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Expanding the Genotypic Spectrum of Congenital Sensory and Autonomic Neuropathies Using Whole-Exome Sequencing

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Abstract

Objective

To test the hypothesis that many patients presenting with congenital insensitivity to pain have lesser known or unidentified mutations not captured by conventional genetic panels, we performed whole-exome sequencing in a cohort of well-characterized patients with a clinical diagnosis of congenital hereditary sensory and autonomic neuropathy with unrevealing conventional genetic testing.

Methods

We performed whole-exome sequencing (WES) in 13 patients with congenital impaired or absent sensation to pain and temperature with no identified molecular diagnosis from a conventional genetic panel. Patients underwent a comprehensive phenotypic assessment including autonomic function testing, and neurologic and ophthalmologic examinations.

Results

We identified known or likely pathogenic genetic causes of congenital insensitivity to pain in all 13 patients, spanning 9 genes, the vast majority of which were inherited in an autosomal recessive manner. These included known pathogenic variants (3 patients harboring mutations in *TECPR2* and *SCN11A*), suspected pathogenic variants in genes described to cause congenital sensory and autonomic syndromes (7 patients harboring variants in *NGF*, *LIFR*, *SCN9A*, and *PRDM12*), and likely pathogenic variants in novel genes (4 patients harboring variants in *SMPDL3A, PLEKHN1,* and *SCN10A*).

Conclusions

Our results expand the genetic landscape of congenital sensory and autonomic neuropathies. Further validation of some identified variants should confirm their pathogenicity. WES should be clinically considered to expedite diagnosis, reduce laboratory investigations, and guide enrollment in future gene therapy trials.

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Glossary

CADD = Combined Annotation-Dependent Depletion; **FATHMM** = Functional Algorithm through Hidden Markov Models; **gnomAD** = Genome Aggregation Database; **HSAN** = hereditary sensory and autonomic neuropathy; **LOFTEE** = Loss of Function Transcript Effect Estimator; **NGF** = nerve growth factor; **SIFT** = Sorting Intolerant From Tolerant; **SVM** = support vector machine.

Congenital sensory and autonomic neuropathies are clinically and genetically heterogeneous disorders.[1] Patients have reduced or absent sensation to pain and temperature frequently causing self-mutilations and ulcers, which can result in soft tissue infections or osteomyelitis.[1] In addition, patients have a variable degree of autonomic dysfunction that may include anhidrosis, reduced production of tears, blood pressure fluctuations, or gastrointestinal disturbances.

Historically, syndromes with congenital insensitivity to pain have been classified as part of the hereditary sensory and autonomic neuropathies (HSANs). The current classification of HSAN is numerical, based on age at onset, inheritance pattern, and clinical features.[2] Up to 8 types of HSANs have been now described. Each HSAN is caused by one or several mutations that affect specific aspects of sensory and autonomic neuronal development, resulting in variable phenotypes. Genetic causes of HSANs include pathogenic variants in *SPTLC1*, *SPTLC2, RAB7, ATL1, DNMT1, ATL3* (HSAN1, usually adult onset), *WNK1, KIF1A, FAM134B, SCN9A* (HSAN2),[3,4] *IKBKAP* (HSAN3),[5] *NTRK1* (HSAN4),[6] *NGF* (HSAN5),[7,8] *dystonin* (HSAN6),[9,10] *SCN11A* (HSAN7),[11] and *PRDM12* (HSAN8).[12] However, described pathogenic variants in the above-mentioned genes are not found in many patients presenting with congenital sensory and autonomic neuropathies, suggesting that additional genes are likely associated with HSAN.[7] The HSANs can be transmitted as either autosomal dominant or recessive traits. Patients with autosomal dominant HSAN (HSAN1) usually present in adulthood, whereas autosomal recessive HSANs (HSAN2 to HSAN8) typically express at birth.[13]

Whole-exome sequencing, the analysis of the protein-coding exons of genes, has the potential to accelerate the diagnosis of patients with rare inherited neuropathies.[14,15] We hypothesized that many patients presenting with a congenital sensory and autonomic phenotype have lesser known or unidentified mutations. To test this hypothesis, we performed whole-exome sequencing in a cohort of well-characterized patients with a clinical diagnosis of congenital sensory and autonomic neuropathy that had undergone unrevealing conventional genetic testing. Discovering genetic variants involved in the etiology of patients with congenital insensitivity to pain could expand the genotype-phenotype correlations and contribute to the development of personalized gene therapies.

