Int J Clin Exp Med 2018;11(12):13936-13940
www.ijcem.com /ISSN:1940-5901/IJCEM0080592

Case Report

No mutation in *NTRK1* or *NGF* in a Chinese patient with hereditary sensory and autonomic neuropathy type IV

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Received May 30, 2018; Accepted August 4, 2018; Epub December 15, 2018; Published December 30, 2018

Abstract: Hereditary sensory and autonomic neuropathy type IV (HSAN IV), or congenital insensitivity to pain with anhidrosis (CIPA), is a rare autosomal recessive disease which is characterized by childhood onset of a lack of pain sensation, anhidrosis, and mental retardation. Mutations in the neurotrophic tyrosine kinase receptor type 1 (NTRK1) responsible for neurotrophin signaling pathway have been associated with this disorder. In this study, we performed genetic analysis on the *NTRK1* and *NGF* genes in a Chinese patient with HSAN IV. The patient had classical clinical manifestations of HSAN IV, including anhidrosis, recurrent fever, absent pain perception, and mild developmental delay. No possible pathogenic mutation in *NTRK1* or *NGF* genes was found. The result indicates that genetic heterogeneity may be present in HSAN IV and further research is necessary to clarify the disorder.

Keywords: Congenital insensitivity to pain, hereditary sensory and automatic neuropathy type IV, neurotrophic tyrosine kinase receptor type 1, nerve growth factor, mutation

Introduction

Hereditary sensory and autonomic neuropathies (HSAN) are a clinically and genetically heterogeneous group of congenital peripheral neuropathies, primarily affecting the peripheral sensory and autonomic neurons. They are characterized by sensory and autonomic dysfunction due to faulty development of autonomic and sensory neurons. Patients usually exhibit prominent distal sensory loss manifesting insensitivity to pain. Based on the age of onset, inheritance pattern and additional features, HSAN is classified into 5 different subtypes (HSAN I-V) [1], with a new type added to the list recently [2]. HSAN IV, also referred as congenital insensitivity to pain with anhidrosis (CIPA, OMIM 256800), is an autosomal recessive disorder characterized by recurrent episodes of pyrexia, anhidrosis, insensitivity to noxious stimuli, self-mutilating behavior and mental retardation [3, 4]. Morphometric studies of peripheral nerves reveal loss of unmyelinated C-fibers and small-diameter myelinated Aδ fibers of afferent neurons [5, 6]. Innervations of sweat glands were absent resulting in anhidrosis and hyperthermia [7]. Insensitivity to pain is associated with defective development of small nociceptive neurons in dorsal root ganglia (DRG) [8, 9].

Mutations in the gene of the neurotrophic tyrosine kinase receptor type 1 (*NTRK1*) have been regarded causative for HSAN IV [10]. The human *NTRK1* gene, located on chromosome 1 (1q21-22), encodes a receptor tyrosine kinase for the nerve growth factor (NGF) that is autophosphorylated in response to NGF stimulation [11, 12]. The NGF/NTRK1 pathway is responsible for innervations of the skin by sensory and sympathetic axons, as well as for the development and survival of the DRG neurons carrying pain sensation [13].

To date, approximately 50 different missense, nonsense, and frameshift, as well as splice site mutations in *NTRK1* gene have been identified in patients with HSAN IV. The known mutations in the *NTRK1* gene are listed in http://www.molgen.ua.ac.be/CMTMutations/. In this study, we

present a Chinese girl with typical clinical features consistent with HSAN IV. *NTRK1* gene analysis revealed one intronic homozygous deletion, two heterozygous mutations, but on the basis of mRNA analysis, no possible pathogenic mutation was predicted. Since *NGF* mutation has been found in a patient with HSAN IV-like symptoms [14], *NGF* gene was also sequenced in this patient. But no mutation was found. Therefore, the data in this study indicate that genetic heterogeneity may be present in HSAN IV and further research is necessary to clarify the disorder.

Materials and methods

Case report

A 6-month-old girl, born to a healthy non-consanguineous Chinese couple, first presented to the hospital with a history of repeated episodes of high fever which responded to antipyretics poorly. This girl as well as her parents was examined. The clinical data were recorded from clinicians' observations.

Molecular genetic tests

After receiving informed consent from the child's parents and the approval from the University Hospital Ethics Committee, genetic analysis was done to the patient and her parents. Genomic DNA was extracted from whole blood using the SE Blood DNA Kit (Omega Bio-Tec) according to the manufacturer's instructions. Polymerase chain reaction (PCR) and sequencing of all 17 exons and their flanking sequences of the *NTRK1* gene were performed. Sequence variations were analyzed with reference to the wild-type sequence (GenBank Accession No. NG_007493). Furthermore, full length *NGF* gene was amplified from genomic DNA and sequenced.

Total RNA was isolated from peripheral blood leukocytes of the patient by using TRIzol according to the manufacturer's instructions (Invitrogen). RNA samples were reverse transcribed with Takara PrimeScript RT reagent Kit (Takara Biotechnology) using two pairs of *NTRK1* specific primers. The product was directly sequenced with both forward and reverse primers. All the primer sequences and experiment protocol used in this study are available upon request.

