Published in final edited form as:
J Neurol. 2012 August ; 259(8): 1673–1685. doi:10.1007/s00415-011-6397-y.

Frequency of mutations in the genes associated with hereditary sensory and autonomic neuropathy in a UK cohort

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Conflicts of interest GL Davidson, JM Polke, M Laura, MAM Salih, J Blake, S Brandner, N Davies, R Horvath, S Price, M Donaghy, M Roberts, N Foulds, G Ramdharry, D Soler, MP Lunn, H Manji, MB Davis have no disclosures. SM Murphy is the recipient of a Post Doctoral training fellowship from the Inherited Neuropathy Consortium Rare Disease Clinical Research Consortium supported by the NINDS/ORD (1U54NS065712-01). F Muntoni serves on a scientific advisory board for AVI BioPharma, Inc.; serves on the editorial boards of Neuromuscular Disorders and Neuropaediatrics; is listed as an author on a pending patent re: Tailed antisense nucleotides to redirect splicing; receives publishing royalties for Duchenne Muscular Dystrophy (Oxford University Press, 2003); and receives research support from AVI BioPharma, Inc., PTC Therapeutics, Inc., GlaxoSmithKline, Wellcome Trust, the European Union, Medical Research Council UK, Muscular Dystrophy Campaign, Muscular Dystrophy Association USA, Spinal Muscular Atrophy Foundation, the NIH, the Association Francaise contre les myopathies (AFM), the NIHR, and the Great Ormond Street Hospital Children's Charity. H Houlden receives research support from The Medical Research Council (MRC fellowship, G108/638 and G0802760), The Brain Research Trust, Ataxia UK, The BMA Vera Down Award and the Muscular Dystrophy Campaign UK and association USA. MM Reilly receives research support from The Medical Research Council, the Muscular Dystrophy Campaign and NINDS/ORD (1U54NS065712-01). This work was undertaken at University College London Hospitals/University College London, which received a proportion of funding from the Department of Health's National Institute for Health Research Biomedical Research Centres funding scheme.

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Abstract

The hereditary sensory and autonomic neuropathies (HSAN, also known as the hereditary sensory neuropathies) are a clinically and genetically heterogeneous group of disorders, characterised by a progressive sensory neuropathy often complicated by ulcers and amputations, with variable motor and autonomic involvement. To date, mutations in twelve genes have been identified as causing HSAN. To study the frequency of mutations in these genes and the associated phenotypes, we screened 140 index patients in our inherited neuropathy cohort with a clinical diagnosis of HSAN for mutations in the coding regions of *SPTLC1*, *RAB7*, *WNK1/HSN2*, *FAM134B*, *NTRK1* (*TRKA*) and *NGFB*. We identified 25 index patients with mutations in six genes associated with HSAN (*SPTLC1*, *RAB7*, *WNK1/HSN2*, *FAM134B*, *NTRK1* and *NGFB*); 20 of which appear to be pathogenic giving an overall mutation frequency of 14.3%. Mutations in the known genes for HSAN are rare suggesting that further HSAN genes are yet to be identified. The p.Cys133Trp mutation in *SPTLC1* is the most common cause of HSAN in the UK population and should be screened first in all patients with sporadic or autosomal dominant HSAN.

Introduction

The hereditary sensory and autonomic neuropathies (HSAN, also known as the hereditary sensory neuropathies, HSN) are a clinically and genetically heterogeneous group of disorders. The cardinal clinical feature is a predominantly sensory axonal neuropathy characterised by loss of sensation including pain and temperature and in some subtypes positive sensory symptoms such as pain and paraesthesiae. The sensory loss frequently results in complications such as ulcers, infections, osteomyelitis, selfmutilation and spontaneous or surgical amputations. Motor and autonomic involvement occur to a variable degree [8, 21]. HSAN has been traditionally classified into five groups (HSAN I–V) [2] based on mode of inheritance and clinical features. More recently the classification has included the known causative genes. To date, mutations in 12 genes have been identified as being responsible for HSAN (Table 1). Genetic analysis is essential in the diagnosis of patients with HSAN and has clarified the phenotypic spectrum and helped further the understanding of the mechanisms of sensory neuron degeneration. Rotthier et al. [24] investigated a cohort of 100 European patients with HSAN and found mutations in 19%; most frequently in *RAB7* and *NTRK1*. Mutations were also found in HSN2/WNK1 and *SPTLC1*; however, no mutations were present in CCT5 or *NGFB*.

This study was designed to determine if the frequency of mutations in the genes associated with HSAN is similar in our mainly UK cohort and to describe the associated phenotypes. A total of 140 index cases with HSAN/sensory predominant CMT2 were screened for mutations in the coding regions of *SPTLC1*, *RAB7*, *WNK1/HSN2*, *FAM134B*, *NTRK1* and *NGFB*. We did not test for IKBKAP or CCT5 as none of our patients had the distinctive phenotype of Riley-Day syndrome (HSAN III) and none had spastic paraplegia. We did not screen SPTLC2, *ATL-1*, *DNMT1* or *KIF1A* as these recently described genes were reported subsequent to completion of this project.