Methods

Study Design

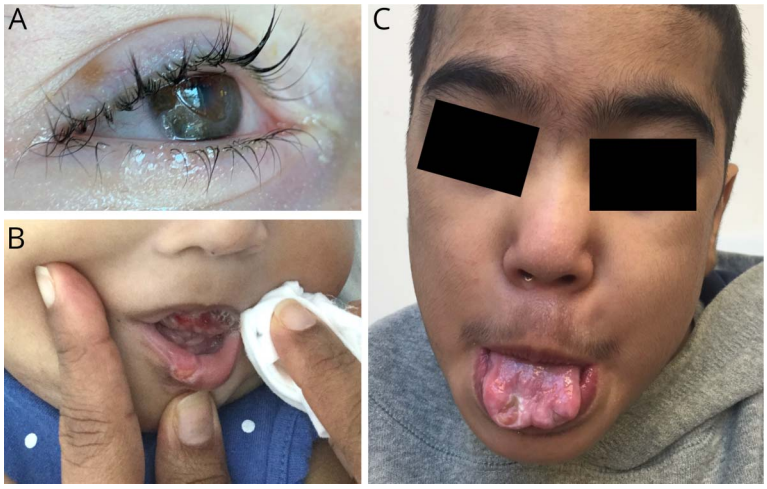
Consecutive patients with a phenotype consistent with congenital impaired sensation to pain and temperature with variable degree of autonomic dysfunction referred to the New York University Dysautonomia Center between July 1, 2013, and July 1, 2019, were eligible. Inclusion criteria were (1) congenital impaired or absent sensation to pain and temperature; (2) negative screening for mutations in genes causing early-onset HSAN including *WNK1*, *FAM134B*, *KIF1A*, *IKBKAP* (familial dysautonomia), and *NTRK1* (congenital insensitivity to pain with anhidrosis) performed with a conventional genetic panel ordered by local pediatrician or geneticist; and (3) preserved muscle strength with no signs of motor neuropathy. All patients underwent a comprehensive medical and family history, physical and neurologic examinations, and cardiovascular autonomic (including venous plasma catecholamine levels in the supine resting position) and ophthalmologic evaluations. Neuroimaging, nerve conduction studies, polysomnography, and sural nerve biopsy were performed in some patients. We also reviewed the patients' history obtained from medical records including birth history, developmental history, and clinical/genetic metabolic evaluations.

Genetic Analysis

We reviewed all clinical genetic testing performed for each case. When a genetic diagnosis was not available, we performed research-based whole-exome sequencing. DNA extracted from blood obtained from the index case and both parents (trio analysis) underwent whole-exome sequencing using either the Agilent Technologies (Santa Clara, CA) SureSelect XTHuman All Exon v4 or Illumina (San Diego, CA) Rapid Capture Exome kit. Sequencing of 100 bp paired-end reads was obtained using Illumina HiSeq. Coverage was >90% or >80% meeting 20× coverage with the 2 methods, respectively. Read alignment, variant calling, and annotation were performed on a pipeline based on Burrows-Wheeler Aligner.

We called variants using human genome 19 coordinates. For undescribed variants, we used inheritance patterns and in silico predictions (PolyPhen2[16]; Sorting Intolerant From Tolerant [SIFT],[17] Combined Annotation-Dependent Depletion [CADD],[18] Loss of Function Transcript Effect Estimator [LOFTEE],[19] MutationTaster,[20] Meta-support vector machine (Meta-SVM),[21] and the Functional Algorithm through Hidden Markov Models [FATHMM][22]) to assess potential pathogenicity. The occurrence frequencies were obtained from control databases (including the Genome Aggregation Database [gnomAD],[23] 1000 Genomes database,[24] and dbSNP). We excluded variants with allele frequency >0.5% in control populations. For homozygous mutations, we excluded homozygous variants in the latest version of gnomAD (v2.1.1).

Figure 1 Manifestations of Impaired Sensation to Pain in Patients With Congenital Sensory and Autonomic Neuropathies



(A) Severe corneal ulceration in 15-year-old patient with a variant in the newly described gene *PLEKHN1* (patient 9). (B) Lip and tongue automutilation in a 1-year-old patient with a novel likely pathogenic variant in *PRDM12* (patient 13, this patient had an absent corneal reflex as documented in the online video 1). (C) Severe tongue automutilation in a 12-year-old patient with a novel likely pathogenic variant in *LIFR* (patient 6, note also the facial hirsutism, a described feature of patients with Stüve-Wiedemann syndrome caused by *LIFR* mutations).

