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SHORT REPORT

Exome sequencing reveals *HINT1* mutations as a cause of distal hereditary motor neuropathy

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Distal hereditary motor neuropathies (dHMNs) are a heterogenous group of genetic disorders with length-dependent degeneration of motor axons. Obtaining a genetic diagnosis in patients with dHMN remains challenging. We performed exome sequencing in a diagnostic setting in 12 patients with a clinical diagnosis of dHMN. Potential disease-causing variants in genes associated with dHMN and other forms of inherited neuropathies/motor neuron diseases were validated using Sequenom. The coverage in the genes studied was >95% with an average coverage of >50 times. In none of the patients a mutations was found in genes previously reported to be associated with dHMN. However, in 2/12 patients a recessive mutation in *histidine triad nucleotide binding protein 1* (*HINT1*, recently discovered as a cause of axonal neuropathy with neuromyotonia) was identified. Our results demonstrate the diagnostic value of exome sequencing for patients with inherited neuropathies. The phenotypic spectrum of recessive mutations in *HINT1* includes dHMN. *HINT1* should be added to the list of genes to check for in dHMN.
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INTRODUCTION

Hereditary neuropathies are a clinically and genetically heterogenous group of disorders.[1,2] Mutations in >50 genes have been identified to date and the inheritance pattern can be autosomal dominant, recessive or X-linked.[3] The most common clinical phenotype is Charcot-Marie-Tooth (CMT) disease characterized by a length-dependent sensorimotor neuropathy with progressive distal weakness, muscle atrophy, sensory loss and foot deformities.[4–6] The distal hereditary motor neuropathies (dHMNs) are much rarer and present with a progressive distal motor weakness without sensory abnormalities.[7,8] The disease usually starts in the lower limbs. Unusual presentations include dHMN with onset in the upper limbs or with vocal cord or diaphragm paralysis. Despite the degeneration of motor axons in the peripheral nerves, some patients have pyramidal signs due to upper motor neuron involvement. There is clinical and genetic overlap with the axonal form of CMT (CMT2) and with some motor neuron disorders (MNDs).[7,9] The inheritance pattern of dHMN can also be autosomal dominant, autosomal recessive or X-linked. A significant proportion of dHMN patients will have a negative family history and can be classified as apparently sporadic cases. Mutations in the genes *HSPB1*, *HSPB8*, *BSCL2*, *IGHMBP2*, *SETX*, *GARS*, *DYNC1H1*, *DCTN1*, *ATP7A*, *TRPV4*, *REEP1* and *SLC5A7* explain <20% of cases. Hence, 80% of dHMN is caused by mutations in as yet undiscovered genes. Obtaining a genetic diagnosis in dHMN patients in daily clinical practice remains a challenge.

We therefore performed exome sequencing in 12 patients with a clinical diagnosis of dHMN and looked for mutations in genes known to be associated with dHMN, but also checked CMT- and MND-causing genes.[7]

METHODS

Patient selection

Patients with a slowly progressive pure motor length-dependent neuropathy followed at the neuromuscular reference center of the university hospital in Leuven with a clinical diagnosis of dHMN could participate in our study that aimed to better diagnose these patients after signing an informed consent. As a part of their genetic work-up, most patients had already undergone genetic testing for some of the genes involved in dHMN. For most of the patients, mutations in *SMN1* had been excluded as well.

Exome sequencing and bioinformatics analysis

DNA was extracted from blood using standard techniques. The exomes were captured using the TruSeq exome enrichment kit (Illumina, San Diego, CA, USA). A 2×100 base pair paired-end sequencing was performed with the TruSeq SBS kit on an in-house HiSeq 2000 sequencer (Illumina). For all the exomes, BWA[10] was used to align the raw reads to the human reference genome (NCBI Build 37/hg19) using default parameters. PCR duplicates were removed with Picard Mark Duplicates (http://picard.sourceforge.net). Base recalibration, local realignment, around small insertions/deletions (indels) and single-nucleotide variant calling were performed using the Genome Analysis ToolKit (GATK v1.0.4487).[11] Substitutions were called using the Genome Analysis ToolKit (GATK v.1.0.4487) Unified Genotyper,[11] while small

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insertions/deletions (indels) were detected using Dindel v1.01.[12] Substations and indels in the coding region of the captured regions were selected for further analysis.

