CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

Novel In-Frame Deletion *CNOT3* Variant in a Family With Intellectual Developmental Disorder With Speech Delay and Dysmorphic Facies

Cha Gon Lee, MD, PhD, Hyun Jung Kim, MD, PhD, Chang Ahn Seol, MD, PhD, and Chang-Seok Ki, MD, PhD

Correspondence
Dr. Lee
leechagon@hanmail.net

Neurol Genet 2024;10:e200116. doi:10.1212/NXG.0000000000200116

Abstract

Objectives

Intellectual developmental disorder with speech delay, autism, and dysmorphic facies (IDDSADF) is caused by heterozygous *CNOT3* (MIM# 604910) variants on chromosome 19q13. This study aimed to identify and describe the clinical features of a Korean family with maternally inherited speech delay and intellectual and developmental disability to elucidate the underlying genetic mechanism.

Methods

We conducted whole-exome sequencing and confirmatory Sanger sequencing on the proband, the mother, and unaffected grandparents with wild-type genotypes.

Results

The phenotypes of the mother and 2 daughters presented muscular hypotonia, global developmental delay, speech delay, intellectual disability, macrocephaly, facial dysmorphic features, and focal corpus callosum hypoplasia. Whole-exome sequencing identified a novel in-frame deletion, c.2017_2019del (p.Phe673del) in *CNOT3*, located in the C-terminal negative on the TATA-less-box domain.

Discussion

This report presents a new possible mechanism underlying IDDSADF caused by CNOT3 variants—an in-frame deletion. The findings enhance our understanding of early-life neurodevelopment and the genotype-phenotype relationships of IDDSADF caused by *CNOT3* variants. In addition, this report could assist in early diagnosis and facilitate genetic counseling.

Introduction

Intellectual developmental disorder with speech delay, autism, and dysmorphic facies (IDDSADF, MIM# 618672) is a newly identified neurodevelopmental disorder that results from certain pathogenic germline heterozygous variants in the *CNOT3* gene (MIM# 604910).[1] This gene is located on chromosome 19q13 and codes for a component of the carbon catabolite repression 4-negative TATA-less (CCR4-NOT) protein complex.[2] This complex is essential for regulating mRNA levels posttranscriptionally.[2] To date, 22 patients with IDDSADF have been documented in the literature, with 17 pathogenic germline variants in *CNOT3*. These include 8 missense variants, 6 frameshift variants, and 3 nonsense variants (Figure 1A).[1,3,4] In this report, we describe a rare case of autosomal dominant

From the Department of Pediatrics (C.G.L.); Department of Rehabilitation Medicine (H.J.K.), Nowon Eulji Medical Center, Eulji University School of Medicine, Seoul; and GC Genome (C.A.S., C.-S.K.), Yongin, Republic of Korea.

Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

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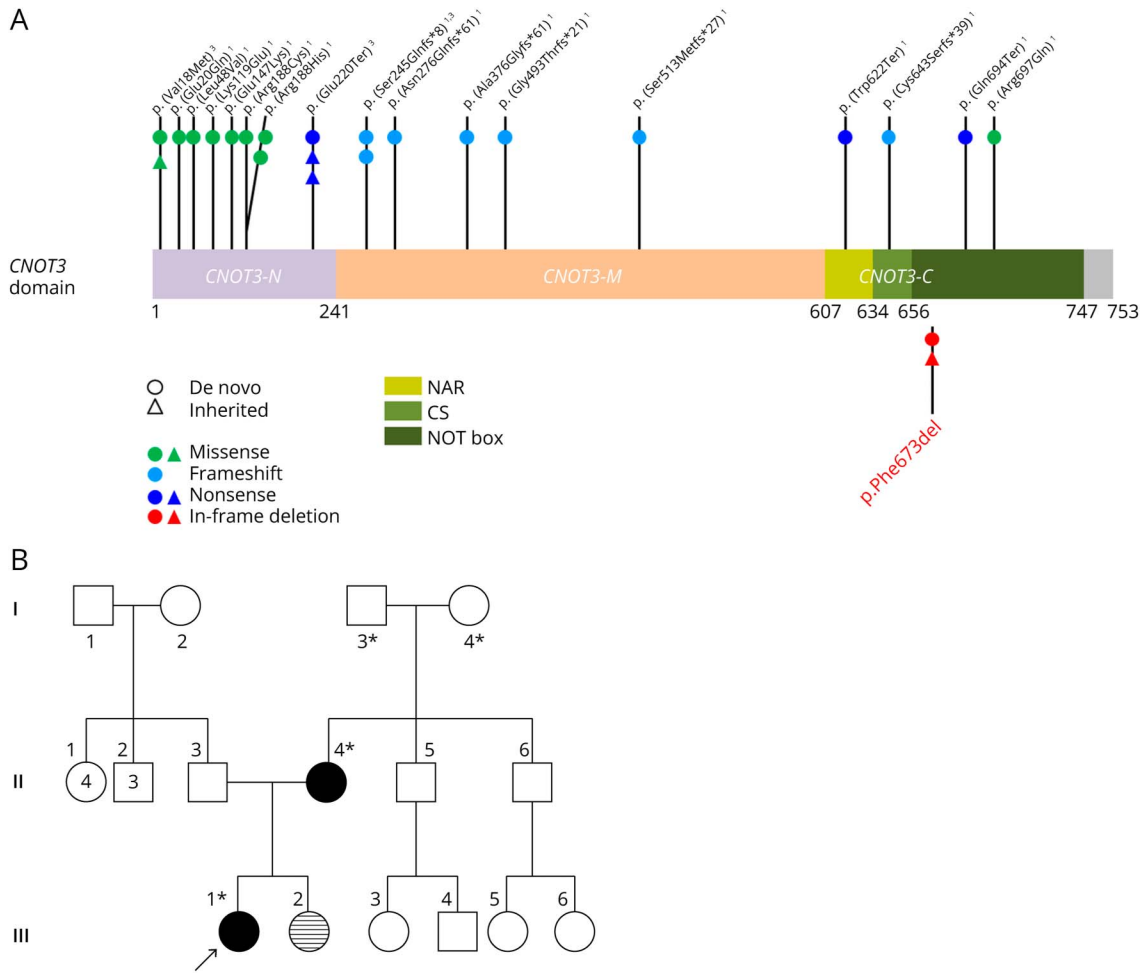
Figure 1 Schematic Summary of *CNOT3* Variants and Pedigree Analyses for the Family



(A) Schematic representation of CNOT3 domains and previously reported *CNOT3* pathogenic variants in patients with IDDSADF (upper panel) and the novel variant identified in our patients (lower panel, indicated in red). Before our report, 17 germline heterozygous pathogenic variants were reported, including 8 missense, 6 frameshifts, and 8 nonsense variants (adapted and used with permission from Martin R et al. Eur J Hum Genet 2019;27:1677-1682[1]; permission conveyed through Copyright Clearance Center, Inc.). (B) Pedigree of the family. The black arrow indicates the proband (III-1). Darkened symbols represent affected family members. A striped symbol indicates an affected family member by clinical features. Asterisks indicate sampled individuals. CNOT3-N = N-terminal coiled-coil domain; CNOT3-M = Middle linker region; CNOT3-C = C-terminal region; CS = Connector sequence; NAR = NOT1 anchor region; NOT-box = Negative on TATA-less box.

inherited familial IDDSADF caused by a novel heterozygous pathogenic in-frame deletion *CNOT3* variant.

Methods

Patients

A family presenting with intellectual and developmental disabilities (IDD) visited the Nowon Eulji Medical Center (Seoul, Republic of Korea). The pedigree chart of the family is shown in Figure 1B. This family comprised 3 identified affected individuals (III-1, III-2, and II-4).

Ethics Statement

The Institutional Review Board of the Nowon Eulji Medical Center (EMCS 2018-11-035) approved using human clinical materials and blood samples in this study. Written informed consent for the publication of medical photographs and genetic test results was obtained from the affected individuals.