Protein Structure Prediction

To further explore the functional outcomes of some of the identified mutations predicted to be pathogenic, we investigated the effect of mutations in genes encoding sodium channels on the protein secondary structures. Wild-type protein sequences, mutated positions, original amino acid letters, and substitute amino acid letters were entered into MUpro (mupro.proteomics.ics.uci.edu).[25] Change of free energy ($\Delta\Delta G$) for each mutation was predicted using the SVM-based method. A negative $\Delta\Delta G$ value denotes decreased stability, whereas a positive value indicates increased stability. For each mutation in genes encoding sodium channels, a sequence of 200 amino acids (from −100 to +99 flanking the mutated amino acid) was extracted and entered into PSIPRED (bioinf.cs.ucl.ac.uk/psipred). Default parameters were used to predict its secondary structure.

Standard Protocol Approvals, Registrations, and Patient Consents

We followed the STrengthening the REporting of Genetic Association Studies (STREGA) statement.[26] The New York University School of Medicine Institutional Review Board approved this study. Written informed consent was obtained from all patients or guardians participating in the study.

Data Availability

Anonymized data will be shared by written request from any qualified investigator.

Results

Cohort Phenotype

We studied 13 patients from 10 families with a referral diagnosis of congenital sensory and autonomic neuropathy without signs of motor neuropathy. We confirmed reduced or absent sensation to pain and temperature in all patients, with mostly preserved sensation to fine touch. The first clinical signs of the disease became manifest between birth and age 6 months, the most common being tongue and lip mutilations in 8 patients (figure 1) and vomiting with aspiration suggesting neurogenic dysphagia in 5 patients. The degree and severity of autonomic involvement was variable and included reduced or absent basal production of tears in 11 patients, gastrointestinal disturbances (dysphagia in 9, gastroesophageal reflux in 4, nausea and vomiting in 4, and reduced gastrointestinal motility and constipation in 7), and either reduced (in 2) or excessive sweating (in 3). Cardiovascular autonomic function and plasma catecholamine levels were normal in all cases, except in patients with *LIFR* mutations who had paroxysmal episodes of hypertension and severe diaphoresis associated with high plasma norepinephrine levels and hyponatremia. The majority of the patients (8/13) had corneal ulcers or other signs of corneal keratopathy with reduced production of basal tears and absent corneal reflex (video 1, shows lack of corneal reflex in patient 13 who had a likely pathogenic variant in *PRDM12*). Delayed developmental milestones were seen in 8 patients, despite no signs of myopathy, motor neuron disease, or peripheral motor neuropathy. Sleep disorders were infrequent except in patients with *TECPR2* and *SCN9A* variants, who had central sleep apnea, and in patients who had *LIFR* and *PRDM12* variants who had predominantly obstructive sleep apnea. The clinical characteristics of these patients are detailed in table 1. All patients were alive at the time of writing.

Identification of Genetic Variants

We identified known or suspected genetic causes of congenital sensory and autonomic neuropathy in all 13 patients including known pathogenic variants (3 patients), suspected pathogenic variants in genes described to cause congenital sensory and autonomic syndromes (7 patients), and likely pathogenic variants in novel, unexpected genes (3 patients). The genetic characteristics of the described variants are listed in table 2.

Described Genetic Pathogenic Variants

TECPR2

Two unrelated patients of Ashkenazi Jewish ancestry had a homozygous known pathogenic variant (p.Leu440Argfs) in

Table 1 Summary and Clinical Characteristics of Patients With Congenital Sensory and Autonomic Neuropathy, by Genetic Subgroup

