Putative low penetrance or susceptibility variants: sodium channel genes in painful neuropathy as an example

Carl Fratter[1]

Kate Sergeant[1], Julie C Evans[1], Anneke Seller[1], David Bennett[2]

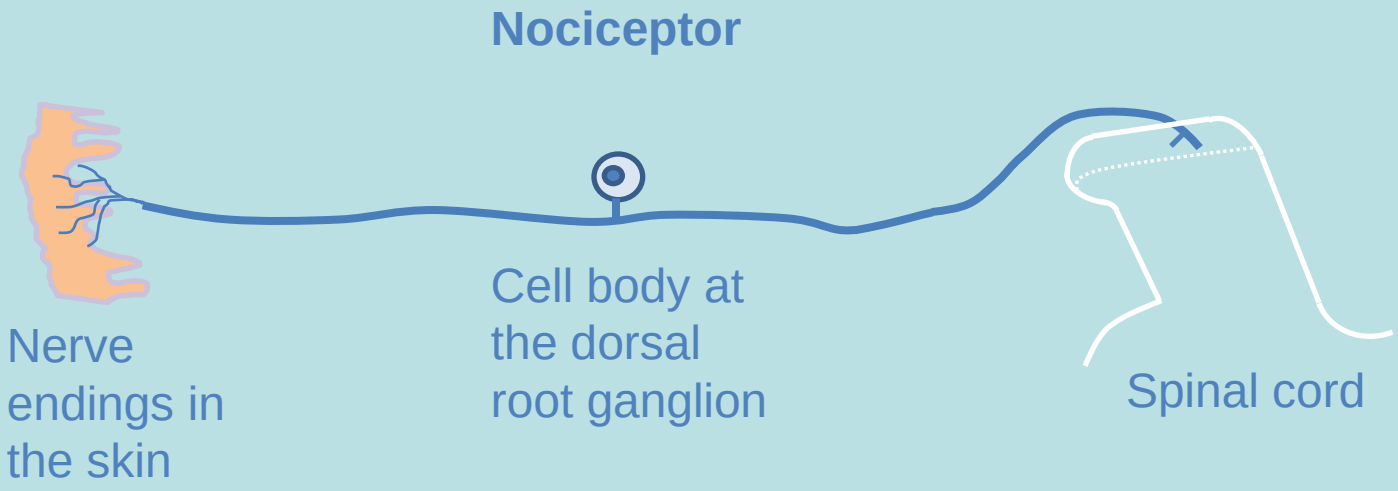
[1]Oxford Medical Genetics Laboratories, Oxford University Hospitals NHS Foundation Trust
[2]Nuffield Department of Clinical Neurosciences, University of Oxford

Oxford University Hospitals NHS
NHS Foundation Trust

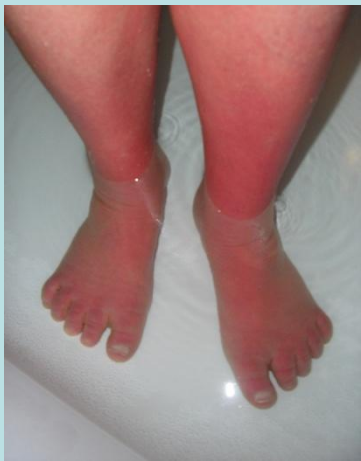
Oxford Biomedical Research Centre
Enabling translational research through partnership

Painful Neuropathies

- Group of disorders with increased pain perception
  - Include erythromelalgia and small fibre neuropathy (SFN)
- Increased/inappropriate signalling by the nociceptors – the sensory neurons responsible for pain perception

