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

Jose-Alberto Palma, MD, PhD, Rachita Yadav, PhD, Dadi Gao, PhD, Lucy Norcliffe-Kaufmann, PhD, Susan Slaugenhaupt, PhD, and Horacio Kaufmann, MD, FAAN

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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.
Congenital sensory and autonomic neuropathies are clinically and genetically heterogeneous disorders. 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. 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. 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), IKBKAP (HSAN3), NTRK1 (HSAN4), NTRK3 (HSAN5), NGF (HSAN6), dystonin (HSAN6), SCN11A (HSAN7), and PRDM12 (HSAN8). 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. 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.

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. 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 XT Human 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; Sorting Intolerant From Tolerant [SIFT], Combined Annotation-Dependent Depletion [CADD], Loss of Function Transcript Effect Estimator [LOFTEE], MutationTaster, Meta-support vector machine [Meta-SVM], and the Functional Algorithm through Hidden Markov Models [FATHMM]) to assess potential pathogenicity. The occurrence frequencies were obtained from control databases (including the Genome Aggregation Database [gnomAD], 1000 Genomes database, 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).

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.
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

![Figure 1 Manifestations of Impaired Sensation to Pain in Patients With Congenital Sensory and Autonomic Neuropathies](image-url)

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

Abbreviations: GERD = gastroesophageal reflux disease; het = heterozygous; hom = homozygous.
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
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 Nav1.9.

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 HSANS, 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.

**SCN9A**

Mutations in SCN9A (encoding the sodium ion channel Na$_v$1.7) have been reported in patients with either painful neuropathy or congenital insensitivity to pain, depending on the effect of the mutation on the sodium channel function.$^{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,$^{30,41}$ 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 cardioliarin 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$_v$1.8, which is highly expressed in sensory neurons. Heterozygous gain of function mutations in SCN10A have been reported in individuals with painful small fiber peripheral neuropathy, characterized by autonomic dysfunction and burning pain in extremities.$^{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 variations on the helices. According to neXiProt,$^{50}$ 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 PRRM2 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). 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. NFG (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. 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. 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). The underlying pathophysiology of dysautonomic crisis in patients with HSAN3 is unrestrained catecholamine release in the context of the TrkA/NGF/p75 complex.

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). 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. 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 Na1.7) and SCN11A (encoding sodium ion channel Na1.9), 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. 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 and has been anecdotally reported in individual cases of congenital sensory and autonomic neuropathy. 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 neuropathies.
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. 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 analgesics, 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 definitive 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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Disclosure
The authors report no disclosures relevant to the manuscript.

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Appendix

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dadi Gao, PhD</td>
<td>Massachusetts General Hospital Research Institute, Boston</td>
<td>Analyzing data, significant contribution to the manuscript, and final approval</td>
</tr>
<tr>
<td>Lucy Norcliffe-Kaufmann, PhD</td>
<td>NYU School of Medicine, New York</td>
<td>Analyzing data, significant contribution to the manuscript, and final approval</td>
</tr>
<tr>
<td>Susan A. Slaugenhaupt, PhD</td>
<td>Massachusetts General Hospital Research Institute, Boston</td>
<td>Analyzing data, significant contribution to the manuscript, and final approval</td>
</tr>
<tr>
<td>Horacio Kaufmann, MD</td>
<td>NYU School of Medicine, New York</td>
<td>Designing research studies, acquiring data, analyzing data, providing reagents, and writing the initial draft of the manuscript</td>
</tr>
</tbody>
</table>

References
Expanding the Genotypic Spectrum of Congenital Sensory and Autonomic Neuropathies Using Whole-Exome Sequencing
Jose-Alberto Palma, Rachita Yadav, Dadi Gao, et al.
Neurol Genet 2021;7;
DOI 10.1212/NXG.00000000000000568

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