Chronic non-paroxysmal neuropathic pain — Novel phenotype of mutation in the sodium channel SCN9A gene

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1. Introduction

Nav1.7 voltage-gated sodium channel is preferentially expressed in sympathetic neurons and nociceptive small-diameter sensory neurons of the dorsal root ganglion [1,2]. It has a cardinal role in pain transmission. This protein, the alpha subunit of a tetrodotoxin-sensitive voltage-gated sodium channel, plays a role in neuronal excitability and repetitive firing properties [3]. Nav1.7 is encoded by the SCN9A gene. Loss of function mutations in the SCN9A gene causes selective absence of pain perception. Affected patients exhibit profound insensitivity to pain of any type, often displaying painless fractures, burns and injuries. This phenotype is inherited in a recessive manner [4].

Gain-of-function mutations cause two distinct paroxysmal pain syndromes: inherited erythromelalgia (IEM) and paroxysmal extreme pain disorder (PEPD). IEM is characterized by episodic burning pain, flushing of the extremities, inflammation and swelling. PEPD is characterized by periodic pain particularly affecting the rectal, perioral and mandibular regions. Both IEM and PEPD are inherited in a dominant manner [4–8]. A recent study reports on a patient with mixed phenotype of both IEM and PEPD caused by A1632E mutation in the SCN9A gene [9].

To the best of our knowledge, no clinical syndromes caused by gain of function other than IEM and PEPD have been described with SCN9A mutations.

We describe a patient with a novel phenotype characterized by chronic non-paroxysmal pain syndrome without cutaneous autonomic manifestations. At onset the pain involved the feet ("sock distribution") typical to polyneuropathy. Only later pain generalized to broad body areas. This patient harbors a novel mutation R1550W in the SCN9A gene with a dominant trait.

2. Material and methods

2.1. Patients

Nine patients with unexplained chronic severe neuropathic pain were screened for SCN9A mutations. All patients were seen in the Neuromuscular Clinic at Wolfson Medical Center during the years 2006–2008.

Mutations were found in one patient (patient 1) whose clinical data is described. Informed consent form was obtained from all patients.

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2.2. Patient 1 (with SCN9A mutation)

69-year-old healthy woman with a negative family history developed burning pain and tingling in her feet at age 56. Symptoms gradually spread proximally to involve the legs and thighs. Two years after onset of symptoms skin biopsies from the ankle and thigh were performed at Johns Hopkins Medical Center in order to evaluate small cutaneous nerve fibers. Epidermal nerve fiber density was normal. The burning pain intensified and gradually spread to the abdomen, trunk, arms and lips. Three years after onset, pain was generalized, writhing and agonizing and occurred constantly during the day and night. The patient always graded her pain as maximal (grade 10) in repeated VAS scales. She did not have rash, swelling, erythema, excessive sweating or other cutaneous manifestations. Neurological examination demonstrated normal muscle mass, strength and tone. Tendon reflexes were brisk with Hoffman’s and Trommer’s signs. Plantar responses were flexor. Light touch, vibration, temperature, proprioception and pinprick sensation were intact. Motor and sensory nerve conduction studies were normal. Quantitative sensory testing (QST) showed normal thresholds to cold, warm, pain and vibratory sensations. Brain and cervical spine MRI was normal. Extensive laboratory workup showed no evidence of collagen diseases, Sjögren syndrome, metabolic abnormalities, HIV infection, Borrelia burgdorferi infection, endocrinopathy, B12 and B1 deficiency, paraproteinemia and paraneoplastic syndromes. Over the years the patients was treated with a wide range of anti-neuropathic pain agents, starting with monotherapy and later with combination therapy including: carbamazepine, gabapentin, amitriptyline, pregabalin, duloxetine, clonazepam, tramadol, valproic acid, topiramate, sertaline and mirtazapine. The patient is currently being treated with pregabalin 450 mg daily, tramadol 200 mg daily, clonaze- pam 2 mg daily and sertaline 100 mg daily. Under this combination therapy she experienced only mild alleviation of pain, and she continued to suffer from generalized constant and agonizing burning pain that significantly interfered with her quality of life.

2.3. Eight patients (without SCN9A mutations)

Two of the eight patients presented familial cases; one Ashkenazi and one Persian Jews. Three patients had similar symptoms as patient 1, although one patient exhibited milder pain. Two patients presented with severe genital pain (vulvodynia) and pain in the feet and legs.