Methods

This study was carried out in the National Hospital for Neurology and Neurosurgery (NHNN) and Institute of Neurology. Ethical approval was obtained from the joint medical and ethics committee at the NHNN. Written informed consent was obtained from all patients.

Patients

We selected a cohort of 140 patients from our inherited neuropathy database. These were patients presenting with a phenotype compatible with any of the forms of HSAN or CMT2 with a predominant sensory phenotype (Supplementary table). Forty-one patients (29%) had definite autosomal dominant (AD) inheritance, 12 patients (9%) had presumed autosomal recessive (AR) inheritance and the remainder were either sporadic cases or the referring physician did not document inheritance. The database includes patients seen in the peripheral neuropathy clinics in the NHNN as well as patients' DNA referred from other hospitals for diagnostic and research testing. External referring physicians were neurologists with experience in neuromuscular disease. Sixty-seven patients (48%) included in this study were seen within our own department, the remainder were external DNA samples. When mutations were found in patients we had not seen personally, we either reviewed the patient or obtained detailed clinical information from the referring physician. Most patients presented with distal progressive sensory loss, with or without ulceromutilating complications or autonomic dysfunction. Because of the overlap between CMT2B and HSAN I we included patients who had motor involvement; however, sensory features were always predominant. Diagnosis was based on clinical phenotype in addition to neurophysiology.

DNA extraction, PCR and sequencing

DNA was extracted from blood using Flexigene extraction kit and Autopure LS (Qiagen) extraction system. Coding regions and flanking introns were amplified using Roche or Qiagen PCR reagents. Primers and PCR conditions are available upon request. Reference sequences used were *SPTLC1*: NM_006415.2, *RAB7*: NM_0004637.5, HSN2/WNK1: NM_213655.1, *FAM134B*: NM_001034850.1, *NGFB*: NM_002506.2 and *NTRK1* (*TRKA*): NM_002529.3.

Sequence reactions were performed using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and resolved on an ABI 3730xl Sequencer. Sequence variants were confirmed by repeat sequencing. We considered variants pathogenic if they were absent from controls, segregated within families or, in the case of homozygous variants, if unaffected parents were heterozygous. We also considered conservation of amino acids among species and used three commonly used prediction programmes PolyPhen (http://genetics.bwh.harvard.edu/pph/), SIFT (http://blocks.fhcrc.org/sift/SIFT.html) and aGVGD (http://agvgd.iarc.fr/). In addition, we checked the 1000genomes and NHLBI exome sequencing project databases (http://www.1000genomes.org and http://evs.gs.washington.edu/EVS) to determine whether novel variants had been reported previously. One hundred seventy chromosomes from British controls and 180 chromosomes from Asian controls were screened for all novel variants depending on ethnicity of the patient.

Results

We identified 25 patients with sequence variants in our cohort of 140 patients (17.9%), 20 of which (14.3%) are likely to be pathogenic. Mutations were found in all genes tested (Table

2). Details on some of the patients with the *RAB7*, *FAM134B* and *SPTLC1* mutations have been reported previously [9, 10, 18]. Patients with mutations varied in their clinical phenotype, although as expected there was more consistency in the phenotypes associated with individual genes (Table 3). Pedigrees of the families are shown in Fig. 1 (families reported previously by us and newly diagnosed families with p.Cys133Trp *SPTLC1* have not been included). All novel variants reported here were not detected in the control cohorts.

SPTLC1 mutations

We found mutations in *SPTLC1* in 13 index patients with HSAN, all but one of which was the common p.Cys133Trp mutation. Seven of these families have been reported previously [9]. Patients presented in the second or third decades with decreased sensation in the feet; lancinating pain and paraesthesiae were common. Painless ulcers occurred in all families, with Charcot joints and amputations in some individuals, often with motor involvement. Neurophysiology demonstrated absent or reduced sensory action potentials (SAPs) with variable motor studies, usually demonstrating axonal loss but occasionally showing intermediate or demyelinating motor conduction velocities as we have previously described.

There is insufficient evidence of pathogenicity for the novel variant p.Ala310Gly; this amino acid is only moderately conserved among species and two of three prediction programs suggested that this change would be tolerated (Table 4). This patient was adopted and is not available to investigate the pathogenicity of the mutation further with deoxysphingoid base (DSB) levels.

RAB7 mutations

We identified one family with a mutation in *RAB7*, p.Asn161Thr. This family has been described in detail previously [10].

