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Novel nonsense and frameshift *NTRK1* gene mutations in Chinese patients with congenital insensitivity to pain with anhidrosis

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ABSTRACT. Congenital insensitivity to pain with anhidrosis (CIPA; MIM 256800) is a rare autosomal recessive disorder characterized by absence of reaction to noxious stimuli, recurrent episodes of fever, anhidrosis, and mental retardation. It is caused by mutations in the gene coding for neurotrophic tyrosine kinase receptor type 1 (*NTRK1*; MIM# 191315). We screened two Chinese CIPA cases for mutations in the *NTRK1* gene and examined their phenotype. Two novel mutations of the *NTRK1* gene and two known mutations were identified. Including our two novel mutations, there are now 62 different *NTRK1* gene mutations reported in patients with CIPA. We find that a combination of two null alleles usually leads to the severe phenotype, while the mild form of the CIPA disease is associated with at least one mild allele. Thirty-four among the 62 mutations (55%) are located within the tyrosine kinase domain of the NTRK1 protein. We concluded that the tyrosine kinase domain is a hot spot for mutations.

Key words: NTRK1; Congenital insensitivity to pain with anhidrosis; Mutation; Tyrosine kinase domain

INTRODUCTION

Congenital insensitivity to pain with anhidrosis (CIPA; MIM 256800), also known as hereditary sensory and autonomic neuropathy type IV, is a rare autosomal recessive disease characterized by recurrent episodes of unexplained fever, anhidrosis, the lack of response to noxious stimuli, recurrent infection and mental retardation. Pain insensitivity often leads to self-mutilating behaviors such as tongue and hand biting (Vardy et al., 1979; Rosemberg et al., 1994). Abnormality in neutrophil functions and B lymphoblastoid cell lines could explain dysregulation of immune mechanisms and severe infections, which possibly bring about a chronic inflammatory response (Sato et al., 2004; Beigelman et al., 2009). A patient with CIPA has also presented recurrent infections secondary to hypogammaglobulinemia (Kilic et al., 2009). This disease was first described by Swanson in 1963. Indo et al. (1996) identified mutations of the *NTRK1* gene responsible for CIPA among Japanese families. It encodes a protein of 790 or 796 amino acids. A single transmembrane domain divides this protein into an extracellular domain and an intracellular tyrosine kinase domain (TKD). The former is important for NGF binding, and the latter is important for signal transduction (Mardy et al., 1999).

To our knowledge, there is no report of an *NTRK1* gene mutation on the Chinese mainland. In this study, we performed a mutation analysis of the *NTRK1* gene in two Chinese patients with typical CIPA and identified two novel and two known mutations. These mutations may be useful for expanding the database of *NTRK1* mutations. Including our data, 62 different *NTRK1* gene mutations have been reported in patients with CIPA from various ethnicities, including 22 missense, 11 nonsense, 19 frameshift, 9 splice-site mutations, and 1 gross deletion mutation (Figure 1) (Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac/index.php).

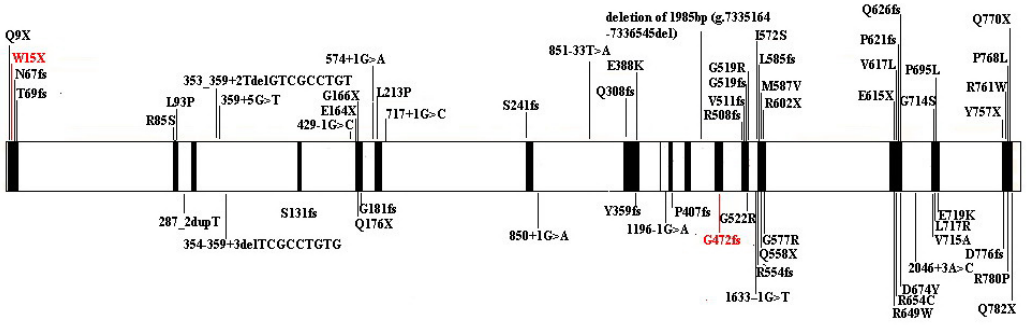

Figure 1. All mutations in the *NTRK1* gene in patients with CIPA. Red letters indicate the novel mutations found in this study.