Commonly occurring substitutions and indels are unlikely to be disease causing and therefore eliminated using a set of publicly available variant data sets. These databases included: all common SNPs from dbSNP version 132 (defined as SNPs with a minor allele frequency of >1%), variants identified in the March 2012 release of the 1000 Genomes Project, common SNPs from 126 HapMap3 individuals in 11 populations provided by Affymetrix (Santa Clara, CA, USA) and variants identified in 46 HapMap individuals using the Complete Genomics whole-genome sequencing technology. Stepwise filtering was applied on all variants of each exome. As such, we were able to detect all novel variants in each patient. A list of these filtered variants, as well as the rough data, have been submitted to EGA (accession number EGAS00001000576). For diagnostic purposes, we looked for known disease-causing mutations in these lists of variants for each patient.

Validation of genetic variants identified

Standard Sequenom MassARRAY genotyping experiments were performed to validate candidate variants according to the manufacturer's conditions, a strategy previously used.[13] Variants that failed to be successfully genotyped in the first round of validation were subsequently redesigned for a second attempt using a new set of Sequenom primers (e.g, by designing new extension primers that annealed on the other DNA strand as the extension primer from the first validation round).

RESULTS

We performed exome sequencing in 12 patients followed at the neuromuscular reference center of the university hospitals in Leuven with a clinical diagnosis of dHMN. All patients had undergone genetic testing to some extent as a part of their work-up, but the results were negative in all patients. None of the patients belonged to extended pedigrees. The 12 patients had a pure motor phenotype, reported no sensory complaints and had a normal sensory neurological examination. Some of them had pyramidal signs with hyperreflexia, but always without Babinski signs. None of the patients had vocal cord involvement. The clinical and electrodiagnostic findings of the patients are summarized in Table 1. Conduction blocks or other signs of demyelinization were absent in all patients. The sural sensory nerve action potential was normal in all patients.

The diagnostic value of exome sequencing in this cohort of genetically unexplained dHMN patients was evaluated. We looked for mutations in genes known to be associated with dHMN. Since there is clinical overlap with other inherited neuropathies such as CMT and with some forms of MND, we also assessed whether mutations in genes underlying CMT or MND were present. The list of 97 genes studied can be found in Supplementary Table 1. This list includes all genes known to be associated with dHMN, CMT and other inherited neuropathies, but also other phenotypes with lower motor neuron involvement.

On average, all mappable reads covered 93.0% of the TruSeq captured regions, with an average coverage of 62.2×. The total substitutions and indels count ranged from 51 018 to 52 805 and from 21 807 to 34 843, respectively, in the different patients. Among these variants, on average, for each patient, 22 587 subsititions and 498 indels are located in the coding region while 557 substitutions and 35 indels were detected as possible disease-causing mutations after eliminating commonly occurring variants. Within the 97 studied disease-causing genes, a coverage of 95.8% of the coding regions was obtained with an average coverage of 50.2×, suggesting that exome sequencing can pick up coding mutations with a considerable sensitivity in the diagnostic setting (Supplementary Table 1 gives an overview of the list of all genes studied, with the coverage per gene).

First, we looked for known disease-causing mutations in genes known to be associated with dHMN. In none of the typical dHMN-causing genes known mutations were found, illustrating the low frequency of occurrence of mutations in each of these genes. Next, we also checked genes associated with other forms of inherited neuropathies or MND (Supplementary Table 1). Likewise, no pathogenic mutations were identified in genes underlying CMT, hereditary sensory and autonomic neuropathies (HSANs) or MND. In addition, we also looked for any variants that were not previously reported as causative mutations in the selected genes, as these variants could potentially be pathogenic. For genes with recessive inheritance only homozygous variants or compound heterozygous variants were taken into account and validated using Sequenom MassARRAY. In total, seven variants in five genes occurring in five patients could be validated. These variants are listed in Table 2, however, the pathogenicity of these variants remains uncertain.