WNK1/HSN2 mutations

We identified two novel frameshift mutations in *WNK1/HSN2*; a compound heterozygous mutation p.[Cys20TrpfsX18] + [Thr390CysfsX21] in the index patient of family 15, and homozygous p.Thr390CysfsX21 in the proband of family 16. Unaffected parents were heterozygous (Fig. 1, families 15 and 16). The proband from family 15 had congenital insensitivity to pain (CIP) and is from a non-consanguineous Maltese family. She had loss of toe-nails due to repeated trauma, and fractured her left leg without pain. The index patient from family 16 was also of Maltese descent with CIP. He had trophic ulcers on the fingers which were shortened due to repeated trauma. His parents were not consanguineous, he had an affected sister and cousin; DNA was not available from the other affected members of this family.

FAM134B mutations

We found a homozygous nonsense mutation in *FAM134B* (p.Gln145X), in a patient with HSAN II, which we and others have previously reported [13, 18]. A second variant, p.Gly216Arg, is most likely a rare polymorphism as the affected sibling did not carry the variant (Fig. 1, family 18).

NTRK1 mutations

Five variants were found within the *NTRK1* coding region; all were homozygous and two are novel. Four of these are considered pathogenic.

One patient from Saudi Arabia (family 19, V.1), with consanguineous parents, had a homozygous nonsense mutation (p.Gln176X) [11]; parents were heterozygous. Sural nerve

biopsy demonstrated a reduction in small myelinated fibres and unmyelinated fibres, consistent with HSAN V [20]. In family 20, also from Saudi Arabia, there was a homozygous nonsense mutation (c.1069_1076dup-GGCAACTA) leading to a stop mutation, p.Tyr359X [17] in the proband; both his consanguineous, unaffected parents were heterozygous carriers. The third Saudi Arabian family, family 21, had a homozygous missense mutation (p.Arg654Cys) [17]. His parents were first cousins and unaffected, they as well as his unaffected sister were heterozygous (Fig. 1).

The index patient from family 22 was found to have a novel apparently homozygous missense mutation, p.Glu492Lys. Born to an unrelated American mother and British father, this patient had a congenital sensory neuropathy with associated anhidrosis, seizures, deafness and developmental delay. Neurophysiology demonstrated a sensory neuropathy with additional involvement of the central sensory pathways. Family members were unavailable for segregation analysis; however, this amino acid is highly conserved across species and all prediction programs suggested that this amino acid change would be damaging (Table 4). This variant has been published on the http://evs.gs.washington.edu/EVS website as being present in eight of 7,020 European/American and three of 3,738 African/American alleles (i.e. 0.1% of chromosomes); thus this may represent a rare recessive pathogenic allele.

The variant p.Arg6Trp is unlikely to be relevant as the family have dominant inheritance and have subsequently been found to have a mutation in *DNMT1* [12]. In addition, this variant has been reported to occur in 15/3,668 (0.4%) Europeans (http://evs.gs.washington.edu/EVS).

NGFB mutations

A heterozygous duplication, p.Gly161_Glu162dup, was found in a British female (Fig. 1, family 24). This patient presented at 48 years with a progressive sensory axonal neuropathy with Charcot joints. There was a dominant family history of a similar neuropathy; however, all affected family members were deceased. Although we cannot prove that this duplication is pathogenic the phenotype is similar to the previously reported heterozygous *NGFB* patients.

A second heterozygous variant (p.Ser187Asn) was found in a patient with CIP, family DNA was not available. We are unsure of the pathogenicity of this variant; we cannot rule out a non-coding mutation or deletion on the other allele.

Discussion

Our genetic screen of 140 patients with HSAN found 25 index patients with mutations in six genes (*SPTLC1*, *RAB7*, *WNK1/HSN2*, *FAM134B*, *NTRK1* and *NGFB*), of which at least 20 are considered pathogenic; a frequency of 14.3%, similar to the 19% described by Rotthier et al. [24]. Our control groups were negative for all variations described, suggesting that none of these variants are common polymorphisms. None of the variants were reported on the 1000genomes database, although two of the *NTRK1* variants were reported on the NHLBI database. We did not have enough evidence to support pathogenicity for five of the variants found (*SPTLC1* p.Ala310Gly, *FAM134B* p.Gly216Arg, *NTRK1* p.Arg6Trp and *NGFB* p.Gly161_- Glu162dup and p.Ser187Asn).

The purpose of this study was to determine the frequency of mutations in the genes known to cause HSAN in a mainly UK cohort. Mutations were most frequent in *SPTLC1* (12%), found in British Caucasian patients with dominant inheritance and adult-onset disease. Mutations in *NTRK1* were next most common (6%) and found predominantly in patients of

Saudi Arabian descent with consanguineous parents. Mutations in all other genes were rare, accounting for <2% each. These results suggest that many more genes responsible for causing HSAN have yet to be identified. Since this study was completed, mutations in four further genes (*ATL-1* [6], SPTLC2 [23], *DNMT1* [12] and *KIF1A* [22]) have been reported to cause HSAN; it is unknown whether mutations in these additional genes would contribute significantly more patients than that found in our cohort given the small number of patients reported to date.

