ORIGINAL ARTICLE

Identification of a Novel Variant in CC2D1A Gene Linked to Autosomal Recessive Intellectual Disability 3 in an Iranian Family and Investigating the Structure and Pleiotropic Effects of this Gene

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Zahra RASHVAND PhD [1],
Hossein NAJMABADI PhD [2],
Kimia KAHRIZI MD [2],
Hossein MOZHDEHIPANAH MD [3],
Mohammad MORADI BS [4],
Zohreh ESTAKI DDS [5],
Khadijeh TAHERKHANI MS [4],
Nooshin NIKZAT MS [2],
Reza NAJAFIPOUR MD [2],
Mir Davood OMRANI PhD [1,6]

1. Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Genetics Research Center, the University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
3. Depatment of Neurology Boali Hospital, Qazvin University of Medical Sciences, Qazvin, Iran
4. Cellular and Molecular Research Centre, Qazvin University of Medical Sciences, Qazvin, Iran

Abstract

Objectives

Intellectual disability (ID) represents a significant health challenge due to its diverse and intricate nature. A multitude of genes play a role in brain development and function, with defects in these genes potentially leading to ID. Considering that many of these genes have yet to be identified, and those identified have only been found in a small number of patients, no complete description of the phenotype created by these genes is available. *CC2D1A* is one of the genes whose loss-of-function mutation leads to a rare form of non-syndromic ID-3(OMIM*610055), and four pathogenic variants have been reported in this gene so far.

Materials & Methods

In the current study, two affected females were included with an initial diagnosis of ID who were from an Iranian family with consanguineous marriage. Whole-exome sequencing was used to identify the probable genetic defects. The Genotypic and phenotypic characteristics of the patients were compared with a mutation in the *CC2D1A* gene, and then the structure of the gene and its reported variants were investigated.

Results

The patients carried a novel homozygous splicing variant (NM_017721, c.1641+1G>A) in intron 14, which is pathogenic according to the ACMG guideline. Loss-of-function mutations in *CC2D1A* have severe phenotypic consequences such as ID, autism spectrum disorder (ASD), and seizures. However, missense mutations lead to ASD with or without ID, and in some patients, they cause ciliopathy.

5. Department of Pediatric Dentistry, School of Dentistry, Qazvin University of Medical Sciences, Qazvin, Iran
6. Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding Author
Omrani MD. PhD
Professor of Medical Genetics, Department of
Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Koodakyar St, Daneshjoo Bld, Evin, Chamran Highway, Tehran, Islamic Rep. of Iran,
Email: davood_omrani@ sbmu. ac.ir
And head of Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Najafipour R. MD
Associate Professor of medical genetics, Genetics Research Center, the University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
Email: re.najafipour@uswr.ac.ir
Mir Davood Omrani and Reza Najafipour are equal contributions to this work and designated as co-Corresponding Authors

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Conclusion

This study reports the fifth novel, probably pathogenic variant in the CC2D1A gene. Comparing the clinical and molecular genetic features of the patients with loss-of-function mutation helped to describe the phenotype caused by this gene more precisely. Investigating the CC2D1A gene's mutations and structure revealed that it performs multiple functions. The DM14 domain appears more pivotal in triggering severe clinical symptoms, including ID, than the C2 domain.

Keywords: CC2D1A, Intellectual disability, Whole-exome sequencing, Mutation, Iran
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Introduction

CC2D1A (coiled-coil and c2 domain containing 1a) (MIM * 610055) is a protein-coding gene located on chromosome 19 (19p13.12) (1). This gene was first identified by studying HEK293 cells to find human genes that activate the nuclear factor-ƙB(NF-Ƙb) (2-3). CC2D1A is composed of a "C2" domain (protein kinase C conserved region 2), four "DM14" domains (Drosophila melanogaster 14), and a predicted helix-loop-helix DNA binding domain (4). Numerous studies have shown that this gene plays many functions in cells. Signaling and endosomal trafficking are regulated by this protein (5-7). On the other hand, the role of CC2D1A as a molecular scaffold for accumulating signaling proteins enables this gene to affect biochemical pathways significantly (8-9). These pathways include nuclear factor-κB (NF-κB((10-11), cAMP-responsive element-binding protein (CREB), protein kinase A (PKA) (12), protein kinase B (PKB/AKT), bone morphogenetic protein

(BMP) (13), and Notch (7,14).

