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Clinical Spectrum of Three Patients with Congenital Insensitivity to Pain with Anhidrosis

울 산 대 학교 대 학 원
의 학 과
조 준 희

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지 도 교 수　이 범 희

이 논문을 의학 석사 학위 논문으로 제출함

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울 산 대 학교　대 학 원
의　학　과
조 준 희

조준희의 의학 석사학위 논문을 인준함

심사위원 염 미 선 인

심사위원 이 범 희 인

심사위원 곽 윤 해 인

울 산 대 학 교 대 학 원

2024년 2월

영문요약

Congenital insensitivity to pain with anhidrosis is a genetic disease which is caused by the pathologic variants of the *NTRK1* gene.[1] Cardinal features of this disease is characterized by recurrent fever, pain insensitivity, inability to sweat and intellectual disability. *NTRK1* gene encodes Tropomyosin-related kinase A (TrkA), a specific receptor for Nerve growth factor (NGF), and binding of NGF activates several signaling pathways involved in neuronal survival during embryonic development.[2] As survival and development of small-diameter primary afferent fibers and sympathetic postganglionic neurons are dependent on this signaling, CIPA patients who have defects in TrkA show pain insensitivity and inability to sweat. Inability to sweat reduces heat dissipation through evaporation, which leads to recurrent fever.[3] Also, defects in TrkA signaling causes dysfunction of NGF-dependent neurons in the brain, resulting in intellectual disabilities, hyperactivity, and emotional lability.[4] However, heterogeneity exists in disease severity. Some patients showed normal body temperature or normal intellectual ability.[5] Though several groups tried to reveal the genotype-phenotype correlation, it is yet controversial.[6, 7] In this study, we described clinical features of three Korean CIPA patients whose diagnosis was confirmed by genetic sequencing and investigated the genotype-phenotype correlation in our results. We also conducted literature review and compared our results with those of published data.

The body temperature of patient 1 was high since birth and he was suffered from recurrent fever. He did not respond to nociceptive stimuli. He did not sweat at all until childhood. However, the symptom alleviated in the upper body as he grew up. He showed global developmental delay and self-mutilation behavior. He got decompression surgery for paravertebral abscess that compressed T11-L5 spines. Although he got

emergent surgery, he became paraplegic and wheelchair bound. Patient 2 showed recurrent fever and decrease in sweating. He showed self-mutilating behavior such as tongue biting. He did not cry during venipuncture or muscular injection. He was diagnosed as developmental delay. His height was about 25th percentile and weight was about 3rd percentile throughout the growing period. Unlike patient 1, his symptoms did not alleviated and persisted. Patient 3 showed recurrent fever, anhidrosis, insensitivity to pain but she had normal intelligence and did not show self-mutilating behaviors. However, she had impulsive and aggressive tendencies. She had left distal tibia non-ossifying fibroma, left talonavicular joint deformity and left distal tibia fracture. Clinical features observed from our study were consistent with those of previous studies. In genetically diagnosed CIPA patients, the most common symptom was anhidrosis (97%), followed by pain insensitivity (94%), recurrent fever (89%), intellectual disability (75%), self-mutilation (75%) and musculoskeletal abnormalities (64%). Whole exome sequencing was performed for patient 1,2 and their parents. Whole genome sequencing was performed for patient 3 and her parents. Homozygous c.851-33T>A was identified in patient 1 and both of his parents were carriers. The c.851-33T>A causes aberrant splicing which produces truncated proteins lacking transmembrane and intracellular domains including tyrosine kinase domain.[8] Compound heterozygous c.851-33T>A and c.2020G>T were identified in patient 2. Each variant was inherited from his father and mother, respectively. The c.2020G>T is located in tyrosine kinase domain and was known to reduce activity of *NTRK1* receptor. Considerable receptor function was observed in cases homozygous for this variant and disease expression was only identified when the counterpart allele was completely inactivated.[9] Compound heterozygous c.2303C>T and

g.7071_11990del were identified in patient 3. His mother and father were carriers for each variants. The c.2303C>T is located in tyrosine kinase domain, and was reported to present mild symptoms. Patients with this variant showed preserved cognitive function and partial pain sensation.[10] The g.7071_11990del is a novel gross deletion variant and located in exon 5-7. The g.6995_11999del which is located near g.7071_11990del was reported to cause premature translation termination and truncated protein synthesis. Compound heterozygote with g.1-1258_10169del showed mild symptoms of normal intelligence and no self-mutilation. However, compound heterozygote with c.851-33T>A showed intellectual disability and self-mutilation.[11]

All of three patients showed recurrent fever, anhidrosis, and pain insensitivity, while developmental delay, intellectual disability, and self-mutilation were not observed in patient 3. Also, patient 2 did not show musculoskeletal symptoms such as fracture or joint deformity which were observed in patient 1. Patient 1 had homozygous c.851-33T>A which lacks tyrosine kinase domain while patient 2 had c.851-33T>A and c.2020G>T which was reported to reduce activity of *NTRK1* receptor. Patient 3 had c.2303C>T which was reported to present mild symptoms and g.7071_11990del which is expected to show different severity according to the counterpart allele. As CIPA is an autosomal recessive genetic disorder, it seems that patient 2 and patient 3 presented mild symptoms because they had one 'mild' variant while patient 1 had homozygous c.851-33T>A. Our study expanded the spectrum of the *NTRK1* variants and provided additional clues of genotype-phenotype relationship in CIPA.

차례

INTRODUCTION

Congenital insensitivity to pain with anhidrosis (CIPA, MIM #256800) is a rare autosomal recessive genetic disease which was initially reported by Swanson in 1963 from two male siblings who were insensitive to pain and did not sweat.[12] The main clinical manifestations of this disease are impaired perception of pain, inability to sweat, recurrent febrile episodes, and intellectual disability.[13] Impaired perception of noxious stimuli results in recurrent trauma, burn, and self-mutilating behaviors such as biting tongue, fingers and toes. Repeated trauma or self-mutilating behaviors increase the risk of fracture, arthropathy, and musculoskeletal infection.[14, 15] Anhidrosis disturb the homeostatic thermoregulation in hot environments, which result in recurrent febrile episodes. Most patients with CIPA shows varying degree of psychiatric disability, such as intellectual disability, attention deficit hyperactivity disorder (ADHD).[4]

CIPA is caused by the recessive mutations in the *NTRK1* gene, which maps to chromosome 1q21-q22, spans over 25kb and consists of 17 exons and 16 introns. It encodes a Tropomyosin-related kinase A (TrkA) protein, a membrane-bound receptor tyrosine kinase which has high affinity for Nerve growth factor (NGF),[2] and is involved in nociceptive reception and sweating regulation.[1] Binding of NGF to TrkA activates several signaling pathways that involved in survival and maintenance of peripheral sensory and autonomic neurons during embryonic development.[16, 17] Most of the primary afferent neurons with Ad-, C- fibers and the sympathetic postganglionic neurons are dependent on the NGF-TrkA signaling. As primary afferent fibers of small-diameter (Ad and C) transmit pain sensation and sympathetic postganglionic neurons innervate eccrine glands, CIPA patients show deficit in pain sensation and sweating.[3]

More than 115 variants were reported to be pathogenic or likely pathogenic for *NTRK1*. (http://www.hgmd.cf.ac.uk/ac/). Nonsense, frameshift, splice-site, missense, gross deletion have been reported in the extracellular and intracellular domains.[2] There have been several studies regarding genotype-phenotype correlation. While some groups reported no correlation,[18, 6] several groups reported heterogenous clinical manifestations and analyzed genotype-phenotype correlation based on molecular structure and function of *NTRK1* gene. Patients with homozygous M581V variation showed normal body temperature and described as 'mild clinical symptoms'.[5, 19, 7] It is yet controversial whether correlation between specific genetic variations and clinical manifestations exists.