When we analysed the number of cases with a genetic diagnosis based on inheritance pattern, we found causative mutations in 10/41 (24.4%) patients with AD inheritance and 5/12 (41.7%) patients with AR inheritance, versus 5/87 (5.7%) patients with unknown or sporadic inheritance; thus the likelihood of establishing a genetic diagnosis is higher in patients with a definite family history.

Mutations in *SPTLC1* accounted for 12% of our patients with HSAN. All but one were the common p.Cys133Trp mutation, presenting with the typical HSAN I phenotype. We have previously demonstrated that this mutation occurs due to a founder effect in the UK [9]. Of the known disease- causing mutations in *SPTLC1*, all but two lie within exons 5 and 6, a region of the protein important for substrate specificity [19]. Altered substrate-specificity of the enzyme results in the formation and accumulation of toxic DSBs [3, 19]. Of the three reported mutations outside exons 5 and 6, p.Gly387Ala was subsequently demonstrated to be a polymorphism [7]. Our finding of mutations in *SPTLC1* being the commonest cause of HSAN is in contrast to Rotthier et al. [24] who found mutations in *RAB7* to be the most common in a European cohort. We only found one patient with a *RAB7* mutation in our cohort [10]. This likely reflects the fact that in the European cohort, there was a founder effect for the p.Leu129Phe *RAB7* mutation in Austrian patients [24], while in the UK population there is a founder effect for the common p.Cys133Trp *SPTLC1* mutation [9].

Two patients with recessive mutations in HSN2/WNK1 were found. Both mutations cause a shift in the open reading frame, resulting in premature stop codons which likely render the protein non-functional. The phenotype of these patients was similar to previously reported patients with CIP [14]. Both of these mutations are novel but their absence in controls and segregation within both families supports pathogenicity.

The homozygous nonsense mutation in *FAM134B*, p.Gln145X [18], was previously reported in a Turkish individual [13]. Both patients had onset in the first decade, with impaired nociception, ulcerations and amputations. Our patient did not have any autonomic dysfunction, and had significant motor involvement, in contrast to the patient previously reported.

Mutations in *NTRK1* accounted for 6% of our HSAN cohort. The phenotype in the four families with pathogenic mutations was consistent with that reported in the literature with congenital insensitivity to pain with anhidrosis (CIPA), learning disability and sensory complications. Three of the mutations were heterozygous in unaffected parents/siblings and homozygous in affected individuals, supporting pathogenicity. p.Tyr359X, found in a Saudi Arabian patient with CIPA, was previously described in a Japanese patient [17]. It causes a frameshift, resulting in a premature stop codon which likely renders the protein nonfunctional. p.Arg654Cys, found in a consanguineous Saudi Arabian family, was homozygous in the affected individual while unaffected family members were heterozygous. This mutation was previously described by Miura et al. as p.Arg648Cys, using an alternative amino acid numbering system, who demonstrated that this amino acid is conserved among the three human TRK families, as well as among at least 14 other tyrosine kinase receptors [17]. The homozygous p.Gln176X *NTRK1* mutation has been described previously in two

patients from Kuwait with CIPA [11]. This nonsense mutation lies within the extracellular domain of the *TRKA* protein and would likely disrupt its function as a receptor for NGF [11]. Of the two novel variants, p.Glu492Lys is considered pathogenic. This was found in a patient with CIPA and cognitive delay; the amino acid is highly conserved across species and all three prediction programs suggested a damaging effect of the change, supporting pathogenicity.

We did not find any patients with homozygous mutations in *NGFB*, but found two patients with heterozygous variants. To date only one *NGFB* point mutation has been described in a family with HSAN V (p.Arg221Trp) [4, 16], one family with HSAN IV was reported with a homozygous frameshift mutation due to a point mutation and two base pair deletion on the same allele (p.Val232fs) [1] and one patient with a sensory and autonomic neuropathy has been described with heterozygous deletion of 12 genes one of which was *NGFB* [5]. Although HSAN V is usually recessive, there is a suggestion that heterozygotes have a higher incidence of Charcot joints and neuropathy, as reported in a large family with p.Arg221Trp [15]. Our patient with a heterozygous duplication (p.Gly161_Glu162dup) has a similar phenotype; however, it is currently not possible to confirm whether this variant is pathogenic.

Mutations in the genes known to cause HSAN remain rare. Only 14.3% of this cohort of patients had a pathogenic mutation in a causative gene; thus there are clearly many more HSAN genes which remain unknown. Rapid advances in genetics have already led to the discovery of four additional HSAN genes since the completion of this study. The results of this study have allowed us develop an algorithm for genetic testing of patients with HSAN in the UK (Fig. 2). Although the majority of the mutations found in our cohort are likely to be pathogenic, the exact mechanisms of how these mutations disrupt neural processes require further investigation. Functional studies may provide further understanding of these genetic effects giving insight into possible treatments.

