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Phenotypic heterogeneity of intellectual disability in patients with congenital insensitivity to pain with anhidrosis: A case report and literature review

Zhenlei Liu[1,2,*], Jiaqi Liu[1,3,*], Gang Liu[1,4,*], Wenjian Cao[5], Sen Liu[1,4], Yixin Chen[1], Yuzhi Zuo[1,4], Weisheng Chen[1], Jun Chen[1], Yu Zhang[6], Shishu Huang[7], Guixing Qiu[1,4], Philip F. Giampietro[8], Feng Zhang[4,5], Zhihong Wu[4,9] and Nan Wu[1,4]

Abstract

Congenital insensitivity to pain with anhidrosis (CIPA) is a rare autosomal recessive heterogeneous disorder mainly caused by mutations in the neurotrophic tyrosine receptor kinase 1 gene (*NTRK1*) and characterized by insensitivity to noxious stimuli, anhidrosis, and intellectual disability. We herein report the first north Han Chinese patient with CIPA who exhibited classic phenotypic features and severe intellectual disability caused by a homozygous c.851-33T>A

[1]Department of Orthopaedic Surgery, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, PR China
[2]Department of Neurosurgery, Xuanwu Hospital, Capital Medical University, Beijing, PR China
[3]Breast Surgical Oncology, Cancer Hospital of Chinese Academy of Medical Sciences, Beijing, PR China
[4]Beijing Key Laboratory for Genetic Research of Skeletal Deformity, Beijing, PR China
[5]State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai, PR China
[6]Berry Genomics Co., Ltd., Beijing, PR China
[7]Department of Orthopaedic Surgery, West China Hospital, Sichuan University, Chengdu, PR China
[8]Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
[9]Department of Central Laboratory, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, PR China

*These authors contributed equally to this work.

Corresponding author:
Nan Wu, Department of Orthopaedic Surgery, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences; Beijing Key Laboratory for Genetic Research of Skeletal Deformity, No. 1 Shuaifuyuan, Dongcheng District, Beijing 100730, PR China.
Email: dr.wunan@pumch.cn

mutation of *NTRK1*, resulting in aberrant splicing and an open reading frame shift. We reviewed the literature and performed *in silico* analysis to determine the association between mutations and intellectual disability in patients with CIPA. We found that intellectual disability was correlated with the specific Ntrk1 protein domain that a mutation jeopardized. Mutations located peripheral to the Ntrk1 protein do not influence important functional domains and tend to cause milder symptoms without intellectual disability. Mutations that involve critical amino acids in the protein are prone to cause severe symptoms, including intellectual disability.

Keywords
Congenital insensitivity to pain with anhidrosis, genetics, intellectual disability, neurotrophic tyrosine receptor kinase 1, north Han Chinese, *in silico* analysis

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Introduction

Congenital insensitivity to pain with anhidrosis (CIPA, OMIM #256800), also known as hereditary sensory and autonomic neuropathy IV, is an autosomal recessive disorder caused by loss-of-function mutations in the neurotrophic tyrosine receptor kinase 1 gene (*NTRK1*, OMIM *191315),[1] nerve growth factor beta gene (*NGFβ*, OMIM *162030),[2,3] and necdin gene (*NDN*, OMIM *602117).[4] The vast majority of mutations are observed in *NTRK1*.[5] CIPA is characterized by insensitivity to noxious stimuli, anhidrosis, and intellectual disability.[2,6] Classic symptoms of CIPA include recurrent unexplained fevers without sweating, self-mutilating behavior (e.g., biting of the tongue, lips, and distal extremities), recurrent bone fractures and Charcot arthropathy, nonhealing ulcers and injuries, and psychomotor retardation.[5,7,8] The pain insensitivity in patients with CIPA is caused by an absence of small-diameter thinly myelinated primary afferent or unmyelinated neurons, and the anhidrosis is caused by a deficiency of sympathetic postganglionic neurons.[5,9]

Phenotypic heterogeneity in the occurrence of intellectual disability has been reported in patients with CIPA. *Ntrk1*-knockout mice lack basal forebrain cholinergic neurons and striatal cholinergic neurons.[9] No autopsy data in humans with CIPA are available to validate these animal observations. Additionally, cranial magnetic resonance imaging has revealed no structural brain alterations in patients with CIPA.[10] We herein present a case involving a patient with CIPA with classic phenotypic features and severe intellectual disability caused by a previously reported homozygous c.851-33T>A mutation of NTRK1 gene. We performed a literature review and *in silico* analysis to determine the association between mutations and intellectual disability in patients with CIPA.

