Clinical Case Report

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Phenotypic and genotypic features of a pair of Chinese identical twins with congenital insensitivity to pain and anhidrosis

A case report

Ningbo Li, MD, Jiaoli Sun, MD, Shanna Guo, MD, Yi Liu, MSc, Cong Wang, MSc, Changmao Zhu, MSc, Xianwei Zhang, MD*

Abstract

Rationale: Congenital insensitivity to pain with anhidrosis (CIPA) is a rare autosomal recessive genetic disorder characterized by insensitivity to noxious stimulus and the absence of sweating. Fractures and joint destruction are common complications, but detailed studies on mineral and skeletal homeostasis are not available. Mental retardation is often reported, but detailed observations during childhood are lacking.

Patient concerns: A pair of 46-month-old Chinese identical twin brothers was presented at our hospital. The brothers had the typical manifestations of insensitivity to noxious stimulus, inability to sweat, and recurrent episodes of unexplained fever. Fortunately, they did not present common complications such as self-mutilation, trauma, bruise, and repeated bone fractures.

Diagnoses: Two novel compound heterozygous variants of *NTRK1* (c.632T > A and c.1253_1254delTC) were identified.

Interventions: The patients were subjected to routine and specialist clinical examinations. Daily care and symptomatic treatment were given.

Outcome: X-ray films of proband 2 showed a fracture in the first metatarsal. Decreased bone mineral density (BMD) and mild-to-moderate retardation of the Gesell developmental schedules (GDS), especially language and adaptability, were observed. Evaluation results for BMD and GDS in proband 2 were worse than those in his brother.

Lessons: The current findings expand our knowledge about the spectrum of phenotypic and genotypic features of CIPA, which will help facilitate future genotype–phenotype association studies. Daily care by parents promotes favorable outcomes in patients.

Abbreviations: BMD = bone mineral density, CIPA = congenital insensitivity to pain with anhidrosis, DQ = development quotient, GDS = Gesell developmental schedules, NGF = nerve growth factor, NTRK1 = neurotrophic receptor tyrosine kinase 1, QST = quantitative sensory testing, TrkA = tropomyosin-related kinase A.

Keywords: bone miner density, congenital Insensitivity to pain with anhidrosis, mental retardation, NTRK1, phenotype, variant

1. Introduction

Congenital insensitivity to pain with anhidrosis (CIPA) (OMIM #256800), also referred to as hereditary sensory and autonomic neuropathy type IV (HSAN IV), is a rare, autosomal recessive genetic disorder that is caused by a failure of nerve growth factor (NGF)-dependent primary afferents and sympathetic postganglionic neuron development.[1,2] The main clinical features are insensitivity to noxious stimulus, the absence of sweating (anhidrosis), recurrent episodes of unexplained fever, self-mutilating behavior, and variable mental retardation.[3]

One of the characteristic features of CIPA is multiple accidental injuries of the bone system, such as bone and joint fractures, and dislocation of the hip during physical development, which is the most common reason for physical disability in patients.[4] They often occur in the long bones and the calcaneus of the limbs, resulting in osteomyelitis, malformation, claudication, and even amputation.[5] Further key features of CIPA are mental retardation to variable degrees. Patients have intellectual disability (or learning disabilities) and severe attention-deficit-hyperactivity disorder.[6,7] Their behaviors are characterized as emotionally labile, irritable, hyperactive, and erratic. Susceptibility to infections is a common symptom in CIPA patients. Studies have revealed that patients are vulnerable to *Staphylococcus aureus* infection, and neutrophil chemotactic activity is impaired owing to lack of the NGF-tropomyosin-related kinase A (TrkA) signaling pathway.[8,9] In addition to the above-mentioned common symptoms, each patient will also have unique clinical features, for example, thin hair, eczema, or glaucoma.[10]

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Department of Anesthesiology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

** Correspondence: Xianwei Zhang, Department of Anesthesiology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095 Jie-Fang Road, Wuhan 430030, China (e-mail: ourpain@163.com).*

