

Inherited Peripheral Neuropathies: testing of more genes provides answers and triggers new questions

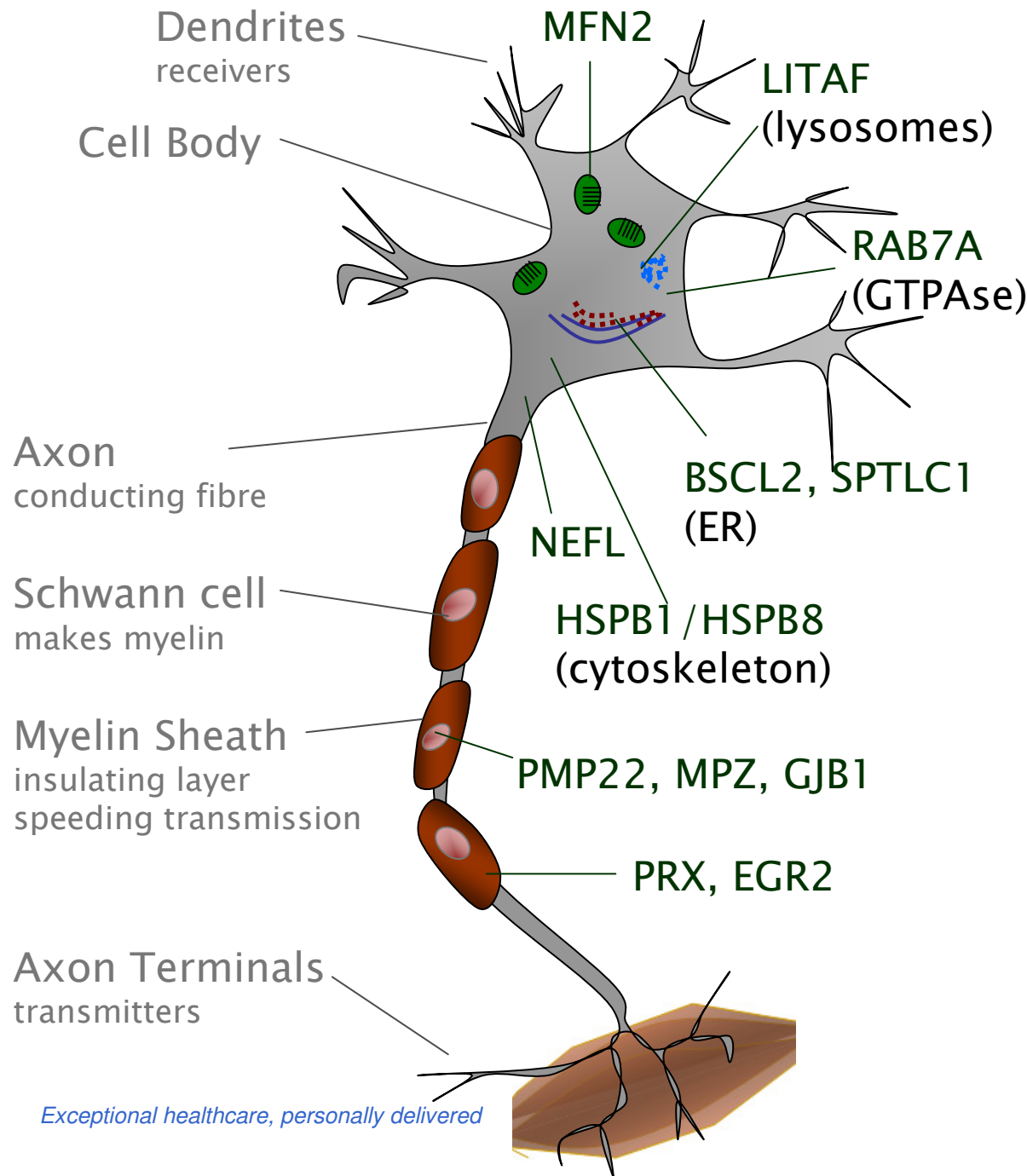
CMGS Spring Meeting
Durham, April 2011
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Overview

- Disease and service background
- Review of the service
- Results
- Novel sequence variants investigation
- Examples
- Conclusions

Charcot–Marie–Tooth

- Affect approximately 1 in 2,500 in the population
 - Clinically and genetically heterogeneous group of disorders
 - Clinical features
 - distal muscle wasting
 - weakness and atrophy
 - distal sensory loss
 - hyporeflexia
 - foot deformity
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- Clinical classification is primarily based on the motor and sensory involvement:
 - Hereditary Motor and Sensory Neuropathy (HMSN or CMT)
 - Hereditary Motor Neuropathy (HMN)
 - Hereditary Sensory Neuropathy (HSN)
 - Hereditary Neuropathy with Liability to Pressure Palsies (HNPP) is genetically related to HMSN and is also considered under the inherited neuropathies.