MATERIAL AND METHODS

Patients

Patient 1 was a 5-year-old boy who presented manifestations of recurrent fever, insen-

sitivity to pain, anhidrosis, and dry skin from early infancy. He was born at term by normal delivery and had cerebral hemorrhage five weeks after birth. The skin of his palms and soles was thickened and hyperkeratotic. His developmental milestones such as crawling, sitting, standing, walking, and talking were normal. He had mild mental retardation and destructive behavior, but no history of osteomyelitis or other infections.

Patient 2, a 5-year-old girl, also presented the characteristic features of CIPA, including insensitivity to pain, inability to sweat, and self-mutilating behaviors. She was born after normal pregnancy and normal delivery, and had recurrent unexplained fever from the first week after birth. After teething, she showed serious self-mutilating behaviors such as biting her lips, fingers, and toes due to the absence of pain perception. On examination, she was found to have multiple self-inflicted wounds on the tongue and the fingers from biting. She had a history of osteomyelitis of both knees at 3 years old. Her father and mother were carriers of the *NTRK1* gene mutations, but they did not exhibit any neurological or immunological problems. The two families exhibited autosomal recessive inheritance.

Mutational analysis

This study protocol was approved by the Ethics Committee of Xinhua Hospital. After informed consent, peripheral blood samples were obtained from the two patients. We also collected blood from the parents of patient 2. In addition, samples from 100 unrelated population-matched controls were sequenced for mutation to exclude the possibility that polymorphism of the *NTRK1* gene was involved. We extracted DNA according to standard methods. We designed primers flanking all 17 coding exons and intron-exon boundaries of the *NTRK1* gene using the web-based version of the Primer 3.0 program (htttp://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). After amplification, the products were purified using a QIAquick PCR Purification kit (Qiagen). We sequenced the *NTRK1* gene using an ABI PRISM® 3730 automated sequencer (Applied Biosystems). Sequence comparisons and analysis were performed using Phred-Phrap-Consed Version 12.0 program. Mutations were identified by comparison with the reported cDNA reference sequence (GenBank accession No. NM_002529).

RESULTS

Patient 1 had one missense mutation and one nonsense mutation (Figure 2). The missense mutation (c.1945C>T, R649W) was located in the TKD of the NTRK1 protein. This mutation had been reported by Mardy et al. (1999). The nonsense mutation (c.44G>A, W15X) is a novel mutation. The patient had inherited the c.1945C>T and c.44G>A mutations from his mother and father, respectively. Patient 2 had a novel frameshift mutation due to the deletion of 1 bp in exon 12 (c.1415delG; p.Gly472fs). She had a splice-site mutation in intron 7 (c.851-33T>A), which has previously been proven to be a disease-causing mutation in Japanese and Korean patients with CIPA (Indo et al., 1996; Mardy et al., 1999; Lee et al., 2009). A family study found that the patient had inherited the c.851-33T>A and c.1415delG mutations from her mother and father, respectively (Figure 3). The clinical and molecular findings of the 2 patients analyzed in this study are summarized in Table 1.