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Genetic studies have indicated that mutation of human neurotrophic receptor tyrosine kinase 1 (NTRK1), also known as TrkA, is associated with CIPA. *NTRK1* is located on chromosome 1q21-q22 and encodes 3 isoforms, the longest of which has 796 amino acids.[11] TrkA is a high-affinity receptor for nerve growth factor (NGF) and contains a single transmembrane domain.[12] The extracellular domain of TrkA specifically binds to NGF, which causes the dimerization of 2 TrkA molecules and autophosphorylation of the TrkA kinase domain tyrosine residues in the intracellular domain. A loss-of-function variant of NTRK1 may result in the absence of small myelinated (A-fibers) and unmyelinated nerves (C-fibers) and a loss of sympathetic innervation of the eccrine sweat glands.[11,13] Since the first report of the link between TrkA mutation and CIPA in humans by Indo in 1996,[14] more than 90 variants (http://www.hgmd.cf.ac.uk/ac/index.php), mostly missense and nonsense variants, but also small insertions, small deletions, and splice variants, have been identified in CIPA patients.

Although CIPA has been studied for half a century, detailed clinical data are still lacking. Here, we report the first Chinese identical twins affected by CIPA. We describe their medical history and clinical characteristics in detail, and assessed their bone development and intellectual development. Two novel variants of *NTRK1* were identified.

2. Methods

This study was approved by the Institutional Ethics Committee at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (20130501). Fully informed written consent was obtained from the patients' parents for publication of this case report and accompanying images.

2.1. Subjects and phenotyping

A pair of identical twins (proband 1 and proband 2) was recruited in this study at 46 months of age. The clinical features of the twins were assessed through a detailed questionnaire that covered a summary of CIPA symptoms, and previous medical records provided by their parents. The patients were interviewed and physically examined to confirm diagnosis. Quantitative sensory testing (QST),[15,16] including pressure pain, thermal pain, and acupuncture, and a perspiring test were conducted. Fasting blood samples were collected. Blood routine biochemical and serum immunization items were measured using an automatic biochemistry analyzer. X-ray imaging of the left metatarsal bone was performed for proband 2. Bone mineral density (BMD) was measured with an Ultrasonic Bone Densitometer (Omnisense 7000P; Sunlight Medical Inc., Israel). Mental development was evaluated using Gesell developmental schedules (GDS), which measure neurodevelopmental status on the basis of development quotient (DQ) scores in 4 domains (motor, adaptive, language function, and personal/social function).[17,18]

2.2. Genetic tests

Peripheral blood samples collected from the children and parents were anticoagulated with anti-EDTA and frozen at –80°C. DNA was extracted using TIANamp Genomic DNA Kits (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. The patients were tested using a targeted next-generation sequencing (NGS) gene panel provided by the Beijing Genomics Institute that covers 57 genes associated with known causes of inherited peripheral neuropathy, including *NTRK1*, *NGF*, *SCN9A*, and *DMT*. Library preparation and sequencing were performed using the Illumina MiSeq platform (Illumina Inc., San Diego, CA), according to the manufacturer's standard protocol. Sequencing data were analyzed in Sequencing Analysis, version 5.1.1, and were compared to a reference sequence (RefSeq ID, NM 002529.3) available in the NCBI database.

2.3. Functional analysis

Missense variant amino-acid substitution scores were assessed online (http://www.russell.embli-heidelberg.de/aas/), whereas conservation was determined using Clustal Omega. The pathogenicity of missense variants was predicted using PolyPhen-2 and SIFT. Mutated residues were mapped to the three-dimensional structure of NTRK1 deposited in Protein Data Bank (http://www.rcsb.org/pdb/home/home.do, PDB ID, 4F0I) in PyMOL version 1.8 (http://www.pymol.org/).

3. Case report

Proband 1 (the elder brother) and proband 2 were identical twin brothers 46 months old from nonconsanguineous parents. As the first symptom of CIPA, the patients developed high fever. Recurrent episodes of fever with unknown causes were uncontrolled during 15 days after birth, and antipyretics were ineffective. They were diagnosed as having severe pneumonia and were admitted to the intensive care unit at 1 month of age. Proband 2 experienced hyperpyretic convulsion 3 times at 3 months of age. The probands were insensitive to noxious stimulus, but both presented abdominal pain occasionally. The twins were more likely to fall down than their peers.

