Autosomal Dominant Erythermalgia Associated With a Novel Mutation in the Voltage-Gated Sodium Channel α Subunit Nav1.7

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Background: Autosomal dominant primary erythermalgia is a rare disorder characterized by recurrent attacks of red, warm, and painful hands and/or feet.

Objective: To describe the phenotypes and molecular data of a 10-member family with 5 symptomatic living patients with erythermalgia.

Results: The clinical phenotype of this family was featured by episodic or continuous symmetrical red swelling, irritating warmth, and burning pain of feet and lower legs provoked or aggravated by warmth and exercise, and relief was always obtained by application of cold, such as putting feet in (ice-) cold water. The symptoms in this family were only partially controlled by analgesics and sedatives. All affected family members were heterozygous for a novel mutation (S241T) of the voltage-gated sodium channel α subunit Nav1.7.

Conclusion: Primary erythermalgia may be a neuropathic disorder of the small peripheral sensory and sympathetic neurons, and may be caused by hyperexcitability of Nav1.7.

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PRIMARY OR IDIOPATHIC ERYthermalgia (Online Mendelian Inheritance in Man 603415) spontaneously arises during early childhood and adolescence in the absence of any detectable underlying disorder.[1-3] Primary erythermalgia is characterized by attacks or episodes of symmetrical red congestion and vasodilatation and burning pain of feet and lower legs provoked by exercise, long standing, and exposure to warmth that usually compel patients not to wear socks or closed shoes even in wintertime and to search for relief by immersion of feet in ice-cold water. The symptoms are refractory to treatment, and although many therapeutic options have been tested, there is no drug that consistently alleviates symptoms in these patients. Primary erythermalgia has been recognized as a separate clinical entity and proved to be a congenital disorder with documented autosomal dominant inheritance,[1,3-7] but sporadic cases have been reported as well.

A positional cloning effort located the gene for autosomal dominant erythermalgia on chromosome 2q.[4,5] Subsequently, Yang and colleagues[7] identified mutations in a gene named *SCN9A* in a family and in a single sporadic case with primary erythermalgia.[7] *SCN9A*, found at 2q24.3, is a 26-exon gene and encodes the voltage-gated sodium channel α subunit Nav1.7. The α subunits of the voltage-gated sodium channel form the sodium pore and are associated with accessory β subunits that modulate channel properties and interact with cytoskeletal and extracellular matrix proteins. Nav1.7 is found predominantly in dorsal root ganglia and sympathetic ganglia neurons.

Interestingly, Nav1.7 is located at the terminal of sensory neurons, and it is thought that its expression is regulated by inflammatory mediators. Most neurons that express Nav1.7 are nociceptors, and are characterized by slow recovery from inactivation and by slow closed-state inactivation that results in relatively large responses to small subthreshold depolarizations.[8] There is evidence to suggest that Nav1.7 likely plays some role in the transmission of nociceptive information. Indeed, small-diameter sensory neurons are thought to play a crucial role in several chronic painful neuropathies that arise from injury to peripheral nerves, such as those secondary to trauma, nerve compression, and diabetic neuropathy and those associated with postherpetic neu-