Results

Clinical data

This is a case of a Chinese girl who first came to the hospital because of recurring episodes of fever without obvious etiology when she was 6 months old and was clinically diagnosed with HSAN IV. There has been no similar patient within the family.

At birth the patient looked normal with the birth weight 3200 g delivered at term. Since she was 56 days old, she presented episodes of fever of unknown origin and an absence of sweating. The parents noticed that the patient exhibited an absence of normal reaction to painful stimuli such as falls and injections (e.g. for vaccinations). She began biting her tongue, lips, and the tips of her fingers after her teeth erupted at the age of 5 months. Emotional tearing was normal. Her developmental milestones such as lifting of the head, rolling and sitting were slightly delayed. The patient had hyperactivity and emotional lability. When the patient was 15 months old, she developed a long-lasting period of fever and died probably from intracranial infection.

On examination when she was 6 months old, her height and weight were a little below normal for her age and she was mildly mentally retarded. She had multiple autoamputations of the fingers and self-inflicted wounds on the tongue and lips from biting. Fungiform papillae of the tongue, corneal reflexes, and overflow tears were present. No corneal lesions were found. The skin of her palms and soles was thickened, dry, and mildly hyperkeratotic. Neurological examination revealed a generalized absence of response to painful stimuli. Deep tendon reflexes and plantar responses were normal. The remainder of her physical examination was unremarkable.

On investigation of this six-month girl, her serum uric acid level was normal. Complete hemogram revealed iron deficiency anemia. Other hematological and biochemical investigations were within normal limits and MRI of the brain was normal. The parents refused all invasive tests including nerve conduction study, intra-dermal histamine test, as well as skin and nerve biopsy.

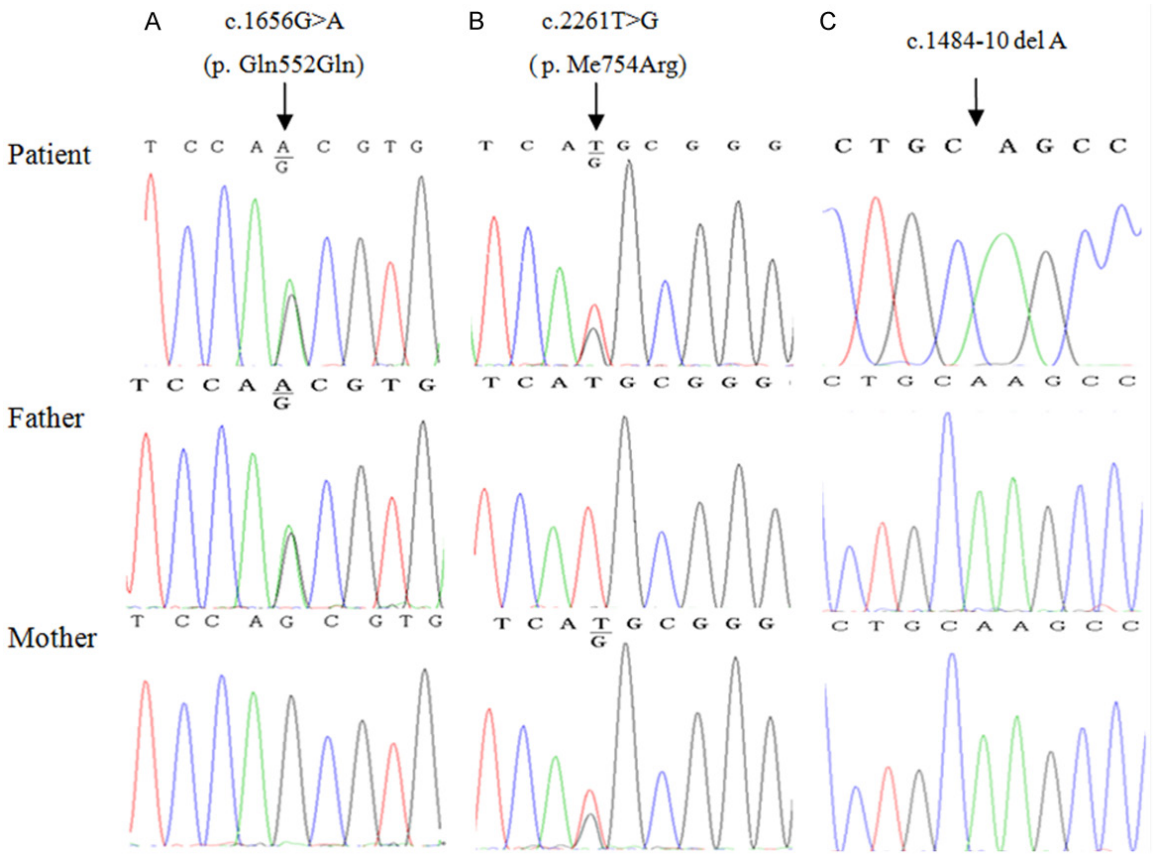

Figure 1. Partial sequencing results of the patient diagnosed as HSAN IV and her parents. A. Direct sequencing result (part of exon 14) showed that the patient had the mutation of Gln552Gln in heterozygous state and she inherited it from her father. B. The patient inherited the heterozygous missense mutation of p. Met754Arg from her mother as shown by the sequencing result (part of exon 17). C. The patient had a homozygous 1-base deletion in intron 12 (c.1484-10 del A), while neither of her parents carried it.