Myelinopathy (CMT1)

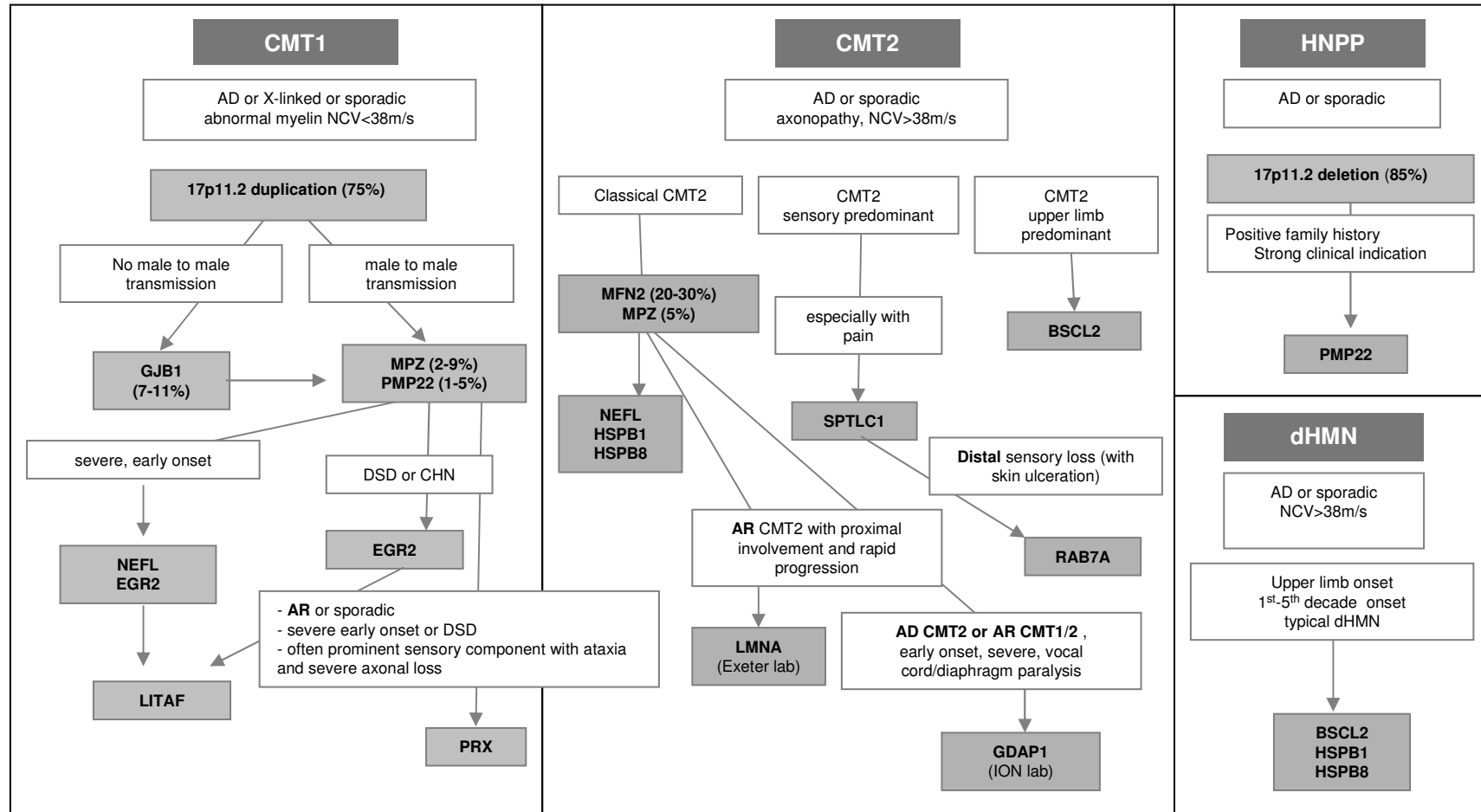
– destruction of myelin (or of the Schwann cells) which leaves the axon intact, but causes an acute failure of impulse conduction

Axonopathy (CMT2)

– impairment of axon function

- *distal*: progression from the center toward the periphery
- *proximal*: from the periphery toward the center

Genetic testing pathway



Review of the service

- Service offered on local, national and international level; UKGTN
 - Reviewed the referrals of the last three years (April 07–Aug 10)
 - Included one index case per family
 - Excluded cases with confirmed genetic diagnosis in the family

 - Included:
 - 775 patients referred with ?query neuropathy/ ?CMT/ HNPP; tested for the common duplication/deletion

 - 488 patients tested for point mutations in *PMP22, MPZ, GJB1, NEFL, EGR2, PRX, MFN2*

