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ORIGINAL ARTICLE

Novel NTRK1 mutations cause hereditary sensory and autonomic neuropathy type IV: demonstration of a founder mutation in the Turkish population

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Abstract Hereditary sensory and autonomic neuropathy type IV (HSAN IV), or congenital insensitivity to pain with anhidrosis, is an autosomal recessive disorder characterized by insensitivity to noxious stimuli, anhidrosis from denervated sweat glands, and delayed mental and motor development. Mutations in the neurotrophic tyrosine kinase receptor type 1 (NTRK1), a receptor in the neurotrophin signaling pathway phosphorylated in response to nerve growth factor, are associated with this disorder. We identified six families from Northern Central Turkey with HSAN IV. We screened the NTRK1 gene for mutations in these families. Microsatellite and single nucleotide polymorphism (SNP) markers on the Affymetrix 250K chip platform were used to determine the haplotypes for three families harboring the same mutation. Screening for mutations in the NTRK1 gene demonstrated one novel frameshift mutation, two novel nonsense mutations, and three unrelated kindreds with the same splice-site mutation. Genotyping of the three families with the identical splice-site mutation revealed that they share the same haplotype.

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This report broadens the spectrum of mutations in NTRK1 that cause HSAN IV and demonstrates a founder mutation in the Turkish population.

Keywords Congenital insensitivity to pain with anhidrosis · NTRK1 · CIPA · Hereditary sensory and autonomic neuropathy type IV · HSAN IV · Mutation · Founder effect

Introduction

Hereditary sensory and autonomic neuropathies (HSANs) are a group of rare congenital disorders, all characterized by widespread sensory and variable autonomic dysfunction due to faulty development of autonomic and sensory neurons. Though clinically similar, they are genetically distinct entities resulting from mutations in different genes. The current classification of the HSAN group of disorders divides the syndromes up into five subtypes [1, 2]. Of these subtypes, the only autosomal dominant forms are HSANI/IB (OMIM # 162400/608088), which display minimal to no autonomic dysfunction [1, 2].

Hereditary sensory and autonomic neuropathy type IV (HSAN IV) (OMIM # 256800), also known as congenital insensitivity to pain with anhidrosis (CIPA), is an autosomal recessive disorder characterized by recurrent episodes of unexplained pyrexia, anhidrosis, mental retardation, and an insensitivity to pain with subsequent self-mutilating behavior [3–5]. Ultrastructural analysis of nerve biopsies in HSAN IV patients demonstrates an absence of small myelinated (A-fibers) and unmyelinated nerves (C-fibers) [6, 7]. Furthermore, eccrine sweat glands demonstrate a lack of autonomic innervation [8, 9], and the epidermis of patients is devoid of nerve branches and endings [10]. The genetic basis of CIPA is well described. The observation that knockout mice lacking the TrkA gene, a receptor tyrosine kinase for nerve growth factor (NGF) [11], shared multiple phenotypic characteristics with CIPA patients led ultimately to the identification of mutations in the human homologue, neurotrophic tyrosine kinase receptor type 1 (NTRK1), among affected individuals, demonstrating that this was the causative gene in HSAN IV [12].

The NTRK1 gene is composed of 17 exons, and at least three splice variants exist [13, 14]. To date, different reports have demonstrated various mutations in different ethnic groups along the entire length of the gene, both in the extracellular, NGF-binding domain, and the intracellular, tyrosine kinase domain [15–22]. The absence of differentiated small myelinated, unmyelinated, and peripheral autonomic nerve fibers from defective NGF signaling likely leads to impaired pain perception and autonomic dysfunction. The combination of mental impairment with insensitivity to pain frequently leads to the patients' self-mutilating behavior.

We present four novel mutations in the *NTRK1* gene found among six patients of Turkish descent with HSAN IV, all products of consanguineous marriages. Interestingly, three of the patients share an identical splice site mutation. Additional genotyping analysis of nearby markers performed on these patients demonstrated that they share the identical extended haplotype, demonstrating for the first time a founder effect among patients of Turkish descent with HSAN IV.