Methods

Patient

This study was approved by the Ethical Review Board of the Peking Union Medical College Hospital. Written informed consent was obtained from the patient's parents.

A 27-year-old Han Chinese woman was referred to our hospital in 2013 and diagnosed with CIPA. Her clinical

history was reviewed and a physical examination was performed. Her intelligence quotient was measured with the Wechsler Adult Intelligence Scale (WAIS IV, 2008 version). A nerve biopsy was not conducted because of the minimal benefit to the patient.

Genetic analysis

After obtaining informed consent, we drew 4 ml of peripheral blood from the patient and her parents. Genomic DNA was extracted with the TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's protocol.

All 17 exons and intron–exon boundaries were amplified by polymerase chain reaction (PCR) with primers used in previous reports.[6,11,12] Sanger sequencing was conducted by Berry Genomics Co., Ltd. (Beijing, China). The sequences were analyzed with Chromas software (version 2.1.1) and BLAT to UCSC Human Genome (Feb 2009, GRCh37/hg19, http://genome.ucsc.edu/) to call mutations.

The oligonucleotide-based comparative genomic hybridization (CGH) microarray is an efficient and reliable assay for copy number variation studies. The Agilent 1x1M Human Genome CGH microarray (Agilent, Santa Clara, CA, USA) was employed in this study. DNA processing, microarray handling, and data analysis were conducted by following the Agilent oligonucleotide array CGH protocol (version 6.0).

Literature review

The MeSH terms "congenital insensitivity to pain with anhidrosis" and "ntrk1 receptor" were used to search for articles pertinent to CIPA caused by *NTRK1* mutation. Title and abstract screening was conducted to retrieve genetic studies, which elucidated genetic mutations causing CIPA. All mutations reported up to October 2017 were reviewed. Furthermore, using the known Ntrk1 protein structure (available at the RCSB Protein Data Bank, http://www.rcsb.org/pdb/home/home.do) and PyMOL software (Version 4.3.0), we attempted to interpret the heterogeneity of the intellectual disability in patients with CIPA.

Results

Clinical findings

The patient in the present case was a 27-year-old Han Chinese woman (Figure 1(a), III:3) born to nonconsanguineous parents (Figure 1(a), II:6 and II:7). Her mother had a history of a terminated pregnancy (Figure 1(a), III:1) and fetal death at 7 months of gestation (Figure 1 (a), III:2). The proband had a 27-year history of anhidrosis and insensitivity to pain and had experienced recurrent unexplained fevers (maximum body temperature, 42°C) during her first year of life. Bilateral dislocation of the hip joints had been present since age 2 years. Although her body development was normal, she showed cognitive impairment at a young age. Self-mutilating behavior was first observed at age 6 years and resulted in finger, ankle, tibia, hip, and femur fractures. She developed osteomyelitis due to poor healing following trauma or surgical treatment of fractures. She required a wheelchair for ambulation because of deformation of her right hip joint, right knee, and left ankle (Figure 1(b)). Her intelligence quotient was about 44 (WAIS IV, 2008 version). She showed emotional instability and communication difficulty. The clinical characteristics of patients with CIPA with the hotspot c.851-33T>A mutation are summarized in Table 1.