CC2D1A regulated the expression of brain receptors such as serotonin and dopamine (4, 15-16). Zhao M. et al. reported the essential function of CC2D1A in neuronal development. They observed the death of CC2D1A knockout mice after birth, probably because of a disturbance of synaptic maturation (10). Oaks A. et al. showed that defects in CC2D1A impair early postnatal brain development when social and cognitive behaviors are developing in humans. Disruption of this pathway leads to intellectual disability (ID) and autism spectrum disorder (ASD). Moreover, they observed that the production of mice with a defect in the CC2D1A gene was fatal in the perinatal period (17). From these studies, it can be concluded that multiple functions of this gene have significant effects on neuronal differentiation and cognitive functions (17).

Loss-of-function mutation in CC2D1A has been linked to severe autosomal recessive non-syndromic ID-3 (# 608443) (18). Furthermore, in addition to ID, it can cause a spectrum of neurodevelopmental diseases, including seizures and ASD (17). So far, only three pathogenic variants have been reported in this gene (clinVar). The first two cases were reported (19, 20) and the last case has yet to be published. This gene appears to be highly conserved; therefore, mutations are typically incompatible with life. Recent studies have shown a link between the CC2D1A gene and ciliopathy (21, 22).

This study reports a novel homozygous splicing variant (NM_017721, c.1641+1G>A) in the CC2D1A gene in an Iranian family with consanguineous marriage. Mutation in this gene is reported for the first time in Iran. A comparison was made between the genetic characteristics of the mutations in this gene and the clinical presentation of the patients with these mutations. Due to the small number of mutations reported, this comparison helps to better understand the genotype-phenotype correlation. According to the literature, the structure of the CC2D1A gene and its reported mutations were evaluated to determine the importance of domains in the occurrence of phenotypes.

Materials & Methods

Editorial policies and ethical considerations

The present study was performed at Shahid Beheshti University of Medical Sciences (SBUMS), Tehran, Iran, in collaboration with the University of Social Welfare and Rehabilitation Sciences, Tehran, Iran, and Qazvin University of Medical Sciences, Qazvin, Iran. The project protocol was approved by the local medical ethics committee of SBUMS (IR.SBMU.MSP.REC.1398.798) . In addition, this study was supported by the Iran National Science Foundation (INSF) . Written informed consent was taken from the parents of the two patients for molecular analysis and evaluation of patient images.

A consanguineous couple with two affected children was enrolled in this study. The family was from Qazvin province, Iran. After obtaining informed consent, genetic counseling and a complete clinical examination were performed. Karyotyping, molecular methods, and tandem mass spectrometry were used to exclude chromosomal abnormalities, fragile X syndrome, and metabolic diseases. Finally, the proband underwent whole-exome sequencing.

Genetic analysis

Genomic DNA was extracted from the blood samples of the patients, their parents, and unaffected siblings to perform genetic testing according to standard procedures (Miller et al., 1988) . The concentration of DNA was quantified by a Nanodrop 2000 C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and its quality was checked on 1 % electrophorese agarose gel.

Whole-exome sequencing was accomplished on the proband's DNA, following a method described in a previous study (23). Mode of inheritance of disorder, location, function, and minor allele frequency of variants (MAF) were considered for filtering steps. Databases including 1000 Genomes Project (www.1000genomes.org), ESP6500 (evs.gs.washington.edu/EVS/), Exome Aggregation Consortium (ExAC) (exac.broadinstitute.org), and Genome Aggregation Database (gnomAD) (gnomad.broadinstitute.org), and an in-house database of 800 Iranian control samples published in Iranome (www.iranome.com) were used to find variants with frequencies < 1 %. The sorted variants were examined for the presence of the genes involved in ID / developmental disabilities (24-25). Finally, the pathogenicity of the selected variants was investigated using in silico predictive tools such as Mutation Taster (www.mutationtaster.org), CADD (cadd.gs.washington.edu), FATHMM-MKL (fathmm.biocompute.org.uk/fathmmMKL.htm), BayesDel addAF (26), DANN (27), EIGEN (www.columbia.edu/~ii2135/eigen.html), and in silico nucleotide conservation from Genomic Evolutionary Rate Profiling (GERP) scores (mendel.stanford.edu/SidowLab/downloads/gerp). To assess genotype-phenotype correlation, an extensive search was performed in the literature, OMIM database (www.omim.org), ClinVar database (www.ncbi.nlm.nih.gov/clinvar), and locus/disease-specific databases, which finally led to the identification of the causal variant that was further validated by conventional Sanger sequencing and co-segregation analysis in the available family members.