Genetic Studies

The genomic DNA of the 2 affected individuals (III-1 and II-4) and 2 unaffected individuals (I-3 and I-4) in the family was extracted from the peripheral blood leukocytes. However, we failed to draw peripheral blood from the affected individual (III-2). Initially, a 750 K high-resolution genotyping SNP microarray (Affymetrix, Santa Clara, CA) analysis was performed on the proband (III-1), which revealed normal results. Subsequently, whole-exome sequencing (WES) was performed on the proband (III-1). SureSelect Human All Exon V5 (Agilent Technologies, Santa Clara, CA) was used for library preparation, and sequencing was performed using an Illumina NextSeq500 platform (Illumina Inc., San Diego, CA) at GC Genome (Yongin, Republic of Korea), generating 2 × 150 bp paired-end reads. Alignment of sequence reads indexing of the reference genome (hg19) and variant calling using a pipeline based on GATK Best Practices were performed by GC

Genome. Sanger sequencing was performed to determine whether the variants detected by WES were segregating with the disease phenotype in this family.

Results

Clinical Descriptions

Individual 1 (Proband, III-1)

A 15-year-old female patient (III-1) visited Nowon Eulji Medical Center (Seoul, Republic of Korea) for IDD and recurrent headaches. She was born to nonconsanguineous Korean parents at term with a weight of 2.7 kg, which is an appropriate weight for gestational age. Down syndrome was suspected, owing to facial features and hypotonia at birth; thus, karyotyping was performed, which revealed normal results. The patient had shown global developmental delay from infancy. She had undergone active speech therapy for developmental speech articulation disorder, dysarthria, and anarthria between the ages 5 and 9 years. At a follow-up examination at age 15 years, she showed mild intellectual disability with a full-scale IQ (FSIQ) of 59 on the Korean Wechsler Intelligence Scale for Children-V and a borderline adaptive behavior composite score of 70 on the Korean Vineland II scale. During a physical examination at age 15 years, she presented with a body weight of 61.6 kg (SD score [SDS] 1.10), a height of 159.7 cm (0.04 SDS), and macrocephaly with a head circumference of 55.4 cm (2.93 SDS).[5] She had a high broad forehead, short palpebral fissures, thick ala nasi, anteverted nares, small chin, and large, posteriorly rotated ears (Figure 2A). Neurologic examination results were normal. MRI showed a focal thinning at the body-splenium junction of the corpus callosum, and magnetic resonance angiography (MRA) of the brain was normal (Figure 2B).

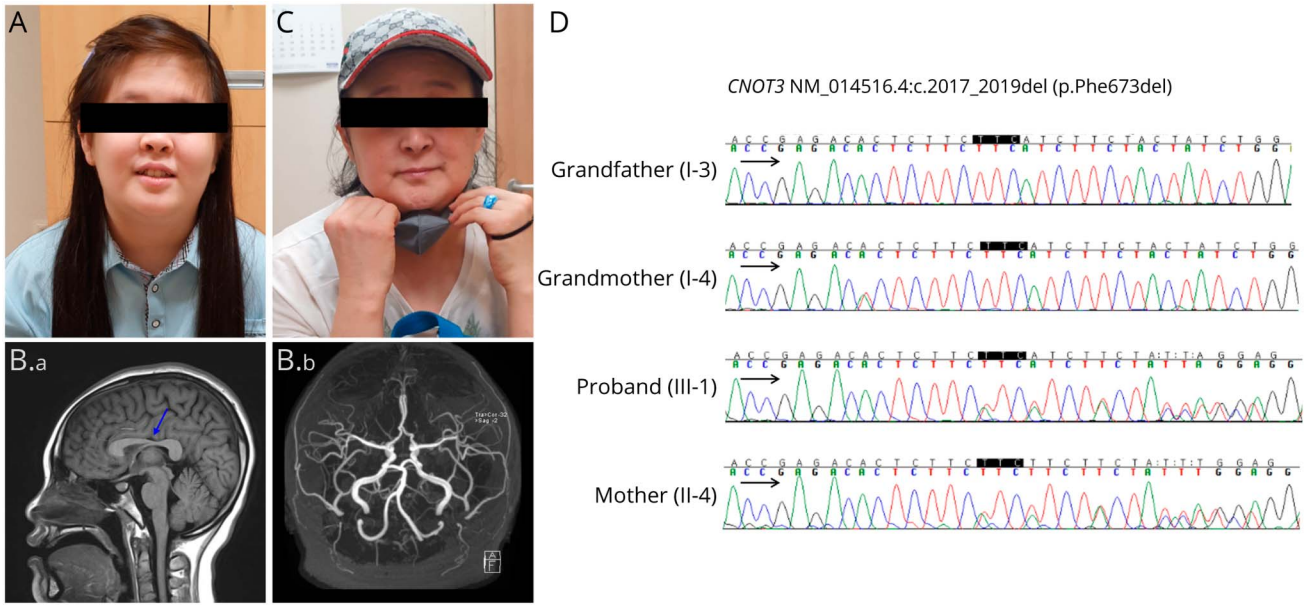
Individual 2 (Mother, II-4)