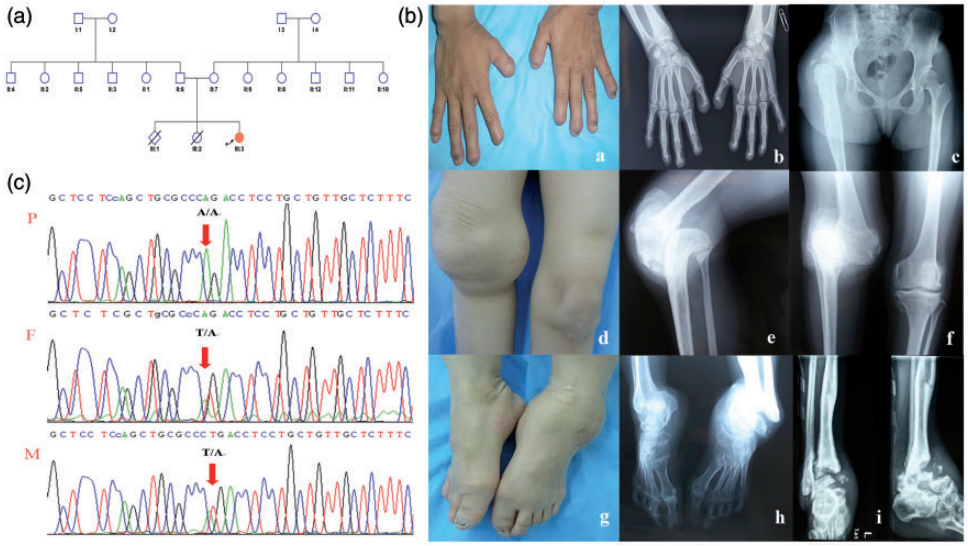

Figure 1. Patient's pedigree and clinical and imaging characteristics. Panel A: Pedigree of the patient (P), who is indicated by an arrow. Panel B: Clinical characteristics and imaging of the proband. (a, b) Hypoplasia of the distal phalanges of both hands due to self-mutilation and osteomyelitis. (c) Deformity of the hip joint. (d–f) Malunion of the right knee. (g–i) Deformity and multiple fractures of the ankle and tibia. Panel C: Sequencing of *NTRK1* reveals the homozygous c.851-33T>A mutation in the patient (P) and heterozygous c.851-33T>A mutation in her father (F) and mother (M).

Genetic analysis

PCR followed by Sanger sequencing of *NTRK1* was completed. Because previous reports have indicated that point mutations in the intron cause abnormal splicing and resultant CIPA, we repeated the analysis and confirmed the presence of a homozygous c.851-33T>A mutation in intron 7 in the patient and a heterozygous c.851-33T>A mutation in both of her nonconsanguineous parents (Figure 1, Panel C). The CGH microarray showed no evidence of copy number variation in her genome (data not shown), validating the homozygosity of the c.851-33T>A mutation. This mutation was previously verified to result in aberrant splicing and a reading frame shift and can reportedly cause CIPA.[13–19] To our knowledge, this is the first reported patient homozygous for the c.851-33T>A mutation in the north Han Chinese population.

Literature review

Structure of Ntrk1 protein

Crystal structures of the extracellular and intracellular parts of the Ntrk1 protein were identified by Wehrman *et al.*[20] and Bertrand *et al.*,[21], respectively (Figure 2). The Ntrk1 protein contains 796 amino acids and consists of three leucine-rich repeats, two immunoglobulin-like domains (Ig-C1 and Ig-C2), a transmembrane domain, an adenosine triphosphate (ATP) binding site, and a tyrosine kinase domain (TKD). Once the nerve growth factor (NGF) homodimer binds to Ntrk1, it causes Ntrk1 dimerization and autophosphorylation of tyrosine residues 675, 679,

Table 1. Clinical characteristics of patients with CIPA with the c.851-33T>A mutation