Their development milestones were delayed: they walked at 17 months and talked at 26 months. At 46 months, they could not speak complete sentences, and their "sentences" were limited to 3 words. There was no family history of these symptoms, and the probands have an unaffected sister.

3.1. Phenotypic features

At intake (46 months), the probands showed mild physical signs and symptoms. No obvious damage or bruises were found on their body. Palmoplantar hyperkeratosis was observed as significant fissuring of the plantar skin (Fig. 1). Fortunately, they had no bone fracture or dislocation and they show no self-mutilating behaviors, such as biting of the tongue, lips, and fingertips. For proband 2, mild malformation was found in the left foot (Fig. 1). Interestingly, both patients were extremely heat intolerant; they could not eat hot food or stay in a high-temperature environment. Irritability, hyperactivity, impulsivity, and acting-out behaviors were observed.

3.2. Clinical examination

Except for mildly elevated leukocytes and complement 4 slightly below the lower limit of normal, routine clinical blood parameters were within normal ranges (Table 1). The results of electrocardiography and electroencephalography were within normal ranges.

X-ray radiography indicated deformity of the first metatarsal in the left foot in proband 2. BMD at the mid-tibia was 3356 m/s (Z-score: –0.8, 20%) and 3270 m/s (Z-score: –1.6, 6%), in proband 1 and proband 2, respectively (Table 1).

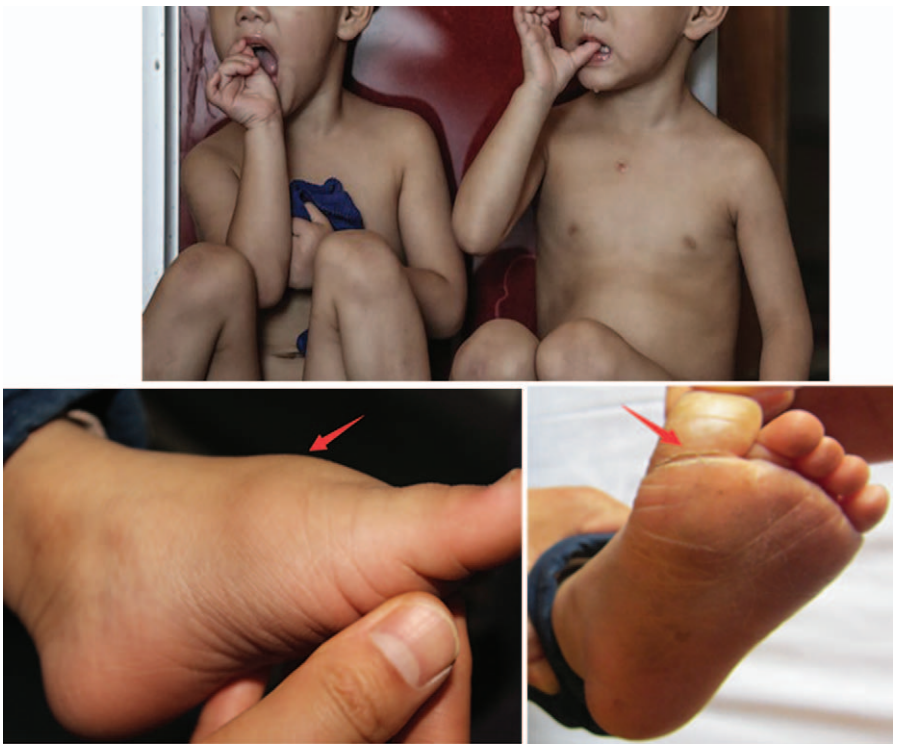

Figure 1. Photographs showing certain clinical features of the probands.

Evaluation of mental development of the twins on the basis of GDS (Table 2) indicated that proband 1 had motor and personal/social functioning abilities in the normal ranges. However, the DQ score of language functioning was only 47 and was within the moderate mental retardation range ($40 < DQ < 54$). The development of proband 2 was significantly worse than that of his older brother, and all 4 DQ scores were in the mild mental retardation range ($54 < DQ < 75$).