Materials and methods

Family collection

This study was approved by the Yale University Human Investigations Committee (Protocol No. 7680) and by the Istanbul University Cerrahpasa School of Medicine HIC. We identified six unrelated families from Northern Turkey. All of these families had two or more family members diagnosed with HSAN IV based upon clinical findings or pathological analysis. Blood samples were collected from all available family members after the attainment of informed consent. Total genomic DNA was isolated according to standard protocols.

Mutational analysis

Exon–intron boundaries of the NTRK1 gene (NTRK1; MIM# 191315) were determined based on the University of California at Santa Cruz (UCSC) Genome Browser (NCBI Build 36.1) and previous reports [13]. Exon–intron boundaries were determined based upon the cDNA sequence for NTRK1 (NM_002529). PCR primers were designed using PRIMER3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). All known exons were amplified and sequenced for each index case (n=6) and their parents using standard techniques.

Genotyping using microsatellite short tandem repeat and single nucleotide polymorphism markers

Microsatellite short tandem repeat (STR) markers were identified near the *NTRK1* gene using the physical map data from the UCSC Genome Browser (May 2004, genome.ucsc.edu; HG17: chr1:153,643,744–153,664,715). The parents and affected child were genotyped in each family harboring the shared splice-site mutation. All genotyping for microsatellite analysis was performed by PCR, with detection of fluorescent products on an ABI 3700 sequencer, and analyzed with Genescan and Genotyper software (version 3.5) (ABI, Norwalk, CT, USA). Allele sizes were

determined by an investigator blinded to patient characteristics. Single nucleotide polymorphisms (SNPs) were identified within the introns and exons of the NTRK1 gene and were directly sequenced for the proband and the parents.

Array-based SNP allele analysis

Affected-only whole-genome SNP allele analysis was performed using the Affymetrix 250K SNP Array (Affymetrix, Santa Clara, CA, USA) containing over 250,000 SNP markers for genome-wide analysis, according to the company's protocols using patients 72–1, 73–1, and 80–1. Affymetrix GeneChip Operating Software package (Affymetrix) was utilized to obtain raw microarray feature intensities, the results of which were processed to derive SNP genotypes.

Results

Patient characteristics

Family NG-46

The index case is a 10-year-old male, the product of a consanguineous marriage between first cousins. He presented with anhidrosis, insensitivity to pain, psychomotor retardation, and multiple recurrent long bone fractures. On examination, he was found to have multiple autoamputations of the fingers and self-inflicted wounds on the tongue from biting. His physical exam was notable for the aforementioned lesions and microcephaly (Fig. S1). MRI of the brain was normal. Upon questioning, the family reported another relative with similar symptoms, but details on the degree of relatedness were not available.