Pt.	Genetic variants	Race	Sex	Age (y)	Insensitivity to pain	Charcot arthropathy	Recurrent bone fracture	Self-mutilation	Compromised proprioceptive sensation	Anhidrosis	Recurrent unexplained fever	Intellectual disability	Reference
1	Homo.	Chinese	F	27	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Present report
2	Homo.	Korean	M	28	Yes	NA	Yes	Yes	NA	Yes	NA	Yes	Nam et al., 2017[13]
3	Co.Het. and IVS14+3A>T	Korean	F	17	Yes	NA	Yes	Yes	NA	Yes	NA	Yes	Nam et al., 2017[13]
4	Co.Het. and IVS14+3A>T	Korean	F	16	Yes	NA	Yes	Yes	NA	Yes	NA	Yes	Nam et al., 2017[13]
5	Co.Het. and IVS14+3A>T	Korean	M	14	Yes	NA	Yes	Yes	NA	Yes	NA	Yes	Nam et al., 2017[13]
6	Co.Het. and c.2303C>T	Korean	M	16	No	No	Yes	No	No	NA	No	No	Jung et al., 2013[14]
7	Co.Het. and c.1415delG	Chinese	F	5	Yes	NA	NA	Yes	No	Yes	Yes	Yes	Li et al., 2012[15]
8	Homo.	Korean	M	2.7	Yes	NA	No	Yes	NA	Yes	Yes	Yes	Lee et al., 2009[16]
9	Co.Het. and NA	Korean	M	1.6	Yes	NA	No	Yes	NA	Yes	Yes	Yes	Lee et al., 2009[16]
10	Co.Het. and NA	Korean	F	2.4	Yes	NA	No	Yes	NA	Yes	Yes	Yes	Lee et al., 2009[16]
11	Co.Het. and NA	Chinese	M	20	Yes	Yes	Yes	NA	Yes	Yes	Yes	Yes	Guo et al., 2004[17]
12	Co.Het. and NA	Chinese	M	18	Yes	Yes	Yes	NA	Yes	Yes	Yes	Yes	Guo et al., 2004[17]
13	Co.Het. and c.2281C>T	Chinese	M	5.8	Yes	Yes	Yes	NA	NA	Yes	Yes	NA	Lv et al., 2017[18]
14	Co.Het. and c.1652delA	Chinese	M	20	Yes	Yes	Yes	NA	NA	Yes	Yes	NA	Lv et al., 2017[18]
15-19	Co.Het. and NA	Japanese	NA	NA	Yes	NA	NA	NA	NA	Yes	Yes	NA	Miura et al., 2000[19]

All patients had the c.851-33T>A mutation. Pt., patient; F, female; M, male; Homo., homozygous; Co.Het., compound heterozygous; NA ,not available.