In quantitative sensory testing, the scores reached the upper limit of normal, and the probands showed had no evasive response in pressure pain testing of the skin and muscles. Their temperature sensation was normal, and they could clearly distinguish between cold and heat stimuli. However, cold pain tolerance threshold was absent. Interestingly, both patients withdrew their fingers within 4 seconds from under the thermal radiation stimulator and claimed less than 41°C to be "painful." Touch, vibration, and position senses were intact.

Table 1

Biochemical parameters and bone mineral density of the probands.

	Proband 1	Proband 2	Reference range
WBC	13.12	12.05	4.0–10.0 × 10^9/L
PMN (%)	52.5	41.1	
LY (%)	38.9	50.3	
MNC (%)	6.2	6.2	
IgA, g/L	0.78	1.01	0.82–4.53
IgG, g/L	7.7	9.6	7.51–15.6
IgM, g/L	0.76	0.67	0.46–3.04
C3, g/L	0.82	0.83	0.65–1.39
C4, g/L	0.11	0.12	0.16–0.38
IL-6, pg/mL	3.19	<1.5	< 7
Ca, mmol/L	2.39	2.48	2.15–2.55
BMD, m/s	3356	3270	
BMD (*Z*-score)	−0.8	−1.6	
BMD (%)	20	6	

BMD = bone mineral density, C3 = complement 3, Ig = immunoglobulin, IL-6 = interleukin 6, LY = lymphocyte, MNC = monocyte, PMN = polymorphonuclear, WBC = white blood cell.

3.3. Genetic analysis

A targeted NGS gene panel sequencing analysis was performed for the family. Two compound heterozygous variants of *NTRK1*, c.632T > A in exon 6 and c.1253_1254delTC in exon 11, were found, which were inherited from the father and the mother, respectively (Fig. 2). The missense variant, c.632T > A leads to the substitution of valine with glutamate at amino acid position 211 (p.V211E). Sequence alignment showed that the Val211 residue is highly conserved (Fig. 3). PolyPhen-2 and SIFT analyses both predicted that these missense substitutions were probably damaging. However, in the 3D structural protein model, the variant Val211 caused no apparent structural change (Fig. 3). The variant c.1253_1254delTC in exon 11 results in the

Table 2

Gesell developmental schedules scores and developmental age of the probands.

	Proband 1		Proband 2	
Domain of GDS	DQ	DA, months	DQ	DA, months
Motor	84	39	64	30
Adaption	68	31.5	54	25
Language functioning	47	22	58	27
Personal/social functioning	84	39	59	27.5

DA = developmental age, DQ = developmental quotient, GDS = Gesell Developmental Schedules.