In this study, 3 patients from 3 unrelated families were recruited and underwent a comprehensive evaluation. Using whole exome sequencing or whole genome sequencing, we identified 4 different *NTRK1* variants [c.851-33T>A, c.220G>T (p.Asp674Tyr), c.2303C>T (p.Pro768Leu), g.7071_11990del] in 3 unrelated Korean families. We compared the disease severity between 3 patients. Also, we compared the disease severity of patients from previous studies who had variants identified in our patients and investigated whether genotype-phenotype correlation exists in our results. This study expands the clinical and genetic spectrum and helps understanding of this rare condition.

BODY

Ethical considerations

This study was approved by the Institutional Review Board of Asan Medical Center, Seoul, Korea. (IRB number: 2023-1562). Written informed consents were obtained from all individuals. Parental consent was obtained from participants aged under 18 years old.

Clinical presentation of three patients

A total of 3 Korean patients from 3 unrelated non-consanguineous families were diagnosed as CIPA at Asan Medical Center, Seoul, Korea from September 2015 to August 2023. Medical histories, findings of physical examination, developmental test, blood biochemistry, and imagings such as x-ray, CT, MRI were reviewed based on electronic medical record (EMR). A pedigree of each families was drawn based on clinical assessment and genetic test results. (Figure 2)

All three patients developed clinical manifestations consisted with CIPA. The median age at presentation was 12 months (range, 4 months to 8 years old). The median age at diagnosis was 8 years (range, 9 months to 16 years old). The clinical features of each patient are described in table 1.

Patient 1.

He was a single child from healthy, non-consanguineous Korean parents. He was born at a gestational age of 38 weeks, weighing 2750 gm (5-10th percentile). His parents observed that his basal body temperature was slightly high since birth and he experienced recurrent fever episodes. He did not sweat at all until childhood. However, the symptom alleviated as he grew up. At age 18 months, he abruptly showed limping gait, and diagnosed as developmental dysplasia of the hip. At age 2 years, he jumped from the table and his right hip was dislocated, but he did not show any expected normal pain responses and actively moved his legs. His height and body weight was under 3 percentile throughout the growing period. At age 3 years, his developmental status was 24 months for cognition, 16 months for language expression, 24 months for language comprehension, 24 months for social, and 11 months for motor development.

Although he started language, cognitive rehabilitation, he was diagnosed as mild mental retardation at age 6 years old and his grades in schools were very low. He also started to bite his fingers and toes, and repetitive hospitalization was required due to recurrent soft tissue and bone infection, such as thigh abscess, hand or knee cellulitis and foot osteomyelitis. At age 16 years, infectious spondylitis with paravertebral abscess was noted, with compressed T11- L5 spines. Despite the immediate decompression surgery, he became paraplegic and wheelchair bound. His upper extremities length was within normal range, while his lower extremities were relatively short.

Patient 2.

He was born at gestational age of 40 weeks, weighing 2850 gm (10-15th percentile) from healthy, non-consanguineous Korean parents. He was the only child of this family. At birth, high arched palate and hypotonia were noted. Recurrent episodes of fever with decrease in sweating were noted without any other symptoms such as cough or rhinorrhea. He showed self-mutilating behavior of tongue biting. No pain response was noted during venipuncture or muscular injection. At age of 7 months, his developmental status was 3 months for fine motor, and 5 months for personal-social area. Although he started rehabilitation therapy, he was diagnosed as global developmental delay at 11 months of age. His developmental status was 8 months for gross motor, 6 months for fine motor, 8 months for personal social, 9 months for language, and 8 months for cognitive adaptive area. At age 4 years, his height was 103cm (25th percentile) and weight was 14.4kg (3rd percentile). Anhidrosis, mild fever, self-mutilation of tongue biting, and poor weight gain persisted.

Patient 3.

She was born at gestational age of 40 weeks, 3560 gm (50-75th percentile) from healthy, non-consanguineous Korean parents. Her two elder sisters were healthy. Recurrent episodes of fever with decrease in sweating were noted with no definite evidence of infection. At age 7 years, she wrenched her left ankle, but she did not feel the pain or have limitation of motion. She went to the hospital 1 week after the accident because of sustained left foot swelling and was diagnosed as left distal femur fracture and left foot Kohler's disease. At age 8 years, a new hypolucent fibro-osseous lesion at left distal femur, destruction of both talonavicular joints, and the avascular necrosis of left navicular bone was identified. She received several subsequent surgeries due to persistent non-union of destructive bones. At age 10 years, her height was in the 85th percentile and weight was in the 90th percentile. Her intelligence was normal without any self-mutilating behaviors, but her parents reported her impulsive and aggressive tendencies.

Genetic analysis

Peripheral blood sample was obtained from each probands and their both parents. Genomic DNA was extracted from blood using Allprep DNA/RNA kits (Qiagen, Venlo, Netherlands). For whole-exome sequencing (Family #1 and #2), exon regions of genomic DNA was captured using xGen Exome Research Panel v2 (Integrated NDA Technologies, Coralville, Iowa, USA). DNA libraries were generated using TruSeq DNA PCR-Free Library Prep Kits (Illumina, San Diego, CA, USA) and sequenced through an Illumina NovaSeq6000 (Illumina) platform (30x mean depth of coverage). Genome sequences (Family #3) were aligned to the human reference genome (GRCh38/hg38) using the BWA-MEM algorithm. PCR duplicates were removed by SAMBLASTER. Initial mutation calling for base substitutions and short-indel calls were made using HaplotypeCaller2 and Strelka2.

Structural variations were identified by Delly. Variant filtering and their Mendelian inheritance were checked. Detection of *de novo* mutations and prediction of mutational impact were conducted, and classification of the variants based on American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guidelines (Seo et al. 2020; Richards et al. 2015). Genome sequencing, analysis and interpretation were conducted by RareVision system (Genome Insight, San Diego, Ca, USA) or EVIDENCE (3billion Inc, Seoul, South Korea). The final evaluation of variant pathogenicity was determined by medical geneticists based on patient's phenotype and familial history.

Whole exome sequencing was done for patient 1, 2 and their parents, and whole genome sequencing for patient 3 and her parents. Figure 2 shows pedigrees of three families and identified variants. Patient 1 was homozygous for c.851-33T>A in the *NTRK1* gene and both of his parents were carriers for c.851-33T>A. This variant is a known pathogenic variant by ACMG classification which causes aberrant splicing and reported in several preceding studies.[7] Patient 2 had compound heterozygous pathogenic variants, c.851-33T>A and c.2020G>T. c.851-33T>A was inherited from his father, and the missense variant, c.2020G>T(p.Asp674Tyr), classified as Likely pathogenic by ACMG classification was inherited from his mother. c.2020G>T was a previously reported one.[12] In patient 3, compound heterozygous variants, c.2303C>T and g.7071_11990del were identified. c.2303C>T (p.Pro768Leu) was inherited from his mother and gross deletion of g.7071_11990del was inherited from her father. c.2303C>T (p.Pro768Leu) was reported previously,[10] while g.7071_11990del was a novel variant.