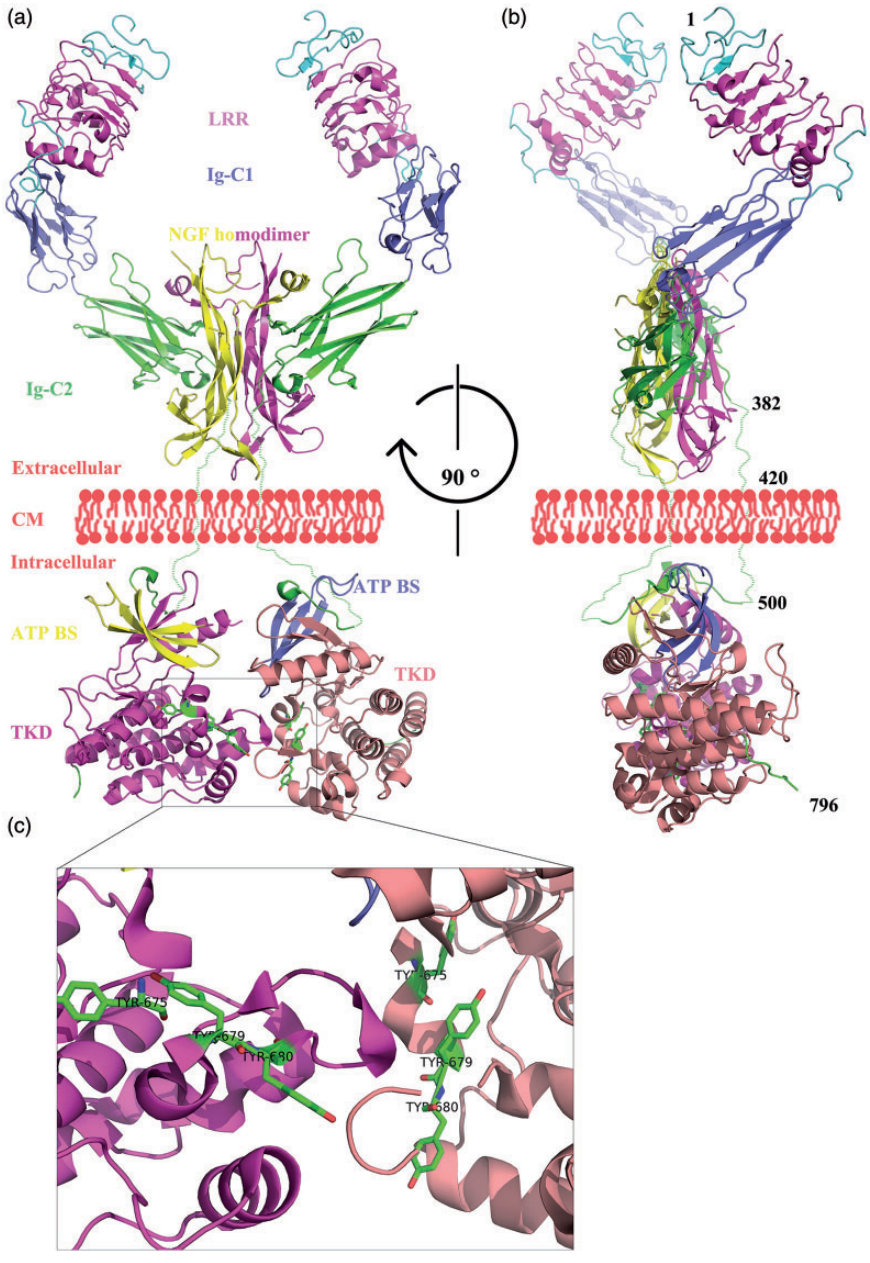

Figure 2. Whole structure of Ntrk1 dimer binding with NGF homodimer. Panel A: Front view. Panel B: Lateral view. Panel C: Details of the interfaces of the cytoplasmic domains and three tyrosine residues phosphorylated during signaling transduction. Stereo visualization is realized by shading the further parts of the molecule. Residue numbers were labeled approximately in Panel B. Dotted lines were drawn manually to connect the extracellular and intracellular portions because no exact crystal structure for the transmembrane domain is available.

LRR, leucine-rich repeats; Ig-C1 and Ig-C2, immunoglobulin-like domains; NGF, nerve growth factor; CM, cytomembrane; ATP BS, adenosine triphosphate binding site; TKD, tyrosine kinase domain.

and 680.[22] This initiates the cytoplasmic signaling cascade that is essential for the survival and growth of neurons.[23]

Heterogeneity of intellectual disability in patients with CIPA

According to our literature review, 90% (99/110) of previously reported patients with CIPA had documented intellectual disability. However, about 10% (11/110) of the patients did not have intellectual disability (44 additional patients with CIPA whose intellectual disability status was not mentioned in the literature were excluded from the statistical analysis). We attempted to determine whether an association exists among CIPA mutation, its effect on Ntrk1 protein, and the presence of intellectual disability (Table 2).

In silico investigation of heterogeneity

We hypothesized that if the mutation on one of the two *NTRK1* alleles is benign or tolerable, the patient may have less severe symptoms. We used the Polyphen2 (Harvard Medical School, Boston, MA, USA; http://genetics.bwh.harvard.edu/pph2/) and SIFT (J. Craig Venter Institute, La Jolla, CA, USA; http://sift.jcvi.org/) scores to predict the influence of a mutation. As shown in Table 2, these predictions were not correlated with the patients' phenotype with respect to intellectual disability, as shown in italics. We then examined whether any observable association was present between the mutations and intellectual disability with an available Ntrk1 protein structure.

Figure 3(a) shows missense mutations (Table 2, Patients 1–5) in patients with CIPA without intellectual disability. As shown in the table, the point mutations p.Leu213Pro, Arg760Trp, and p.Pro767Leu were all located peripherally without apparent involvement in NGF binding, ATP binding, or interfaces of the TKD. In addition, leucine and proline are of similar structure with respect to their nonpolar side chains, which may have less effect on protein function. Because CIPA is a recessive disease, one copy of near fully functional genes will relieve the patient's symptoms. The premature stop mutation p.Tyr756Ter caused Ntrk1 protein truncation of 40 amino acids at the C-terminal. This part of the Ntrk1 protein is located at a region distal to the kinase interface, which may be another reason that these children were not intellectually compromised.