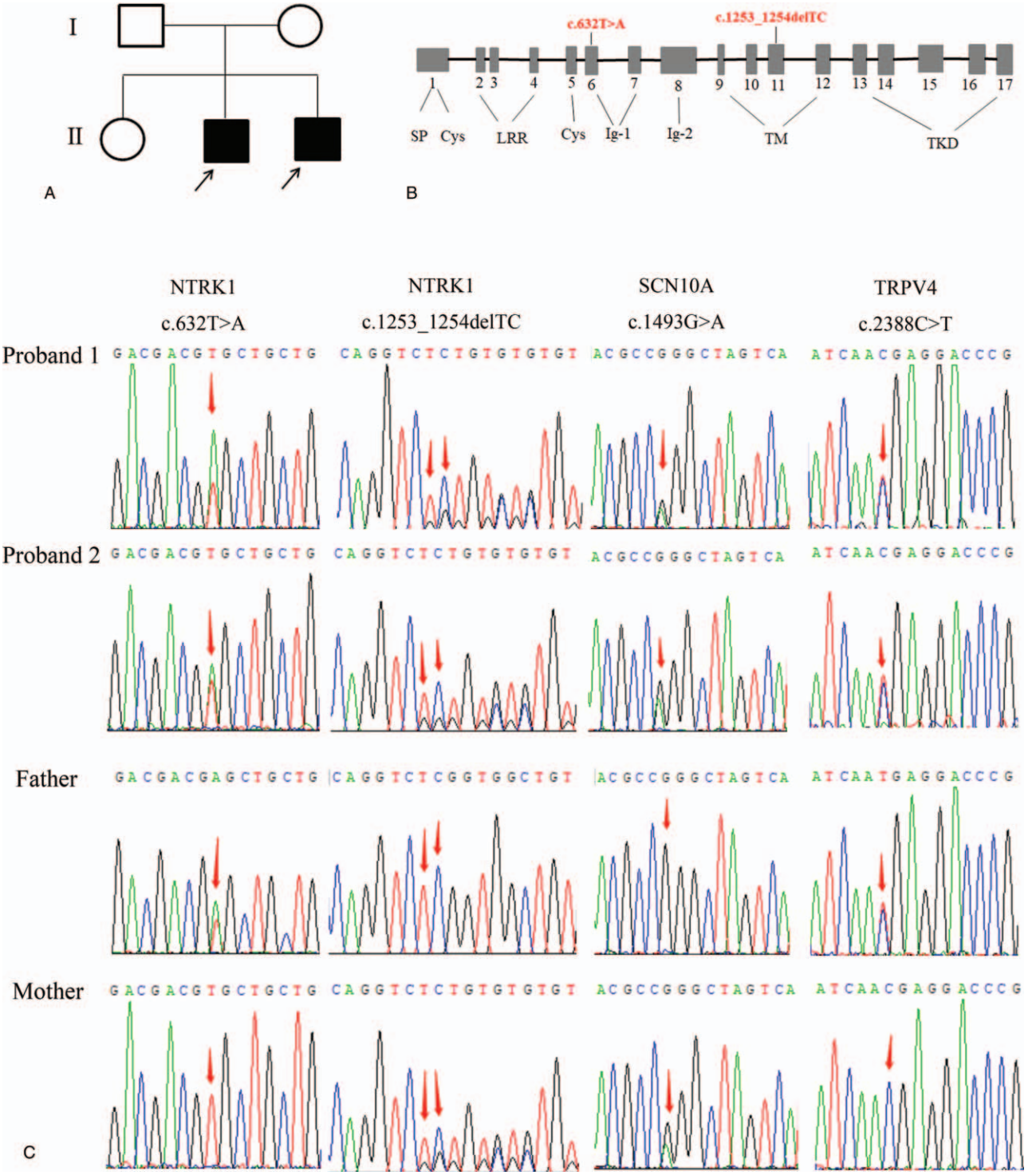

Figure 2. Genetic information of the twins. (A) Pedigree of the family. (B) Schematic illustration of *NTRK1* and the location of the 2 novel variants, c.632T > A and c.1253_1254delTC. (C) *NTRK1* sequencing results revealing that the patients had 2 compound heterozygous variants, c.632T > A and c.1253_1254delTC, inherited from the mother and the father, respectively, which might explain the symptoms in the patients. Variants of *SCN10A* (c.1493G > A) and *TRPV4* (c. 2388C > T) were identified in the family.

substitution of serine with glycine at position 419, leading to a frameshift that ultimately truncates the TrkA protein to 78 amino acids (p.S419Gfs*80).

Furthermore, heterozygous variants c.1493G > A (p.R498Q) in *SCN10A*, and c.2388C > T (p.N796N, rs116685089) in *TRPV4*, were found in the twins (Fig. 2C). PolyPhen-2 and SIFT suggested that the variant c.1493G > A is probably damaging. The frequency of the synonymous variant c.2388C > T in *TRPV4* is 0.0031 and 0.0009 according to the dbSNP database and the 1000 Genomes Project, respectively.

The identical twins were diagnosed with CIPA based on their phenotypic and genotypic features. However, they did not

