

# Genetic heterogeneity and exclusion of a modifying locus at 2q in a family with autosomal dominant primary erythermalgia.

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## Summary

**Background** Primary erythermalgia is a rare disorder characterized by recurrent attacks of red, warm and painful hands and/or feet. In a previous study we reported localization of a gene for primary erythermalgia to a 7Æ94-cM region on chromosome 2q. A recent study reported voltage-gated sodium channel gene SCN9a sequence variants in a family and a single individual with primary erythermalgia.

**Objectives** To describe the clinical characteristics of a large three-generation family with primary erythermalgia and to test for genetic linkage to chromosome 2q.

**Methods** We collected clinical data of a 10-member three-generation family with autosomal dominant primary erythermalgia. In addition, we performed linkage analysis and searched for SCN9a variants using a restriction fragment length polymorphism assay.

**Results** We established the diagnosis of autosomal dominant primary erythermalgia in six of 10 family members. We excluded linkage to chromosome 2q and could not detect SCN9A variants in this family.

**Conclusions** In this family with autosomal dominant primary erythermalgia, exclusion of linkage to chromosome 2q is strongly suggestive for genetic heterogeneity.

Primary erythralgia (MIM 133020) is an autosomal dominant condition that is characterized by intermittent attacks of red, warm, swollen extremities.<sup>1,2</sup> Patients generally develop complaints at a young age, mostly within the first decade of life. Typically, attacks are provoked by warmth and exercise, but in more advanced cases, symptoms can be constantly present.<sup>3</sup> The pathology of primary erythralgia is unknown, although recent studies have suggested that increased microvascular arteriovenous shunting in deep dermal plexa inducing hypoxia is responsible for the pain.<sup>4</sup> It is not known, however, whether this actually underlies primary erythralgia or if it arises as a secondary phenomenon. Treatment of primary erythralgia is challenging, as a great variety of therapeutic options have been proposed but none has brought uniform relief.

A recent genome-wide linkage study placed the gene for primary erythralgia on chromosome 2q.<sup>5</sup> An analysis of recombinant events in one family identified marker D2S2370 at the centromeric end and marker D2S1776 at the telomeric end as flanking markers. More recently, Chinese investigators detected two sequence variants in a sodium channel  $\alpha$  subunit of SCN9a, a gene that is located in this interval in patients with primary erythralgia.<sup>6</sup> SCN9a encodes a voltage-gated sodium channel  $\alpha$  subunit and is predominantly expressed in sensory and sympathetic neurones.<sup>7</sup> These investigators found a T2573A mutation that segregated with the disease in a three-generation Chinese family, while a T2543C mutation was present in a sporadic case. This suggests that SCN9a mutations cause primary erythralgia, although confirmation is lacking to date.

We report a family with autosomal dominant primary erythralgia in whom we excluded linkage to chromosome 2q, strongly suggesting genetic heterogeneity.

## **Materials and methods**

### **Subjects**

Blood samples for DNA analysis were obtained from subjects belonging to a large three-generation Massachusetts family with primary erythralgia after informed written consent, in accordance with Lahey Clinic Institutional Review Board-approved protocol (LCID 2002-041). In the present study the diagnosis of primary erythralgia was confirmed by the patient's history and the clinical findings.<sup>3</sup>

### **Genotyping**

Linkage analysis to the disease interval on chromosome 2 was performed with seven microsatellite markers. We genotyped DNA samples by polymerase chain reaction (PCR) amplification of genomic DNA in the presence of  $\alpha$ -<sup>32</sup>P deoxycytidine triphosphate, electrophoresis on a 6% polyacrylamide gel and exposure to radiographic film.

### **Linkage analysis**

Linkage analysis and calculation of the two-point logarithm of odds (Lod) scores between the disease locus and each individual marker were performed using the MLINK and ILINK programs of the LINKAGE package (version 5.1).<sup>8</sup> Multipoint analysis was performed using the LINKMAP program by subsequent three-point linkage analysis on all tested markers. Haplotypes were constructed in such a way as to minimize the number of crossovers.

### **SCN9a mutation detection**

We searched for the T2543C and T2573A variants within the SCN9a gene using PCR followed by restriction fragment length polymorphism (RFLP). PCR was performed with the primers SCN9a exon 15 forward (5' -AAGATTTTCATAGTGATTAACAT- 3' ) and SCN9a exon 15 reverse (5' -GAGAAATTAATAATGCATGAAC- 3' ). The 437-bp PCR product was digested with BsrI resulting in a 108-bp and 329-bp fragment when the T2543C mutation is present or with MnlI resulting in a 142-bp and 295-bp fragment when the T2573A mutation is not present. For single-strand conformational polymorphism (SSCP), PCR products (4  $\mu$ L) of all exons were diluted 1 : 1 with loading buffer, denatured at 95 C, cooled on ice, and loaded on a 12% polyacrylamide gel with 10% glycerol. SCN9a primers are available upon request.