Characteristic	*SMPDL3A* hom variants; n = 2 (twin sisters)	*NGF* hom variants; n = 2 (siblings)	*LIFR* hom variants; n = 2 (siblings)	*TECPR2* hom variants; n = 2
Sex	F, F	F, F	M, M	F, M
Origin	Irish	Indian	Pakistani	Ashkenazi
Age at genetic testing	Both 14 y	8 y, 14 y	8 y, 12 y	3 y, 4 y
Age at first sign or symptom identified	Both 6 mo (corneal insensitivity)	Both 6 mo (tongue and lip mutilations)	At birth (GERD and respiratory complications)	At birth (vomiting and aspiration)
Sensation to pain and temperature	Severely reduced	Absent	Reduced	Reduced
Sensation to fine touch	Reduced	Preserved	Preserved	Preserved
Deep tendon reflexes	Absent	Present	Present	Reduced
Sensory nerve conduction velocities	Absent sensory nerve action potentials	Normal	N/A	N/A
Sural nerve biopsy	Loss of large and small myelinated and nonmyelinated neurons	N/A	N/A	N/A
Basal tears	Absent	Absent	Reduced	Reduced
Emotional tears	Present	Present	Present	Present
Corneal abrasions	Present	Absent	Present	Present (punctate keratitis)
Self-mutilation in the tongue, lips, or fingers	Yes 2 (100%)	Yes 2 (100%)	Yes 2 (100%)	No
Sweating	Normal	Absent	Paroxysmal hyperhidrosis with hyponatremia	Normal
Cardiovascular abnormalities	No	No	Paroxysmal hypertension	Normal
Plasma catecholamines levels	Normal	N/A	Elevated norepinephrine during paroxysmal hypertension	Normal
Gastrointestinal abnormalities	Neurogenic dysphagia and sialorrhea 1 (50%)	Reduced motility 2 (100%). Necrotizing enterocolitis 1 (50%) requiring colostomy	Neurogenic dysphagia, sialorrhea, gastroparesis, and nausea accompanied by hyperhidrosis and hypertension	Neurogenic dysphagia, and constipation
Sleep disordered breathing	No	No	Obstructive sleep apnea	Central sleep apnea, snoring, and stridor
Orthopedic abnormalities	Frequent fractures and neuropathic joints (ankles) 2 (100)	Frequent fractures	Bowing of legs, camptodactylia, and frequent fractures	No
Hyperlordotic spine	No	No		
Sensorineural hearing loss	1 (50%)	No	No	1 (50%)
Muscle tone	Normal	Normal	Normal	Reduced
Motor development	Normal	Normal	Delayed, did not walk until 2 years old	Delayed, did not walk until 2.4 years old
Other features	Reduced olfaction in 1 (50%)	Vesicoureteral reflux 2 (100%)	Hirsutism	Neurodevelopmental delay
	UPSIT: 34/40		Lung disease (bronchiectasis, asthma, and restrictive lung disease)	Proprioceptive ataxia
	Mild dysarthria			Strabismus
	Normal brain MRI			Thin corpus callosum 1 (50%)

Abbreviations: GERD = gastroesophageal reflux disease; het = heterozygous; hom = homozygous.

Table 1 (continued)

PLEKHN1 **hom variant; n = 1**	***SCN9A*** **hom variant; n = 1**	***SCN10A*** **het variant; n = 1**	***SCN11A*** **het variant; n = 1**	**PRDM12 het variant; n = 1**
F	M	M	M	F
German	German/Irish	Caribbean	German/Italian	Caribbean
15 y	9 y	7 y	16 mo	1 y
At birth (hypotonia, GERD, and vomiting)	6 mo (insensitivity to pain with falls or injuries)	6 mo (tongue and lip mutilations)	6 mo (tongue and lip mutilations)	6 mo (tongue and lip mutilations)
Absent	Reduced	Absent	Absent	Absent
Reduced	Preserved	Preserved	Preserved	Preserved
Absent	Reduced	Reduced	Present	Reduced
Absent sensory nerve action potentials	N/A	Normal	N/A	Normal
Loss of large and small myelinated and nonmyelinated neurons	N/A	Loss of large and small myelinated and nonmyelinated neurons	N/A	N/A
Absent	Present	Absent	Present	Reduced
Present	Present	Present	Present	Present
Present	Absent	Present	Absent	Absent
Yes	Yes	Yes	Yes	Yes
Normal	Normal	Normal	Increased	Normal
No	No	No	No	No
Normal	Normal	Normal	Normal	Normal
Neurogenic dysphagia, sialorrhea, GERD, vomiting, and gastroparesis requiring Nissen fundoplication.	No	GERD	Neurogenic dysphagia, GERD, and frequent vomiting requiring Nissen fundoplication.	Neurogenic dysphagia, GERD, requiring G-tube
No	Central sleep apnea	No	No	Predominantly obstructive with some central apneas
Frequent hip dislocation and scoliosis	Frequent fractures	Frequent fractures and neuropathic joints–osteomyelitis		
1 (100%)	No	No	No	No
Reduced	Normal	Reduced	Reduced	Reduced
Delayed, did not walk until 3 years old	Normal	Delayed, did not walk until 2 years old	Delayed, unable to walk at age 2; small for age	Delayed; unable to crawl at age 1.
Reduced olfaction: UPSIT: 28/40	—	Neurodevelopmental delay	Normal brain MRI	Neurodevelopmental delay
Lung disease		Normal brain MRI		Strabismus
Mild dysarthria and mild proprioceptive ataxia				Plagiocephaly
Normal brain MRI				

TECPR2. Given their Ashkenazi Jewish ancestry, these 2 patients were initially tested for familial dysautonomia (HSAN3) with negative results. Mutations in *TECPR2* have been shown to cause a familial dysautonomia-like syndrome.[27,28] Our 2