Figure 3(b) shows missense mutations (Table 2, Patients 9–20) in patients with CIPA with apparent intellectual disability. All mutations were located in the functional domains; i.e., NFG binding site, ATP binding site, and interfaces of the TKD. Thus, we conclude that mutations located in functional domains of *NTRK1* contribute to the observed intellectual disability.

In the present case, the c.851-33T>A mutation of *NTRK1* caused a frame shift after the 283rd amino acid and a premature stop codon at the 319th codon,[41] deleting the whole transmembrane domain, ATP binding site, and TKD. This mutation is highly deleterious and would be expected to cause intellectual disability in this patient.

Discussion

To our knowledge, this is the first case of CIPA in a north Han Chinese patient caused by a homozygous c.851-33T>A mutation that resulted in aberrant splicing and an open reading frame shift. We also reviewed all published Ntrk1 CIPA mutations in the literature and postulate that the presence of intellectual disability is determined by the location of the Ntrk1 mutation with respect to the functional protein domain. Missense mutations located

Table 2. *NTRK1* mutations reported in patients with CIPA with or without intellectual disability

Pt.	Mutations	Polyphen2 score and prediction*	SIFT score and prediction*	Patients' information			Reference
				Race	Inheritance model	Intellectual disability	
1	c.722T>C; p.Leu213Pro	*1.000, Pr.D*	*0.00, D*	American	Co.Het.	No	Bonkowsky et al., 2003[24]
	c.1556delG; p.G519fs28X	-	-				
2	c.2347C>T; p.Arg760Trp#	0.653, Po.D	*0.00, D*	Italy	Co.Het.	No	Indo, 2001[25]
	c.2337C>G; p.Tyr756X#	-	-				
3	c.2303C>T; p.Pro767Leu#	*1.000, Pr.D;*	*0.03, D;*	Japanese	Co.Het.	No	Ohto et al., 2004[26]
	c.1621G>C; p.Gly516Arg#	*1.000, Pr.D*	0.23, T				
4	c.2303C>T; p.Pro767Leu#	*1.000, Pr.D*	*0.03, D*	Japanese	Co.Het.	No	Tanaka et al., 2012[27]
	c.1660delC; p.Arg554Gly fs104X	-	-				
5	c.2303C>T; p. Pro767Leu#	*1.000, Pr.D*	*0.03, D*	Korean	Co.Het.	No	Jung et al., 2013[14]
	c.851-33T>A	-	-				
6	c.574 + 1G>A	-	-	Spanish	Co.Het.	No	Sarasola et al., 2011[28]
	c.2206-2A>G	-	-				
7	c.1633–1G>T	-	-	Turkish	Homo.	No	Tuysuz et al., 2008[10]
8	c.1633–1G>T	-	-	Turkish	Homo.	Yes	Tuysuz et al., 2008[10]
9	c.G1247A; p.Glu388Lys	*0.883, Po.D*	*0.66, T*	Swedish	Co.Het.	Yes	Wieczorek et al., 2008[29]
	c.C2429T; p.Gln782X	-	-				
10	c.1561T>C; p.Phe520Leu#	0.993, Pr.D;	0.00, D;	Chinese	Co.Het.	Yes	Gao et al., 2013[30]
	c.2057G>A; p.Arg685His#	*0.103, B*	0.00, D				
11	c.1635G>C; p.Ala526Pro#	0.999, Pr.D;	*0.13, T;*	Chinese	Co.Het.	Yes	Wang et al., 2015[31]
	c.2197G>A; p.Gly713Asp#	1.000, Pr.D	0.00, D				
12	c.1715T>G; p.Ile571Ser#	1.000, Pr.D	0.00, D	Turkish	Homo.	Yes	Li et al., 2012[15]
13	c.1825A>G; Met586Val#	0.987, Pr.D	0.01, D	Japanese	Homo.	Yes	Yotsumoto et al., 1999[32]
14	c.1945C>T; Arg648Trp#	1.000, Pr.D	0.00, D	Chinese	Co.Het.	Yes	Li et al., 2012[15]
	c.44G>A; Trp15X	-	-				
15	c.2001C>T; p.Arg653Cys#	0.989, Pr.D	0.00, D	Turkish	Homo.	Yes	Guven et al., 2014[33]
16	c.2150C>T; Pro689Leu	1.000, Pr.D	0.00, D	Israeli	Homo.	Yes	Shatzky et al., 2000[34]
17	c.2209G>C; Val709Leu#	No Data;	*0.33, T;*	Malaysia	Co. Het.	Yes	Shalimar et al., 2007[35]
	c.2236G>A; Glu718Ser#	0.997, Pr.D	0.01, D				

