Primary Erythermalgia as a Sodium Channelopathy

Screening for SCN9A Mutations: Exclusion of a Causal Role of SCN10A and SCN11A

Joost P. H. Drenth, MD, PhD; Rene H. M. te Morsche, MS; Sahar Mansour, BMBS, FRCP; Peter S. Mortimer, MD, FRCP

**Objectives:** To elucidate the rate of missense mutations in the SCN9A gene (which encodes sodium channel Na_1.7) (OMIM 603415) among patients with primary erythermalgia and to examine the possibility that other sodium channels can cause the disease.

**Design:** Case series.

**Setting:** Department of Medicine, Radboud University Nijmegen, the Netherlands.

**Participants:** Six patients with sporadic and 9 with unique familial primary erythermalgia.

**Interventions:** Questionnaire to determine clinical profile and sequencing of all coding exons from SCN9A and those of SCN10A (OMIM 604427) and SCN11A (OMIM 604385) in 2 selected cases with a clear family history of the disease.

**Main Outcome Measures:** Detection of SCN9A mutation.

**Results:** We identified 1 patient with an SCN9A mutation. This mutation (I848T) has been associated with primary erythermalgia. Sequencing of 2 other candidate genes did not show mutations in 2 patients with familial primary erythermalgia.

**Conclusions:** The Na_1.7 voltage-gated sodium channels are related to syndromes of altered nociception. We detected a low SCN9A mutation rate in patients with primary erythermalgia, suggesting that pain syndromes in the skin may have a polygenic basis.

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Primary erythermalgia is a syndrome characterized by intermittent attacks of red, warm, painful, swollen extremities. Patients have complaints at a young age, mostly within the first decade of life, although in some the onset can be in middle age. The pain is usually described as intense burning without dysesthesia or numbness and is localized in the extremities, with the feet being more commonly affected. The disease is autosomal dominantly inherited, but sporadic cases do occur.

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Primary erythermalgia is sometimes termed erythromelalgia, but the latter is reserved for a condition that is caused by arteriolar inflammation resulting from platelet-rich thrombi in the end-arterial microvasculature. The platelet count is invariably elevated to more than 400 x 10^9/L (to convert the platelet count to x 10^9/L, multiply by 1), and aspirin relieves symptoms. Recent studies have shown that the gene responsible for primary erythermalgia is located on chromosome 2q, and that heterozygous mutations in SCN9A cause the disease. The sodium channel Na_1.7 is a member of a subfamily of sodium channels encoded by SCN9A that is part of a cluster of synthetic genes. This similarity is the reason why the various mammalian sodium channels share more than 50% of their amino acid composition of transmembrane and extracellular domains. The tissue expression of the sodium channel subtypes differ, and they can be located in central nervous system, skeletal muscle, and heart. The Na_1.7 is preferentially expressed within primary sensory nerves such as nociceptive dorsal root ganglion and sympathetic ganglion neurons. The Na_1.7 mutations that have been characterized so far can broadly be categorized as gain-of-function mutations. They cause hyperpolarizing shifts in activation, slow deactivation, and enhanced response to small depolarization. These changes cause increased response of Na_1.7 sodium channels to small stimuli, a conceptual explanation as to why patients with primary erythermalgia experience incapacitating burning pain.

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There appears to be evidence of genetic heterogeneity in cases of primary erythromelalgia, giving reason to explore the possibility that other genes encoding sodium channels might cause the disease. Apart from Na,1.7, at least 2 other mammalian sodium channels are located in the peripheral nervous system; Na,1.8 (encoded by SCN10A) and Na,1.9 (encoded by SCN11A) have a more restricted distribution pattern than does Na,1.7, are mainly expressed in small sensory neurons of dorsal root ganglia, and appear to fulfill a key role in the perception of pain. Interestingly, no phenotype has been assigned to either Na,1.8 or Na,1.9.

We undertook this study with 2 goals in mind. First, we wanted to detect the mutation rate among a mixed sample of patients with sporadic and familial cases of peripheral burning pain caused by primary erythromelalgia. In addition, we wanted to explore the possibility that sodium channels other than Na,1.7 can cause the disease.

METHODS

SUBJECTS

Blood samples for DNA analysis were obtained from subjects with familial or sporadic primary erythromelalgia. The study was approved by the institutional review board at the University Medical Center St Radboud, and written informed consent was obtained from the subjects. All patients included in this study had been clinically examined by experienced physicians. All patients had completed a structured questionnaire establishing the patient's history and clinical findings. Family history was considered positive if there was at least 1 relative with the disorder.