Literature review

Review of literature was done as follows: The biomedical and life science database

(PubMed) was searched using the MeSH Terms (congenital insensitivity to pain with anhidrosis[MeSH Terms]) OR (Hereditary sensory autonomic neuropathy type 4[MeSH Terms]). All English articles between November 1951 and August 2023 were assessed. Articles reporting three or more of pain insensitivity, anhidrosis, recurrent fever, and intellectual disability in genetically diagnosed CIPA patients were included. Symptoms not described were considered absent for the patient. When genetic analysis was not conducted for all reported patients, only results from genetically diagnosed patients were included. Articles reporting the surgical, anesthetic procedures for previously diagnosed CIPA patients were excluded.

Among 2114 searched articles, 47 were included and clinical features of total 178 patients were reviewed. The results of our study were consistent with those of previous literatures. Clinical features of these patients are shown in Table 2. The most common symptom shown in genetically diagnosed CIPA patients was anhidrosis (97%), followed by insensitivity to pain (94%) and recurrent fever (89%). Intellectual disability or self-mutilation was shown in 75% of patients and musculoskeletal problems such as bone fracture or joint deformity were shown in 64% of patients. Recurrent or severe infection such as osteomyelitis was reported in 24% of patients.

Discussion

NTRK1 was first identified in 1986 as a new tyrosine kinase family isolated from human colon carcinoma cells.[13, 14] *NTRK1* encodes TrkA protein which found to be a signal transducing receptor for NGF.[15, 16] Binding of NGF induces the phosphorylation and homodimer formation of TrkA, which activates following intracellular downstream signal transductions.[17, 2] NGF-TrkA signaling plays crucial roles in survival and growth of

neurons, neuronal differentiation, neurite outgrowth, and synaptic plasticity.[18] Primary afferent neurons and sympathetic postganglionic neurons in the peripheral nervous system which constitute afferent and efferent pathways in thermal regulation are regulated by NGF.[19, 4] These NGF-dependent neurons are also reported to have influence on inflammation by secreting pro-inflammatory or anti-inflammatory cytokines.[20, 21, 4, 3] Modulation of NGF is associated with sensitization of nociceptor and altered transcription of nociceptor genes which could lead to hyperalgesia.[22] In addition, NGF-dependent neurons are considered to have crucial role in emotions and feelings through their capacity to regulate interoceptive system.[23, 24, 4]

TrkA exists as two isoform of 790 or 796 amino acid residues. Except the presence of six amino acid residues located near the extracellular transmembrane region, two isoforms have similar biologic properties including the NGF binding.[25] However, as the isoform of 796 amino acid residues is abundantly expressed in neuromuscular tissues while the other one primarily expressed in non-neuronal cells, it is regarded as more relevant isoform associated with the pathophysiology of CIPA.[25, 26] TrkA protein is composed of three parts : an extracellular domain, an intracellular domain, and a single transmembrane domain dividing them.[25, 26] The extracellular domain consists of a signal peptide, two cysteine clusters, three tandem leucine-rich motifs, and two immunoglobulin-like domains important for NGF binding.[25, 27] The intracellular domain is important for signal transduction in response to NGF binding and includes a juxtamembrane region, a tyrosine kinase domain, and a 15 amino acid carboxy-terminal tail.[25] TrkA tyrosine residues – 490, 670, 674, 675, and 785 are identified as autophosphorylation sites, which serve as docking sites for downstream intracellular

signal transduction molecules.[28, 25]

Cardinal symptoms and signs of CIPA, insensitivity to pain, anhidrosis, and mental retardation, result from the absence or deficient functional TrkA proteins.[3] Binding of NGF activates TrkA tyrosine kinase activity which results in neuronal growth and prevention of neuronal apoptosis.[29] CIPA patients are lack of NGF-dependent primary afferents and sympathetic postganglionic neurons.[3] NGF-dependent primary afferent neurons with small diameters and thinly myelinated Ad-fibers or unmyelinated C-fibers transmit nociceptive and interoceptive stimuli, while sympathetic postganglionic neurons innervate various exocrine glands including sweat glands.[4] Thus, CIPA patients lack neurons involved in pain sensation and maintaining homeostasis, which lead to insensitivity to pain, anhidrosis and recurrent fever.[3, 4] In brain, TrkA is principally expressed in cholinergic neurons that are involved in learning and memory.[30] TrkA density was relatively low in mild cognitive impairment or Alzheimer's disease patients compared with no cognitive impairment group.[31] Selective TrkA ligand treatment showed improvement of memory and learning, *in vivo*.[32] NGF-TrkA signaling is reported to associated in pathogenesis of Alzheimer's disease by modulating metabolism of amyloid precursor protein and synaptic functions in cholinergic neurons.[33] Also, brain TrkA mRNA level was low in patients who suicided due to major depression disorder compared with patients who died due to traffic accident.[34] These findings suggest that TrkA-expressing, NGF-dependent neurons in the brain are involved in various intellectual abilities and dysfunction of NGF-TrkA signaling could result in mental retardation and psychiatric problems such as hyperactivity or emotional lability.[3, 4] Although insensitivity to pain, anhidrosis, and mental retardation is the typical findings of

CIPA patients, heterogeneity exists in terms of phenotype and disease severity.[35] Several variants are reported to show 'mild' clinical manifestations in CIPA patients.[10, 36, 37] A patient with compound heterozygote variants of p.Gly513Arg and p.Pro762Leu did not show severe mental retardation or self-mutilation.[36] Also, patients with p.Pro768Leu are reported to have partially preserved pain sensation and normal intelligence.[10, 37] An in silico study which investigated the phenotypic heterogeneity of CIPA patients reported that p.Leu213Pro, Arg760Trp, and p.Pro767Leu are associated with normal intelligence. Structural similarity between proline and leucine that have a non-polar side chains is suggested to contribute to the mild clinical symptoms.[8] Another in vitro study to reveal the phenotype-genotype relationship investigated the effect of three *NTRK1* variants on protein structures and cellular functions, and identified that C300stop variant is associated with normal intelligence. Soluble misfolded conformers resulting from C300stop are not severely neurotoxic, and it could be immediately eliminated through autophagy.[9]

In our study, four individual variants were identified : c.851-33T>A, c.2020G>T, c.2303C>T, and g.7071_11990del. The c.851-33T>A was reported as the second most common variant in Japanese CIPA patients and is regarded as the founder variants in East Asian populations.[38, 39, 7] It causes aberrant splicing of intron 7 and an insertion of a 137-bp additional section resulting in premature stop codon at 319th codon.[38, 8] Proteins translated from these frameshift variants lack transmembrane domain and extracellular domain including juxtamembrane region and tyrosine kinase domain.[8] The c.2020G>T is located in tyrosine kinase domain, and was reported to reduce activity of *NTRK1* receptor instead of inactivating it. Proper neural development was observed in

cases homozygous for this variant and a complete inactivation of the counterpart allele was required for the disease expression.[9] The frequency of this variant is extremely low in the gnomAD v2.1.1 dataset (total allele frequency: 0.0000041, PM2) and it has been observed as compound heterozygote with other variants, which is regarded as the result of mild nature of this variant.[12, 40, 38] The other variant located in tyrosine kinase domain, c.2303C>T, was reported to show mild symptoms of preserved cognitive function and pain sensation.[10, 36, 37] While preserved cognitive function was common in three case, there was a difference in the degree of pain sensation. Patients reported by Tanaka et al and Jung et al showed partial pain sensation whereas the patient reported by Ohto et al showed pain insensitivity.[10, 36, 37]

The g.7071_11990del is a novel gross deletion variant that encompasses exon 5-7 and is located in extracellular domain. A similar gross deletion of g.6995_11999del was previously reported and was expected to cause premature termination of translation, which could result in absent or truncated protein synthesis.[11] Compound heterozygote g.6995_11999del and g.1-1258_10169del showed normal intelligence and no self-mutilation, while compound heterozygote with c.851-33T>A showed mental retardation and self-mutilation.[11] As g.7071_11990del is located near g.6995-11999del, we expected that g.7071_11990del would have similar effect on structure and function of *NTRK1*. However, we could not confirm this prediction because RNA sample collection was unavailable.