Overall, this study helps broaden the spectrum of HSAN, confirms the predominance of the p.Cys133Trp *SPTLC1* mutation in the UK population, provides additional insights for molecular and clinical diagnosis and illustrates the need for further study into disease-causing mutations in HSAN.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to the patients and families who support our research. We would like to thanks the following for essential grant support; The Medical Research Council (MRC) MMR and HH (MRC fellowship, G108/638 and G0802760), The Brain Research Trust (BRT) (HH), Ataxia UK (HH), The BMA Vera Down Award (HH) and the Muscular Dystrophy Campaign UK and association USA (HH and MMR). SMM and MMR are grateful to the NINDS/ORD (1U54NS065712-01) for their support. MAS was supported by the College of Medicine Research Center (CMRC, Project No. 05-495), College of Medicine, King Saud University, Riyadh, Saudi Arabia. This work was undertaken at University College London Hospitals/University College London, which received a proportion of funding from the Department of Health's National Institute for Health Research Biomedical Research Centres funding scheme. This study was funded by a UCLH/UCL Comprehensive Biomedical Research Centre (CBRC) Grant. We would like to thank the following for essential grant support; The Medical Research Council (MRC) MMR and HH (MRC fellowship, G108/638 and G0802760), The Brain Research Trust (BRT) (HH), Ataxia UK (HH), The BMA Vera Down Award (HH) and the Muscular Dystrophy Campaign UK and association USA (HH and MMR). SMM and MMR are grateful to the NINDS/ORD (1U54NS065712-01) for their support. MAS was supported by the College of Medicine Research Center (CMRC, Project No. 05-495), College of Medicine, King Saud University, Riyadh, Saudi Arabia. FM is supported by the Great Ormond Street Hospital Children's charity. This work was undertaken at University College London Hospitals/University College London, which received a

proportion of funding from the Department of Health's National Institute for Health Research Biomedical Research Centres funding scheme.

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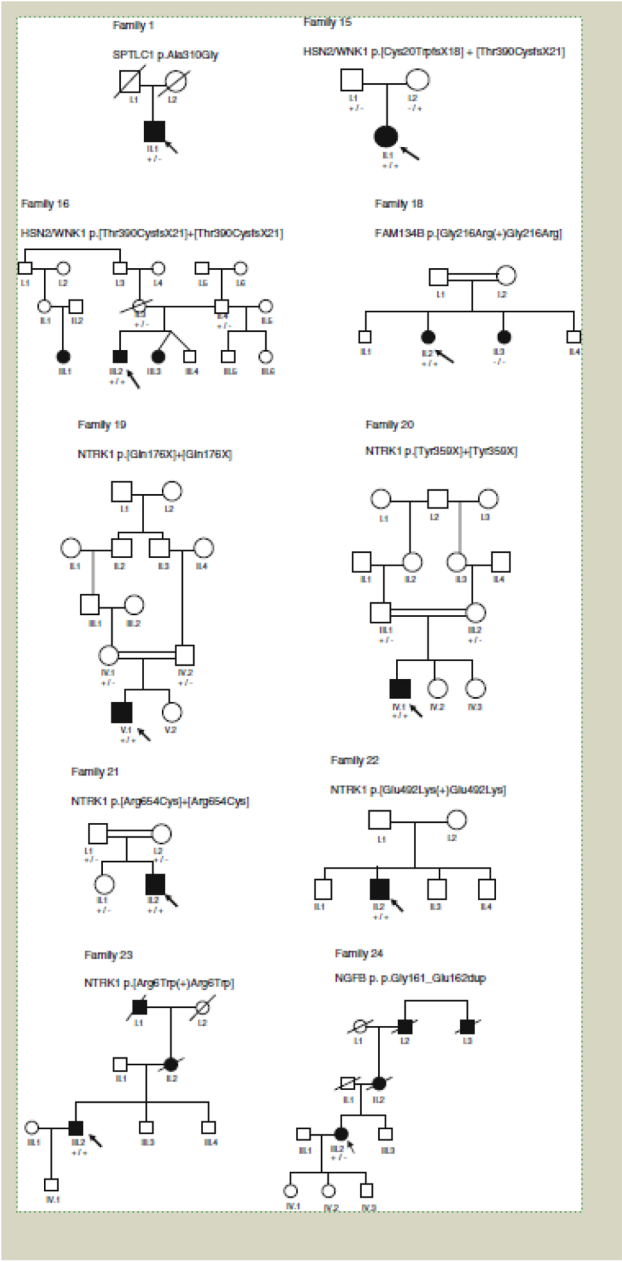

Figure 1.