## **Results**

### **Clinical assessment**

Patient I2 developed symptoms of erythralgia at 76 years of age. Walking triggered attacks of red, hot and very painful feet. Symptoms progressed to be constant yet more severe with walking (Fig. 1). Pain was partially relieved by cold water and elevation of the legs. Patient II3 first developed symptoms at 47 years of age with symmetric burning pain in the feet and hands associated with redness and swelling, initiated by standing and exercise and partially relieved by cold and elevation of the legs. Nerve conduction studies were normal. Patient II2 described milder but similar symptoms in the feet and hands since the age of 48 years. Patient III5 developed typical symptoms of erythralgia in the hands and feet at 29 years of age. Nerve conduction studies were normal. Skin biopsy of the distal leg revealed normal epidermal and dermal nerve fibre density and morphology. Patient III3 developed similar symptoms in the hands and feet at 24 years of age. Patient III4 developed milder symptoms at age 21 years. Affected subjects had all the clinical criteria for erythralgia and no alternative aetiology was found. Affected subjects were largely refractory to symptomatic treatment.

### **Genetic heterogeneity**

This 10-member American family fulfilled the complete criteria for primary erythralgia but two-point linkage analysis with markers surrounding the gene locus on chromosome 2q yielded only negative Lod scores. We therefore decided to subject the results to multipoint analysis. Seven markers encompassing 11- 3 cM were used to generate a multipoint map of the region. Multipoint analysis revealed only negative Lod

scores, making it unlikely that this region harbours the gene for primary erythralgia. The results were corroborated by inspection of haplotypes showing that no single block of markers was shared among affected family members. In order to exclude the remote possibility that SCN9a is involved in the pathogenesis of primary erythralgia in this family, we performed SSCP of all 26 SCN9a exons, and were unable to detect differences in mobility of the PCR products among the affected and nonaffected family members. Lastly, we amplified exon 15 of SCN9a followed by specific RFLP. We could not detect the T2543C and T2573A variants that have earlier been implicated in primary erythralgia in any of the samples tested. All in all, this suggests that primary erythralgia is genetically heterogeneous and that more than a single gene can be responsible for the disease.

## **Discussion**

The absence of positive linkage to the chromosome 2q locus in our family with autosomal dominant primary erythralgia probably points to genetic locus heterogeneity. This is important information, as it was assumed that hereditary erythralgia was a monogenic disorder caused by a single gene defect.<sup>5</sup> This hypothesis was corroborated by the finding that the phenotype of the patients from our family is similar to that found in patients from other families that do link to the chromosome 2q locus. All affected members fulfil the requirements for primary erythralgia, as they have attacks of bilateral or symmetrical burning pain in the hands or feet, with initiation or aggravation of attacks by standing, exercise or exposure to heat, and relief by elevation and cold. Affected parts are warm, flushed and congested during attacks, and the disorder is refractory to treatment.<sup>1</sup> Recently, a preliminary study suggested that mutations in a sodium channel gene (SCN9a) are responsible for the disease.<sup>6</sup> We could exclude SCN9a using SSCP and RFLP as the causative gene in our family. Although the association of SCN9a and primary erythralgia needs confirmation, it is possible that voltage-sensitive sodium channels play a pathological role in primary erythralgia.<sup>7</sup> The voltage-sensitive sodium channel complex is composed of a subunits of 260 kDa that form the voltage-sensitive and ionselective pore and smaller auxiliary b subunit(s) of 33–36 kDa, which can modulate the kinetics and voltage dependence of channel-gating of the a subunits. The voltagegated sodium channel subunit encoded by SCN9a is denoted as Nav1.7, and is predominantly expressed in the peripheral nervous system.<sup>9,10</sup> It is likely that mutations in a gene other than SCN9a are responsible for the phenotype in our family. In the light of our results, it would seem logical to focus our attention on the other voltage-gated sodium-channel subunits. In this respect, the SCN10a gene seems a good candidate because the encoded protein, Nav1.8, is present in the peripheral sensory nervous system. Genes for the sodium channel auxiliary b subunit may also be good candidates.

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