Table 2 Summary of Suspected Pathogenic Variants for a Series of 13 Patients With Congenital Sensory and Autonomic Neuropathy

ID	Gene	Suspected or known pathogenic mutation	Zygosity	Inheritance	PolyPhen2 prediction effect (score)	SIFT (score)	Comment
1	*SMPDL3A*	c.791T>G (p.Ile264Ser)	Homozygous	AR	Probably benign (0.006)	Neutral (0.53)	Novel potential candidate gene
2	*SMPDL3A*	c.791T>G (p.Ile264Ser)	Homozygous	AR	Probably benign (0.006)	Neutral (0.53)	Novel potential candidate gene
3	*NGF*	c.433delG (p.Asp145Ilefs*13)	Homozygous	AR	Probably damaging (1)	Deleterious (0)	Novel potential pathogenic variant
4	*NGF*	c.433delG (p.Asp145Ilefs*13)	Homozygous	AR	Probably damaging (1)	Deleterious (0)	Novel potential pathogenic variant
5	*LIFR*	c.1718dupT (p.Ser574Ilefs*6)	Homozygous	AR	Probably damaging (1)	Deleterious (0)	Novel potential pathogenic variant
6	*LIFR*	c.1718dupT (p.Ser574Ilefs*6)	Homozygous	AR	Probably damaging (1)	Deleterious (0)	Novel potential pathogenic variant
7	*TECPR2*	c.1319delT (p.Leu440Argfs)	Homozygous	AR	N/A	N/A	Described pathogenic variant[28]
8	*TECPR2*	c.1319delT (p.Leu440Argfs)	Homozygous	AR	N/A	N/A	Described pathogenic variant[28]
9	*PLEKHN1*	c.A1091T (p.Asp364Val)	Homozygous	AR	Probably benign (0.02)	Deleterious (0.02)	Novel potential candidate gene
10	*SCN9A*	c.2686C>T (p.Arg896Trp)	Homozygous	AR	Probably damaging (1)	Deleterious (0)	Novel potential pathogenic variant
11	*SCN10A*	c.2367C>A (p.Asn789Lys)	Heterozygous	AD	Probably damaging (0.992)	Deleterious (0)	Novel potential candidate gene
12	*SCN11A*	c.1187T>C (p.Leu396Pro)	Heterozygous	De novo	Probably damaging (0.991)	Deleterious (0)	Described pathogenic variant[29]
13	*PRDM12*	c.503 G>A (p.Arg168His) inherited from the father	Compound heterozygous	AR	Probably damaging (0.994)	Deleterious (0)	Novel potential pathogenic variants
		c.794A>C (p.His265Pro) inherited from the mother			Probably damaging (0.999)	Deleterious (0)	

patients (patient 1 and patient 2) share the same *TECPR2* pathogenic variant and phenotype with 3 previously reported patients.[28] Patient 2 had a thin corpus callosum, which has been reported before in other patients with the same *TECPR2* pathogenic variant.[28]

SCN11A

We identified a heterozygous, de novo, *SCN11A* variant (p.Leu396Pro) in 1 patient (patient 3) of German/Italian ancestry. The same variant has been previously described in another patient with the similar syndrome.[29] *SCN11A* encodes the sodium ion channel $Na_v1.9$.

Suspected Pathogenic Variants in Previously Described Genes

Nerve Growth Factor

Heterozygous mutations in the nerve growth factor (NGF) (encoding the polypeptide NGF, key in the development of sensory and autonomic neurons) are described to cause HSAN5, a disorder characterized by congenital insensitivity to pain.[8] We identified a homozygous previously undescribed potential pathogenic variant (p.Asp145Ilefs*13) in 2 siblings (patients 3 and 4) of Indian ancestry. Both patients had a similar phenotype to that described in the literature, but they also had anhidrosis, which is not typical of most patients with *NGF* mutations.

LIFR

Biallelic mutations in the *LIFR* gene, encoding the receptor for leukemia-inhibitory factor, a polyfunctional cytokine that affects the differentiation, survival, and proliferation of a wide variety of cells during embryologic development, cause Stüve-Wiedemann syndrome, a disorder characterized by skeletal changes, bowing of the lower limbs, episodic changes in temperature, and respiratory infections.[30,31] Reductions in pain and temperature sensation as well as autonomic dysfunction have been described as well, although they are not usually listed as classic features of the syndrome.[32–34] We identified a homozygous, previously undescribed potential pathogenic variant (p.Ser574Ilefs*6) in 2 siblings (patients 5 and 6) of Pakistani ancestry, who had a similar phenotype to that described in the literature. Of note, these 2 siblings had paroxysmal episodes of hypertension and diaphoresis associated with high plasma norepinephrine levels and

hyponatremia, requiring frequent visits to the emergency department. These episodes of diaphoresis were so severe and dramatic as to sometimes requiring the patients' clothes be changed 7–10 times a day.