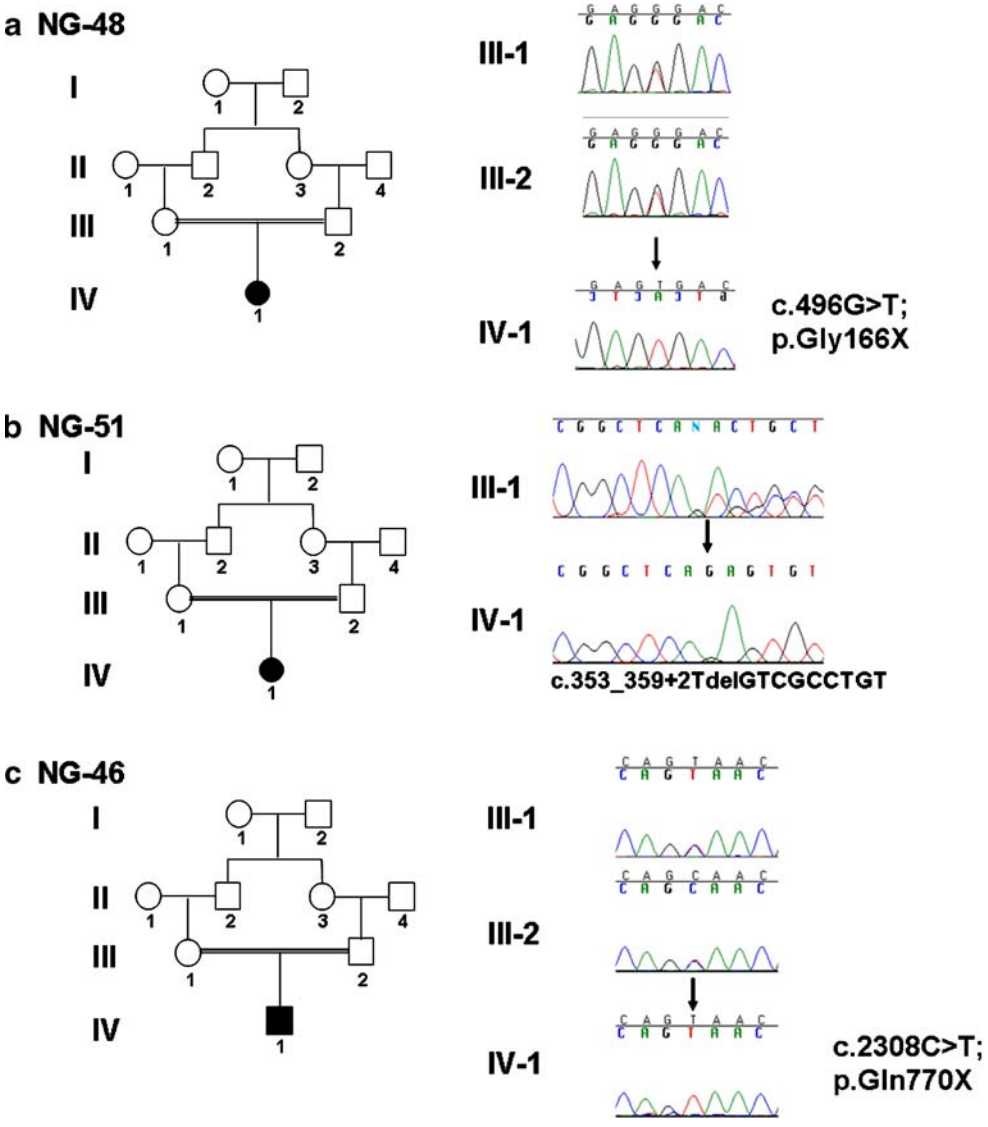

Fig. 1 Family pedigrees and mutation analysis. **a** Family NG-48. Mutation analysis demonstrated the index case was homozygous for the c.496G>T mutation. Both parents were heterozygous. **b** Family NG-51. The index case was homozygous and the parents were heterozygous (data only shown for III-1) for the c.353_359+2TdelGTCGCCTGT. **c** Family NG-46. The index case was homozygous and the parents were heterozygous for the c.2308C>A mutation