The proband's mother (II-4) visited the clinic with her daughter at age 50 years. She was the third child of healthy, nonconsanguineous Korean parents. At the time of her birth, her father (I-3: 40 years) was older than her mother (I-4: 23 years). No family history of neurodevelopmental disorders was found. Moreover, II-4 had no history of hospitalization and surgery other than that for the cesarean sections for her 2 childbirths. She reported that she had a noticeable global developmental delay, especially speech delay, which had set her apart from her siblings. Her exact IQ was not measured, but she had apparent intellectual and learning disabilities. During her physical examination at age 50 years, she presented with a height of 161.7 cm (0.13 SDS), a weight of 65.7 kg (1.47 SDS), and macrocephaly with a head circumference of 54.2 cm (2.22 SDS). She had a high broad forehead, short palpebral fissures, thick extended ala nasi, anteverted nares, thin upper lip, small chin, and large, posteriorly rotated ears (Figure 2C).

Individual 3 (Sister, III-2)

The 10-year-old individual, the younger sister of III-2, was born at term with appropriate weight for the gestational age after an uneventful pregnancy. Her psychomotor development was

Figure 2 Facial Features and Sanger Sequencing Chromatograms of the Family



(A) Photograph of the proband (III-1) at age 15 years, exhibiting a high broad forehead, short palpebral fissures, thick ala nasi, anteverted nares, small chin, and large, posteriorly rotated ears. (B.a) T1-weighted sagittal brain magnetic resonance images of the proband (III-1) show a focal thinning at the body-splenium junction. (B.b) Time-of-flight magnetic resonance angiography of the proband (III-1) reveals no significant stenosis and occlusive lesion in intracranial vessels. (C) Photograph of the patient's mother (II-4) at age 50 years, exhibiting a high broad forehead, short palpebral fissures, thick extended ala nasi, anteverted nares, small chin, and large, posteriorly rotated ears. (D) In this report, Sanger sequencing confirmed the CNOT3 variant c.2017_2019del (p.Phe673del) in patients II-4 and III-1 and the wild-type genotype in unaffected grandparents (I-3 and I-4).

delayed—she started independent walking when she was 16 months. She had shown language delay and speech problems and had been assessed for developmental language delay and speech disorder when she was 21–35 months. However, she did not receive any language and speech therapy. At age 4 years, she underwent a frenectomy under general anesthesia. In her recent check-up, she revealed a moderate ID with an FSIQ of 46. In a physical examination at age 10 years, she exhibited macrocephaly with a head circumference of 57 cm (3.82 SDS). She had a high broad forehead, short palpebral fissures, thick ala nasi, anteverted nares, and large, posteriorly rotated ears.