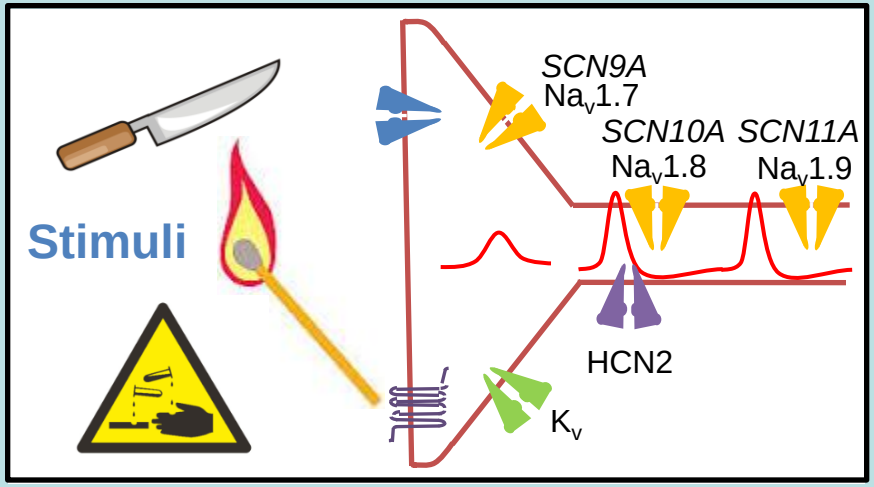


Erythromelalgia



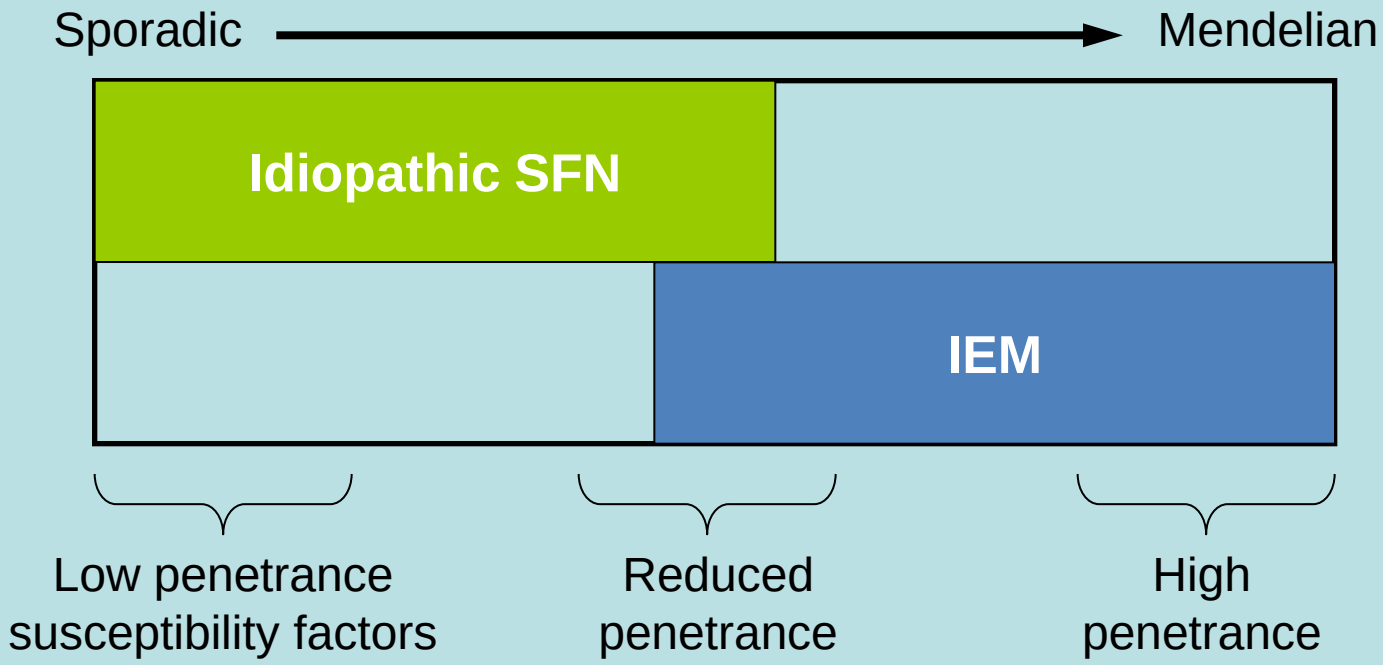
Painful Neuropathies

Inherited Erythromelalgia (IEM) and idiopathic SFN have been associated with gain of function missense variants in *SCN9A*, *SCN10A* and *SCN11A* in some cases



- *In vitro* voltage clamp and current clamp functional studies can be used to demonstrate hyperexcitibility (gain of function)

Spectrum of penetrance & disease risk



IEM - prevalence probably approx. 1 in 500,000
Idiopathic SFN - prevalence unknown, but significantly less than 1 in 1000