2.4. Methods

Genomic DNA was extracted from peripheral blood by the Puregene kit (Gentra, Minneapolis, USA), according to the manufacturer’s instructions. Genomic DNA was used as a template for PCR amplification of each of the 28 exons of the NaV1.7 gene. The reaction was performed in a 50 μl volume containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 250 μM dNTPs, 1 μM of each primer, 100 ng of genomic DNA and 1.25U of AmpliTaq Gold DNA polymerase (Perkin Elmer Applied Biosystem) with an initial denaturation step of 10 min at 95 °C to activate the polymerase followed by 35 cycles of 94 °C; 15 s, 60 °C; 45 s, 72 °C; 45 s and a final elongation of 10 min at 72 °C. Predicted amplicon sizes were confirmed by agarose gel electrophoresis. The amplified PCR products were purified with the ExoSapIT kit (Amersham Pharmacia biotech, Amersham, Buckinghamshire, UK), according to the manufacturer’s instructions, and sequenced with fluorescently labeled dideoxynucleotide terminators and an Applied Biosystem 373A automated sequencer. Sequences were compared with the SCN9A transcript (ENSG00000169432).

3. Results

We have screened nine patients for mutations in the SCN9A gene. Seven patients presented normal sequences, one (patient 1) demonstrated predicted pathological mutation (W1550R) and one harbored a homozgyous substitution (V1625I) predicted to be benign by Polyphen and SIFT prediction softwares and due to its non conserved location. Patient1 had a heterozygous change of n.4648 T-C in exon 27 resulting in a substitution of W1550R (Fig. 1), predicting damage in the transmembrane S2 region, repeat IV (Polyphen and SIFT prediction softwares). This mutation was not detected in 50 controls.

4. Discussion

SCN9A encodes the alpha subunit of the sodium channel protein 9 (voltage-gated sodium channel Nav1.7). Nav1.7 comprises 1988 amino acids organized into four domains, each with six transmembrane segments (S1–6). The sequence contains 4 internal repeats, each with 5
hydrophobic segments (S1, S2, S3, S5, and S6) and one positively charged segment (S4) \([10]\). Segments S4 are probably the voltage-sensors and are characterized by a series of positively charged amino acids at every third position. The channel produces fast inactivating sodium current. Because of its slow closed-state inactivation, Nav1.7 produces depolarizing current in response to small depolarizing stimuli close to resting potential, thus amplifying small depolarization such as generator potentials. SCN9A is expressed strongly in dorsal root ganglion, with only minor levels elsewhere in the body. Isoform 1 is expressed preferentially in the central and peripheral nervous system while isoform 2 is expressed preferentially in the dorsal root ganglion \([3,11,12]\). SCN9A plays a role in pain mechanisms, especially in the development of inflammatory pain.

In IEM the mutations were shown to produce a hyperpolarizing shift in activation and slow deactivation and enhance the channel response to small depolarizing stimuli; changes that can confer hyperexcitability on cells in which the channel is expressed. All mutations show a common feature of hyperpolarizing shift in activation of the channel \([7,8,13–15]\).

Molecular analysis of the SCN9A gene in our patient revealed a dominant mutation: W1550R. This mutation is considered pathological for the following: a. It is predicted to cause a change in protein hydrophobicity, thus predicting damage in the transmembrane S2 region, repeat IV (Polyphen and SIFT prediction softwares). The transmembrane localization of the mutation is presented in Fig. 2. b. Tryptophan 1550 is a conserved amino acid (Fig. 3). c. It has not been found in the control subjects. This mutation is novel and has not been found in IEM and PEPD patients.

We did not find abnormal sequences in the rest of our patients, although some had resembled phenotype. These patients might have a large heterozygous deletion in the same gene which could not be detected by our method and can be detected by the MLPA method. A more plausible possibility is that other loci are involved in these phenotypes.

In contrast to previously known SCN9A phenotypes, our patient exhibited sustained non-fluctuating neuropathic pain without autonomic symptoms. To the best of our knowledge, this is the first report of these clinical features associated with SCN9A mutations.

Our results suggest that the clinical spectrum of SCN9A mutations is broader than previously appreciated. These mutations can probably cause not only the periodic pain-autonomic phenotypes (i.e. IEM-PEPD spectrum), but rather a more diverse spectrum of neuropathic pain syndromes. Our findings warrant the search for SCNA9 mutations in patients with resistant unexplained neuropathic pain.

References