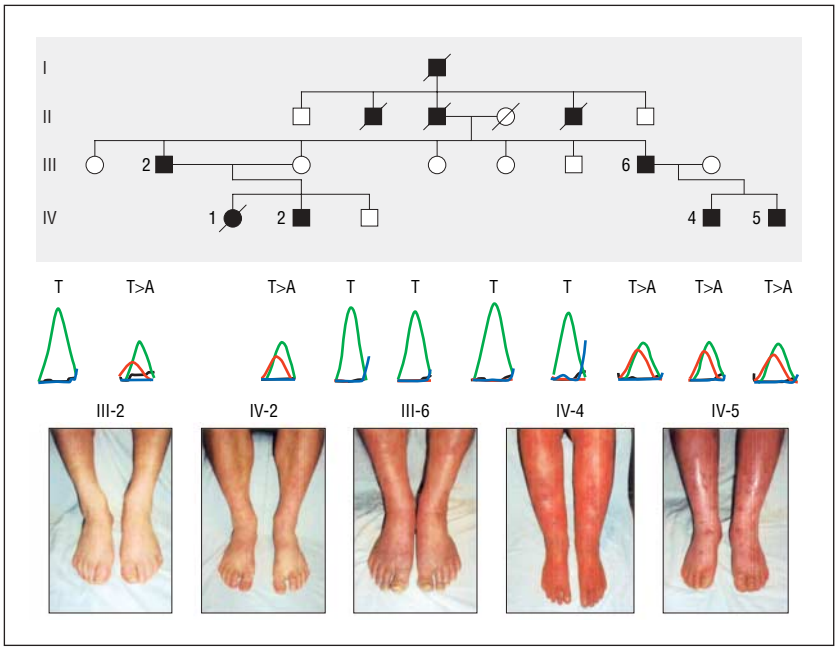

Figure. Pedigree structure, sequencing data, and phenotype. The pedigree and electrophoretograms of part of *SCN9A* exon 6 are shown (*SCN9A* is the gene that encodes the voltage-gated sodium channel α subunit Nav1.7). The DNA sequence for the affected family members revealed a heterozygous substitution of serine for threonine at position 721. There was complete segregation of the mutation with the disorder. Red erythematous lesions in patients with primary erythermalgia demonstrate that the disease is not only restricted to the feet but that it can extend to the lower legs and that cooling can cause ulcerative lesions. Squares indicate males; circles, females; shaded symbols, individuals affected by primary erythermalgia; unshaded symbols, healthy individuals; and symbols with a diagonal line, deceased individuals. T is the nucleotide in the DNA that is present in normal nonaffected individuals. In patients, it is replaced by an A nucleotide.

ralgia.[9] While these neurophysiologic data suggest a putative role of Nav1.7 in those with primary erythermalgia, confirmation of its presence in mutations outside the Chinese population is lacking. To answer this question on the causative role of the *SCN9A* gene in primary erythermalgia, we screened this gene in a Flemish family in whom primary erythermalgia was dominantly transmitted in 10 affected members of 4 generations.

METHODS

CLINICAL EXAMINATION

After approval of a research protocol by the Human Ethical Committee, the extended family was contacted. One of us (J.J.M.) visited the family members in their homes and performed structured interviews with affected and unaffected members. A standardized questionnaire was administered to elucidate symptoms and symptom characteristics and to evaluate measures the patients had taken to minimize symptoms. Standardized light photographs were taken from the available affected family members (**Figure**). A total of 10 participants (7 males and 3 females; mean [SD] age, 44.1 [19.1] years) were available for study, including 5 affected individuals (5 males) with ages ranging from 14 to 64 years.

GENETIC ANALYSIS

Blood was obtained from each subject by venipuncture. Genomic DNA was extracted from white blood cells according to standard procedures. In search for mutations in the *SCN9A* gene, we performed polymerase chain reaction (PCR) for each of the 26 coding exons. Primers were designed according to the published sequences (GenBank accession No. NM_002977.1). The PCR products always included the exon-intron splicing junctions. The PCR protocol included an initial cycle of denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at the melting temperature of the used primer set for 30 seconds and 1 minute at 72°C, and an extension step at 72°C for 5 minutes. The PCR products were purified after electrophoresis on an agarose gel with a kit (QIAEXII Gel Extraction Kit; Qiagen, Hilden, Germany). Both strands of the PCR products were sequenced directly using the chain termination method in the automated DNA sequencing facility at the University Medical Centre St Radboud, Nijmegen, on a sequencer (ABI3700; Perkin Elmer Applied Biosystems, Boston, Mass). We tested sequence variants for segregation among family members and with 100 control alleles.

RESULTS

CLINICAL PHENOTYPE

The pedigree includes 10 affected persons who fulfilled the clinical diagnostic criteria for primary erythermalgia (Figure).[10] Five patients were available for study. Four deceased patients had experienced attacks or episodes of moderate to severe painful red swollen feet. Patient IV:1 was described previously, and experienced the most severe erythermalgia, complicated by excruciating pain and skin lesions; this patient died at the age of 15 years.[3] The clinical features and outcome of treatment for 6 patients (5 alive) are summarized in **Table 1**. Patients III:2 and IV:2 have moderate attacks or episodes (lasting for a few days or a week) of typical erythermalgia elicited by warmth, exercise, and standing, and relief is always provided by cold. A typical episode is featured by extremely red symmetrically swollen feet associated with burning to aching pain for which patient III:2 only takes aspirin, 500 mg, but patient IV:2 needs morphinomimetic agents. Exposure of feet to ice-cold water or after prolonged air cooling is always followed by intense burning to aching pain. Between attacks, patients III:2 and IV:2 cycle between cold blue and red warm discolored feet and hands. An ambient temperature between 16°C and 19°C

Table 1. Clinical Features and Outcome of Treatment in 6 Affected Members of a Family With Autosomal Dominant Erythermalgia

Affected Member	Age, y		Distress Location	Redness Score*	Pain Score*	Skin Lesions	Current Status and Treatment
	Present	At Onset					
III:2	64	5	Feet/lower legs	2	1	No	No treatment, only minor symptoms
IV:1	15†	9	Feet/lower legs up to knees	3	3	Bullae/infections	Excruciating continuing pain, analgesics/sedatives brought no relief, not compatible with life
IV:2	32	2.5	Feet/lower legs/hands	2	2	No	Adaptation of lifestyle, bearable
III:6	48	8.5	Feet/lower legs	2	3	Minor	Methadone hydrochloride and occasional (ice-) cold water baths
IV:4	18	8	Feet/lower legs up to knees	3	3	Severe skin defects/ infections	Methadone/sedatives, air cooling of feet/legs day and night, and daily (ice-) cold water baths
IV:5	14	10	Feet/lower legs up to knees	3	3	Skin defects healed after discontinuation of cold-water baths	Analgesics/sedatives plus day and night air cooling of the feet and lower legs