Pedigrees of families with mutations. An arrow indicates the proband; a square a male; a circle a female; a filled symbol indicates affected; a slanted line through a symbol indicates the individual is deceased; +/+ homozygous for mutation; +/− heterozygous for mutation; −/− homozygous normal

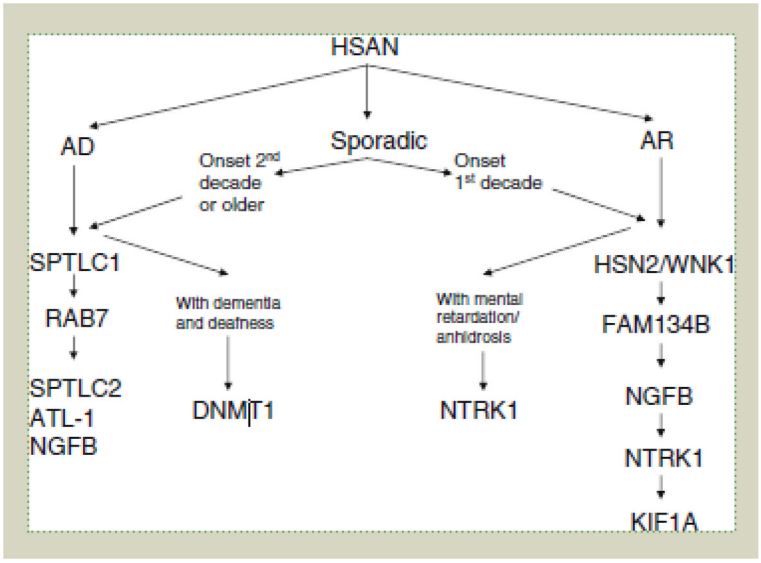

Figure 2.
Algorithm for genetic testing of patients with HSN

Table 1

Classification of the hereditary sensory and autonomic neuropathies

Type	Inheritance	Gene/locus	Specific phenotype
HSAN I	AD	SPTLC1	Predominantly sensory neuropathy, frequent later motor involvement, neuropathic pain,
ulcero-mutilating complications			
HSAN I	AD	SPTLC2	As for SPTLC1
HSAN I	AD	ATL1	Sensory neuropathy without motor involvement, reflexes may be brisk, ulcero-mutilating complications. Spasticity has been described (allelic with hereditary spastic paraplegia
HSP3A)			
HSAN I	AD	DNMT1	Sensory neuropathy, sensorineural deafness with dementia developing in 4th decade
CMT2B	AD	RAB7	Sensorimotor neuropathy, ulcero-mutilating complications
HSAN1B	AD	3p22-p24	Sensory neuropathy, cough, gastro-oesophageal reflux
HSAN II	AR	HSN2/WNK1	Sensory neuropathy, severe ulcero-mutilating complications, frequent autonomic
dysfunction, onset first two decades			
HSAN II	AR	FAM134B	Sensory neuropathy, severe ulcero-mutilating complications, variable autonomic
and motor involvement			
HSAN II	AR	KIF1A	Sensory neuropathy, severe ulceromutilating complications, mild motor involvement
HSAN III	AR	IKBKAP	Familial dysautonomia or Riley-Day syndrome, prominent autonomic dysfunction,
absent fungiform papillae of the tongue			
HSAN IV	AR	NTRK1	Congenital insensitivity to pain with anhydrosis (CIPA), severe sensory neuropathy, anhidrosis, mental retardation, unmyelinated fibers mainly affected
HSAN V	AR	NGFB	Congenital insensitivity to pain, minimal autonomic dysfunction, no mental retardation, mainly small myelinated fibers affected (one case of HSAN V described due to NTRK1 mutations)
HSAN with spastic Paraplegia	AR	CCT5	Mutilating sensory neuropathy with spastic paraplegia

AD autosomal dominant, *AR* autosomal recessive, *SPLTC1* serine palmitoyltransferase, long chain base subunit-1, *SPTLC2* serine palmitoyltransferase, long chain base subunit-2, *ATL1* atlastin-1, *RAB7* member RAS oncogene family, *DNMT1* DNA methyltransferase 1, *HSN2/WNK1* nerve specific isoform of WNK lysine deficient protein kinase 1, *FAM134B* family with sequence similarity 134, member B, *KIF1A* kinesin family member 1A, *IKBKAP* inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein, *NTRK1* neurotrophic tyrosine kinase receptor type 1, *NGFB* nerve growth factor beta polypeptide, *CCT5* chaperonin containing T-complex polypeptide 1, subunit 5