CASE DEFINITION

There is variation in the definitions of erythromelalgia and erythromelalgia in the literature. We have defined the phenotype of primary erythromelalgia as follows: attacks of bilateral or symmetric burning pain in the hands or feet; initiation or aggravation of attacks by standing, exercise, or exposure to heat; relief by elevation and cold; warmth, flushing, and congestion of the affected parts during attacks; and refractoriness to treatment. Erythromelalgia has the following phenotype: burning of the affected parts during attacks; and refractoriness to treatment by standing, exercise, or exposure to heat; resolution of attacks step at 72°C for 5 minutes. The PCR products were subjected to electrophoresis on an agarose gel, and appropriate bands were cut and purified by means of a gel extraction kit (QIAEXII; Qiagen, Hilden, Germany). Purified amplicons were used to perform sequencing by means of a terminator kit (BigDye; PerkinElmer Applied Biosystems, Boston, Massachusetts) according to the manufacturer's manual. Sequences were analyzed on a capillary sequencer (ABI3700; PerkinElmer Applied Biosystems). Sequence electropherograms were visually inspected and compared with gene sequences obtained from GenBank and with control samples. Furthermore, we tested sequence variants for segregation among family members when available (for an example, see the “Clinical Case” subsection of the “Results” section).

RESULTS

PATIENTS

We collected DNA from 15 patients with sporadic and familial disease who completely fulfilled the criteria for primary erythromelalgia (Table 1). The group consisted of 5 men and 10 women, with a mean age of 41.2 years. Eight patients were of Dutch ancestry, while there were 3 Americans, 2 patients from Belgium, and single patients from Sweden and England. The average age at onset of the symptoms was 25 years, with a considerable range (0-51 years) and standard deviation (20 years). The clinical symptoms were restricted to the feet in 6 cases, to the hands in 2 cases, and indiscriminately to hands and feet in 7 cases. The feet were most prominently affected. Nine patients had a family history of the disease. The most prominent phenotype was burning pain of the extremities, which was present in all patients. Patient 5 died at the age of 14 years as a result of exhaustion due to very severe refractory symptoms. None of the patients had elevated platelet counts, effectively ruling out the presence of erythromelalgia.

CLINICAL CASE

A 13-year-old girl (patient IV:3 [the daughter of patient 14]; Figure, A) presented with severe burning pain in the feet and, to a lesser extent, the hands, with intense heat (Figure, C). Symptoms started at 10 years of age and consisted of attacks of red, swollen, tight, and puffy hands and feet. The only relief from the pain was obtained by cooling and particularly from immersion in cold water. The biggest problem was usually at night during the summer, when her feet had to be left uncovered. One peculiar symptom was that she felt pain at the back of her mouth whenever she started to eat, especially with citrus flavors. Her family history was positive. She had 2 less severely affected brothers (patients IV:1 and IV:2; Figure, A) who both had ear involvement. Their mother (patient III:3 in the Figure and patient 14 in Table 1) started to have symptoms at the age of 3 years and had a phenotype similar to those of her daughter and sons.

DNA SEQUENCING AND DETECTION OF SCN9A MUTATIONS

Genomic DNA was purified from leukocytes from fresh drawn blood according to established protocols. We performed mutation screening for all 26 exons that constitute the SCN9A open reading frame. Mutational screening of SCN10A and SCN11A constituted sequencing of 27 and 26 exons, respectively (primer sequences are available on request). Genomic DNA was amplified by polymerase chain reaction (PCR) with oligonucleotide primers complementary to flanking intronic sequences. The 30-µL reaction mixture contained 200 ng of genomic DNA, 10 mM Tris hydrochloride (pH 9.0), 50 mM potassium chloride, 0.1% Triton X-100, 2 mM magnesium chloride, 0.25 mM dideoxynucleotide triphosphates, 100 ng of forward and reverse primers, and 3 U of Taq DNA polymerase. The PCR conditions were 5 minutes at 95°C, then 35 cycles of 30 seconds at 95°C, 30 seconds at the melting temperature (the temperature at which half of the DNA strands are in the double-helical state and half are in the random-coil state) of the used primer set and 1 minute at 72°C, and finally an elongation step at 72°C for 5 minutes. The PCR products were subjected to electrophoresis on an agarose gel, and appropriate bands were cut and purified by means of a gel extraction kit (QIAEXII; Qiagen, Hilden, Germany). Purified amplicons were used to perform sequencing by means of a terminator kit (BigDye; PerkinElmer Applied Biosystems, Boston, Massachusetts) according to the manufacturer’s manual. Sequences were analyzed on a capillary sequencer (ABI3700; PerkinElmer Applied Biosystems). Sequence electropherograms were visually inspected and compared with gene sequences obtained from GenBank and with control samples. Furthermore, we tested sequence variants for segregation among family members when available (for an example, see the “Clinical Case” subsection of the “Results” section).
MUTATIONAL ANALYSIS