In our results, patient 1 and patient 2 showed pain insensitivity, global developmental delay, and self-mutilation while patient 3 showed relatively preserved pain sensitivity, normal development and intelligence, and no self-mutilation. Also, in addition to pain insensitivity,

anhidrosis, mental retardation, and self-mutilation, patient 1 showed severe skeletal and infectious diseases which were not shown in patient 2. Patient 1 had homozygous c.851-33T>A that lacks tyrosine kinase domain.[8] Patient 2 had compound heterozygous c.851-33T>A and c.2020G>T which is located at tyrosine kinase domain and reported to reduce *NTRK1* receptor but not enough to inactivate it.[9] Patient 3 had heterozygous c.2303C>T which is reported to show mild clinical manifestations[10] and a novel gross deletion g.7071_11990del which is expected to show difference in the severity of symptoms according to the counterpart allele. It seems that it is because patient 2 and patient 3 had one 'mild' variant while patient 1 had homozygous c.851-33T>A.

CONCLUSION

We described clinical and genetic features of three Korean CIPA patients. We identified one novel gross deletion variant and there were varying degrees of disease severity. Patients with variants previously reported as 'mild' showed alleviated clinical manifestation. Our study expanded the spectrum of the *NTRK1* variants and provided additional clues of genotype-phenotype relationship in CIPA. More information on the relationship between genotype-phenotype could be the key to understanding the pathogenesis and clinical prognosis of CIPA

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국문요약

선천성 무통각증 및 무한증은 *NTRK1* 유전자의 병적 변이로 인해 생기는 유전병이다.[1] 이 병의 특징적인 증상은 반복되는 발열, 무통각증, 무한증, 그리고 지능 저하이다. *NTRK1* 유전자는 Tropomyosin-related kinase A (TrkA) 를 발현하는데, 이것은 Nerve growth factor (NGF) 에 대한 특이적인 수용체이며 NGF가 이 수용체에 결합할 경우 여러 세포 내 신호전달 체계를 활성화시켜 배아 발달 시기에 신경세포 생존에 관여한다.[2] 작은 직경의 1차 구심 섬유와 교감신경계 신경절이후신경세포는 이 신호전달체계에 의존적이기 때문에 TrkA 에 결함이 있는 CIPA 환자들은 무통각증과 무한증을 나타내게 된다. 무한증은 증발을 통한 열 발산을 감소시키므로 반복적인 발열이 나타난다.[3] 또한 TrkA 의 결함은 뇌 안에 있는 NGF 의존성 신경세포들의 기능 장애를 유발하여 지능 저하, 과민행동, 그리고 정서 불안 등을 유발한다.[4] 그러나 질병의 중증도는 환자마다 조금씩 차이를 보인다. 일부 환자들은 정상 체온과 정상 지능을 보였다.[5] 몇몇 연구자들이 이 질환의 유전자형과 표현형의 관계에 대해 밝히고자 노력하였으나 이에 대해서는 아직 논란이 있다.[6, 7] 이 연구에서는, 유전자 검사로 확인된 세 명의 한국인 선천성 무통각증 및 무한증 환자들의 임상 양상을 기술하였고 이들에서 유전자형과 표현형의 관계에 대해 고찰하였다. 또한 문헌 고찰을 통하여 선행 연구들의 결과를 이 연구의 결과와 비교해 보았다.

1번 환자는 태어날 때부터 체온이 높았고 이후로도 발열이 반복적으로 발생하였으며 통증을 유발할 만한 자극에도 반응하지 않았다. 그는 아동기에는 땀을 전혀 흘리지 않았지만 10대 이후로 성장하면서 상지에는 약간의 땀이 나는 정도로 호전되었다. 그는 범발달 지연을 보였으며 자해 행동을 보였다. 그는 T11-L5 척수를 압박하는 척추주변 농양으로 응급 감압 수술을 받았음에도 불구하고 하반신 마비가 되어 휠체어를 이용하게 되었다.

2번 환자는 반복되는 발열과 무한증, 그리고 혀를 씹는 등의 자해 행동을 보였다. 그는

정맥 주사나 근육 주사를 맞을 때 울지 않았고 범발달장애로 진단 받았으며 성장 기간 동안 지속적으로 신장은 25퍼센타일, 체중은 3퍼센타일 정도로 유지되었다. 1번 환자와 달리, 그의 증상들은 성장함에 따라 호전되지 않고 지속되었다. 3번 환자 역시 반복되는 발열, 무한증, 무통각증을 보였지만 정상 지능을 보였고 자해 행동은 보이지 않았다. 그러나 그녀는 충동적이고 공격적인 경향성을 보였고 좌측 원위 경골에 골절, 비골화성 섬유종 및 좌측 거주상골 변형 소견을 보였다. 이 연구의 환자들이 보인 임상 양상은 이전 보고된 문헌들의 결과와 부합하였는데, 유전 검사로 진단된 CIPA 환자들에서 가장 흔한 증상은 무한증(97%)이었고 무통각증(94%), 반복적인 발열(89%), 지능 저하(75%), 자해 행동(75%), 근골격계 이상(64%) 순이었다. 1번, 2번 환자와 부모는 전장 엑솜 염기서열 분석을 시행하였고 3번 환자와 부모는 전장 유전체 염기분석을 시행하였다. 1번 환자에게는 c.851-33T>A 동형 접합이 확인되었고 부, 모는 각각 보인자였다. c.851-33T>A 는 비정상적인 splicing 을 유발하며 이로 인해 transmembrane domain 및 tyrosine kinase domain 이 포함된 intracellular domain 이 결여된 절단된 단백질이 생성된다.[8] 2번 환자에서는 c.851-33T>A 와 c.2020G>T 의 복합 이형접합이 확인되었는데, 각각의 변이는 그의 부, 모로부터 각각 물려받은 것이었다. c.2020G>T는 tyrosine kinase domain 내부에 위치하며 *NTRK1* receptor 의 활성을 감소시키는 것으로 알려져 있지만 이 변이에 대한 동형접합인 경우도 상당한 정도의 수용체 기능이 가능하여, 질병 발현을 위해서는 상동 염색체의 다른 대립 형질이 완전히 불활성화 되어야 한다.[9] 3번 환자에서는 c.2303C>T 와 g.7071_11990del 의 복합 이형접합이 확인되었으며 각각의 변이는 어머니와 아버지로부터 유전된 것이었다. c.2303C>T는 tyrosine kinase domain 내부에 위치하며 경한 임상 증상을 나타내는 것으로 보고되었는데, 이 변이를 가진 환자들은 정상 인지 능력과 부분적인 통각 인지 능력을 보였다.[10] g.7071_11990del 는 새로 발견된 변이이며 5-7번 엑손에 위치한다. 중국 논문에서 보고된 적 있는 g.6995_11999del 는 g.7071_11990del