(continued)

Table 2. Continued

Pt.	Mutations	Polyphen2 score and prediction*	SIFT score and prediction*	Patients' information			Reference
				Race	Inheritance model	Intellectual disability	
18	c.2150T>G; p.Leu716Arg[#]	0.999, Pr.D	0.00, D	French	Co.Het.	Yes	Huehne et al., 2008[36]
	c.1672C>T; p.Gln557X[#]	-	-				
19	c.2155G>A; p.Glu718Lys[#]	0.995, Pr.D	0.00, D	Korean	Co.Het.	Yes	Lee et al., 2009[16]
	c.287 + 2dupT	-	-				
20	c.2206-11G>A; p.Glu734_Ala735insTrpProGln[#]	-	-	Turkish	Homo.	Yes	Yis et al., 2015[37]
		-	-				
21	c.783_785delGAA; p. 261delLys	-	-	Saudi	Homo.	Yes	Algahtani et al., 2016[38]
22	c.1970T>C; p.Leu657Pro	*0.786, Po.D*	0.00, D	Pakistani	Homo.	Yes	Shaikh et al., 2017[39]
23	c.2311C>T; p.Arg771Cys	1.000, Pr.D	0.00, D	Indian	Homo.	Yes	Shaikh et al., 2017[39]
24	c.963delG; p.Leu322Serfs*148	-	-	Chinese	Co.Het.	Yes	Wang et al., 2016[40]
	c.851-33T>A	-	-				
25	c.1786G>A; p.Asp596Asn	0.999, Pr.D	*0.10, T*	Korean	Co.Het.	Yes	Nam et al., 2017[13]
	c.2350_2363del; p.Leu784Serfs*79	-	-				
26	c.704C>G; p.Ser235*	-	-	Korean	Co.Het.	Yes	Nam et al., 2017[13]
	c.2020G>T, p.Asp674Tyr	*0.879, Po.D*	0.00, D				

CIPA, congenital insensitivity to pain with anhidrosis; Pt., patient; Homo., homozygous; Co.Het., compound heterozygous; Pr.D, probably damaging; Po.D, possibly damaging; D, damaging; T, tolerated; B, benign

Notes: Patients who were Homo. or Co.Het. with frameshift mutations, premature stop mutations, and verified splice site mutations were not included in this table because of their highly deleterious effect with all patients exhibiting intellectual disability.

*Polyphen2 and SIFT scores and predictions (Pr.D, Po.D, D, T, and B) are given only for missense mutation. Discordances (patients without intellectual disability who had highly deleterious missense mutations or those with intellectual disability who had at least one possibly damaging or tolerable mutation) are in italics.

[#]Because of the alternative splicing of *NTRK1*, different nomination systems were employed among the literature. Here, we mapped these mutations to the protein structure, in which the residue number may differ from that in the corresponding article.

-Data not available for frameshift or intron mutations.

