WHOLE-EXOME SEQUENCING DATA ANALYSIS FOR DIAGNOSIS OF PERIPHERAL NEUROPATHIES

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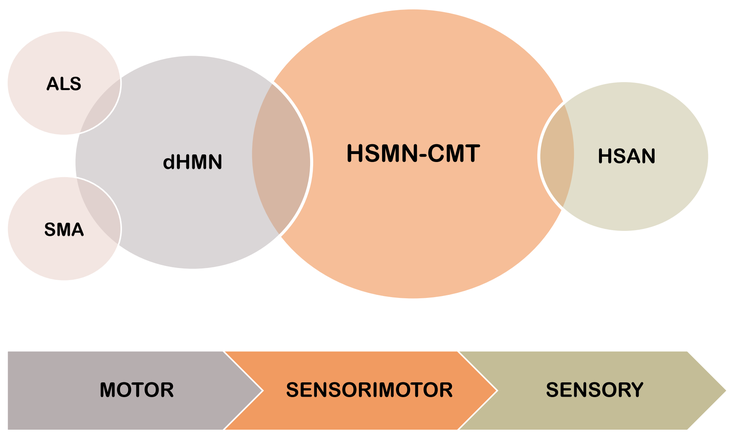
Introduction

Peripheral neuropathies encompasses a group of disorders with genetic and clinical heterogeneity. Charcot-Marie-Tooth disease (CMT), also known as hereditary sensory and motor neuropathy (HSMN), is closely related to distal hereditary motor neuropathies (dHMN), which have only motor involvement. Patients occasionally show additional disorders-related signs of amyotrophic lateral sclerosis (ALS) or spinal muscular atrophy (SMA). Whole-exome sequencing (WES) has become a cost-effective method to examine all known genes implicated in these neuropathies for diagnostic purposes.



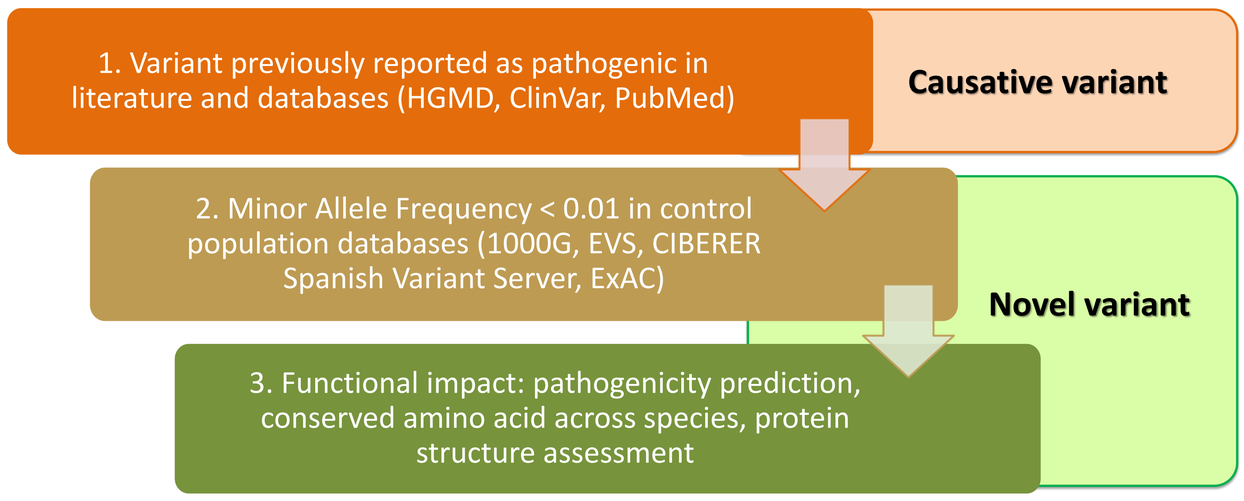
Aim & Study design

- **Aim of this work:** to establish a diagnostic method for genetically heterogeneous peripheral neuropathies based on whole-exome sequencing and subsequent analysis of variants in a list of significant neuropathy-related genes.
- **Study design:**
  - WES was applied to 8 unrelated sporadic cases with predominant signs of CMT or dHMN supervised at the Department of Neurology of Hospital U. I P. La Fe.
  - DNA from individuals was captured using the SureSelect All-Exon Kit V5 (Agilent) and the library obtained was sequenced at Centro Nacional de Análisis Genómico (CNAG) using Illumina technology.