SNC9A

Mutations in *SCN9A* (encoding the sodium ion channel $Na_v1.7$) have been reported in patients with either painful neuropathy or congenital insensitivity to pain, depending on the effect of the mutation on the sodium channel function.[35,36] We identified a de novo homozygous previously undescribed potential pathogenic variant (p.Arg896Trp) in patient 10 with central European ancestry with a similar phenotype to that of previously described patients with congenital insensitivity to pain caused by other *SCN9A* mutations.

PRDM12

Variants in *PRDM12* (encoding a family of transcriptional regulators that participate in the control of neurogenesis) have been described in association with autosomal recessive HSAN8, a disorder characterized by congenital insensitivity to pain, corneal ulcers, and neuropathic joints with reduced lacrimation.[12,37–39] We identified previously undescribed compound heterozygous potential pathogenic variants (p.Arg168His inherited from her father and p.His265Pro inherited from her mother) in a patient of Caribbean ancestry who had a similar phenotype to that described in the literature.

Novel Potential Pathogenic Variants in Novel Genes

We identified novel variants in 3 genes never associated with congenital insensitivity to pain before. These 3 newly identified variants will require further validation.

SMPDL3A

SMPDL3A encodes the protein sphingomyelin phosphodiesterase acid–like 3A. Although the function of this protein remains poorly understood,[40,41] its sequence is homologous to the well-characterized acid sphingomyelinase. Acid sphingomyelinase deficiency causes Niemann-Pick disease, which is characterized by peripheral neuropathy among other manifestations.[42,43] We identified a homozygous *SMPDL3A* variant (p.Ile264Ser) in 2 siblings with similar phenotype (patients 1 and 2). This variant had conflicting results in the in silico tools, with a low CADD score (9.016), a probably benign on PolyPhen2, and neutral SIFT score. However, the allele frequency is <0.3%, and the homozygous state has never been observed in the control population, suggesting a likely pathogenic role.

PLEKHN1

PLEKHN1 encodes the protein pleckstrin-homology N1, also known as cardiolipin phosphatidic acid–binding protein.[44] Its function is yet to be fully defined, but it has been reported to play a role in axonal transport and mitochondrial metabolism.[45] Dysfunction in these systems has been implicated in the pathophysiology of other HSAN.[46,47] We identified a homozygous *PLEKHN1* missense variant (p.Asp364Val) in patient 9, who had a remarkably similar phenotype to patients with the *SMPDL3A* variant described above, including hyposmia, mild dysarthria, and loss of myelinated and unmyelinated fibers in sural nerve biopsy. The *PLEKHN1* variant had conflicting results with in silico predicting tools, with a borderline CADD score (16.25), a deleterious SIFT score, probably benign on PolyPhen2, neutral on MutationTaster, and tolerated in FATHMM. However, it has an allele frequency of <0.04% and the homozygous state has never been observed in control populations, suggesting a likely pathogenic role.

SCN10A

The *SCN10A* gene encodes sodium ion channel $Na_v1.8$, which is highly expressed in sensory neurons. Heterozygous gain of function mutations in *SCN10A* have been reported in individuals with painful small fiber peripheral neuropathy, characterized by autonomic dysfunction and burning pain in extremities.[48] We identified a missense heterozygous *SCN10A* variant (p.Asn789Lys) in patient 11, of Caribbean ancestry. All in silico tools unanimously classified the identified this *SCN10A* missense variant as deleterious and damaging, making it highly likely to be pathogenic. The patient with a heterozygous *SCN10A* variant had inherited it from his mother. A careful neurologic examination of the mother disclosed severely reduced albeit preserved pain and temperature perception without the other sensory or autonomic disturbances that were present in her son, suggesting that heterozygous *SCN10A*-associated variants may have incomplete penetrance or variable expressivity, similarly to what has been described in other hereditary neuropathies.[49]