Results

The family has eight children, two of whom have severe intellectual disabilities. The parents are second cousins. The first patient (proband) is a 35- year- old woman. Her mother had a normal pregnancy and did not report a history of infections, accidents, or taking medication during the pregnancy. Delivery occurred naturally at 38 weeks gestation. The proband was born with a meager birth weight (intrauterine growth restriction or IUGR) and a low Apgar score and received oxygen. She had a developmental delay, so she was able to sit at the age of ten months and walk at the age of two, and she has been able to talk since the age of five years. She still cannot speak properly and utters few words. At the age of nine, she had a recurrent seizure that was controlled with medication. She has shown aggressive behavior and receives drugs to control it. Additionally, she displays signs of autism, such as avoiding eye contact. Upon clinical examination, no distinct facial dysmorphisms were noted, and her limbs appeared normal.

The second patient is a 25- year- old woman who is the proband’s sister. The mother had a normal pregnancy and did not mention any history of taking medication or having accidents or

infections. In the eighth month of pregnancy, due to premature rupture of the amniotic sac (PROM), a cesarean section was performed, and the patient was born with a normal weight and Apgar score. She was hospitalized with severe jaundice after birth. Sitting and walking were normal, but talking was delayed, she and could speak a few words at the age of four. She now speaks well and has been able to attend an exceptional school and graduate. Like her sister, she had a recurrent seizure at the age of nine and took medicine. Moreover, she has aggressive behavior and receives drugs to control it, but she has no autistic behavior. No specific dysmorphism was observed on the face in the clinical examination, and the limbs were completely normal.

Genetic findings

WES analysis was performed on the proband (V5) as described previously. Several filtering steps were used to analyze the obtained data, all summarized in Figure 1b. A novel homozygous splicing variant was identified in exon 14 of the CC2D1A gene (NM_017721, c.1641+1G>A) . This variant obtained PVS1, PM2, and PP3 scores based on the ACMG guidelines and was considered as a pathogenic variant. In silico predictions of pathogenicity performed by Mutation Taster, CADD, FATHMM- MKL, BayesDel addAF, BayesDel noAF, DANN, EIGEN, and EIGEN PC identified this variant as pathogenic. The PhastCons 100- way score (1.000) and the GERP score (5.55) showed that this variant is highly conserved. The results are shown in Figure 1a. The identified variant was further confirmed using Sanger sequencing and was segregated in the family (Figure 2b) .

Discussion

The CC2D1A gene located on chromosome 19 (19p13.12) has 29 exons and a length of about 24,679 bp. The protein encoded by this gene contains two isoforms [long (951aa) and short (388aa)] (19). The long isoform consists of two domains: the C2 domain located at the C-terminal and DM14 domain at the N-terminal. The short isoform only has a C2 domain. Seemingly, the long isoform has the primary function because the loss-of-function mutations of CC2D1A occur in this isoform (19-20).

The C2 motif, located in the C-terminal region of the gene at positions 656-770, is a protein structural domain involved in targeting proteins in cell membranes (28) and calcium-dependent phospholipid binding (29). Zhao M et al., in 2010, demonstrated that CC2D1A activates NF-ƙB through conserved C2 and DM14 domains. They observed that the lack of N-terminal 138 amino acids and the absence of first and second DM14 motifs caused NF-ƙB activity reduction by 50 %. Further deletion, including third and fourth DM14 and C2 domains, reduces the activity to less than 20 %. Therefore, the C2 and DM14 domains, primarily the C2 domain, are essential for NF-ƙB activation (30).