Bioinformatics analysis

- Sequence reads were simultaneously processed using different pipelines developed by CNAG and the Plataforma de Bioinformática para las Enfermedades Raras (BiER).
  - BiER's pipeline is optimized to call variants in exome capture targets only whereas in CNAG's pipeline variants are called in all mapped positions.
- Data obtained from both pipelines were examined for variants in 180 genes previously associated with neuropathies. Mean coverage of the 180 genes was evaluated.
- A prioritization workflow was implemented to reduce the number of gene nucleotide changes.
  - Candidate variants included non-synonymous, stop gained, frameshift, splicing and small indels.
- Selected variants were confirmed by Sanger sequencing and segregation studies were performed where possible.

Variant prioritization workflow

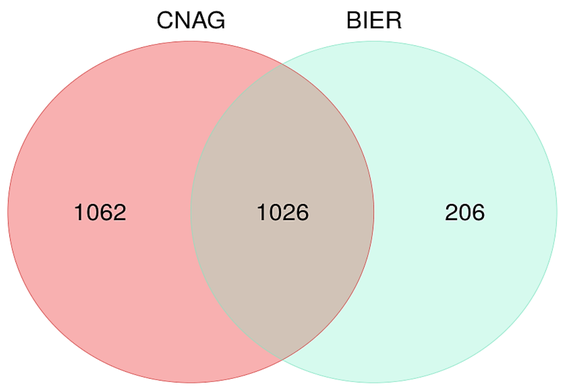


Results

Coverage analysis in neuropathy-related genes

	CNAG pipeline			BiER pipeline		
Samples	mean	% bases > 15	% bases > 30	mean	% bases > 15	% bases > 30
SGT038	74	97	86	35	88	56
SGT077	64	96	82	32	84	48
SGT161	64	96	82	31	84	48
SGT187	70	97	85	34	87	54
SGT230	74	97	87	36	88	57
SGT238	70	96	84	34	86	53
SGT241	71	97	85	34	87	55
SGT274	74	97	87	35	88	57

Unique variants in neuropathy-related genes detected in all samples



Identification of variants in neuropathy-related genes

Sample	Inbreeding	Clinical phenotype	Gene	Inheritance	Mutation	Status	In-silico prediction
SGT038	No	CMT	*FIG4*	Recessive	c.122T>C (p.I41T)	Causative variant	Probably damaging
					c.446+5G>C	Novel variant	Splicing donor site is not recognized
SGT230	Yes	dHMN	*BICD2*	Dominant	c.14G>C (p.S5W)	Novel variant	Probably damaging
SGT238	No	dHMN	*SOD1*	Dominant	c.65A>G (p.E22G)	Causative variant	Benign
SGT241	Yes	dHMN	*DAO*	Dominant	c.958T>C (p.W320R)	Novel variant	Possibly damaging

- In 50% of cases, candidate variants in known neuropathy genes were identified. All of them were detected by CNAG and BiER pipelines.
  - *FIG4* p.I41T and *SOD1* p.E22G were previously reported as pathogenic.
  - These variants were validated by Sanger sequencing. However, validation of *FIG4* c.446+5G>C remains inconclusive.
  - In inbreeding cases, further examination of whole-exome homozygous variants is being carried out to confirm disease's segregation within the family.
- For the remaining cases, novel variants of uncertain significance in genes not previously linked to neuropathies were identified.

Conclusions

- WES is a powerful tool to identify the genetic cause of undiagnosed neuropathies in patients in which candidate gene approaches had failed and genotype-phenotype correlation as well as mode of inheritance are unclear.
- Despite the different methods to call variants in exome sequencing data implemented in CNAG and BiER pipelines, their performances to detect selected candidate variants in neuropathy-related genes have been highly similar.
- Novel variants identified in neuropathy-related genes need additional supporting information as segregation studies and experimental evidence about their pathogenicity to confirm them as probable cause of disease.
- This study has provided us data for further studies to identify novel neuropathy genes increasing our understanding of the biology underlying peripheral neuropathies.