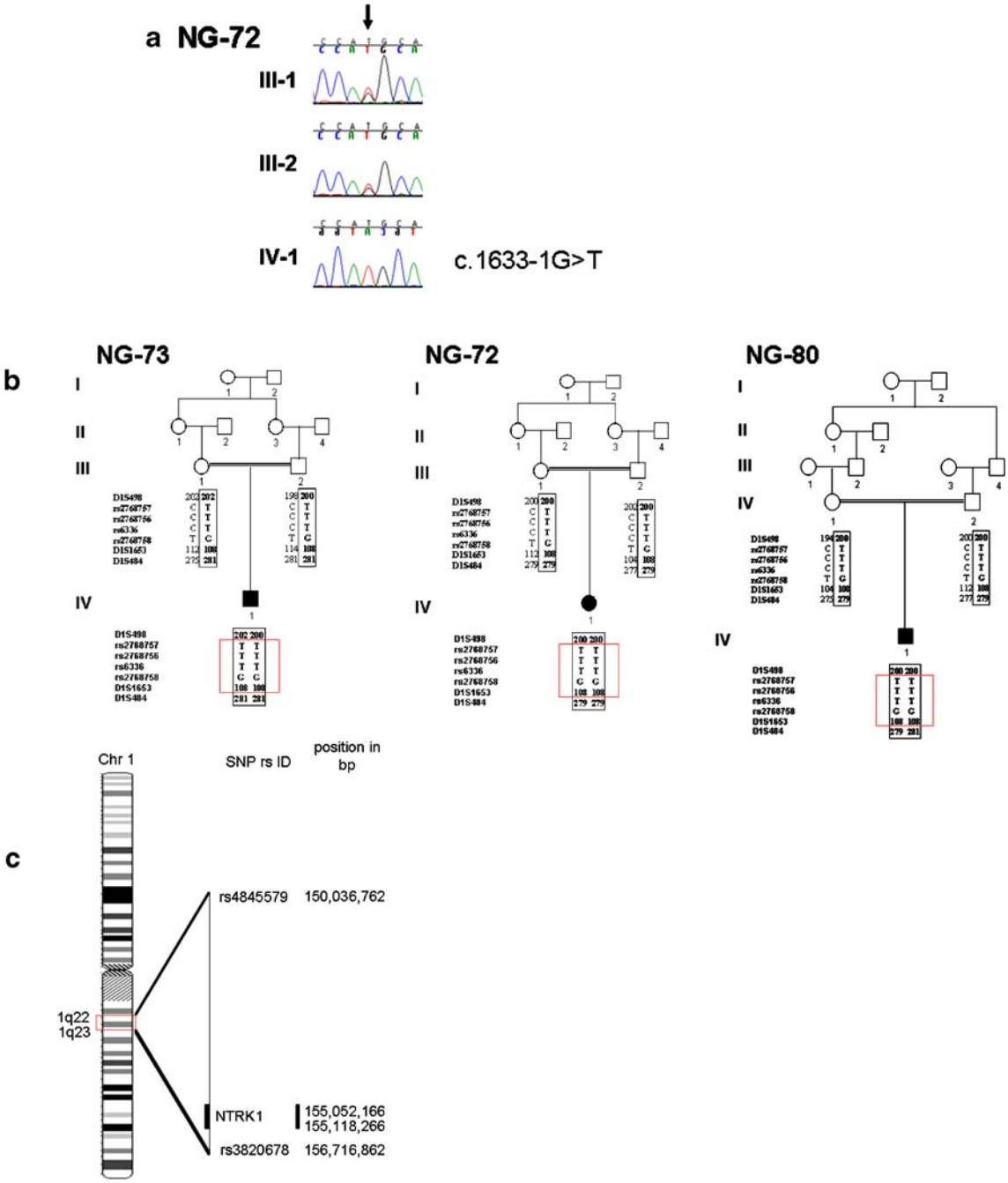

Fig. 2 **a** The three affected children in families NG-72, -80, and -73 share the same splice-site mutation, c.1633–1G>T (*arrow*). **b** Microsatellite and SNP mapping deomonstrates a founder effect. Pedigrees shown for families NG-72 (*left*), -80 (*middle*), and -73 (*right*). The parents were heterozygous and the affected patients were homozygous for the same D1S1653 and SNP haplotype. **c** Diagram demonstrating the span of the 6-Mbp founder effect allele defined by SNP marker rs4845579 at 150,036,762 and marker rs3820678 at 156,716,862. The location of the NTRK1 gene within this homozygous stretch of SNP markers is also shown

Family NG-48

The index case is a young female, diagnosed at age 3.5, the product of a consanguineous marriage between first cousins. She presented with anhidrosis and insensitivity to pain with multiple lesions on her hands, feet, lips, and mouth. Psychomotor development was abnormal with a delayed onset of walking (age 2.5) and an inability to form complete sentences. Her physical exam was notable for the aforementioned lesions and microcephaly (Fig. S2). MRI of the brain was normal. Upon questioning, the family endorsed a second, unspecified relative with similar symptoms.