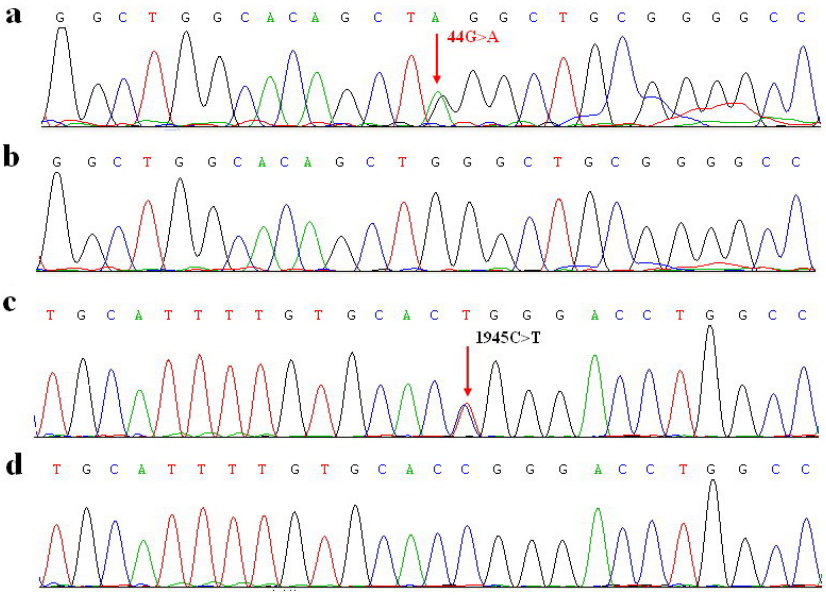

Figure 2. *NTRK1* gene mutations in patient 1. **a.** Heterozygous missense mutation c.44G>A in exon 1. **b.** Sequence of exon 1 of the *NTRK1* gene in normal subjects. **c.** Heterozygous missense mutation c.1945C>T in exon 15. **d.** Sequence of exon 15 of the *NTRK1* gene in normal subjects.

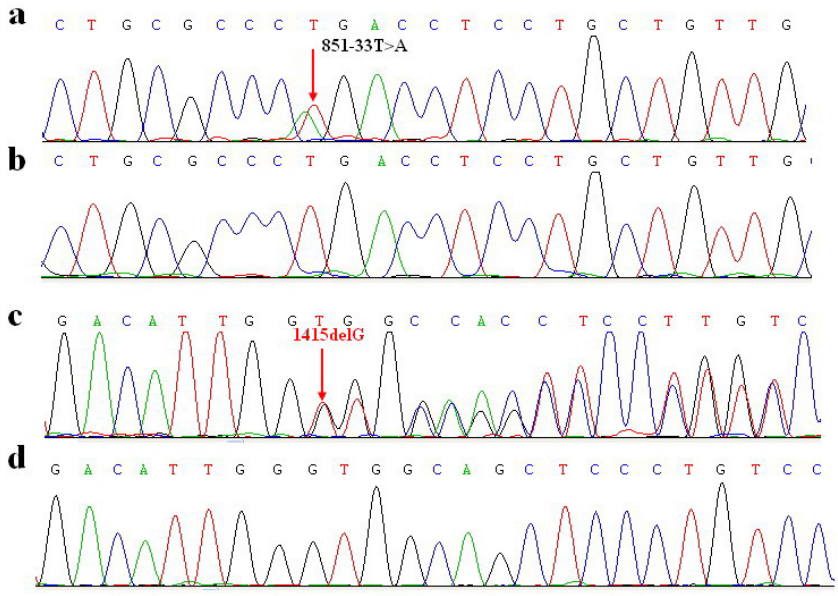

Figure 3. *NTRK1* gene mutations in patient 2. **a.** Heterozygous missense mutation c.851-33T>A in exon 1. **b.** Sequence of intron 7 of the *NTRK1* gene in normal subjects. **c.** Heterozygous frameshift mutation c.1415delG in exon 12. **d.** Sequence of exon 12 of the *NTRK1* gene in normal subjects.

Table 1. The clinical and molecular details of two patients with CIPA.

	Patient 1	Patient 2
Age, years	5	5
Gender	Male	Female
Height, cm	112	110
Weight, kg	19	20
Occipital frontal circumference, cm	48.8	49.4
Family history	-	-
Consanguinity	-	-
Wechsler Intelligence Scale		
Verbal	72	45
Performance	56	<40
Full scale	61	<43
Absent pain perception	+	+
Anhidrosis	+	+
Temperature sensation	+	+
Tactile sensation	+	+
Position sensation	+	+
Vibration sensation	+	+
Recurrent infection	-	+
Bone fractures	-	-
Sepsis	-	-
Recurrent fever	+	+
Developmental delay	-	-
Poor oral intake	-	+
Self-mutilation	+	+
Mutation 1	W15X	c.851-33T>A
Mutation 2	R649W	G472fs

Three known polymorphisms were identified: c.428+78C>T (rs2274497); c.428+197A>G (rs11264577) and c.574+100T>C (rs1800879). We detected 2 novel polymorphic sites at c.1196-68G>A and c.1251+119C>T.