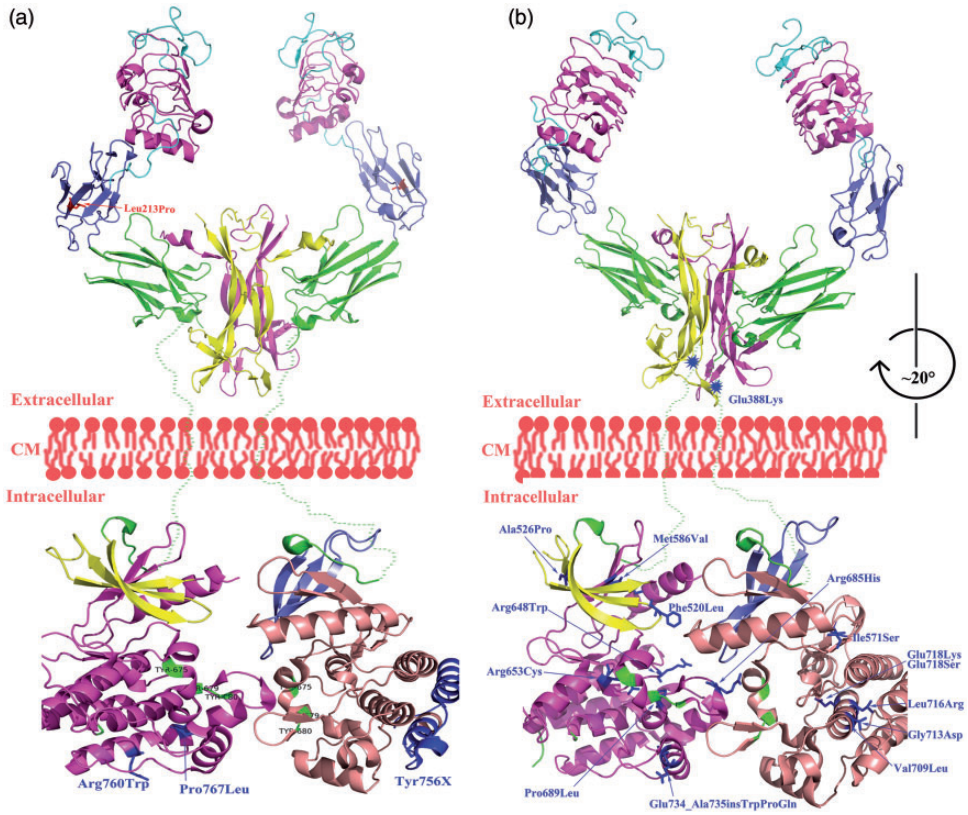

Figure 3. Observable association between mutations and the presence of intellectual disability. Panel A: At least one mutation reported in patients with CIPA without intellectual disability involved peripheral amino acids of the Ntrk1 protein. Panel B: Missense mutations in patients with CIPA with intellectual disability altered critical domains of the Ntrk1 protein. (Rotated by about 20° clockwise from the front view.)

peripheral to the Ntrk1 protein that do not jeopardize important domains (i.e., NGF binding site, ATP binding site, and TKD) tend to cause milder symptoms, usually without intellectual disability. Frame shift mutations, premature stop mutations, splice site mutations, and missense mutations that involve critical amino acids in the protein all cause severe intellectual disability.

However, several reports appear to be discordant. Sarasola *et al.*[28] described a 6-year-old Spanish girl with c.574 + 1G>A and c.2206-2A>G compound heterozygous mutations (Table 2, Patient 6) who was cognitively normal. The author verified the influence of the two splice site mutations with reverse transcription PCR. The c.574 + 1G>A mutation caused exon 5 skipping and predicted Ntrk1 protein truncation after residue 164. The c.2206-2A>G mutation caused two alternative splicings, and the predicted proteins were Ala736_Gln742 interstitial deletion and truncation after residue 735, respectively. Tuysuz *et al.*[10] reported three Turkish patients with CIPA with a homozygous c.1633–1G>T mutation, one 2.5-year-old boy without psychomotor retardation, and one 14-year-old boy and one 8-year-old girl

with psychomotor retardation (Table 2, Patients 7 and 8). This discrepancy indicates that there may be other factors modifying the phenotypes of CIPA. The functional impact of the variant, including dimerization, autophosphorylation, PLCγ activity, and toxicity of the mutated protein to the cell should be validated to confirm the pathogenicity of the variant.

Conclusions

In this study, we observed that intellectual disability in patients with CIPA appears to be determined by which Ntrk1 protein domain a mutation has jeopardized. Although this observation method is qualitative, it provides another alternative to evaluate the function of a missense mutation in addition to the Polyphen2 and SIFT scores. We are awaiting the delineation of additional cases of CIPA to provide additional evidence supporting our observations.

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Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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