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Original Article

Phenotypes and Genotypes in Five Children with Congenital Insensitivity to Pain with Anhidrosis

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ABSTRACT

BACKGROUND: Congenital insensitivity to pain with anhidrosis is an extremely rare hereditary disorder linked to variants in *NTRK1*. Our goal was to characterize the clinical features and the genetic basis of the disorder in Chinese patients. **METHODS:** Patients were enrolled via social networking. Clinical features were investigated by interview, chart review, and physical examination. DNA was extracted from peripheral blood to genotype *NTRK1* in patients and their parents. Variants identified were checked against a control cohort by high-throughput sequencing, and the effects of these variants were assessed in silico. **RESULTS:** Clinical features in five patients were cataloged, and six loss-of-function *NTRK1* variants were identified, including a frameshift variant c.963delG, a nonsense variant c.1804C>T, an intron variant c.851-33T>A, and three missense variants c.1802T>G, c.2074C>T, and c.2311C>T. **CONCLUSIONS:** The results expand the spectrum of clinical and genetic features of congenital insensitivity to pain with anhidrosis and will help facilitate analysis of genotype–phenotype association in the future.

Keywords: congenital insensitivity to pain with anhidrosis, *NTRK1*, phenotype, genotype, variant

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Introduction

Congenital insensitivity to pain with anhidrosis (Online Mendelian Inheritance in Man 256800), also known as hereditary sensory and autonomic neuropathy type 4, is an extremely rare autosomal recessive disorder first described in 1963.[1] It is characterized by profound insensitivity to noxious stimuli and absence of sweating, as well as secondary complications such as repeated injuries, self-mutilation, and recurrent febrile episodes.[2,3] Delayed developmental milestones, hyperactivity, emotional lability, and intellectual disability are also observed to varying degrees.[4] Morphologic studies revealed that insensitivity to pain and anhidrosis result from the absence of sensory and sympathetic postganglionic neurons.[5-7]

Nerve growth factor (NGF) and its high-affinity receptor, tropomyosin receptor kinase A (NTRK1 or TrkA), are essential for the proliferation, differentiation, and survival of sensory and sympathetic neurons.[8] Mice deficient in *NGF* or *NTRK1* has pervasive loss of these neurons.[9,10] Loss-of-function variants in *NTRK1* were first linked to congenital insensitivity to pain with anhidrosis in humans in 1996,[11] and almost all cases of congenital insensitivity to pain with anhidrosis to date are attributed to variants in *NTRK1*.[12]

Although congenital insensitivity to pain with anhidrosis has been defined for more than half a century, clinical data

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remain limited, and the disorder is yet unfamiliar to many clinicians and geneticists because of extremely rare morbidity. For instance, the parents of a ten-year-old boy with unrecognized congenital insensitivity to pain with anhidrosis were under supervision in America for two years because of multiple injuries to the child since early life.[3] Similarly, most affected individuals in China suffered from severe complications and unnecessary expense because of inappropriate care and lack of early diagnosis. Furthermore, some Chinese families eventually had a second affected child in the absence of genetic counseling. In this study, we describe the clinical features and underlying genetic variants in five Chinese children with congenital insensitivity to pain with anhidrosis, in an attempt to gain better insight into the disorder.

Materials and Methods

This study was approved by the Institutional Ethics Committee at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (20130501). Written informed consent was obtained.

Patient enrollment

Patients were enrolled via a group of congenital insensitivity to pain with anhidrosis in QQ (http://im.qq.com/), a Chinese social networking site similar to Facebook. Potential subjects were first asked to complete a Personal Medical Condition Questionnaire, which contained a summary of congenital insensitivity to pain with anhidrosis symptoms. Patients with symptoms consistent with congenital insensitivity to pain with anhidrosis were interviewed and physically examined to confirm diagnosis. Medical records were also reviewed.

NTRK1 screening

DNA from patients and their parents was extracted from peripheral blood using TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing, China). Seventeen exons with at least 50 nucleotides encompassing intron–exon boundaries were amplified by polymerase chain reaction, as were flanking sequences in *NTRK1*_v2 (NM_002529.3), the only splice variant expressed in neurons.[13-15] Amplification products were purified and sequenced on both strands using an ABI3730XL automated sequencer (Applied Biosystems, Foster City, CA). Sequencing data were analyzed in Chromas, version 2.22. Variants identified were named according to Human Genome Variation Society guidelines and were matched against data in dbSNP, dbVar, and ClinVar by using Variation Reporter at National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/variation/tools/reporter/), which generate reports containing cluster ID numbers (rs#), 1000Genome minor allele frequencies, clinical significance, and relevant citations, if any. For novel variants, molecular consequences were inferred by Variation Reporter based on RefSeq transcripts.

Functional analysis in silico

Frameshift variants were interpreted based on NCBI annotation of the genome and on protein sequence and functional information in UniProt (http://www.uniprot.org). Intron variants were interpreted by Automated Splice Site and Exon Definition Analyses (Cytognomix Inc, London, Ontario, Canada, https://www.mutationforecaster.com). Missense variants were characterized by location in the protein, amino acid substitution score, and conservation of the mutated residue. Mutated residues were mapped in PyMOL, version 1.8 (http://www.pymol.org/) to the three-dimensional structure of NTRK1 deposited in Protein Data Bank (http://www.rcsb.org/pdb/home/home.do, PDB ID = 4F0I). Amino acid substitution score was assessed online (http://www.russell.embli-heidelberg.de/aas/), whereas conservation was determined by the Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) was also used to predict the impact of missense variants.

Screening in unaffected volunteers

Variants deemed to be pathogenic were screened by high-throughput sequencing against a control cohort of 1007 unaffected Chinese.

Results

A total of eight patients answered the questionnaire, and their symptoms were consistent with congenital insensitivity to pain with anhidrosis. We then interviewed and examined all these eight patients and performed gene screening for them. In three patients, pathogenic variants were identified in just one allele. These three patients will be investigated further and are not discussed in this report. In the other five patients from four unrelated families, pathogenic variants were detected in both alleles. Physical examination revealed that patients could not sense pain from noxious stimuli and did not sweat but had unimpaired touch, vibration, and position senses. They were sporadic in families. Their parents were not consanguineous. Six pathogenic variants were identified in these five patients, including a frameshift variant c.963delG, a nonsense variant c.1804C>T, an intron variant c.851-33T>A, and three missense variants c.1802T>G, c.2074C>T, and c.2311C>T. Currently, available NCBI data on these variants are summarized in Table 1, and results from our screening are presented in Table 2. Clinical features and pathogenic variants in each patient were described in the following sections.

Patient summaries

Patient 1 was a nine-year-old boy, an only child in his family. Fever developed 28 days after birth, with body

TABLE 1.
Currently Available NCBI Data on the Six Variants Identified in This Study

Variants	Amino Acid Changes	NCBI ID	1000Genomes Minor Allele Frequency	Clinical Significance	PubMed References
c.1802T>G	p.Leu601Arg	N/A	N/A	N/A	N/A
c.1804C>T	p.Arg602*	rs763758904	N/A	N/A	N/A
c.851-33T>A	No changes	rs80356674	N/A	Pathogenic	Three articles[16-18]
c.963delG	p.Leu322Serfs*148	rs778670571	N/A	N/A	N/A
c.2074C>T	p.Arg692Cys	rs761967383	N/A	N/A	N/A
c.2311C>T	p.Arg771Cys	N/A	N/A	N/A	N/A

Abbreviation:
N/A = Not available