DISCUSSION

CIPA is a rare hereditary disease characterized by anhidrosis, insensitivity to noxious stimuli, and mental retardation (Vardy et al., 1979). Indo et al. (1996) first demonstrated that the *NTRK1* gene is responsible for CIPA by identifying mutations in a region encoding the intracellular TKD of *NTRK1* responsible for CIPA in one Ecuadorian and three Japanese families. *NTRK1*, also called *TRKA* (tyrosine kinase receptor A), encodes a high-affinity receptor for nerve growth factor (NGF) that induces neurite outgrowth and promotes survival of embryonic sensory and sympathetic neurons (Mardy et al., 1999). The *NTRK1* gene spans >23 kb and contains 17 exons. The NTRK1 protein is composed of 790- or 796-amino acid residues.

So far, a total of 62 different mutations of the *NTRK1* gene have been reported in patients with CIPA (Greco et al., 2000; Miura et al., 2000b; Shatzky et al., 2000; Bodzioch et al., 2001; Indo, 2001; Indo et al., 2001; Mardy et al., 2001; Miranda et al., 2002; Bonkowsky et al., 2003; Guo et al., 2004; Huehne et al., 2008; Suriu et al., 2009; Lee et al., 2009; Lin et al., 2010). TKD of the NTRK1 protein is located in the codons from 501 to 796, which is approximately 37% of the full length of the TKD protein. Thirty-four mutations among the 62 mutations (55%) are located within the TKD of the NTRK1 protein. These results suggest that TKD may be a hot spot for mutations.

In this study, we found two novel mutations, including one nonsense mutation and one

frameshift mutation. The nonsense mutation (W15X) in patient 1 would result in a truncated protein lacking 781 amino acids. The frameshift mutation (p.Gly472fs) of patient 2 generated a pre-terminating codon (PTC) at 74 codons downstream of deletion site, and the NTRK1 protein synthesis should end there without translating the TKD in exons 13-17. These two mutations almost certainly cause defects in NGF signal transduction. One missense mutation (R649W) was detected in patient 1. This mutation has been reported by Mardy et al. (1999). R649W is highly conserved among the RTK family members. TRKA is a receptor TK that is phosphorylated in response to NGF. The mutant R649W in TKD has shown significantly diminished autophosphorylation in both neuronal and non-neuronal cells (Lin et al., 2010). The other known mutation (c.851-33T>A) of patient 2 was reported in Japanese and Korean patients with CIPA. Perhaps it is a hotspot mutation in Asian populations. In this mutation, A substitutes for a conserved T (U in the transcript) at the fourth position of the branch-site and causes aberrant splicing of intron 7 *in vitro*. Alternative splicing observed in the mutant allele results in an insertion of 137 bp between exon 7 and exon 8 (Miura et al., 2000a).

The correlation between genotype and phenotype is not straightforward in CIPA patients. We summarized the phenotype of patients 1 and 2, and found that the IQ of patient 1 was higher than that of patient 2 (Table 1). Patient 2 had a history of poor oral intake and osteomyelitis, whereas patient 1 did not. These results indicated that the combination of two null alleles usually leads to the severe phenotype, while the mild form of CIPA disease is associated with at least one mild allele that produces some residual function of the NTRK1 protein. Of course, more cases should be investigated because the data are still limited.

To date, *NTRK1* mutations causing CIPA have been identified in various ethnic groups. In this study, we found four mutations in patients 1 and 2. To the best of our knowledge, patients 1 and 2 are the first mainland Chinese patients with an *NTRK*1 gene mutation detected. It confirms that they have the same genetic background as other ethnic groups.

In conclusion, we have reported two novel mutations and two recurrent mutations of the *NTRK1* gene involved in CIPA in this study. These are the first *NTRK1* mutations reported in mainland Chinese. We enlarged the spectrum of mutations in the *NTRK1* gene and speculate the correlation between the phenotype and genotype.

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