**SCN9A**

We analyzed DNA from a total of 15 patients with primary erythermalgia for SCN9A mutations by automated bidirectional sequencing of PCR-amplified genomic DNA for each of the 26 SCN9A exons including the exon-intron junctions. We detected only 1 positive nucleotide substitution; it was at place 2543 of the SCN9A sequence of a single patient (patient 14) and resulted in a replacement of thymine by cytosine (Figure, B). As a consequence, the corresponding amino acid composition changed, with a substitution of isoleucine to threonine at codon 848. This mutation is predicted to be located within the sodium channel pore region at the S4-S5 linker region of domain II (DII-S4-S5) of the Nav1.7 channel.

We verified that this variant was a true mutation by screening for it in a sample of 100 controls derived from the same background population; screening confirmed that it was absent from this pool.

**SCN10A and SCN11A**

Given that erythermalgia is now defined as a small-fiber neuropathy due to sodium channel mutations, we proceeded to sequence 2 other candidate genes. We sequenced all coding exons and intron-exon boundaries of the SCN10A and SCN11A genes in 2 patients with a positive family history in whom DNA from affected and unaffected family members was available in our laboratories. Sequencing DNA from both patients failed to yield any detectable mutation in either gene.

**COMMENT**

Although the clinical phenotype was similar among patients from this cohort, sequencing did not disclose any other SCN9A mutation in the remaining 14 patients.

**Table 1. Demographic Data and Symptoms Reported by Patients With Primary Erythermalgia**

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Age at Onset, y</th>
<th>Ethnicity</th>
<th>Affected Part</th>
<th>Burning Pain</th>
<th>Family History</th>
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<td></td>
<td></td>
<td></td>
<td>Hands</td>
<td>Feet</td>
<td></td>
</tr>
<tr>
<td>1/M/52 (38)</td>
<td></td>
<td>Dutch</td>
<td>N</td>
<td>Y&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Y</td>
</tr>
<tr>
<td>2/F/47 (38)</td>
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<td>American</td>
<td>Y&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>3/F/44 (5)</td>
<td></td>
<td>American</td>
<td>N</td>
<td>Y&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Y</td>
</tr>
<tr>
<td>4/F/41 (4)</td>
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<td>American</td>
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<td>Y</td>
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<tr>
<td>6/F/37 (22)</td>
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<td>Y</td>
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<td>Y</td>
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<tr>
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<td>Dutch</td>
<td>Y&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Y</td>
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<tr>
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<td>Y&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td>N</td>
<td>Y&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

Abbreviations: N, absent; Y, present.

<sup>a</sup>Indicates the most severely affected extremity.
tation. The validity of this mutation was verified because it was absent from a pool of 100 controls and because it was earlier reported to be associated with primary erythermalgia in a French family and in 3 Chinese patients with sporadic disease.5,8,11 So far, it is the most common mutation found in patients with primary erythermalgia. This might reflect a founder effect, might suggest that this position within SCN9A is a mutational hot spot, or might be due to chance. The diversity of the population that harbors the mutation (Western European and Chinese) excludes a founder mutation. DNA has so-called hot spots where mutations occur up to 100 times more frequently than the normal mutation rate, but data available so far are too scarce to refute or support evidence that there are erythermalgia-specific SCN9A hot spots.11

Our study sample was well mixed with respect to sex, ethnicity, age at onset, and presence or absence of a positive family history. The diagnosis was based on well-defined criteria that have been used previously to classify patients. For example, these criteria allowed us to discern affected patients from unaffected family members in a previous linkage study. The presentation and clinical course of the patient with the mutation did not differ in any respect from those of the other (mutation-negative) patients. Thus, on the basis of these criteria, we are unable to predict which patients will carry an SCN9A mutation. One of the implications from these findings is that genes other than SCN9A may be implicated in primary erythermalgia. A literature search showed that 9 SCN9A mutations have so far been shown to cause primary erythermalgia (Table 2). Most mutations have been found in large families with a clear autosomal dominant inheritance pattern. Eight patients from our cohort had a positive family history but did not have any novel or previously discovered SCN9A mutations. Indeed, data from the literature suggest that primary erythermalgia is a genetic heterogeneous disorder, and our findings underscore this point.9 Because there is strong support for the notion that upregulation of sodium channel function is an essential component of the hyperalgesic response, we considered other peripheral nerve system sodium channels such as Na1.8 and Na1.9 to be candidate genes. There is experimental evidence that these genes are implicated in the mediation of pain.17 On the other hand, inflammatory mediators, including prostaglandins, serotonin, and adenosine, decrease the activation threshold, increase the rates of activation and inactivation, and increase the magnitude of the sodium current of tetrodotoxin-resistant channels such as Na1.8 and Na1.9.8,19 Despite the fact that Na1.8 and Na1.9 are not implicated in primary erythermalgia in our sample, these channels remain attractive candidates for neuropathic disorders.