*A score of 1 indicates mild; 2, moderate; and 3, severe.
†This patient is deceased.

is best for coping with the symptoms. Patient III:6 experienced severe erythermalgia, and his 2 sons (patients IV:4 and IV:5) continuously experience severe erythermalgia to a similar degree as patient IV:1. Patient III:6 could reasonably handle the distress during adult life when at work as a factory laborer at an ambient temperature of 16°C to 18°C, but used to cool his feet during lunchtime on a metal plate and at home by air cooling or immersion in cold-water baths. Patients IV:4 and IV:5 cool their feet by ventilator, day and night, and are treated with analgesics and sedatives by a multidisciplinary pain team. Patient IV:5 developed severe skin lesions due to daily cold-water baths in February 2004, but managed to stop this practice with analgesics, sedatives, and a lumbar-implanted neurostimulator, which resulted in healing of the skin defects of the involved lower legs and feet.

PEDIGREE AND GENETIC ANALYSIS

Pedigree structure and disease status are shown in the Figure, demonstrating an autosomal dominant pattern of inheritance. There were 5 affected subjects and 5 directly related unaffected family members available for study. All affected subjects were examined by sequencing and had a missense mutation at position 721 of the *SCN9A* gene. This mutation results in a serine to threonine replacement at codon 241 (S241T). There was 100% penetration, because the mutation was only present in affected family members (Figure). None of the unaffected subjects possessed the mutation. Furthermore, the S241T mutation was located on a region of the Nav1.7 protein that is highly conserved among species (**Table 2**).

Table 2. Amino Acid Sequence of the *SCN9A* Gene Mutation Region in Various Species*

Species	Amino Acid Sequence†		
	235, 236, 237	238, 239, 240	241, 242, 243
HS	VGA	LIQ	SVK
RN	VGA	LIQ	SVK
MM	VGA	LIQ	SVK
TN	VGA	LIQ	SVR
BG	VAA‡	LIQ	SVK
TP	VGA	VIE‡	SVK
Present patients	VGA	LIQ	TVK‡§

Abbreviations: BG, *Blattella germanica*; HS, *Homo sapiens*; MM, *Mus musculus*; RN, *Rattus norvegicus*; TN, *Tetraodon nigroviridis*; TP, *Takifugu pardalis*.
**SCN9A* is the gene that encodes the voltage-gated sodium channel α subunit Nav1.7. The amino acid composition of Nav1.7 from codon 255 to 263 is given for various species.
†The numbers correspond with the codon numbering of Nav1.7.
‡Different amino acids were found for this species.
§This is the S241T Nav1.7 mutation detected in our patients with primary erythermalgia. For comparison, the corresponding amino acids from various species are included. The S at position 241 is highly conserved among species.

COMMENT

Until recently, the cause of primary erythermalgia was completely enigmatic. The advances of the human genome project and previous linkage of this disorder to chromosome 2q have facilitated the discovery of the molecular pathophysiological features. We detected a novel missense *SCN9A* mutation (S241T) in our family. This confirms the essential role of Nav1.7 in mediating pain and indicates that primary erythermalgia is a neuropathic disorder of sodium channel dysfunction. Several lines of evidence suggest that S241T represents an activating mutation causing primary erythermalgia. First, there is perfect cosegregation of the mutation with the disease in our family. Second, the mutation affects the sodium ion transport–associated domain that determines ion selectivity and is central to Nav1.7 function. Third, the serine at position 241 is highly conserved among species, underscoring the functional importance of the change (Table 2). Thus, it is likely that mutation of serine 241 may cause a conformational perturbation

of the Nav1.7 protein. The mutations that previously have been associated with primary erythermalgia are located in the highly conserved II/S5 segment (L858H) and loop region between II/S4 and II/S5 (I848T) of Nav1.7.[7] The latter mutations seem to have functional consequences because transfected cells possess a hyperpolarizing shift in activation and slow deactivation.[8] Furthermore, they also cause an increase in amplitude of the current produced by Nav1.7 in response to slow small depolarizations. This gain of function is compatible with the notion that hyperexcitability of peripheral sensory and sympathetic neurons contributes to symptoms in those with primary erythermalgia. This hypothesis may be compatible with data from occasional cases indicating that treatment with mexiletine hydrochloride, a sodium channel–blocking agent, was favorable in reducing burning pain and red swelling to bearable proportions.[11] The specific physiological effect of the S241T mutation, however, needs to be determined by functional studies of mutated Nav1.7.[6,12] In summary, we report a novel mutation in the *SCN9A* gene associated with autosomal dominant primary erythermalgia. This not only extends the spectrum of mutations of this gene but provides firm confirmation that Nav1.7 sodium channel dysfunction underlies primary erythermalgia.

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