Table 2

Variants found in HSAN patients

Gene	Total patients screened[a]	No. of patients with mutations (%)	Family no.	Mutation(s)
SPTLC1[b]	107	13 (12.1)	1	c.929C[G; p.Ala310Gly (1 patient)[c]
			-	c.399T[G; p.Cys133Trp (12 index patients) [9]
RAB7[b]	115	1 (0.9)	–	c.482A[C; p.Asn161Thr [10]
HSN2/WNK1	129	2 (1.5)	15	c.[60_61delTG] ? [1168_1171delACAG]; p.[Cys20TrpfsX18] ? [Thr390CysfsX21]
			16	c.[1168-1171delACAG] ? [1168-1171delACAG]; p.[Thr390CysfsX21] ? [Thr390CysfsX21]
FAM134B[b]	108	2 (1.9)	17	c.[433C[T(?)433C[T]; p.[Gln145X(?) Gln145X] [18]
			18	c.[646 G[A(?)646 G[A]; p.[Gly216Arg(?)Gly216Arg][c]
NGFB	138	2 (1.4)	24	c.482_487dupGAGAGG; p.Gly161_Glu162dup[c]
			–	c.560G[A; p.Ser187Asn[c]
NTRK1 (TRKA)	88	5 (5.7)	19	c.[526C[T] ? [526C[T]; p.[Gln176X] ? [Gln176X]
			20	c.[1069_1076dupGGCAACTA] ? [1069_1076dupGGCAACTA]; p.[Tyr359X] ? [Tyr359X]
			21	c.[1960 C[T] ? [1960 C[T]; p.[Arg654Cys] ? [Arg654Cys]
			22	c.[1474G[A(?)1474G[A]; p.[Glu492Lys(?)Glu492Lys]
			23	c.[16C[T(?)16C[T]; p.[Arg6Trp(?)Arg6Trp][c]

Genetic sequence variations are described according to the Human Genome Variation Society's recommended nomenclature (http://www.hgvs.org/mutnomen)

[a]Total number of patient samples fully sequenced for each gene

[b]Some of these patients have previously been reported as referenced above

[c]Uncertain significance

Table 3

Phenotypes of index patients with genetic variants

Gene	Amino acid change	Origin	AAO (year)	Inheritance	Sensory involvement	Motor involvement	Reflexes	Ulceromutilating complications	Autonomic symptoms	NCS	Other features	References
SPTLC1 family 1	p.Ala310Glyb[a,b]	UK	50s	U	Pin prick absent throughout, vibration reduced to costal margins Sensory ataxia	None	Present	5th digits of both feet amputated	No	Sensory axonal neuropathy		
SPTLC1 family 2	p.Cys133Trp	UK	20	AD	Pinprick reduced to forearm and knee, vibration to ankles	Distal UL and LL weakness	Absent in LL	Ulcers on feet	No	Sensory motor axonal neuropathy		[9]
SPTLC1 family 3	p.Cys133Trp	UK	29	AD	Sensory loss in feet	None	All reduced	None	No	Sensory motor axonal neuropathy	Lancinating pain	[9]
SPTLC1 family 4	p.Cys133Trp	UK	24	AD	Pinprick reduced above elbows and thighs, vibration to hips	Severe distal UL and LL weakness Hand contractures, Wheelchair	Absent in LL	Osteomyelitis	No	Sensory motor demyelinating neuropathy	Lancinating pain	[9]
SPTLC1 family 5	p.Cys133Trp	UK	16	AD	Pinprick reduced in hands and feet	Distal UL and LL hand	Absent at ankle	Ulcers on feet	No	Sensory motor axonal neuropathy		[9]
SPTLC1family 6	p.Cys133Trp	UK	20	AD	Pinprick reduced to wrists and thighs, vibration to ankle	Distal weakness UL and LL	Absent at ankle	Ulcers on hands and feet, fingers amputated	No	Sensory motor axonal neuropathy	Lancinating pain	[9]
SPTLC1family 7	p.Cys133Trp	UK	18	AD	Sensory loss in UL and LL	Sever UL and LL weakness	Absent	Ulcers on feet, toes amputated	No	Sensory motor axonal neuropathy	Lancinating pain	[9]
SPTLC1 family 8	p.Cys133Trp	UK	60	AD	Pinprick reduced above elbows and mid thigh, vibration to costal margin	Severe distal UL and LL weakness Wheelchair	Absent in LL	Ulcers on feet	No	Sensory motor demyelinating neuropathy	Lancinating pain	[9]
SPTLC1 family 9	p.Cys133Trp	UK	18	AD	Pinprick reduced above elbows and upper thighs, vibration to hips	Severe distal wasting and weakness UL and LL	Absent in LL	Ulcers on feet	No	Sensory motor axonal neuropathy	Lancinating pain	[9]
SPTLC1 family 10	p.Cys133Trp	UK	Teens	AD	Sensation reduced in feet	Present[c]	–	Trophic changes in fingers	No	Sensory motor demyelinating neuropathy		
SPTLC1 family 11	p.Cys133Trp	UK	Teens	AD	Present[c]	Present[c]	–	Trophic changes in fingers	Mild bladder and bowel disturbances	N/A	Lancinating pain	
SPTLC1 family 12	p.Cys133Trp	UK	–	AD	Present[c]	–	–	–	–	N/A		
SPTLC1 family 13	p.Cys133Trp	UK	18	U	Pinprick reduced to elbows and knees, vibration to ankle	Distal weakness and wasting UL and LL	Absent in LL	Ulcers in lower legs and eye bulb	Mild bowel disturbances	Sensory motor axonal neuropathy	Lancinating pain	
RAB7 family 14	p.Asn161Thr	UK	16	AD	Pinprick reduced to ankles, vibration to costal margin	None	Absent at ankles	Scoliosis amputation middle left toe, deformity left foot	No	Sensory motor neuropathy		[10]
HSN2 family 15	p.[Cys20TrpfsX18] + [Thr390CysfsX21][a]	Malta	Congenital	AR	CIP	No	–	Ulcers un noticed fractures osteomecrosis of ankle	No	Sensory motor axonal neuropathy		
HSN2 family 16	p.[Thr390CysfsX21] + [Thr390CysfsX21][a]	Malta	Congenital	AR	CIP	No	–	Ulcerations, Finger tips amputated due to repeated trauma	–	N/A		
FAM134B family 17	p.[Gln145X(+) Gln145X]	Somalia	5	AR	Glove and stocking loss to pin	Significant weakness requiring wheelchair	Absent/diminished	Ulcers, right forefoot amputation scoliosis, ankle deformity acro-osteolysis of finger and toes	No	Sensory motor axonal neuropathy		[18]
FAM134B family 18	p.[Gly216Arg(+) Gly216Arg][a,b]		10	AR	Pin prick reduced in toes	No	Diminished at ankles	Ulcerations, pes cavas scoliosis	No	Sensory axonal neuropathy		
NTRK1 family 19	p.[Gln176X] + [Gln176X]	Saudi Arabia	1st month	AR	CIP	No	Normal	Osteomyelitis, injury to lips and tongue	Anhidrosis bouts of fever	N/A	Cognitive delay	