Family NG-51

The index case is a 6-year-old female, the product of a consanguineous marriage between first cousins. She presented with insensitivity to pain and dry skin. On further exam, she was noted to have ulcerated lesions of her fingers and the soles of her feet and hyperkeratotic, dry skin (Fig. S3). Her psychomotor development was delayed as she first began to walk at 2 years of age, and could not form sentences at the age of 6. MRI of the brain revealed diffuse cortical and cerebellar atrophy. Upon questioning, the family reported another relative with similar symptoms.

Family NG-72

The index case is an 8-year-old female, the daughter of a consanguineous marriage between first cousins. She presented with anhidrosis and recurrent attacks of unexplained fever. She has two third-degree cousins with similar symptomatology. On exam, she was found to have multiple autoamputations of the fingers, toes, and tongue, along with multiple ulcerated lesions on these sites (Fig. S4). The remainder of her physical examination was unremarkable. MRI of the brain demonstrated moderate periventricular leukomalacia.

Family NG-73

The index case is a 2.5-year-old boy who presented to medical attention with anhidrosis, insensitivity to pain, and self-mutilating behavior (Fig. S5). He is the son of a consanguineous marriage between first cousins. He has a first cousin with similar symptoms. The remainder of his physical examination was unremarkable. Psychomotor development, MRI of the brain, and EMG are normal.

Family NG-80

The index case is a 14-year-old male, the son of a consanguineous marriage between second cousins. He presented to medical attention with anhidrosis and insensitivity to pain. He has delayed psychomotor development (walked at 3 years of age) and is unable to form sentences with speech. Physical exam demonstrated severe ulcerative lesions on his ears, fingers, and toes (Fig. S6). MRI of the brain was unremarkable. Upon questioning, the family reported another relative with similar symptoms.

Mutation analysis

Mutational analysis of the *NTRK1* gene in the six index cases and the patients' parents demonstrated a nonsense mutation in exon 5 (c.496G>T; *p.Gly166X*) in family NG-48; a nine-base-pair deletion in family NG-51 in exon 3 (c.353_359+2TdelGTCGCCTGT), resulting in a splice site mutation; and a nonsense mutation in exon 17 (*c.2308C>T*; *p.Gln770X*) in family NG-46 (Fig. 1). Families NG-72, NG-73, and NG-80, though unrelated according to available records, demonstrated an identical, novel mutation at the 5′ splice donor site on exon 14 (c.1633–1G>T) (Fig. 2). In all cases, both parents of the affected patients had a heterozygous mutation, while the affected patient was homozygous. The genomic variants and clinical features are summarized in Table 1.

Analysis of possible founder mutation using STR and SNP markers

We examined three available, informative STR markers (D1S498, D1S1653, and D1S484) located near the *NTRK1* gene and four SNP markers (rs2768756, rs2768757, rs2768758, and rs6336) within the gene in all three affected children and their parents for families NG-72, NG-73, and NG-80. All three affected members with the identical c.1633–1G>T mutation shared the same homozygous haplotype at marker D1S1653 (Fig. 2a,b). All affected patients were homozygous for the SNPs. To confirm these initial results and further delineate this region of shared homozygosity, all three affected patients were analyzed using Affymetrix 250K SNP arrays. The allele calls for the

Table 1 Summary of genomic variants and clinical features

Family no.	Age of index	Pain insensitivity	Anhidrosis	Hyperthermia	Psychomotor retardation	Abnormal brain MRI	Mutation
NG-48	4	+	+	−	+	−	c.496G>T; p.Gly166X
NG-46	10	+	+	−	+	−	c.2308C>T; p.Gln770X
NG-51	6	+	+	−	+	+	c.353_359+2TdelGTCGCCTGT
NG-72	8	+	+	+	+	+	c.1633–1G>T
NG-80	14	+	+	−	+	−	c.1633–1G>T
NG-73	5	+	+	−	−	−	c.1633–1G>T

Mutation notation based upon Human Genome Variation Society convention (http://www.hgvs.org/mutnomen/) and published gene structure [13]

three affected children in families NG-72, 73, and 80 demonstrated a region of identical, shared, homozygous alleles across a ~6-Mb stretch from marker rs4845579 at 150,036,762 to marker rs3820678 at 156,716,862 (Fig. 2c), which represents the common founder effect allele.