Genetic Results

After variant prioritization considering the individual's phenotype, a heterozygous in-frame deletion variant in *CNOT3*, c.2017_2019del (p.Phe673del), was identified based on the reference sequence NM_014516.4. The *CNOT3* variant in the proband and her mother (III-1 and II-4) and the wild-type genotype in unaffected grandparents (I-3 and I-4) were confirmed by Sanger sequencing (Figure 2D). The variant was classified as likely pathogenic based on the following evidence: PS2 (*De novo*), in PM2 (absent from population data contained in dbSNP, gnomeAD, ExAC, 1 KGP, and KRGDB), PP1 (co-segregation in affected family members), and PP4 (highly specific for a disease with a single genetic etiology), according to the sequence interpretation guidelines of the American College of Medical Genetics and Genomics.[6]

Discussion

In the Deciphering Developmental Disorders (DDD) cohort study, de novo heterozygous germline variants in *CNOT3* were discovered as a cause of a new developmental disorder.[7] IDDSADF was recognized as a novel neurodevelopmental syndrome through reverse phenotyping of 16 patients with de novo variants.[1] The main consistent phenotypes in previously described patients are early muscular hypotonia, global developmental delay, prominent speech delay, intellectual disability, behavioral disorders including autism spectrum disorder, and facial dysmorphic features.[1,3,4] Brain MRI abnormalities are reported in >60% of patients, mainly in the corpus callosum.[1,3,4] Here, we report 2 additional patients in a Korean family with consistent phenotypes of muscular hypotonia, global developmental delay, speech delay, intellectual disability, macrocephaly, facial dysmorphic features, and focal corpus callosum hypoplasia. Obvious but variable facial dysmorphic features have been observed in all reported patients, including ours, but there is no overall consistent diagnostic facial gestalt. Although the clinical phenotypes are distinctive and consistent with reverse phenotyping in this syndrome, it is difficult to specify *CNOT3*-related neurodevelopmental syndromic disease based on the clinical phenotype alone. Thus, targeted multigene panel sequencing, including for *CNOT3*, is the most effective method for early diagnosis and informing family genetic counseling for all pediatric patients with variable neurodevelopmental delay.

Rare de novo variants in *CNOT3* have been identified in patients with moyamoya angiopathy (MMA).[8] The disruption of *CNOT3* is a potential predisposing factor for non-atherosclerotic occlusive cerebrovascular disease.[8] Our proband initially visited the hospital with a headache. She was confirmed to have no significant stenosis or occlusive lesion in intracranial vessels in her MRA (Figure 2B). In addition, we also checked that there are no rare variants of *RNF213*, the other genetic predisposing factor of increased incidence of MMA in Asian countries.[8] In future follow-ups, the reported family will be closely monitored about neurologic symptoms of the cerebrovascular disorder.

Twenty-three sequence variants in *CNOT3* are listed on Decipher and 98 on ClinVar. This study analyzed 17 germline variants in *CNOT3* with demonstratable clinical details documented in the literature. The distribution of the 17 reported pathogenic germline variants in *CNOT3* revealed no evidence of mutational hotspots (Figure 1A). However, when the distribution of variants was examined, missense variants were mainly found in the N-terminal, and most of the frameshift variants were distributed sporadically thereafter (Figure 1A). Both truncating (frameshift and nonsense) and missense variants have been described; thus, loss-of-function of the protein has been suggested as a disease-causing mechanism. Our patients were identified with an in-frame deletion variant, p.Phe673del, in *CNOT3*. Microindels (insertions or deletions shorter than 21 bp) represent the second most frequent type of genetic variation in human genomes after single-nucleotide variants.[9,10] The damaging effects of nonframeshifting microindels on protein structure and function are largely unstudied and difficult to predict.[9,10] The phenotypic effects of a nonframeshifting indel variant can lead to changes in the resulting protein's structure or function and its impact on biological pathways; nonframeshifting microindels can be damaging and contribute to disease susceptibility.[9,10] Lin et al. conducted research to assess the functional effects of indel variations observed in the 1000 Genomes Project population: nonframeshift indels were more commonly found in transcription-related proteins, especially in N-terminal and C-terminal regions, coils, and disordered regions, whereas they were depleted in helix and strand secondary structures, contributing to genetic variations and phenotypic diversity.[9] *CNOT3* encodes the CCR4-NOT transcription complex subunit 3, which is a component of the CCR4-NOT deadenylase complex, a large, highly conserved throughout evolution, multifunctional assembly of proteins that acts at different cellular levels to control gene expression.[2] The crystal structure of the human CNOT1-CNOT2-CNOT3 ternary complex is formed by the CNOT1, CNOT2, and CNOT3 C-terminal (-C) regions.[2] CNOT1-C provides a rigid scaffold consisting of 2 perpendicular stacks of HEAT-like repeats.[2] CNOT2-C and CNOT3-C heterodimerize through their SH3 (src Homology-3)–like NOT-box domains.[2] The heterodimer is stabilized and tightly anchored to the surface of CNOT1 through an unexpected intertwined arrangement of peptide regions that lack a defined secondary structure.[2] Our patients had an in-frame deletion variant