Protein Structure Prediction

To further explore the functional outcomes of the sodium channel mutations, we investigated the effect of mutations in *SCN* genes on the protein secondary structures. We first applied MUpro[25] and found that all 3 mutations in *SCN9A* (p.Arg896W), *SCN10A* (p.Asn789Lys), and *SCN11A* (p.Leu396Pro) resulted in decreased protein stability, with predicted changes in Gibbs free energy ($\Delta\Delta G$) as −0.518, −0.966, and −1.777, respectively. These observations indicated that these *SCN* variations might impair the protein structures. These 3 proteins are sodium channels, with transmembrane domains, and we tested the effect of these variation on the helixes. According to neXtProt,[50] the variation p.Arg896W of *SCN9A* is inside an extracellular domain, whereas p.Asn789Lys in *SCN10A* is the last amino acid of a cytoplasmic domain followed by a transmembrane domain. Only p.Leu396Pro of *SCN11A* is located inside a transmembrane domain. All these 3 variations were predicted to alter the protein helices by PSIPRED[51] (figure e-1, links.lww.com/NXG/A389). These results suggested all these *SCN* mutations might interfere the organization of alpha-helices resulting in the disruption of the transmembrane or extracellular protein functions.

Discussion

Our results provide insight into the genetic landscape of congenital sensory and autonomic neuropathies. We show that whole-exome sequencing has a high probability of identifying

the genetic cause of undiagnosed patients with congenital sensory and autonomic neuropathies that have failed earlier candidate gene approaches. Because all patients had negative results from earlier candidate gene testing, we anticipated that novel causal genes were highly likely to be identified. Likely or known pathogenic variants in known genes explained more than 60% of cases. These included novel likely pathogenic variants in *NGF*, *LIFR*, *SCN9A*, and *PRDM12* and known pathogenic variants in *TECPR2* and *SCN11A*.

Our results expand the phenotype-genotype correlation of genes involved in congenital sensory and autonomic neuropathies. For instance, our 2 siblings harboring novel *NGF* variants had anhidrosis, which is not typical of HSAN5. Anhidrosis has so far been reported only in 1 family with a specific *NGF* mutation (c.661C>T).[52] In contrast, anhidrosis is a defining feature of congenital insensitivity to pain with anhidrosis caused by *NTRK1* mutations (HSAN4). The anhidrosis in our 2 patients with *NFG* mutations strongly suggested that they had mutations in *NTRK1* instead, contributing to the delay of the genetic diagnosis. NGF (encoded by *NGF*) engages 2 structurally distinct transmembrane receptors, TrkA (encoded by *NTRK1*) and p75, which create a high-affinity NGF binding site through the formation of a TrkA/NGF/p75 complex.[53] It is tempting to hypothesize that the *NGF* mutations we here describe may cause a dysfunctional NGF-TrkA resulting in anhidrosis, as seen in patients with HSAN4.

Mutations in the *LIFR* gene cause Stüve-Wiedemann syndrome, a disorder characterized by skeletal changes with bowing of the lower limbs, hirsutism, thermoregulation abnormalities, and frequent respiratory infections.[30,31] Our patients with *LIFR* mutations also had paroxysmal episodes of hypertension and severe diaphoresis associated with high plasma norepinephrine levels and hyponatremia, requiring frequent visits to the emergency department, resembling the dysautonomic crisis of patients with familial dysautonomia (HSAN3).[54] The underlying pathophysiology of dysautonomic crisis in patients with HSAN3 is unrestrained catecholamine release in the context of afferent baroreflex failure.[55,56] It is tempting to hypothesize that a similar mechanism could underlie hyperadrenergic episodes in Stüve-Wiedemann syndrome. Although Stüve-Wiedemann syndrome is not included in the classic classification of HSAN, our results strongly argue for the inclusion of *LIFR* mutations in the differential diagnosis of patients with congenital sensory and autonomic neuropathy.[57]

TECPR2 mutations have been described to cause a familial dysautonomia-like HSAN. The disorder was initially described in 3 unrelated Jewish Bukharan families with a different mutation (p.Leu1139Argfs).[27] Because some of these patients developed spasticity in older age, the disease was initially classified as a subtype of hereditary spastic paraparesis and named SPG49. A different mutation in the same gene (p.Leu440Argfs) was reported in 3 non-Bukharan patients with prominent features of sensory and autonomic neuropathy, prompting the reclassification of the disease as a HSAN.[28] Our 2 unrelated patients (patients 7 and patient 8), both from Jewish Ashkenazi families, share the same mutation and the phenotype previously described in the 3 non-Bukharan patients. Familial dysautonomia (HSAN3) and the disorder caused by *TECPR2* mutations share similar characteristics, including neurogenic dysphagia, proprioceptive ataxia and central sleep apnea. However, in contrast to the marked blood pressure abnormalities characteristic of HSAN3, patients with *TECPR2* mutations appear to have preserved cardiovascular autonomic function.