근처에 위치하며 단백 합성의 조기 종결과 이로 인한 절단 단백질 생성을 초래한다. g.6995_11999del 와 g.1-1258_10169del 복합 이형접합을 가진 환자의 경우 정상 지능과 자해 행동이 없는 경한 임상 증상을 보였으나 c.851-33T>A 를 복합 이형접합으로 가진 경우 지능 저하와 자해행동을 보였다.[11]

세 명의 환자 모두 반복되는 발열, 무한증, 무통각증을 보였으나 발달 지연, 지능 저하, 그리고 자해 행동은 3번 환자에서는 보이지 않았다. 또한 2번 환자는 1번 환자와는 달리 골절이나 관절 변이 등 근골격계 증상을 보이지 않았다. 1번 환자는 tyrosine kinase domain이 결여되는 c.851-33T>A 동형 접합을 가진 반면 2번 환자는 c.851-33T>A 와 *NTRK1* 수용체 활성을 부분적으로 감소시키는 c.2020G>T 를 가졌다. 3번 환자에서 보인 c.2303C>T 는 경한 임상 증상을 나타낸다고 보고되었고 g.7071_11990del 는 대립유전자 변이에 따라 중증도에 차이를 보일 것으로 생각되었다. 이를 통하여, 2번, 3번 환자는 하나의 '경한' 변이를 가진 반면 1번 환자는 c.851-33T>A 동형접합을 갖고 있기 때문에 phenotype 에 차이를 보인 것으로 생각된다. 이 연구는 *NTRK1* 변이의 영역을 확장하였고 표현형-유전자형의 연관성에 대한 추가적인 단서를 제공하였다.

Table 1. Clinical and genetic features of three CIPA patients

	Patient 1	Patient 2	Patient 3
Gestational age	38+2 wks, 2750 gm	40 wks, 2850 gm	40 wks, 3560 gm
Age at fisrt visit	12 months	4 months	8 yr
Age at diagnosis	2 yr (clinical) 16 yr (gene confirmed)	9 months	8 yr
Gender	male	male	female
Insensitivity to pain	(+) lower extremities	(+) from birth	(+) but partially preserved
Anhidrosis **Recurrent fever**	(+) lower extremities	(+) from birth	(+) no sweating except back
Development **Intellectual delay**	global development delay mild MR	global development delay	normal development normal intelligence aggressive tendency
Bone fracture **Joint deformity**	DDH, Rt.hip dislocation osteodystrophy paraplegia disproportionate short stature		Lt.distal tibia Fx Lt.Talonavicular jt.deformity Lt.distal tibia non ossifying fibroma
Self-mutilation	(+) nail biting	(+) nail biting	
Infection	paravertebral abscess osteomyelitis		
Mutation 1	c.851-33T>A	c.851-33T>A	c.2303C>T
Mutation 2	c.851-33T>A	c.2020G>T	g.7071_11990del

Table2. Clinical features of patients in this study and previously reported patients with genetically diagnosed CIPA

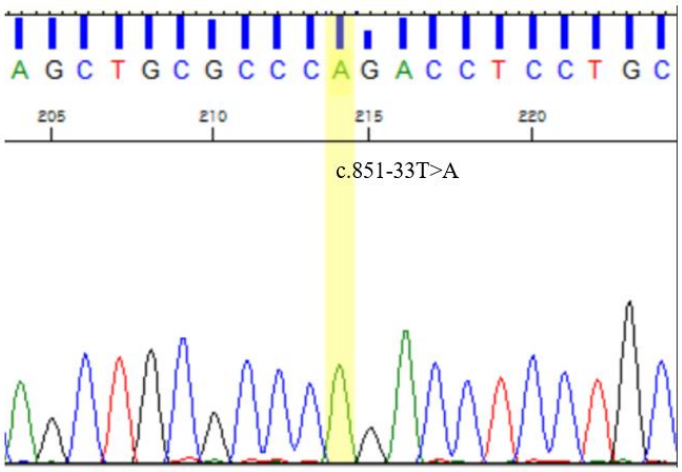
	Our study	Literature	Total
Patients	3	178	181
Male	2/3 (66)	118/178 (66)	120/181 (66)
Female	1/3 (33)	60/178 (34)	61/181 (34)
Insensitivity to pain	3/3 (100)	168/178 (94)	171/181 (94)
Anhidrosis	3/3 (100)	172/178 (97)	175/181 (97)
Recurrent fever	3/3 (100)	158/178 (89)	161/181 (89)
Intellectual disability	2/3 (66)	133/178 (75)	135/181 (75)
Bone fracture **Joint deformity**	2/3 (66)	113/178 (63)	115/181 (64)
Self-mutilation	2/3 (66)	133/178 (75)	135/181 (75)
Recurrent Infection	1/3 (33)	42/178 (24)	43/181 (24)

Table 3. Genetic variations identified in three CIPA patients

	Nucleotide change	Amino acid change	Zygosity	Exon Intron	ACMG classification	Inheritance
Patient 1	c.851-33T>A		Homozygote	Intron	Pathogenic	Both parents
Patient 2	c.851-33T>A		Heterozygote	Intron	Pathogenic	Paternal
	c.2020G>T	p.Asp674Tyr	Heterozygote	Exon15	Likely pathogenic	Maternal
Patient 3	c.2303C>T	p.Pro768Leu	Heterozygote	Exon17	Pathogenic	Maternal
	g.7071_11990del		Heterozygote	Exon5-7 del	Likely pathogenic	Paternal

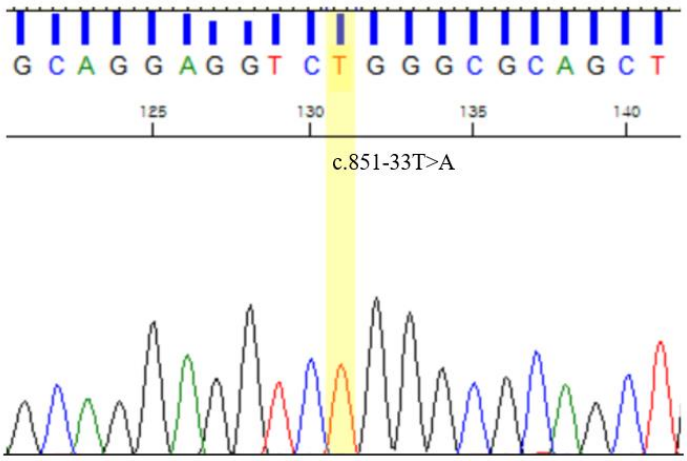
Figure 1. Sequencing chromatograms of three CIPA patients and their parents