The DM14 motifs located at N-terminal at position [1:(138-195), 2:(257-315), 3:(349-407), 4:(494-552)] (https://smart.embl.de/smart/show_motifs.pl?ID=Q6P1N0), and their role are unknown (Figure 2c). The number of DM14 repeats is four times in the human CC2D1A gene and three times in the Caenorhabditis elegans orthologue sequence. The DM14 motifs are present only in the long isoform of CC2D1A (19). The research by Tawashi

et al. has shown that the CC2D1A gene is a novel regulator of phosphodiesterase 4D (PDE4D). The CC2D1A bound to PDE4D could fine-tune cyclic adenosine 3', 5'-monophosphate (cAMP), a key regulator of various cellular signaling, especially in neuronal systems. Although the DM14 motifs, particularly the first motif, are essential for CC2D1A-PDE4D5 binding, they cannot prevent increased PDE4D5 activity (PKA-dependent phosphorylation) alone. Besides, the C2 domain has no role in this connection. The absence of second and third DM14 domains causes PDE4D hyperphosphorylation at the serine 126 positions through the cAMP-dependent protein kinase A (PKA). This hyperphosphorylation causes continuous PDE4D activity and eventually disrupts the homeostasis of the cAMP and the downstream signaling pathway, especially phosphorylation at S133 in CREB. This finding highlights the importance of the role of the second and third DM14 motifs. When the first three motifs from four DM14 motifs are absent in the CC2D1A gene, it causes ID without physical defects. Still, when only the first motif is present, it leads to death in mice at eight to twelve hours after birth, suggesting that the regulatory role of the CC2D1A gene is critical for neuronal function (31).

So far, three pathogenic mutations have been reported in this gene, recorded in ClinVar: one splicing mutation and two deletion variants that lead to a frameshift mutation (32). McSherry et al. also reported one deletion mutation in 2018 that was not recorded in ClinVar (33). In 2006, Basel-Vanagaite et al. first identified the CC2D1A gene as the cause of non-syndromic ID (NSID) in nine consanguineous families with severe ID. Using homozygosity mapping and sequencing, they could detect deletion in this gene (IVS13-16DEL). The resulting protein lacks one of the four DM14 and C2 domains. Researchers identified two protein isoforms, one with 951 amino acids and another with 388, in healthy individuals. Both isoforms contain the C2 domain. This finding suggests that the lack of the C2 domain in the truncated protein found in patients is not the cause of intellectual disability (ID). Instead, the role of the DM14 region is likely more critical in this context (19).

Patients in this study had a psychomotor developmental delay during early childhood and could not speak except for a few words. They all had severe ID and normal physical conditions (19). The next researchers who studied the association of the CC2D1A gene with ID were Manzini et al. in 2014. They detected "c.748+1G>T mutation" in the CC2D1A gene in three families and "c.346delA mutation" in one family with ID, ASD, and seizure. This change (c.748+1G>T) leads to the complete removal of exon 6, disrupts the reading frame at position 172 in the protein, creating an early stop codon at position 223 (p. Thr172Valfs*51). Truncated protein contains N-terminal fragments and lacks DM14 and C2 domains. It can be concluded that the loss of function of CC2D1A is the results from this mutation. The next mutation (c.346delA) is a 1bp deletion in exon 3, resulting in an early frameshift (p.Lys116Argfr*81). This mutation creates N-terminal fragments without any known domains, resulting in CC2D1A loss of function (20). Patients (16 individuals affected in four families) in that study had a spectrum of cognitive disorders and social impairments, including ASD, ID, aggressive behavior, and seizure, suggesting

shared developmental mechanisms (20). McSherry et al., in 2018, also used whole-exome sequencing on 21 Turkish families with non-syndromic ID, finding causative variants in known genes. The CC2D1A (c.811delG, p.A271Pfs*30) was one of those genes. This variant causes frameshift mutation by deleting one nucleotide in exon seven and creating an early stop codon in exon eight at position 301. The resulting truncated protein lacks the third and fourth DM14 and C2 domains and is predicted to cause a loss of function of the CC2D1A gene. The family with this causative variant has three females affected with NSID. All of them suffer from ID, ASD, and seizures and have normal physical conditions without facial dysmorphism (33). In the current study, the variant found in patients is (c.1641+1G>A), a novel splicing variant in exon 14 of the CC2D1A gene. Assumedly, this variant disrupts normal splicing, could eliminate the fourth DM14 and C2 domains, and probably results in the loss of function of the CC2D1A gene. Performing a functional study would shed more light on the functional consequences of this genome and its variants. The patients in this study had ID, ASD, speech delay, and seizures. They were normal in the physical examination and showed no dysmorphic features. The clinical characteristics of all the patients are summarized in Table 1.