Strikingly, in 2/12 patients (~17%, a proportion close to the total proportion of explained dHMN cases in other cohorts) a recessive mutation in *histidine triad nucleotide binding protein 1* (*HINT1*) was identified. In patient dHMN4 a homozygous c.250T>C (p.(Cys84Arg)) mutation was found, patient dHMN11 was

Table 1 Patient characteristics

Patient ID	Sex	Onset age	Onset site	UL involvement	Reflexes	Pes cavus	Inheritance pattern	Sural SNAP amplitude (μV)	EDB CMAP amplitude (mV)
dHMN1	F	29	UL and LL	Yes	Areflexia	Yes	AD	10	1.1
dHMN2	M	45	UL	Yes	Hyperreflexia	Yes	AD	15	3.7
dHMN3	F	26	LL	Yes	Hyporeflexia	Yes	AR	7.5	1.6
dHMN4	F	17	LL	No	Hyporeflexia	Yes	Apparently sporadic	20	2.8
dHMN5	M	1	LL	Yes	Hyporeflexia	+/−	AR	19	0.5
dHMN7	M	17	LL	No	Hyperreflexia	Yes	AD	16	5.3
dHMN8	F	54	LL	No	Hyporeflexia	Yes	Apparently sporadic	10	3.7
dHMN9	M	30	LL	Yes	Hyporeflexia	Yes	AD	4	0.9
dHMN10	M	19	LL	Yes	Hyperreflexia	Yes	Apparently sporadic	>10	<5
dHMN11	M	28	LL	No	Hyperreflexia	Yes	AR	38	NR
dHMN12	F	16	LL	Yes	Hyperreflexia	Yes	Apparently sporadic	14	NR
dHMN13	F	1	LL	Yes	Hyporeflexia	+/−	Apparently sporadic	13.1	0.1

Abbreviations: F, female; M, male; LL, lower limbs; UL, upper limbs; AD, autosomal dominant; AR, autosomal recessive; SNAP, sensory nerve action potential; EDB CMAP, extensor digitorum brevis compound muscle action potential; NR, no response.

compound heterozygous for the same p.C84R mutation and the c.341A>G (p.(His114Arg)) mutation. These mutations were confirmed using standard Sequenom MassARRAY genotyping experiments. The non-consanguineous parents of these patients were asymptomatic and heterozygous carriers of the p.(Cys84Arg) or p.(His114Arg) mutation (Figure 1a). Both the p.(Cys84Arg) mutation and the p.(His114Arg) mutation were predicted to be damaging by SIFT (Craig Venter Institute, San Diego, CA, USA) and Polyphen-2 software (Harvard University, Cambridge, MA, USA) (with a score of 0.992 and 1.000 for p.(Cys84Arg) and p.(His114Arg), respectively). Both amino-acid substitutions were affecting a highly conserved residue (Figure 1b). Our data demonstrate that exome sequencing is useful for diagnostic purposes in patients with dHMN and reveal that recessive mutations in *HINT1* are a cause of dHMN.

DISCUSSION

In this study, using exome sequencing for diagnostic purposes in patients with dHMN, we identified mutations in *HINT1* in a considerable proportion of patients. Recessive loss-of-function mutations were recently reported[14] in patients with a rare phenotype of axonal neuropathy with neuromyotonia[15] and CMT. The gene was identified in a family with CMT with neuromyotonia and in a consanguineous family with CMT, but mutations in *HINT1* were also observed in rare patients with autosomal recessive or sporadic CMT. Most patients from the original report had a sensorimotor axonal neuropathy that was associated with action myotonia in the hands and/or neuromyotonic discharges on needle electromyography. The p.(Cys84Arg) mutation was one of the loss-of-function mutations described in the original study.[14] The patients described here had a phenotype of dHMN without evidence of sensory involvement or neuromyotonia and extend the phenotypic spectrum of *HINT1* mutations. These findings warrant the inclusion of *HINT1* to the list of genes to be tested in the diagnostic work-up of patients with dHMN. HINT1 binds and hydrolyses adenosine 5′-monophosphoramidate substrates. It is a ubiquitously expressed haploinsufficient tumor suppressor gene[16] that promotes apoptosis,[17] inhibits the transcriptional activity of beta-catenin/TCF4, USF2 and NFkappaB, and inhibits the expression of endogenous cyclin D1 and TGFbeta2.[18] How loss-of-function mutations in *HINT1* can cause an axonal neuropathy is unknown. HINT1 knockout mice have been shown to have an increased susceptibility to spontaneous tumor development and to tumor induction. Whether these mice have an axonal neuropathy remains unexplored.[16]