Patient 1, forward



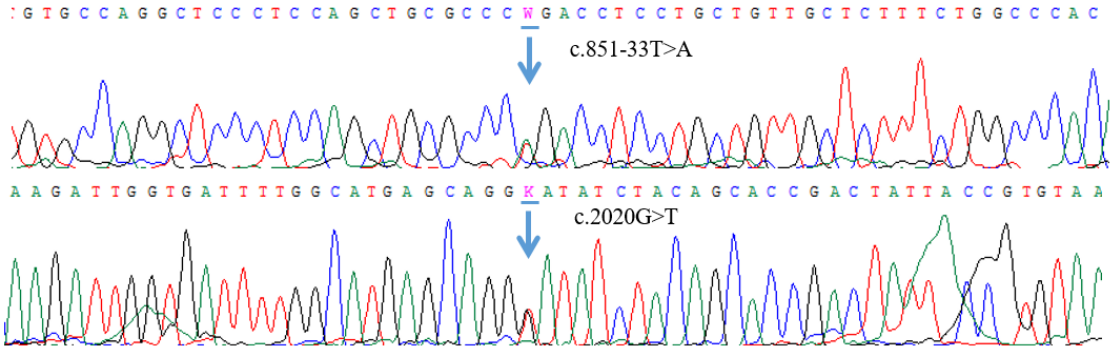
c.[851-33T>A](p.[/splicing])

Patient 1, reverse



c.[851-33T>A](p.[/splicing])

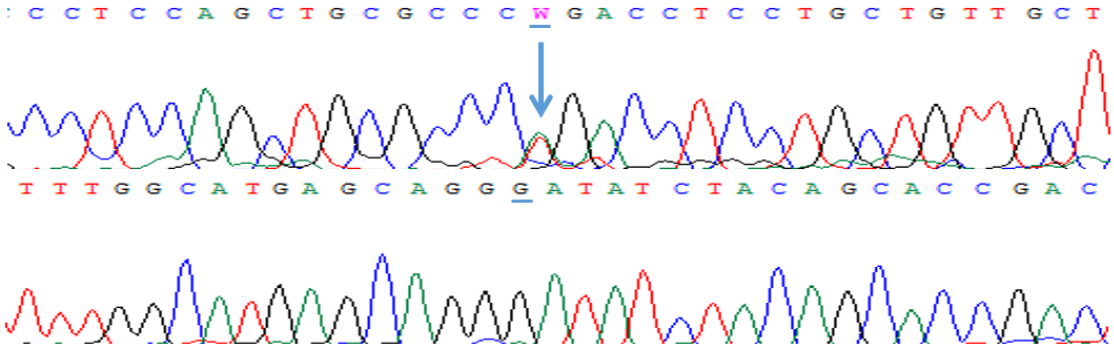
Patient 2



c.[851-33T>A];[2020G>T] (p.[/splicing];[Asp674Tyr])

W = T and A
K = G and T

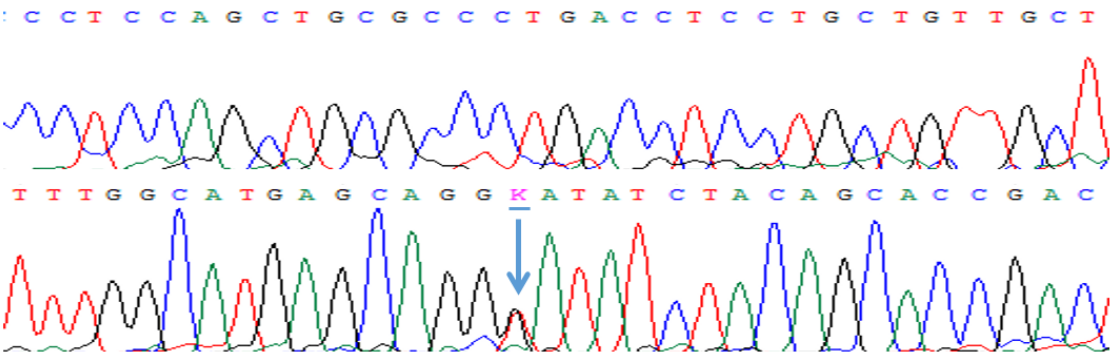
Father of Patient 2



c.[851-33T>A];[=] (p.[/splicing];[=])

W = T and A
K = G and T

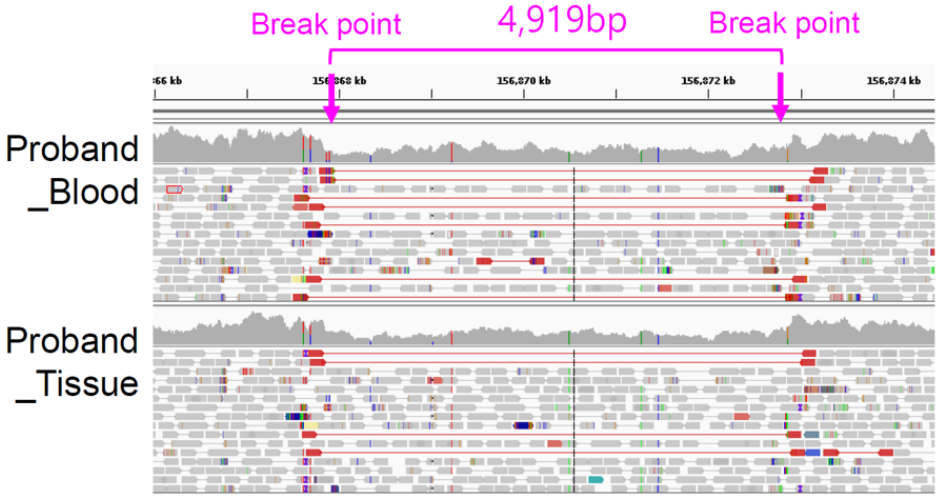
Mother of Patient 2



c.[2020G>T];[=] (p.[Asp674Tyr];[=])

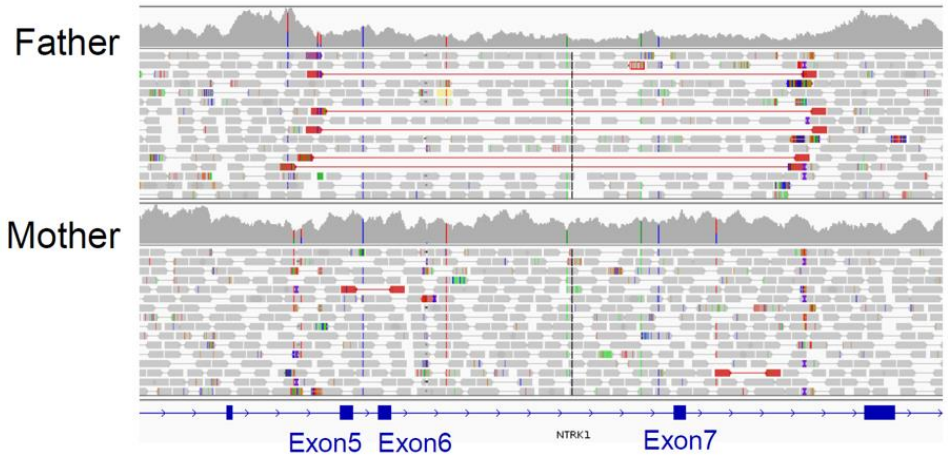
W = T and A
K = G and T

Patient 3



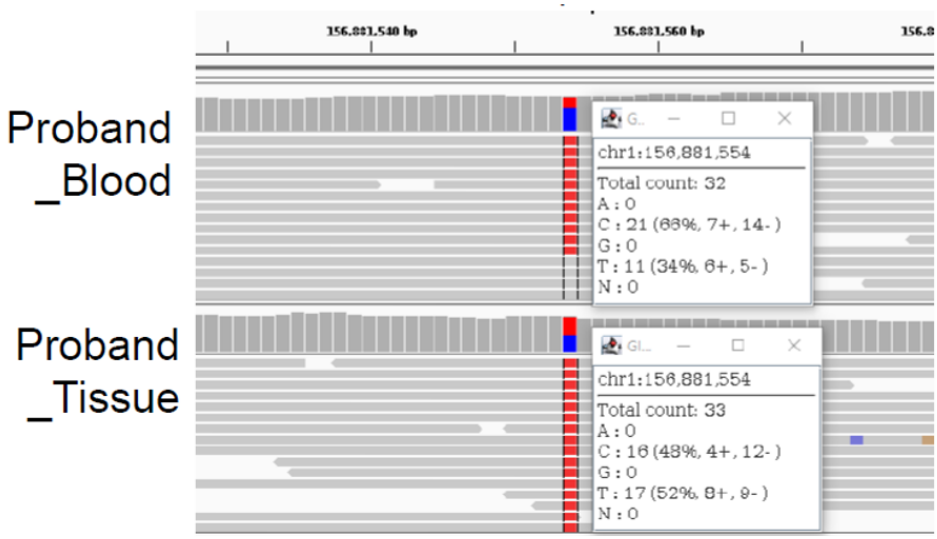
g.7071_11990del (Exon5-7 del)