One of our patients reported that she felt pain at the back of her mouth when eating. Symptoms in primary erythermalgia have been thought to be restricted to the extremities, although patients with severe cases also reported burning pain in body parts such as the tip of the nose, chin, and earlobes.10 This reminds us of another disorder, paroxysmal extreme pain disorder, which was formerly known as familial rectal pain. This syndrome is characterized by paroxysmal episodes of burning pain of the eye, rectum, and submandibulum accompanied by skin flushing. This syndrome appears to be allelic to primary erythermalgia because a recent study identified missense mutations in SCN9A in all affected family members with paroxysmal extreme pain disorder.20 Interestingly, the genetic basis of a peculiar autosomal recessive disorder, formerly known as congenital indifference to pain, has recently been elucidated. The most distinctive feature is that individuals have a congenital absence of sense of pain. Homozygosity mapping in 3 Pakistani families led again to identification of SCN9A as the culprit, but in this disorder the mutations produce complete loss of functional Na1.7 channels.21 These data emphasize the critical role of Na1.7 in human pain.

The Na1.7 is expressed within dorsal root ganglia neurons and corresponding sensory terminals and within sympathetic ganglia neurons, where it controls excitability by amplifying small depolarizing inputs. Functional analysis showed that the I848T Na1.7 mutation causes a hyperpolarizing shift in activation of the channel and a slower deactivation, phenomena that are accompanied by an enhanced response to small depolarizing stimuli. Collectively, the I848T mutation causes hyperexcitability and

### Table 2. SCN9A Mutations in Primary Erythermalgia Reported in the Literature

<table>
<thead>
<tr>
<th>Source</th>
<th>Type</th>
<th>Ethnicity</th>
<th>Exon</th>
<th>Nucleotide</th>
<th>Amino Acid</th>
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<td>Lee et al15</td>
<td>Familial</td>
<td>Taiwanese</td>
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<tr>
<td>Drenth et al5</td>
<td>Familial</td>
<td>French</td>
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<td>T647C</td>
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<tr>
<td>Michiels et al14</td>
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<td>Flemish</td>
<td>6</td>
<td>T721A</td>
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<td>Dutch</td>
<td>9</td>
<td>C1185A</td>
<td>N395K</td>
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<tr>
<td>Zhang et al5</td>
<td>Familial</td>
<td>Chinese</td>
<td>15</td>
<td>T2543C</td>
<td>I848T</td>
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<tr>
<td>Current report</td>
<td>Familial</td>
<td>English</td>
<td>15</td>
<td>T2573A</td>
<td>L858H</td>
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<td>Drenth et al5</td>
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<td>French</td>
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<td>L858F</td>
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<td>Yang et al,14 Zhang et al11</td>
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<td>A863P</td>
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<td>Han et al,12 Drenth et al6</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hardy et al19</td>
<td>Familial</td>
<td>American</td>
<td>23</td>
<td>T4393G</td>
<td>F1449V</td>
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</tbody>
</table>

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may lead to premature depolarization of the neuronal cell membrane, lowering the threshold for activation of other sodium channels located in the vicinity.22

Our genetic findings emphasize that inherited primary erythermalgia is a neuropathic disorder, suggesting that pain syndromes in the skin may have a polygenic basis.23 This corroborates with clinical findings obtained by neurophysiologic tests of small-fiber nerve function, which show that there is a high prevalence of small-fiber neuropathy in these patients.24

All in all, it appears that nerve voltage–gated sodium channels are associated with nociception, as gain of function partial mutations correlate with erythermalgia and paroxysmal extreme pain disorder, whereas null mutations lead to a syndrome of loss of pain sensation. Because Na1.7 is not a cardiac or central nervous system channel, systemic drugs may have fewer side effects and may have a role in the treatment of cutaneous pain syndromes such as neuropathic pain. Ultimately, the rapidly expanding knowledge on this specific channelopathy should lead to a targeted therapy for patients with painful primary erythermalgia.

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Author Contributions: Dr Drenth had full access to all the data in the study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Drenth. Acquisition of data: Drenth, te Morsche, Mansour, and Mortimer. Analysis and interpretation of data: Drenth, te Morsche, and Mortimer. Drafting of the manuscript: Drenth and Mortimer. Critical revision of the manuscript for important intellectual content: Drenth, te Morsche, Mansour, and Mortimer. Obtained funding: Drenth. Administrative, technical, and material support: Drenth, te Morsche, and Mansour. Study supervision: Drenth.

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References