 - 21 patients were tested for *SPTLC1* mutations
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17p11.2 Duplication/deletion

	total tested	duplication/ deletion	%
HMSN	443	125	28%
HNPP	332	90*	27%

- 775 patients tested
- Pick up rate very similar for both HMSN and HNPP
- *3 partial deletions were detected, all involving the deletion of exon 2 and 3 only; this represents 1% of all HNPP cases
- Reported pick up rate: AD CMT1A ~71% , 84% HNPP; isolated: 36%¹
- Neuropathy associated samples: 17.4% (743/4,261) dosage abnormality² ; also 0.9% had complex rearrangements

(¹ Nelis E *et al.* Eur J Hum Genet 1996; 4:25–33 ² Zhang F *et al.* Nature Genetics 2009; 41(7):849–853)


Point mutation testing

	patients tested	variants detected	known mutations	novel variants	mutations
PMP22	191	17	7	10	13
MPZ	181	9	4	5	7
GJB1	159	27	20	7	26
MFN2	81	14	4	10	11
NEFL	45	9	2	7	2
EGR2	30	2	0	2	1
PRX	18	3	0	3	0
total	488	81	37	44	60

Novel variants

	novel variants	novel mutations	novel polys	remaining UV
PMP22	10	6	3	1
MPZ	5	3	0	2
GJB1	7	6	1	0
MFN2	10	7	2	1
NEFL	7	0	6	1
EGR2	2	1	1	0
PRX	3	0	0	3
total	44	23	13	8

Novel variants investigation

- Informatics for genetics analysis
 - Literature search
 - DMuDB and IPN db
- 
- Family studies

In house evaluation of informatics tools performance

- Selection of missense variants for each gene
 - Evaluated the data from the publications (family studies, functional studies, normal chromosomes screened etc)
 - Only ‘proven’ mutations and polymorphisms were included; reported more than once if possible
 - Run those through Alamut
 - Record results for each tool
 - Score the overall outcome
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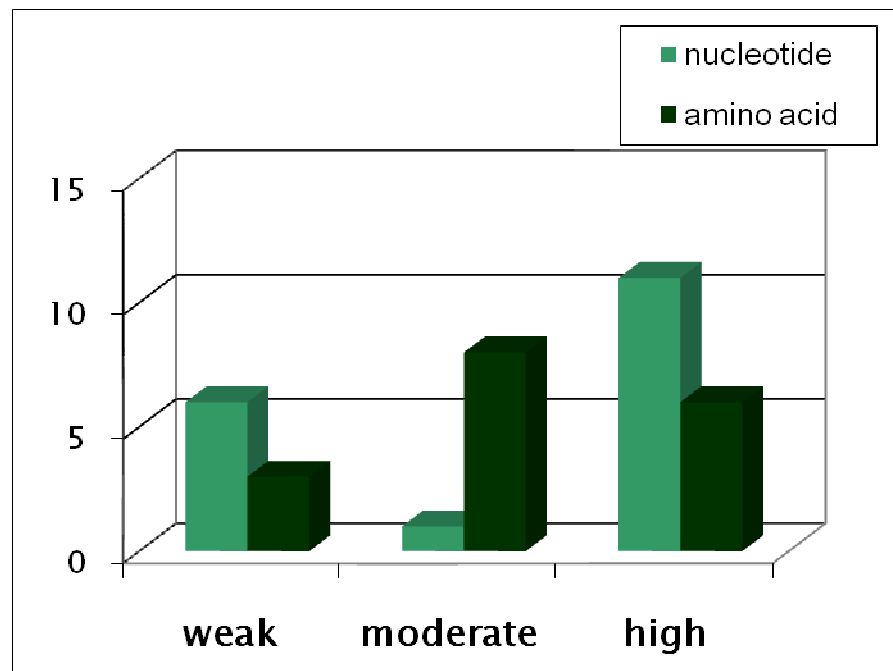
Scoring system

- P1 = pathogenic, all evidence support pathogenicity
- P2 = pathogenic, most evidence support pathogenicity (Polyphen & SIFT have to be in agreement)
- NP = not pathogenic
- Inc = inconclusive