Discussion

The identification of *NTRK1* as the gene mutated in CIPA, or HSAN IV, has underscored the importance of the NGF/TRKA pathway in the development and maintenance of nociceptive and autonomic neurons, dovetailing the clinical constellation of signs and symptoms of patients with the molecular biology of neural crest cell differentiation upon NGF stimulation. The autonomic dysfunction typically seen in CIPA tends to take the form of anhidrosis and impaired thermoregulation with subsequent episodes of unexplained fever frequently leading patients or their families to seek medical attention. Interestingly, autonomic dysregulation in CIPA patients tends to be confined to anhidrosis, frequently sparing remaining sympathetic functions, despite the observation that *Trka*-deficient mice demonstrate miotic pupils and ptosis with no apparent anhidrosis [11]. More research will be needed to explain the differences in the NGF/TRKA pathway between humans and mice.

In this report, we studied six unrelated CIPA patients of Turkish descent, all of whom are the products of consanguineous marriages. Analysis of these patients in the *NTRK1* gene demonstrated four novel mutations. One of these, a nine-pair deletion, results in a splice site mutation in exon 3, while two point (nonsense) mutations led to premature stop codons in exons 5 and 17. It is likely that these mutations will either create a truncated NTRK1 protein or that the transcript is targeted for nonsense-mediated decay. Interestingly, we were unable to correlate the phenotype to the genotype; the three patients harboring the same mutation had different clinical findings (NG-72 had brain MRI abnormalities without psychomotor retardation, while NG-80 had profound psychomotor retardation with a normal MRI).

Two of the patients reported here have abnormal findings on brain MRI (NG-51 and -72). To date, only a single previous description of MRI data in a patient with a syndrome similar to our patients [23] has been reported. In the aforementioned report, serial MRI changes or damage to the white matter are documented and attributed to the high fevers in the patient [23]. It is possible that MRIs of the brain have not been documented within the literature in patients with HSAN IV, and furthermore, our findings might represent later sequelae of the disorder. Two additional patients had microcephaly (NG-46 and -48). Neither microcephaly nor any cranial abnormalities have been previously documented in HSAN IV. Our findings suggest that these clinical features should be added to the heterogenous spectrum of abnormalities in HSAN IV patients. Other findings (long bone fractures) are likely late sequelae of the pain insensitivity. Additionally, we cannot rule out contribution from other recessive genes in these consanguineous families, and certainly, additional mutations may be contributing to the severity of the phenotype seen.

Three unrelated families, NG-72, NG-73, and NG-80, had identical mutations in the donor splice site of exon 14, which likely resulted in this exon being skipped in the mRNA transcript, leading to a truncated protein (frame-shift). Again, further analysis is underway to confirm the precise effect this has on the transcript. These three patients are unrelated to the best of our knowledge, though they do originate from the same geographic area. These data represent a unique possibility of a founder mutation arising in an ancestor of these patients. All three affected children demonstrated loss of heterozygosity and share the same haplotype across a 6-Mbp region on chromosome 1, identifying a founder effect within the Turkish population. Furthermore, all three affected patients were homozygous for the SNPs we sequenced within the NTRK1 gene. Founder mutations in NTRK1 have been described previously within the Japanese [24] and Israeli-Negev Bedouin [25] populations, while others have found common variants suggestive of a founder effect [26].

The results presented herein broaden the spectrum of NTRK1 mutations causing HSAN IV and demonstrate the presence of a founder mutation in Turkey. This study will aid in the structure–function analysis of NTRK1 disease-causing mutations and will aid in genetic counseling of patients.

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Conflict of interest statement The authors report no conflicts of interest or competing interests.

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