Sener EF et al. in 2020 evaluated the association of the CC2D1A gene and ASD. Based on the involvement of the CC2D1A gene in NSID and the presence of ID in one-third of autistic patients, these researchers considered it a new candidate gene in autism. They performed a genetic analysis on the CC2D1A gene in 44 patients with ASD and 27 normal individuals. All the variants identified in the patients were missense and reported as variants of uncertain significance (VUS). Only one variant was splicing and was reported as pathogenic (c. 2520-1G>T). The patient carrying this pathogenic variant had autism without ID or congenital disability. These missense variants are in exons 12, 13, 15, and 19 (Figure 2d) (34). As shown in Figure 2, none of them affected DM14 domains.

Although the mutations found in CC2D1A were connected with NSID, for the first time, Ma ACH in 2020 established a novel association between CC2D1A and ciliary dysfunction. Using whole-exome sequencing on 26 individuals with heterotaxy, they identified novel rare damaging mutations in the CC2D1A gene. A functional study was performed, and heterotaxy phenotypes of the gastrointestinal and cardiovascular systems were observed in zebrafish knockout models. These mutations were missense and considered benign, likely benign, and VUS. These variants and their locations are demonstrated in Figure 2e. Investigation of the positions revealed that only two variants were located in the fourth DM14 domain (exon 14), and the rest were found in exons 6 and 23, which do not interfere with other DM14 domains. On the other hand, exon 14 variants that were likely benign and VUS do not probably have pathogenic effects on the fourth DM14 domain (22). Tuncel G. also reported a patient with Joubert syndrome classified as a ciliopathy disease caused by two homozygous variants, c.1739C>T (rs202057391) in AHI1 and c.1739C>T (p.Thr580Ile) CC2D1A. This variant was also located in exon 15, which is not in DM14 domains (21).

Based on previous studies, the variants of CC2D1A

have a different phenotypic spectrum. Splicing and frameshift mutations cause the loss of function of the CC2D1A gene and disrupt DM14 domains, leading to ID, ASD, and seizures. These mutations are pathogenic according to the ACMG guidelines. On the other hand, variants found in ASD patients were missense with uncertain significance (VUS) according to the ACMG guideline, and the variants detected in ciliopathy were all missense and were reported as benign, likely benign, and VUS. Complete loss of function of the CC2D1A gene appears to create a severe phenotype such as ID plus ASD and, in some cases, seizures and developmental delay. In missense mutations, the function is not lost, and the resulting phenotype is often autism, sometimes accompanied by ID or leading to ciliopathy. Observingly, the effects of the mutated gene, combined with the cumulative impact of other genes, contribute to the characterization of the final phenotype. Comparing the clinical features of the patients with loss-of- function mutations in CC2D1A revealed no phenotype- genotype correlation. Almost all the patients had ID, and a high percentage of them had ASD and seizures. Language impairment is another common symptom in these patients. Some of the patients showed developmental delay. Dysmorphic features were not seen in patients, and all were considered normal after physical examination.

In Conclusion

The present study reported two affected females with ID in an Iranian family with a novel splicing variant in the CC2D1A gene. Investigating the mutations and structure of this gene revealed that the loss- of- function mutation causes a more severe phenotype than the missense mutation, and disruption of DM14 domains has a critical role in the loss- of- function mutation. Comparing the clinical and molecular genetic features of the patients revealed no phenotype- genotype correlation.

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Author's Contribution

Zahra Rashvand: Formal analysis, Writing - original draft, writing review & editing, data curation

Kimia Kahrizi: Conceptualization, writing the original draft, writing the review & editing.

Hossein Najmabadi: Conceptualization, writing the original draft, writing the review & editing.

Reza Najafipour: Conceptualization, writing the original draft, writing the review & editing.

Mir Davood Omrani: Conceptualization, writing the original draft, writing the review & editing.

Mohammad Moradi: participation in sampling and finding families with intellectually- disabled children.

Zohreh Estaki: Participation in editing the article.

Zahra Taherkhani: Participation in sampling and finding families with intellectually- disabled children.

Nooshin Nikzat: Participation in excluding other

Table1: **Demographic** characterizations and clinical presentations of patients.