Interpretation and classification of variant pathogenicity

- Guidelines apply to high penetrance mendelian disorders:
  - ACGS/VGKL guidelines "…do not consider the additive pathogenic effect of multiple low penetrance alleles…"
  - ACMG guidelines (March 2015) "…applicable to variants in all Mendelian genes…"
- What about low penetrance or susceptibility variants?
  - No guidelines as far as I'm aware
  - Waxman *et al.* 2014 (*Lancet Neurol*, **13**, 1152-1160) provide some discussion of this problem for *SCN9A*, *SCN10A* & *SCN11A* variants in pain related disorders

Painful Neuropathies – Oxford Genetics Service

- NGS panel (Agilent HaloPlex):
  - *SCN9A*, *SCN10A*, *SCN11A*, *TRPA1*
    - Sanger sequencing of gaps
  - *NAGLU* recently added
- Established January 2015
- Samples from 80 patients analysed and reported as of May 2016
- Collaboration with Prof Dave Bennett's research group allows the possibility of functional studies to further investigate variants

Results

- Variants identified in 15/80 patients (20%)

Gene	Number of variants		
	Uncertain	Likely Pathogenic	Pathogenic
SCN9A	5	1	0
SCN10A	4	0	0
SCN11A	2	0	0
TRPA1	6	0	0

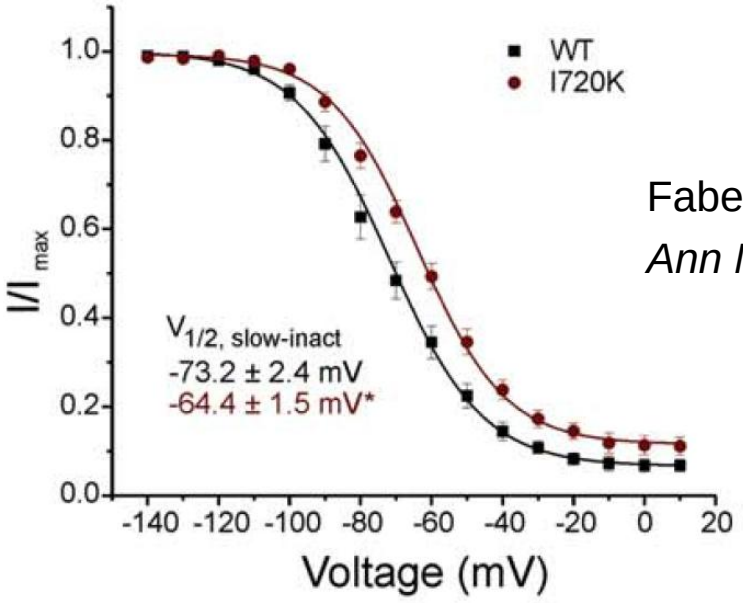
More than one variant identified in some samples/patients.

SCN9A results

- Functional studies have been published in the literature for 5 of the 6 variants identified in our cohort to date
- 2 examples:
  - *SCN9A* c.2159T>A p.(Ile720Lys)
    - 75 year old with small fibre neuropathy
  - *SCN9A* c.4612T>C p.(Trp1538Arg)
    - 52 year old with small fibre neuropathy

SCN9A c.2159T>A p.(Ile720Lys) "likely pathogenic"

- Previously detected in one individual with idiopathic SFN[1] and in one individual with a related pain disorder[2]
- Functional studies: *in vitro* voltage clamp analysis showed impaired slow inactivation and current clamp studies showed dorsal root ganglion (DRG) neuron hyperexcitibility[1]



Faber *et al*., 2012, *Ann Neurol*, **71**, 26-39.