Patient 3



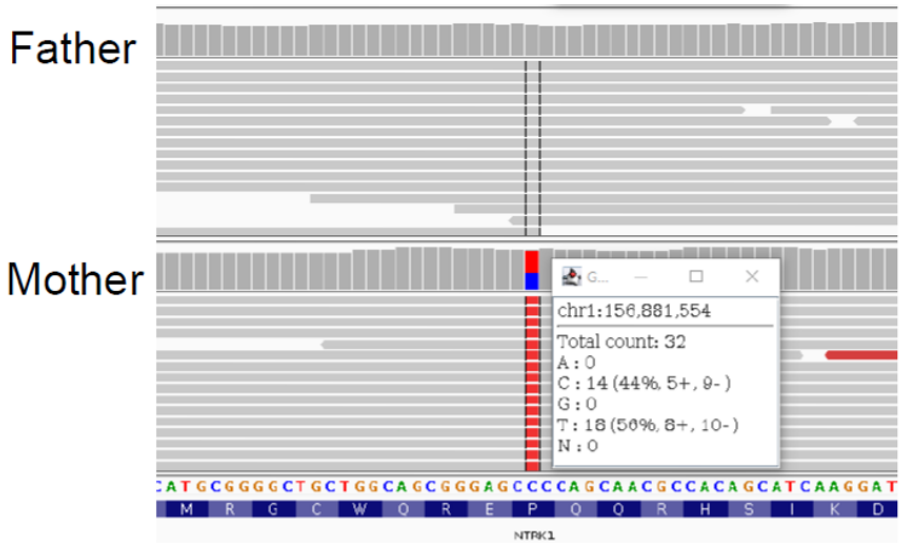
g.7071_11990del (Exon5-7 del)

Patient 3



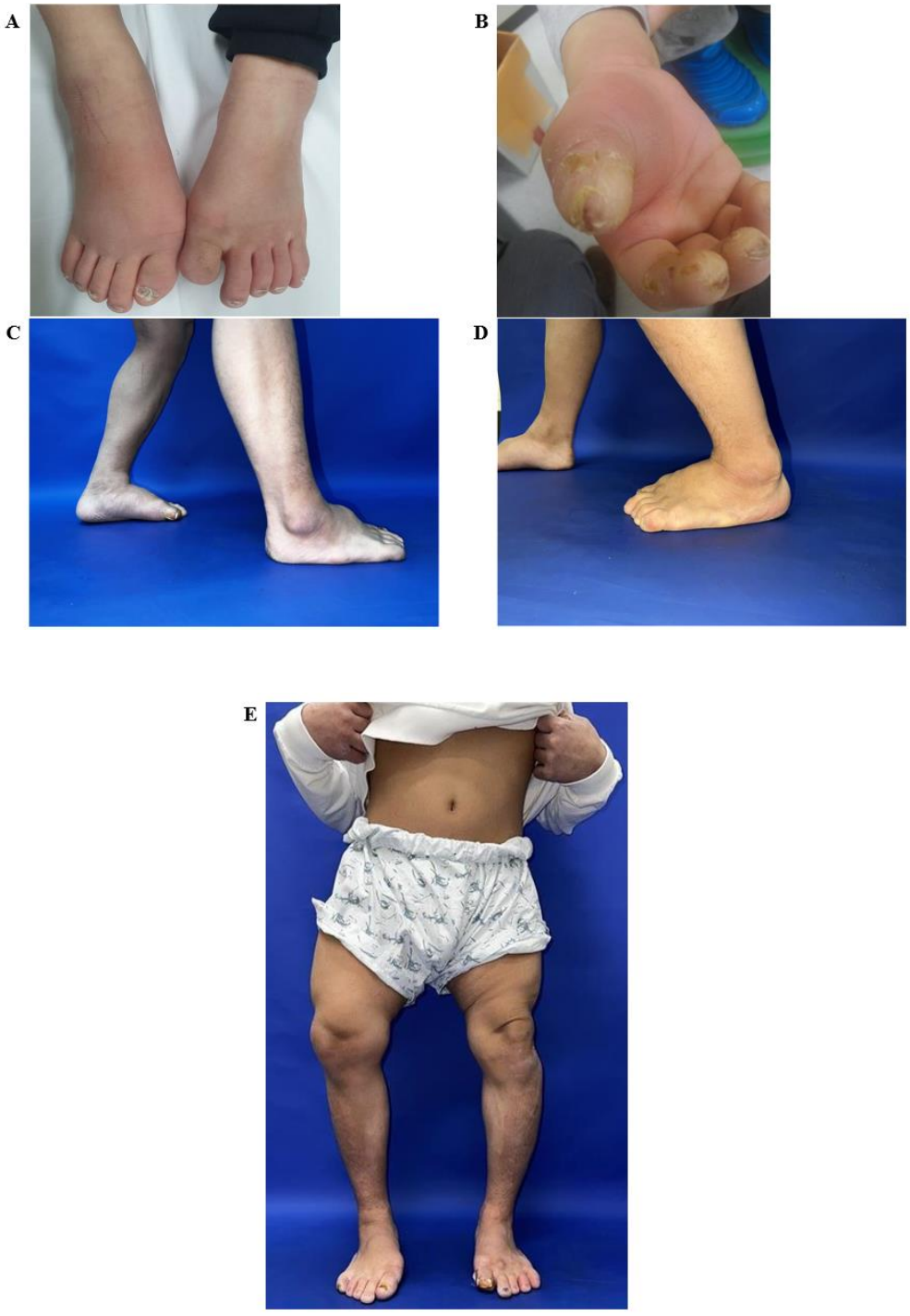
c.[2303C>T] (p.[Pro768Leu])

Patient 3

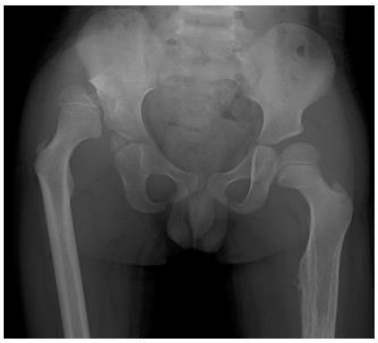


c.[2303C>T] (p.[Pro768Leu])

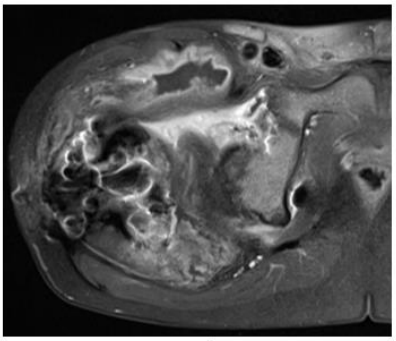
Figure 2. Clinical features of three CIPA patients.