We also identified the potential novel candidate genes *SMPDL3A*, *PLEKHN1*, and *SCN10A*. The patients harboring *SMPDL3A* and *PLEKHN1* variants had a remarkably similar phenotype, whereas the patient with the *SCN10A* variant had a phenotype similar to that of patients with variants in *SCN9A* and *SCN11A* causing dysfunction in sodium channels. Mutations in *SCN10A* causing congenital sensory and autonomic neuropathy expand the phenotype of sodium channelopathies, which also include mutations in genes *SCN9A* (encoding sodium ion channel $Na_v1.7$) and *SCN11A* (encoding sodium ion channel $Na_v1.9$), as the ones here described in patient 10 and patient 12, respectively, which have been reported with both painful neuropathy and congenital insensitivity to pain, depending on the effect of the mutation on the sodium channel function.[48,58] The *SCN10A* variant was unanimously classified as pathogenic by the in silico tools. Also, in silico prediction of the SCN protein function suggested that the *SCN* mutations in our patients might interfere the organization of alpha-helices resulting in the disruption of the transmembrane or extracellular protein functions.

The function of both *SMPDL3A* and *PLEKHN1* remains poorly understood, but both are related to functions or pathways involved in peripheral nerve metabolism and survival. The *SMPDL3A* and *PLEKHN1* mutations had conflicting in silico results, although in both cases, the described frequencies of the alleles were extremely low and the homozygous state has never been observed in control populations, suggesting a likely pathogenic role. Additional cases of congenital HSAN bearing the identified variants in *SMPDL3A, PLEKHN1*, and *SCN10A* should confirm the pathogenicity of these novel candidate genes.

Whole-exome sequencing has been previously used in cohorts of patients with inherited neuropathies[15] and has been anecdotally reported in individual cases of congenital sensory and autonomic neuropathy.[59,60] Here, we provide a comprehensive report of genetic etiologies in a cohort of patients with congenital sensory and autonomic neuropathy syndromes. All patients were referred to the NYU Dysautonomia Center. Although our cohort may have a referral bias, it was likely biased toward enrollment of patients without a clinical explanation. Therefore, we do not think that we have overestimated the role of genetic influences of congenital

hereditary sensory and autonomic neuropathies. None of our patients underwent skin biopsy for the assessment of sensory and autonomic cutaneous innervation, which would have been useful to expand our genotype-phenotype correlations.

In the current era of evolving precision medicine, having an established genetic diagnosis has the potential of influencing treatment. For instance, familial dysautonomia (HSAN3) is caused by a founder mutation in the *ELP1* gene, causing a pre-RNA splicing defect resulting in the expression of a truncated ELP1 protein. Genetic therapies, such as antisense oligonucleotides or U1 snRNAs, as well as small molecules, have shown promise to correct the splicing defect in preclinical models of familial dysautonomia.[61–63] Similar therapeutic mechanisms may be useful to correcting the deleterious consequences of genetic mutations causing other HSAN. Conversely, the identified mutations may be useful to inform drug development of new analgesic drugs, and there are now therapies in the pipeline targeting NGF and sodium channels.

In summary, children with congenital sensory and autonomic neuropathies have identifiable genetic etiologies. Whole-exome sequencing should be considered on a clinical basis to expedite a definite diagnosis, reduce unnecessary laboratory investigations, and eventually guide enrollment in gene-specific clinical trials as they emerge. Genetic diagnosis frequently empowers families with the knowledge to care and advocate for their children and make decisions regarding family planning. In conclusion, our findings suggest that whole-exome sequencing is of high yield in patients with congenital impaired sensation to pain and temperature.

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Jose-Alberto Palma, MD PhD	NYU School of Medicine, New York	Coordination of study design and conduct, designing research studies, acquiring data, analyzing data, and writing the initial draft of the manuscript
Rachita Yadav, PhD	Massachusetts General Hospital Research Institute, Boston	Analyzing data, significant contribution to the manuscript, and final approval
Dadi Gao, PhD	Massachusetts General Hospital Research Institute, Boston	Analyzing data, significant contribution to the manuscript, and final approval
Lucy Norcliffe-Kaufmann, PhD	NYU School of Medicine, New York	Analyzing data, significant contribution to the manuscript, and final approval.
Susan A. Slaugenhaupt, PhD	Massachusetts General Hospital Research Institute, Boston	Analyzing data, significant contribution to the manuscript, and final approval
Horacio Kaufmann, MD	NYU School of Medicine, New York	Designing research studies, acquiring data, analyzing data, providing reagents, and writing the initial draft of the manuscript

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Expanding the Genotypic Spectrum of Congenital Sensory and Autonomic Neuropathies Using Whole-Exome Sequencing

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