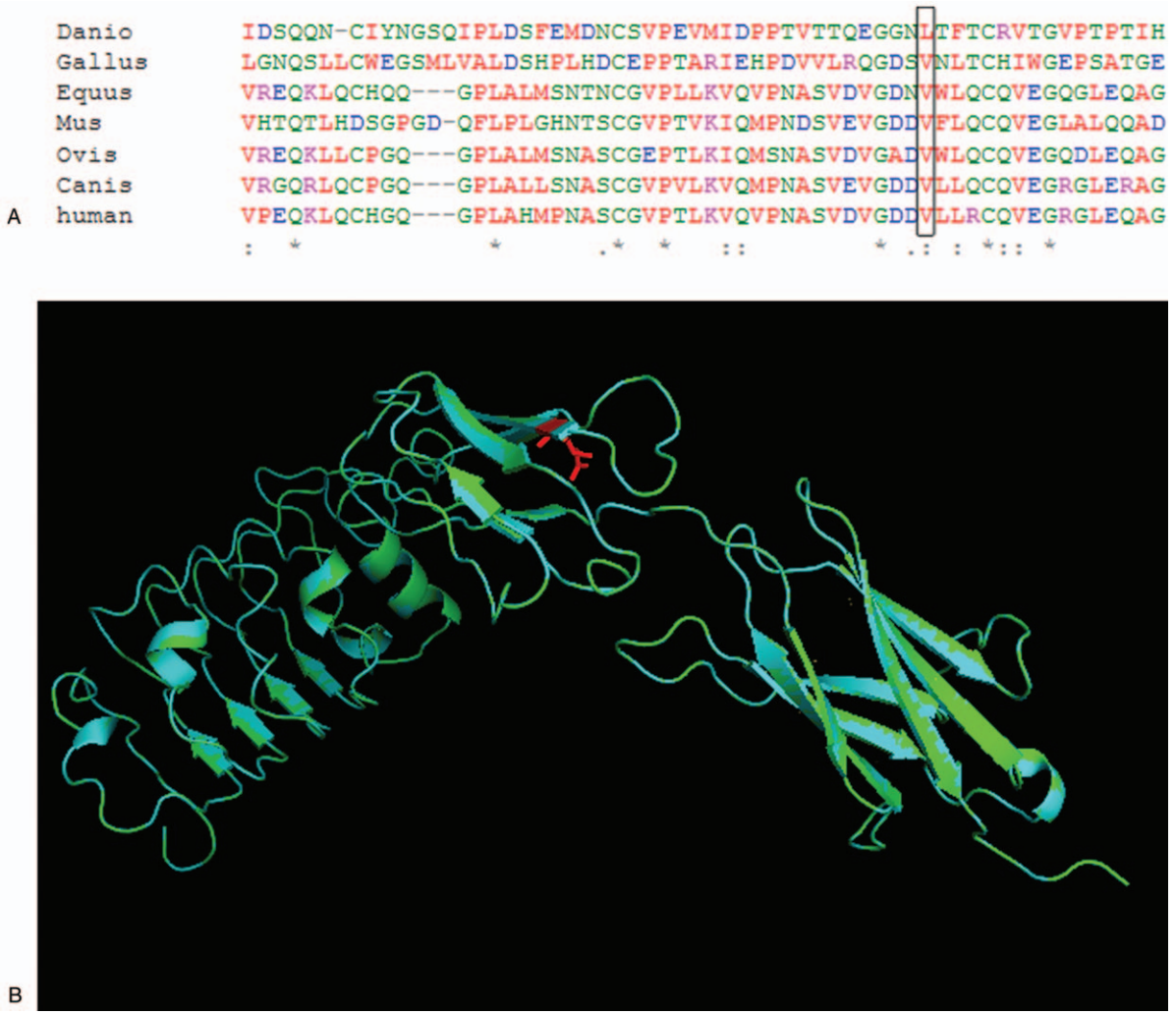

Figure 3. (A) Sequence alignment V211 (c.632T > A) of human *NTRK1* with *NTRK1* genes of 6 model species reveals that the gene is evolutionarily conserved. (B) Crystal structure of amino acids 498–796 of human NTRK1 (PDB ID, 4F0I). The catalytic domain (amino acids 1–498) is shown as green ribbons, in which variant V211 is colored red.

present obvious trauma, severe infection, or bone fracture, which are common and severe complications in CIPA patients. Daily care by the parents crucially promotes favorable outcomes in patients.

4. Discussion

Genetic analysis revealed 2 novel compound heterozygous variants of *NTRK1*: c.632T > A and c.1253_1254delTC. The substitution of valine with glutamate at amino acid position 211 (c.632T > A, p.V211E) results in significant deleterious effects; however, it does not cause significant changes in the tertiary protein structure. The variant c.1253_1254delTC creates a frame shift that produces a truncated protein (p.S419Gfs*80) that lacks the complete tyrosine kinase domain. Shaikh et al[19] demonstrated that pathogenic missense mutations do not necessarily lead to a complete lack of NGF-TrkA downstream signaling function. Thus, both variants are expected to cause loss of function, and the missense variant may have less influence on downstream signaling molecules, resulting in milder symptoms of CIPA.