Molecular genetic analysis

The patient and her parents were screened for mutations in *NTRK1* gene, located at chromosome 1q21-q22. All 17 exons, including the flanking intronic sequences, were sequenced.

In the patient, two heterozygous mutations located in exon 14 and 17 respectively (c.1656G>A, p. Gln552Gln; c.2261T>G, p. Met754Arg) were detected. The family study showed that the patient had inherited the p. Gln552Gln and p. Met754Arg mutations from her father and mother, respectively (**Figure 1A**, **1B**). At the same time, the patient had a homozygous 1-base deletion in intron 12 (c.1484-10 del A), while neither of her parents carried it (**Figure 1C**).

Since HSAN IV is a recessive disease, heterozygous Met754Arg mutation was predicted and was not causative. To verify this and to find whether the mRNA was changed, sequencing of the *NTRK1* RT-PCR product was performed using peripheral blood leukocytes from the patient. The results showed that no change of *NTRK1* mRNA sequence was found. Subsequently, the 3 exons and the flanking intronic sequences of *NGF* gene of the patient were sequenced. The sequence of *NGF* gene of this patient was wild-type and no mutation was found.

Discussion

HSAN IV was first described in 1963 by Swanson in a pair of siblings affected by defect of temperature sensation and insensitivity to pain [4]. The clinical phenotype of HSAN IV is characterized by recurrent episodic fever, anhidrosis, insensitivity to pain, self-mutilating behavior, and mental retardation [10, 15]. It has been

suggested that diagnosis of this disorder requires three clinical criteria, i.e. anhidrosis, decreased pain perception and mental retardation [10]. But the degree of expression of these three clinical features, especially mental retardation, can vary greatly. So clinical diagnosis is primarily based on the fact that HSAN IV is the only HSAN associated with widespread anhidrosis [3]. The patient in this study showed characteristic febrile episodes, anhidrosis, insensitivity to pain, self-mutilating behavior, and mild mental retardation, which were consistent with HSAN IV.

Although defects of the gene *NTRK1*, which is the receptor of NGF, have been regarded as the main cause of HSAN IV, not all patients with this disorder can be traced back to mutations in this gene [16]. Currently, numerous mutations in *NTRK1* gene have been reported. So DNA diagnosis is not routinely used for clinical confirmation of diagnosis for HSAN IV [3].

After no pathogenic mutation was detected in *NTRK1* gene in this patient with typical HSAN IV clinical presentation, other possibilities were considered. HSAN V, caused by the mutations in *NGF* gene on chromosome 1 [17, 18], shares some similar presentations with HSAN IV, but with relatively mild or no mental retardation and significant anhidrosis and it selectively affects nociception [19]. Recently in a family where five affected children had a phenotype resembling HSAN IV, a novel homozygous NGF mutation was detected. Thus the author suggested that both genes should be investigated in families presenting any set of symptoms associated with both neuropathies [14]. So the *NGF* gene was further sequenced in this study, but no mutation was found.

There is also another report that no mutation in *NTRK1* or *NGF* genes could be identified in a patient clinically diagnosed as HSAN IV [16]. Although HSAN has been classified into 5 subtypes, each with its specific gene, the overall mutation rate is relatively low according to reports [20, 21]. Other genes in the process of NGF/NTRK1 signaling complex might be also involved in the mechanism of HSAN IV, such as neurotrophin-3 [22]. Whether peripheral pain transmission or central processing is involved in some patients with clinical presentations as HSAN IV, awaits further research. Furthermore, the difference between HSAN V and HSAN IV is confusing [19]. Mutations in *NTRK1* have been identified in patients with HSAN V-like symptoms [23], while a NGF mutation has also been reported in a family with the phenotype resembling HSAN IV [14]. Whether HSAN IV and HSAN V should be differentiated according to clinical presentations or genes remains unclear. This study requires further confirmation as to whether the patient could be diagnosed pathologically with the sural nerve biopsy and sympathetic skin response testing.

The result in this study indicates that genetic heterogeneity may be present in HSAN IV. Future research is necessary to improve our understanding of the mechanisms of HSAN and more HSAN families and patients are necessary to get a better insight into the molecular basis of these disorders.

Acknowledgements

This work was supported by Norman Bethune Program of Jilin University: 2015216 and grant from Jilin Provincial Science and Technology Department, China: 20180414049GH to Lei Zhang.

Disclosure of conflict of interest

None.

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