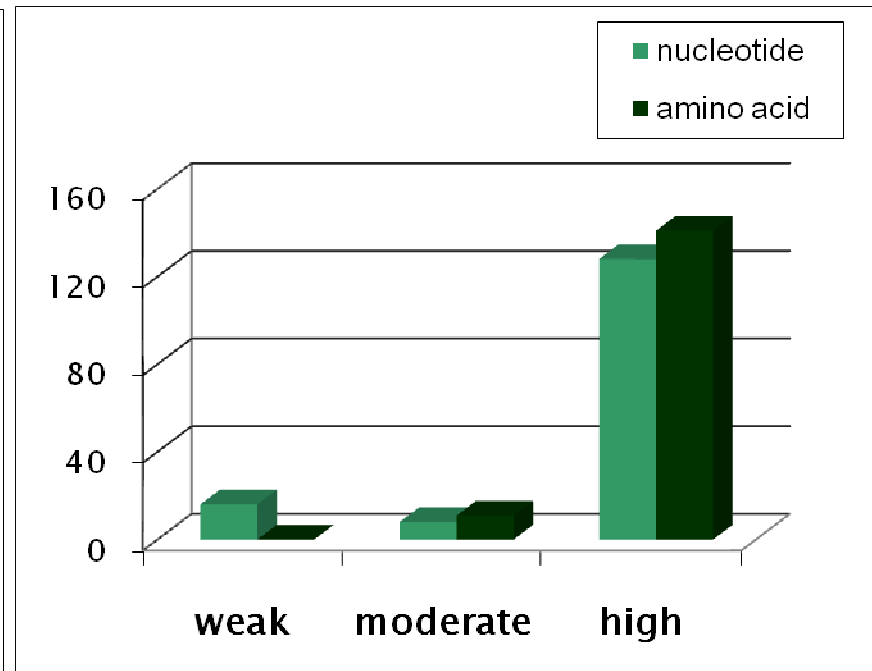
NEFL		nucleotide conservation	amino acid conservation	Grantham Score [0-215]	AGVGD [C0-C65]	Polyphen	SIFT	Informatics suggestion	Literature evidence	in concordance
c.23C>G	p.Pro8Arg	M	H	103	C65	probably	deleterious	P2	MUT	v
c.23C>T	p.Pro8Leu	M	H	98	C65	probably	deleterious	P2	MUT	v
c.64C>T	p.Pro22Ser	W	H	74	C65	probably	deleterious	P2	MUT	v
c.227T>C	p.Val76Ala	H	H	64	C65	benign	deleterious	INC	POLY	
c.268A>G	p.Glu90Lys	H	H	56	C55	probably	deleterious	P1	MUT	v
c.281T>C	p.Leu94Pro	H	H	90	C65	probably	deleterious	P1	MUT	v
c.293A>G	p.Asn98Ser	H	H	46	C45	probably	deleterious	P1	MUT	v
c.642C>G	p.Ile214Met	W	H	45	C35	benign	deleterious	INC	INC	
c.995A>C	p.Gln332Pro	H	H	76	C65	probably	deleterious	P1	MUT	v
c.1186G>A	p.Glu396Lys	H	H	56	C55	probably	deleterious	P1	MUT	v
c.1402G>A	p.Asp468Asn	H	M	23	C0	benign	tolerated	NP	POLY	v
c.1492G>A	p.Ala498Thr	H	M	58	C0	benign	tolerated	NP	POLY	v