[1] Faber *et al*., 2012, *Ann Neurol*, **71**, 26-39.
[2] ClinVar ID RCV000191125.1 (URL: http://www.ncbi.nlm.nih.gov/clinvar/RCV000191125.1)

SCN9A c.2159T>A p.(Ile720Lys) "likely pathogenic"

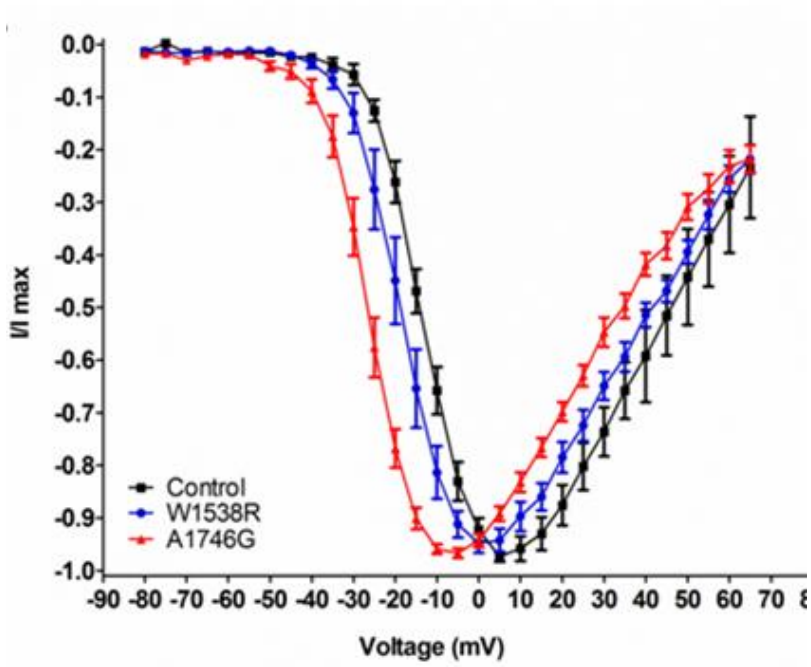
- Exome Aggregation Consortium cohort:
  - 6/21190 European alleles [small numbers, but supported by 1K genomes project data – 2/1006 European alleles]
  - So approx. 1 in 1800 Europeans heterozygous for this variant, which is similar to the prevalence of idiopathic SFN
  - This suggests that this variant is not fully penetrant

CONCLUSION:

Likely to be a pathogenic variant at least as a risk factor for SFN

SCN9A c.4612T>C p.(Trp1538Arg) uncertain clinical significance

- Previously detected in one individual with late-onset erythromelagia[1] and in one individual with a related pain disorder[2]
- Functional studies: *in vitro* voltage clamp analysis showed a small hyperpolarising shift in the current-voltage relationship[1]



Cregg *et al*., 2013, *Neuromol Med*, **15**, 265-278.

[1] Cregg *et al*., 2013, *Neuromol Med*, **15**, 265-278.
[2] Dabby *et al*., 2011, *J Neurol Sci*, **301**, 90-92.

SCN9A c.4612T>C p.(Trp1538Arg) uncertain clinical significance

- Exome Aggregation Consortium cohort:
  - Approx. 0.6% of South Asian alleles (98/16336)
  - Approximately 0.2% of European alleles (125/65600)
  - So > 1 in 100 South Asians heterozygous for this variant, compared to prevalence of idiopathic SFN of < 1 in 1000

CONCLUSION:

<<10% penetrance / susceptibility variant OR not pathogenic

Questions – Susceptibility variants

- How much weight to give to:
  - Allele frequency in population cohorts?
  - Functional studies?
- Proof of pathogenicity?
  - Large replicated association studies required to demonstrate significantly increased risk?
- Clinical importance of identifying susceptibility variants?
  - May have treatment implications
  - Even if proven increased risk, may not be clinically relevant for a given patient, as there could be alternative underlying cause(s)

Summary

- Painful neuropathies provide an example of a group of disorders with inheritance varying from mendelian to sporadic
- Interpretation of reduced penetrance or susceptibility variants is challenging[1]:
  - Functional studies
  - Frequency in population cohorts
  - Correlation with phenotype
  - Association studies
- Guidelines for diagnostic interpretation and reporting are lacking/limited

[1]Waxman *et al.* 2014, *Lancet Neurol*, **13**, 1152-1160.

Acknowledgements

- **Nuffield Department of Clinical Neurosciences, University of Oxford:**
  - David Bennett
  - Andreas Themistocleous

- **Oxford Medical Genetics Laboratories, Oxford University Hospitals NHS Foundation Trust:**
  - Kate Sergeant
  - Julie Evans
  - Kate Gibson
  - Daniel Bowdrey
  - Harriet Hewitt
  - Anneke Seller