Over the last few years, many studies have shown the value of exome sequencing for gene discovery purposes in neurological disorders.[19–23] Next-generation sequencing technologies have also accelerated gene discovery in patients with dHMN,[9,24] CMT[25,26] or other inherited neuropathies.[27–29] More recently, the use of exome sequencing for diagnostic purposes in genetic disorders with a high degree of genetic heterogeneity such as CMT is being explored.[30,31] Although the prize of exome sequencing is declining, the bioinformatic tools for analysis have improved and the coverage of the sequencing appears to be sufficiently high for use in the diagnostic setting, several technical and ethical challenges remain.[32] In particular, establishing the pathogenicity of newly identified genetic variants is often difficult and the possibility of finding unrelated finding requires further attention. A focused analysis of a predefined set of genes, as was done in this study, may circumvent the latter issue. In our study in dHMN patients, we also identified novel variants in genes known to be associated with various forms of inherited neuropathies or related neuromuscular disorders. However, the pathogenic nature of these variants remains uncertain, as the patients did not belong to extended pedigrees allowing segregation analysis.

Several studies have shown that exome sequencing is a reliable first-tier method to screen for mutations in coding regions of a large set of genes in patients with genetically heterogenous neuromuscular disorders.[31,33–35] In our study, we showed that the coverage of a predefined list of genes associated with dHMN and overlapping disorders was sufficiently high to use exome sequencing as an initial diagnostic approach. In addition, the combined use of Sequenom MassARRAY to validate the genetic variants identified appeared to be a fast and powerful strategy. A screening for mutations in genes associated with dHMN did not reveal any hits, confirming that each of the genes is only responsible for a small proportion of dHMN.

Table 2 Novel variants in genes associated with inherited neuropathies

Gene	*Chromosome*	*Position*	*Mutation*	*Patient*
ATL1	chr14	51098982	c.1587C>A (p.(His529Gln))	dHMN9
DNMT1	chr19	10250470	c.3830G>A (p.(Arg1277Gln))	dHMN4
KARS	chr16	75669656	c.801T>G (p.(Phe267Leu))	dHMN10
PRX	chr19	40900171	c.4088G>A (p.(Ser1363Asn))	dHMN9
PRX	chr19	40902776	c.1483G>C (p.(Glu495Gln))	dHMN9, dHMN12
SPG11	chr15	44890530	c.3934A>C (p.(Thr1312Pro))	dHMN3
SPG11	chr15	44888316	c.4399G>C (p.(Asp1467Pro))	dHMN3

Abbreviations: *ATL1*, Atlastin GTPase 1; *DNMT1*, DNA (Cytosine-5-)-Methyltransferase 1; *KARS*, Lysyl-tRNA Synthetase; *PRX*, Periaxin; *SPG11*, Spastic Paraplegia 11.

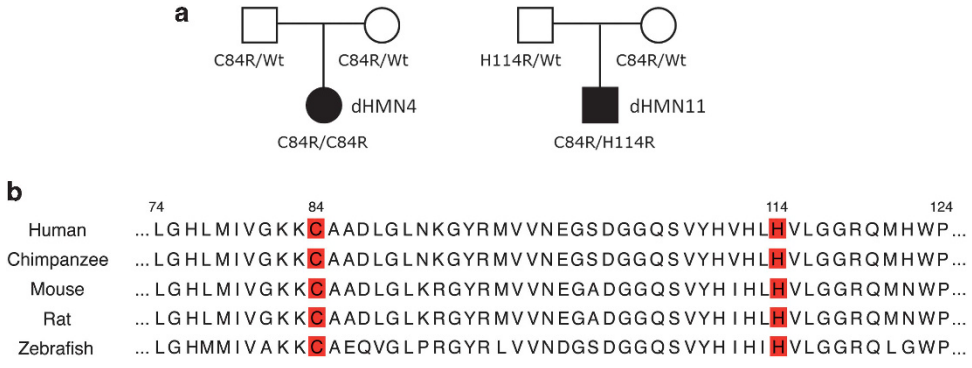

Figure 1 HINT1 mutations observed in dHMN patients. (**a**) Homozygous p.(Cys84Arg) and compound heterozygous p.(Cys84Arg) and p.(His114Arg) mutations observed in patients dHMN4 and dHMN11. (**b**) Conservation of residues 84 and 114 in HINT1 protein across species (marked in red).

The analysis in our study was not limited to genes stringently linked to dHMN. The study of a wider set of genes associated with other forms of inherited neuropathies or more complex phenotypes including neuropathy allowed us to identify recessive mutations in *HINT1* as a cause of dHMN. This comprehensive approach appears to be an advantage of the use of exome sequencing in clinical practice.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)