Conservation comparison for known mutations and polymorphisms

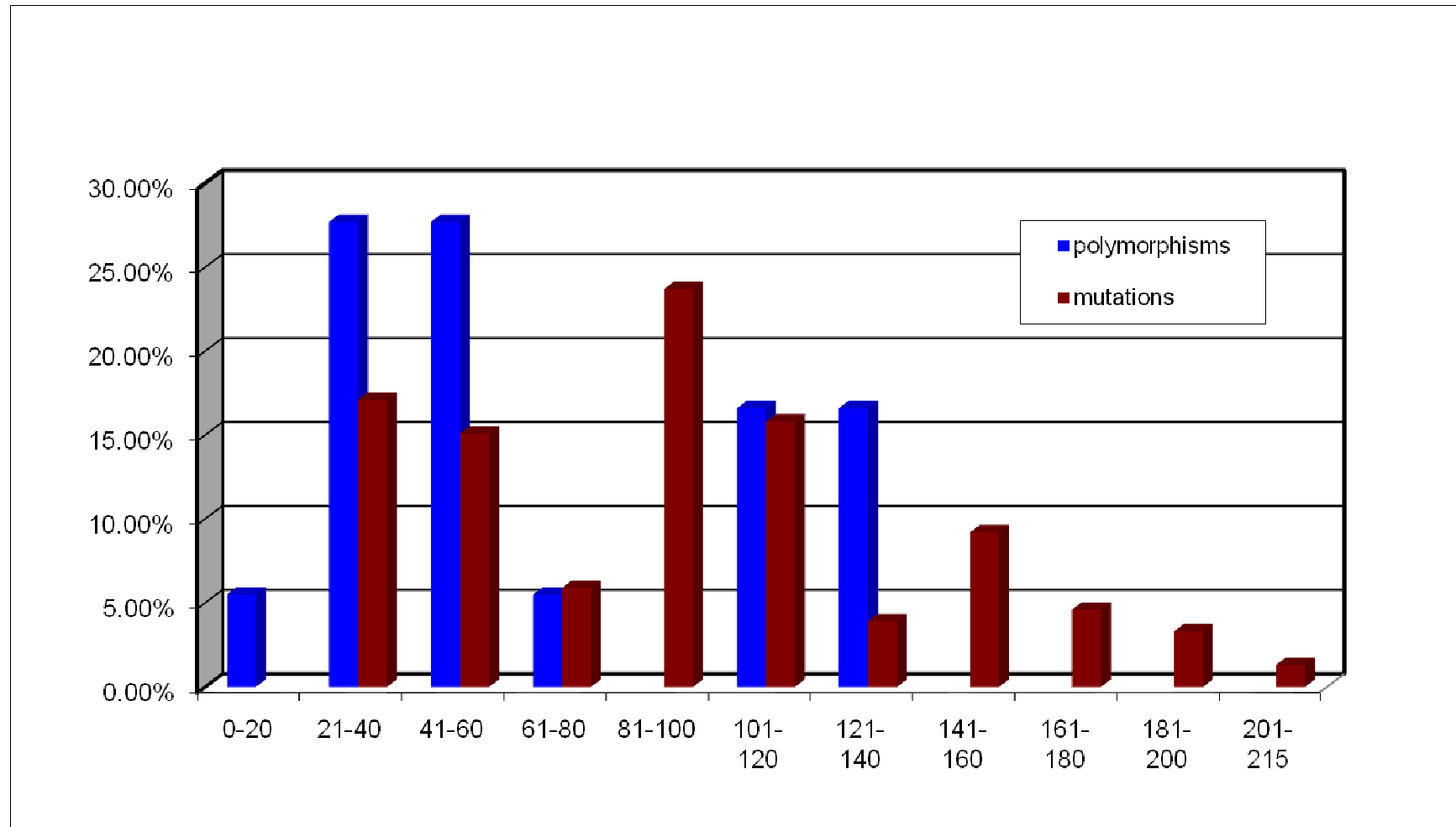
polymorphisms



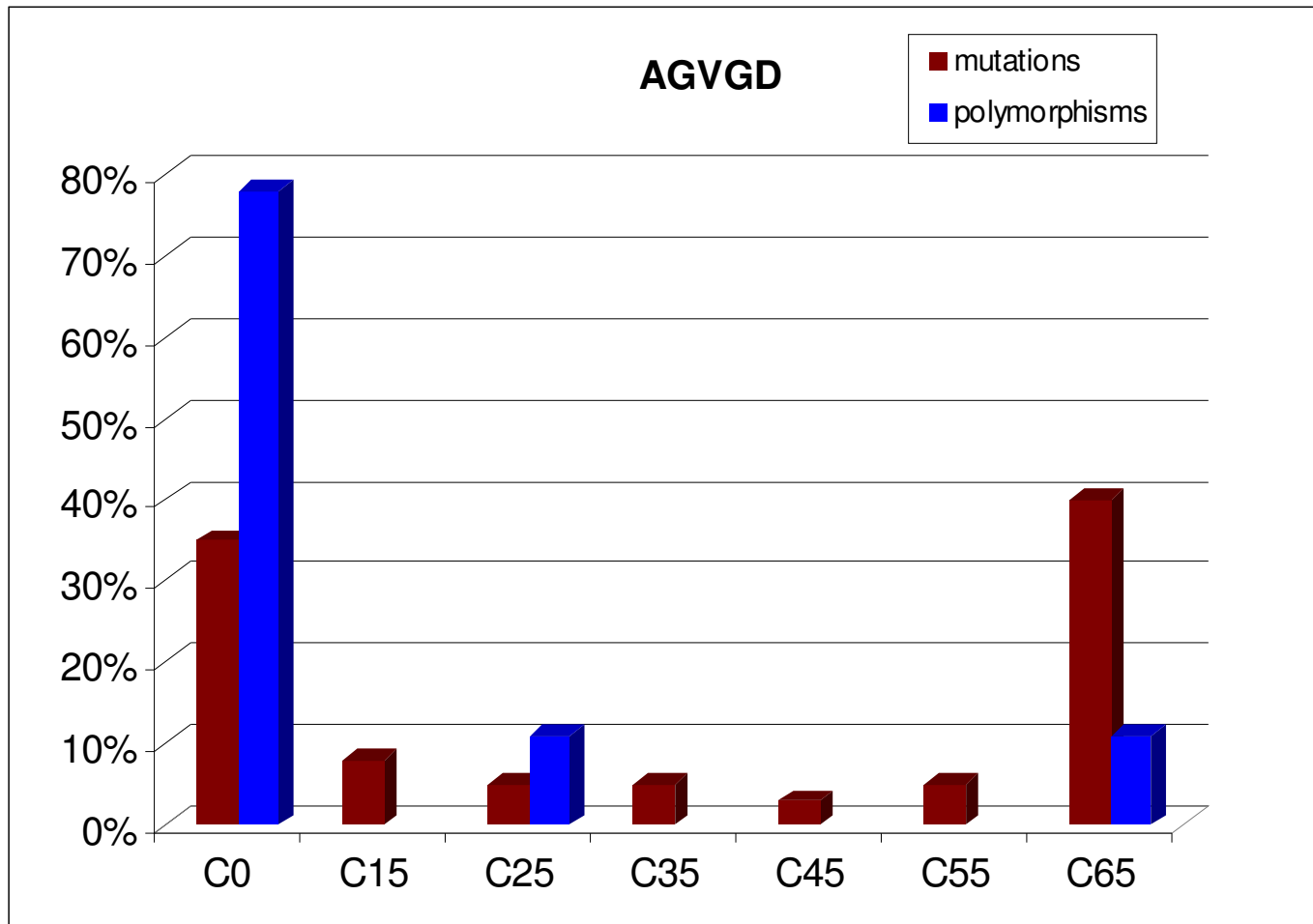
mutations



Grantham Score comparison for known mutations and polymorphisms

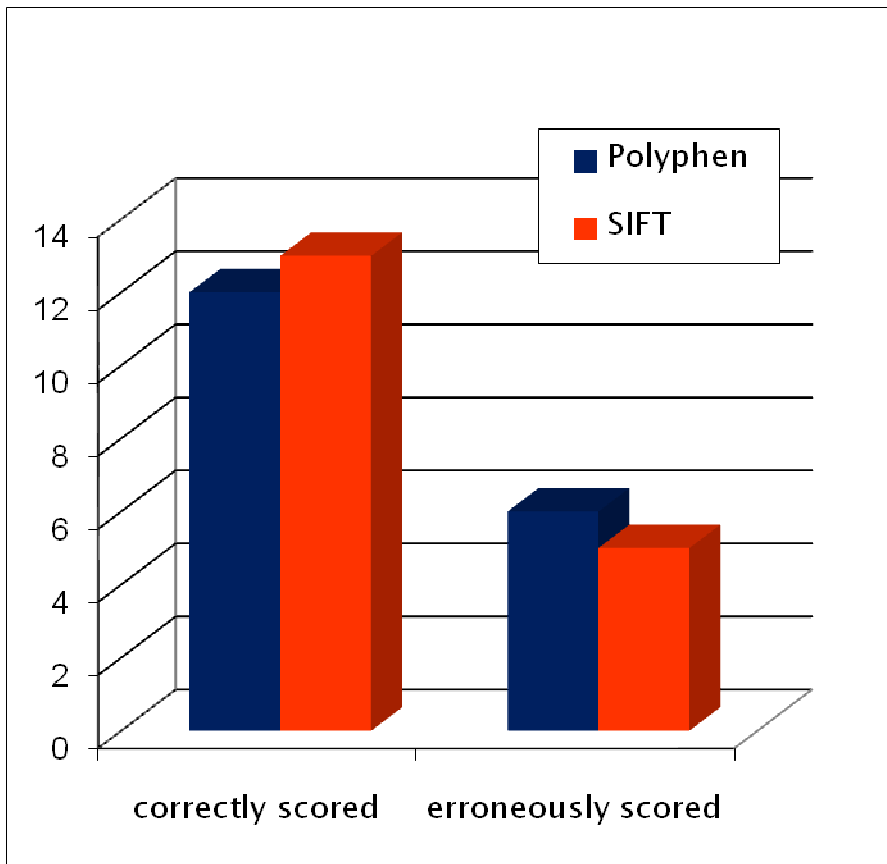


AGVGD comparison for known mutations and polymorphisms

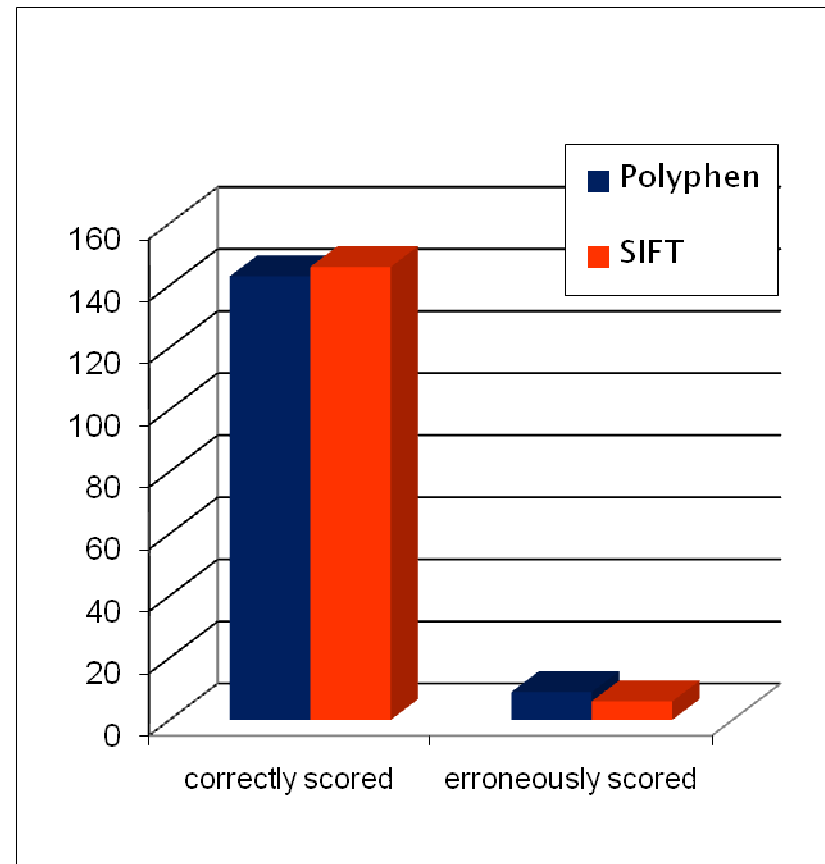


Polyphen and SIFT prediction comparison for known mutations and polymorphisms

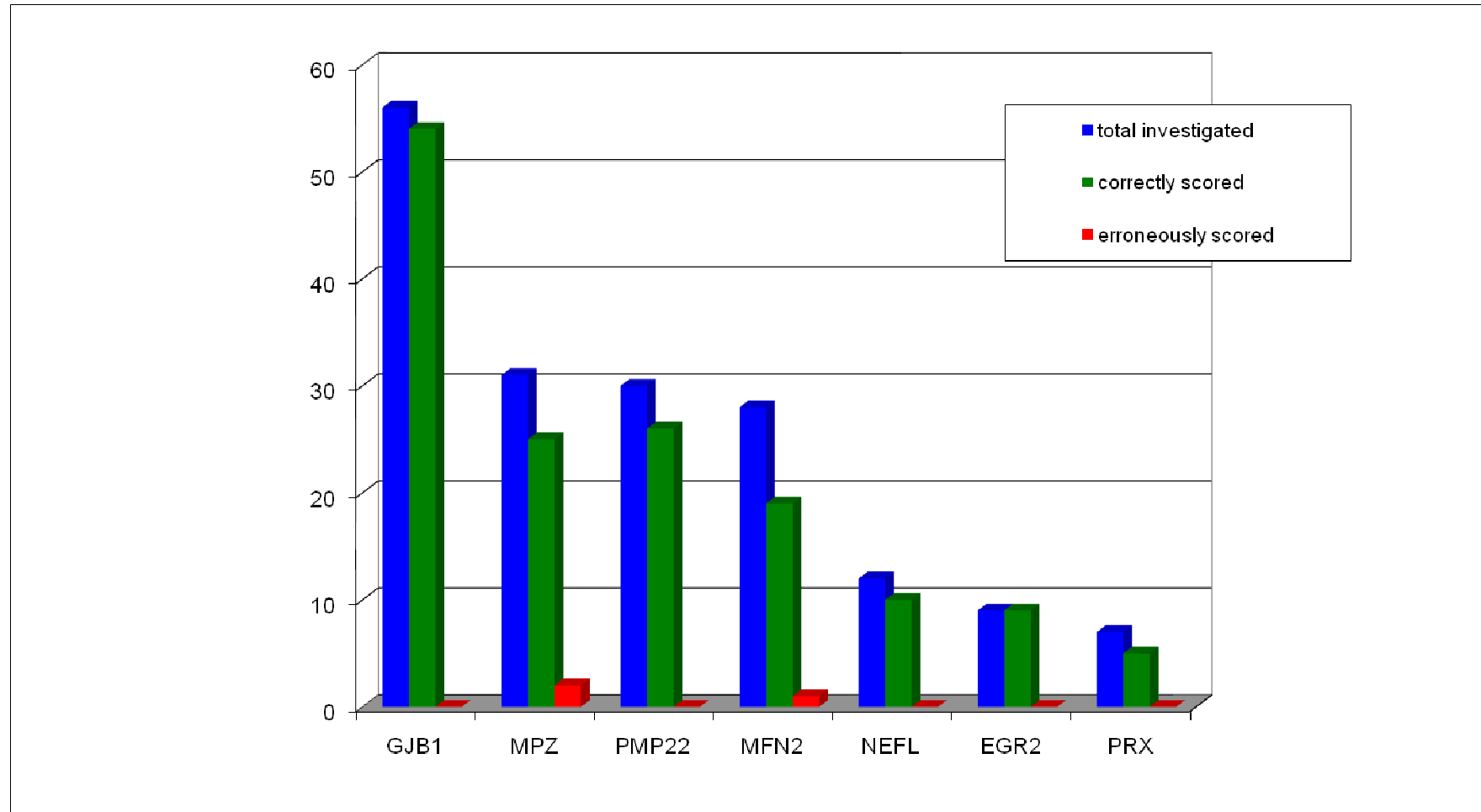
polymorphisms



mutations

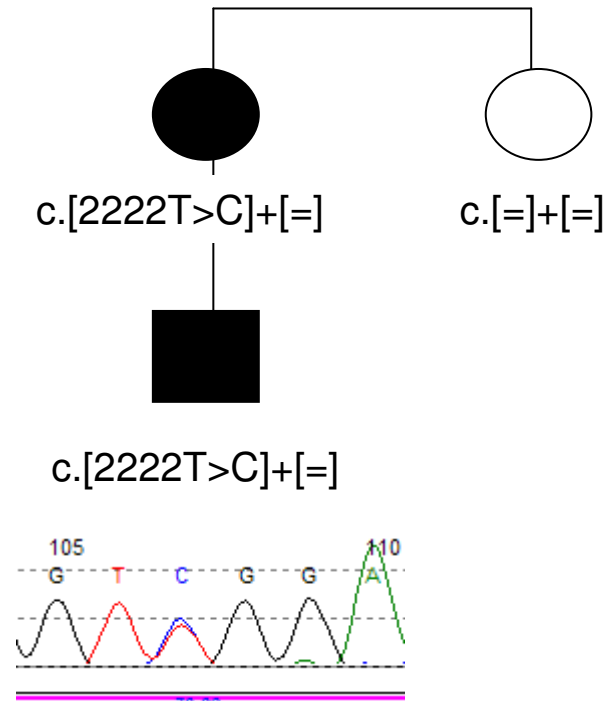


Overview of known mutations and polymorphisms evaluation



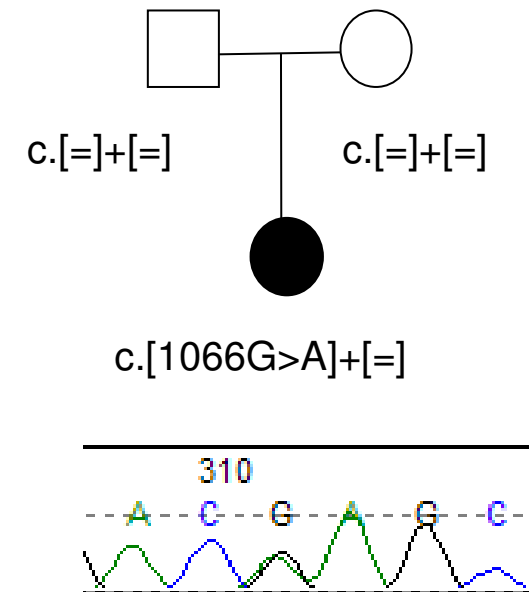