Gene	Amino acid change	Origin	AAO (year)	Inheritance	Sensory involvement	Motor involvement	Reflexes	Ulceromutilating complications	Autonomic symptoms	NCS	Other features	References
NTRK1 family 20	p.[Try359X] + [Try359X]	Saudi Arabia	Birth	AR	CIP	No (wheelchair bound due to osteomylitis resulting in deformities of feet)	Normal	Charcot joint knee, osteomylitis, injury to lips and tongue	Anhidrosis bouts of fever	Sensory axonal neuropathy		
NTRK1 family 21	p.[Arg654Cys] + [Arg654Cys]	Saudi Arabia	Birth	AR	CIP	No	Normal	Injury to lips and tongue	Anhidrosis bouts of fever	Sensory axonal neuropathy	Cognitive delay	
NTRK1 family 22	p.[Glu492Lys] + [Glu492Lys][a]	UK/USA	Congenital	AR	CIP	Delayed motor milestones	Absent/diminished	Multiple injuries and burns on hands	Anhidrosis bouts of fever	Sensory axonal neuropathy	Deafness, cognitive delay	
NTRK1 family 23	p.[Arg6Trp(+)Arg6Trp][a,b]	UK	Adult	AD	Yes	No	–	No	No	N/A	Dementia, deafness	
NGFB family 24	p.Gly161_Glu162dup[a,b]	UK	48	AD	Glove and stocking loss to pin. vibration reduced to costal margins	No	Absent ankle reflexes	Charcot joints in both ankles, deformities in both knees	No	Sensory motor axonal neuropathy		
NGFB family 25	p.Ser187Asn[a,b]	Ireland	Congenital	Sporadic	CIP	–	–	–	–	N/A		

AAO Age at onset, *AD* autosomal dominant, *AR* autosomal recessive, *CIP* congenital insensitivity to pain, *U* unknown, *N/A* not available, *UL* upper limbs, *LL* lower limbs

[a] Novel

[b] Uncertain significance

[c] Limited details available

Table 4

Analysis of novel missense variants

Variant	Amino acid conservation	Amino acid change	Grantham distance	aGVGD	PolyPhen2	SIFT
SPTLC1 c.929C>G; p.Ala310Gly	Moderately conserved	Non-polar/hydrophobic–non-polar/hydrophobic	60	C0	0.827 Possibly damaging	Tolerated
FAM134B c.646 G>A; p.Gly216Arg	Highly conserved	Non-polar/hydrophobic–positively charged/hydrophilic	125	C65	0.997 Probably damaging	Affect protein function
NGFB c.560G>A; p.Ser187Asn	Highly conserved	Polar/hydrophilic–polar/hydrophilic	46	C45	0.048 Benign	Affect protein function
NTRK1 c.1474G>A; p.Glu492Lys	Highly conserved	Negatively charged/hydrophilic–positively charged/hydrophilic	56	C55	0.995 Probably damaging	Affect protein function
NTRK1 c.16C>T; p.Arg6Trp	Highly conserved	Positively charged/hydrophilic-non-polar/hydrophobic	101	C0	0.106 Benign	Affect protein function

aGVGD uses alignment and amino-acid similarity scores to classify variants in order of likelihood of affecting protein function from C65 (most likely to affect protein function); C55; C45; C35; C25; C15; C0 (least likely)

PolyPhen2 uses alignments and (where possible) information on known functional protein domains to score variants from 0 (benign) to 1 (pathogenic)

SIFT uses protein sequence alignments to calculate the probability that an amino acid change will affect protein function