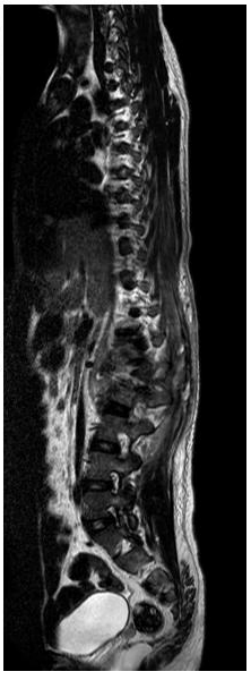


F



G





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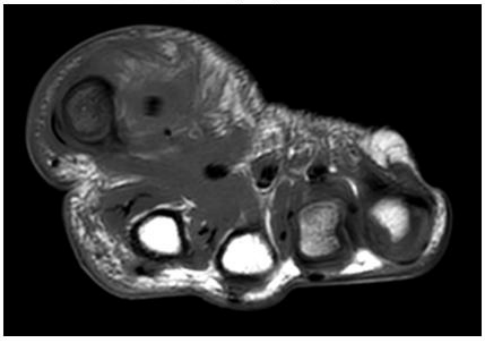

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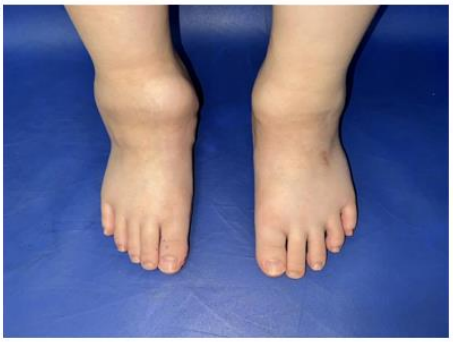

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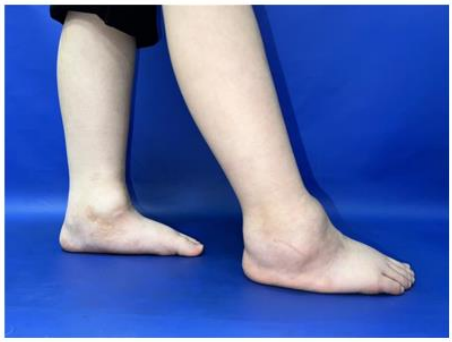

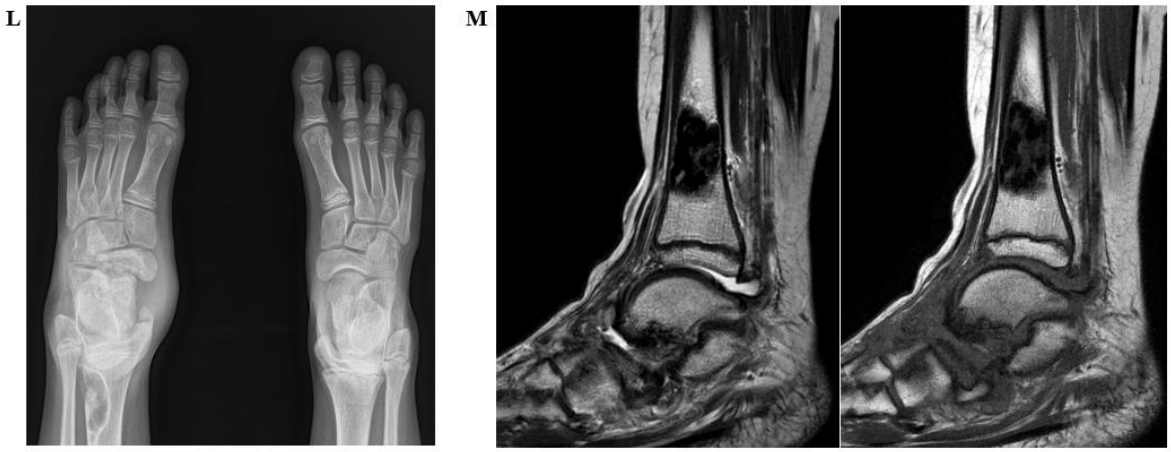

Figure 3. The pedigrees of three families

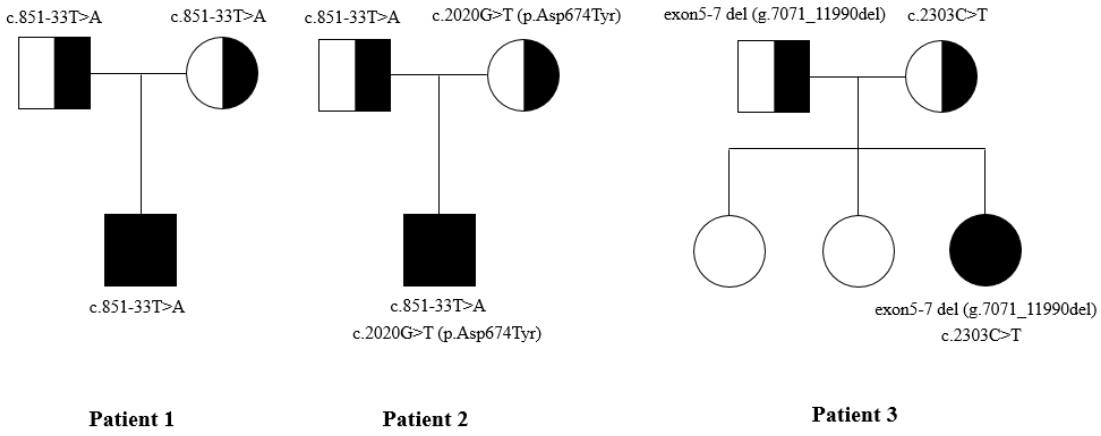

FIGURE LEGENDS

FIGURE 1. Sequencing chromatogram of three patients and their parents. Patient 1 and Patient 2 were conducted whole exome sequencing while Patient 3 underwent whole genome sequencing.

FIGURE 2. Clinical features of CIPA patients. (A-I) Patient 1 : (A,B,C,D) Fingers and toes cellulitis, amputation due to self-mutilating behavior. (E) Recurrent infection and operation history led to osteodystrophy of lower extremities and disproportionate short stature. (F) Right hip joint dislocation and osteodystrophy. (G) Right inguinal abscess. (H) Tenosynovitis of right flexor pollicis lungus tendon. (I) Paravertebral abscess (T11-L5). (J,K,L,M) Patient 3 : (J,K) Both toes deformity due to bone destruction and tissue swelling. (L) Early destruction of both talonavicular joints. (M) Left distal tibia non-ossifying fibroma.

FIGURE 3. The pedigrees of three families. Patient 1 and Patient 2 do not have siblings, while patient 3 has two older sisters without phenotype of CIPA. Patient 1 was a homozygote, while patient 2 and patient 3 were compound heterozygotes. Both parents of each patients were sequenced, and all the parents were identified as asymptomatic carriers. Parents of patient 3 denied the presence of clinical features of CIPA in two older sisters, and sequencing was not performed.