novo*)
- New pathophysiological mechanism : cellular interference



U975

Delphine Bouteiller
Alexis Brice
Eric Leguern



CRICM

UF neurogénétique moléculaire et cellulaire

Oriane Trouillard
Eric Leguern

Cytogénétique

Boris Keren
Baya Benyahia

P3S

Wassila Carpentier
Florent Soubrier

Institut Cochin

Karine Poirier
Jamel Chelly

Cliniciens

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Sophie Meyer, Bordeaux
Rima Nabbout, Paris
Isabelle Py, Cholet
Serge Rivera, Bayonne
François Rivier, Montpellier
Agathe Roubertie, Montpellier
Dominique Steschenko, Nancy
Sandra Whalen, Paris
Et les autres...



Thanks to the families!

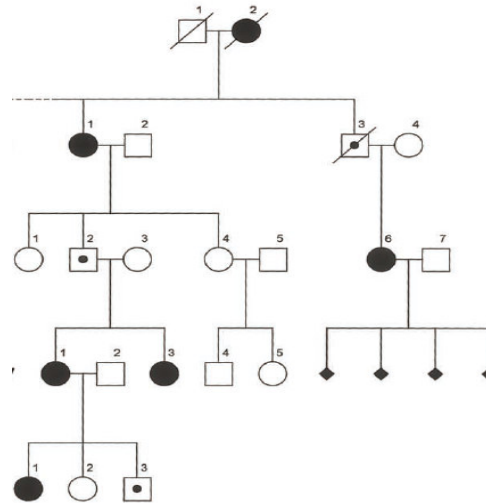
Financements: GIS Maladies rares, AP-HP, INSERM

Craniofrontonasal syndrome

- Another disease with unusual X-linked inheritance



Wieland et al, 2004
Twigg et al, 2004

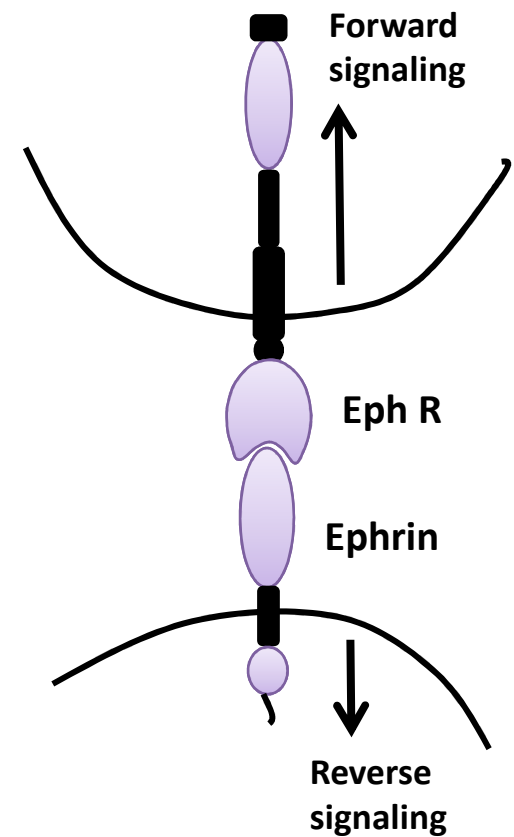


⇒ Mutations in Ephrin B1 (*EFNB1* en Xq12)

- Mouse model supporting cellular interference as the pathological mechanism



Compagni et al, 2003



D'après Wieacker & Wieland, 2008