(p.Phe673del) within the C-terminal region NOT-box domain of CNOT3 (residues 656–747) (Figure 1A). This variant is located within an evolutionarily highly conserved region with PhyloP and PhastCons conservation scores of 7.01 and 1.00, respectively.[11] We suggest that the novel in-frame deletion contributed to the observed phenotype.

Of interest, heterozygous variants in *CNOT1* (MIM#604917) on chromosome 16q21 cause Vissers-Bodmer syndrome (VIBOS, MIM#619033), which is characterized by the global developmental delay with variably impaired intellectual development, speech delay, motor delay, and behavioral abnormalities.[12] A heterozygous variant in *CNOT2* (MIM#604909) on chromosome 12q15 causes intellectual and developmental disability with nasal speech, dysmorphic facies, and variable skeletal anomalies (IDNADFS).[13] VIBOS, IDNADFS, and IDDSADF are associated with the CCR4-NOT transcription complex subunits CNOT1, CNOT2, and CNOT3, respectively. All of them are categorized as neurodevelopmental disorders; thus, all 3 genes encode subunits of the CCR4-NOT transcription complex that contribute to early neurodevelopment. In the future, we suggest an integrated study related to early-life neurodevelopment associated with the CNOT1-CNOT2-CNOT3 ternary complex.

In summary, we present a report on familial IDDSADF in a mother and daughter with a novel *CNOT3* in-frame deletion, c.2017_2019del (p.Phe673del), located in the C-terminal NOT-box domain, which was identified by WES. This new genetic finding is expected to increase our understanding of early-life neurodevelopment and the genotype-phenotype relationships in IDDSADF caused by variants in *CNOT3*. The findings will also help inform early diagnosis and facilitate genetic counseling.

Acknowledgment

The authors thank the patients and their families for their participation in this study.

Study Funding

The authors report no targeted funding.

Disclosure

The authors report no relevant disclosures. Go to Neurology.org/NG for full disclosures.

Publication History

Received by *Neurology: Genetics* April 3, 2023. Accepted in final form October 17, 2023. Submitted and externally peer reviewed. The handling editor was Editor Stefan M. Pulst, MD, Dr med, FAAN.

Appendix Authors

Name	Location	Contribution
Cha Gon Lee, MD, PhD	Department of Pediatrics, Nowon Eulji Medical Center, Eulji University School of Medicine, Seoul, Republic of Korea	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Hyun Jung Kim, MD, PhD	Department of Rehabilitation Medicine, Nowon Eulji Medical Center, Eulji University School of Medicine, Seoul, Republic of Korea	Analysis or interpretation of data
Chang Ahn Seol, MD, PhD	GC Genome, Yongin, Republic of Korea	Analysis or interpretation of data
Chang-Seok Ki, MD, PhD	GC Genome, Yongin, Republic of Korea	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data

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