Case 1: *MFN2* novel variant

male patient , 29yrs old, axonal CMT	<i>MFN2</i>
	c.2222T>C p.Leu741Ser
nucleotide conservation	High
amino acid conservation	High
Grantham score	145
AGVGD	C65
Polyphen	probably
SIFT	affects
informatics score	P1
other evidence	change in adjacent amino acid shown to be important



Case 2: *EGR2* novel variant

	<i>EGR2</i>
female patient, 2yrs old, severe hypomyelinating neuropathy	c.1066G>A p.Glu356Lys
nucleotide conservation	High
amino acid conservation	Moderate
Grantham score	56
AGVGD	C0
Polyphen	possibly
SIFT	affects
informatics score	P2
other evidence	protein domain important



Case 3: *PRX* unclassified variants

male, 67yrs old, CMT1; -ve 17p11.2, PMP22, MPZ	<i>Periaxin</i>		
	c.731C>T p.Ala244Val	c.770C>T p.Ala257Val	c.3373G>A p.Gly1125Ser
nucleotide conservation	Weakly	Weakly	Weakly
amino acid conservation	Highly	Weakly	Weakly
Grantham score	64	64	56
AGVGD	C0	C0	C0
Polyphen	benign	benign	benign
SIFT	affects	tolerated	affects
informatics score	INC	NP	INC
other evidence	all described mutations (12) are nonsense or frameshift		

Detection rate

	tested	positive	%
PMP22 dup	443	125	28.2%

PMP22	114	6	5.3%
MPZ	95	4	4.2%
NEFL	21	2	9.5%
EGR2	30	1	3.3%
GJB1	97	25	25.8%
CMT1			76.3%

PMP22 del	332	90*	27.1%
PMP22	61	7	11.5%
HNPP			38.6%

MFN2	81	11	13.6%
MPZ	65	3	4.6%
NEFL	24	0	0.0%
GJB1	37	1	2.7%
CMT2			20.9%

SPTLC1	21	2	9.5%
HSAN			9.5%

literature

1.2 - 4.7%
2.3 - 9.4%
1.8 - 4.9%
1 - 2.4%
7 - 11%

LITAF (CMT1C)

BSCL2 (dHMN/CMT2)

HSPB1 (dHMN)

HSPB8 (dHMN)

RAB7A (CMT2B/HMN)

17 - 33%
4.8%
1.8 - 4.9%

Conclusions

- Comprehensive service providing diagnosis in affected patients
- High detection rate of novel sequence variants
- Informatics tools proved useful when used in combination/scoring system useful
 - No single tool is accurate enough
 - There is a trend in the tools to over predict pathogenicity
 - Protein specific domains are important and assist in interpretation
- Future: Screening with a ‘neuropathy chip’

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