Previous studies have reported the absence of pain and temperature sensation in individuals with CIPA to result from dysfunction of NGF-dependent primary afferents and sympathetic postganglionic neurons.[20] In this study, the twins presented abdominal pain occasionally. The twins had temperature sensation and even showed sensitivity to thermal stimuli. We tried to explain the interesting individual symptoms from a genetic perspective. We identified one putatively causative single nucleotide polymorphism in each of *SCN10A* and *TRPV4*. Previous studies have shown that increased expression of $Na_v1.8$ mRNA and activity of tetrodotoxin-resistant Na^+ channels in sensory neurons are associated with abdominal pain.[21,22] *TRPV4* is associated with thermal sensation.[23] Although the 2 variants identified have no pathogenic effect on patients, a large number of studies have shown that genetic polymorphisms have a certain influence on the clinical phenotype.[24]

Repeated multiple painless bone or joint fractures are the most common and most serious complications in patients with CIPA.[4] Fortunately, the twins had no history of fracture, as reported by their parents. However, the x-ray films showed that

proband 2 had a deformity of the first metatarsal in the left foot, maybe as a result of bone fracture. Further, increased serum bone resorption marker levels and decreased BMD were observed in the patients. In the past, researchers thought that multiple bone or joint fractures were due to pain-free protection. However, in most patients, the fractured bone was not subjected to strong external impact, and fractures more often occurred inadvertently. Recent studies have revealed that NGF-TrkA is involved in the vascularization and ossification of developing endochondral bone during embryogenesis.[6] Therefore, this study shows that bone fractures in patients with CIPA are not only caused by external forces, but also by the loss of functional TrkA-NGF signal, which leads to abnormal bone development and decreased BMD.[25] It is worth noting that daily meticulous care by the parents can efficiently help preventing fracture occurrence.

The majority of current intelligence assessments, the Wechsler Adult Intelligence Scale, are directed to adult (>18 years old) CIPA patients, and these patients generally have mild to moderate growth retardation.[26,27] Levy Erez et al[7] conducted a formal assessment of intelligence and adaptive behavior in 23 children with CIPA and identified an inverse relationship between age and the intelligence quotient in children. Thus, if mental development status can be fully defined before puberty, this can help us to more clearly and completely understand the impact of the disease on mental development and the positive effects of acquired learning interventions. In this study, we assessed the mental development of the twins, using GDS, which is used to measure the intelligence development level of infants of 0 to 6 years. To our knowledge, this study is the first to evaluate motor, adaption, language functioning, and social functioning status of children with CIPA. We found that the twins were mentally retarded. Language ability was the most affected, and was equivalent to that of normal children at 22 months and 27 months of age, respectively. Thus, the intellectual development of patients with CIPA gradually slows down in the early stages of child development. Proper education and mental exercise in early childhood may have positive effects on mental retardation.

Although the identical twins have the exact same genetic background and living conditions, their clinical phenotypes are different. According to our assessment results, the GDS scores of proband 2 were worse than those of proband 1. One of the reasons may be that proband 2 encountered repeated hypertensive convulsions due to unexplained fever after birth and improper nursing. This may have had profound effects on the development of the nervous system. This highlights that early diagnosis and effective treatment of high fever are very important for patients with CIPA. In addition, proband 2 had poorer bone development than his brother. Although no repeated multiple fractures were reported, his bone density is only 6% of that of his peers. We do not currently have a clear reason for the phenotypic heterogeneity, but perhaps, epigenetic mechanisms are involved.

In summary, our data expand the spectrum of clinical and genetic features associated with CIPA, which may be helpful for future clinical phenotype–genotype association studies.

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Author contributions

Data curation: Jiaoli Sun.
Formal analysis: Jiaoli Sun.
Investigation: Cong Wang, Changmao Zhu.
Methodology: Yi Liu.
Project administration: Xianwei Zhang.
Resources: Shanna Guo, Yi Liu.
Supervision: Ningbo Li.
Writing – original draft: Ningbo Li.
Writing – review & editing: Xianwei Zhang.

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