Family	This study		Basel-Vanagaite et al. (2006)	Manzini et al. (2014)			McSherry et al. (2018)		
Mutation	c.1641+1G>A		IVS13-16DEL	c.748+1G>T, c.346delA			c.811delG		
Gender	female	female	8 females, 8 males	Family1 2mals, 2females	Family22males, 1females	Family3 3males, 1females	Family4	female	**female**	**female**
Age (yrs) at examination	35 years	25 years	NA	NA	NA			11year	**8 year**	**5 year**
Age (yrs) at present	37 years	27 years	NA	NA	NA			14year	**11 year**	**8 year**
ID	+	+	+	+M1, F1, F2	+	+	+	+	+	-
ASD	+	-	-	+M1	+	-	+	+	+	+
Seizures	+	+	-	_	+	-	+	+	-	+
Facial dysmorphism	-	-	-	_	NA	NA	NA	-	-	-
Speech delay	+	+	+	_	+	+	+	-	-	+
Developmental delay	+	+	+	NA	NA	NA	NA	-	+	+
Physical examination	Normal	Normal	Normal except one subject who had progressive microcephaly	NA	NA	NA	NA	Normal	**Normal**	**Normal**
Other phenotype	aggressive behavior	aggressive behavior	NA	M2: cognitive problems and aggressive behavior	NA	NA	NA	NA	**NA**	**NA**

(a)

CC2D1A	Gene
c.1641+1G>A	Change cDNA
Splicing	Type of variant
Damaging	BayesDel addAF
Damaging	BayesDel noAF
Disease- causing	Mutation Taster
Damaging	FATHMM- MKL
Pathogenic	EIGEN
Pathogenic	EIGEN PC
24.2	CADD
0.9939	DANN
1.000	PhastCons100way
5.55	GERP
PVS1, PM2, PP3	ACMG

(b)

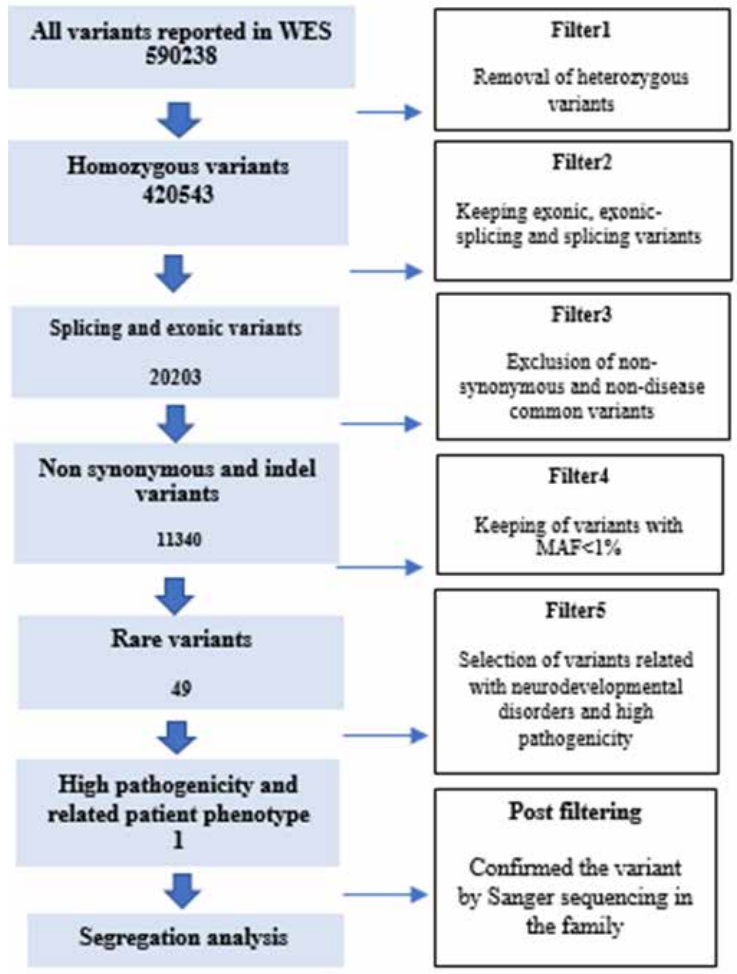
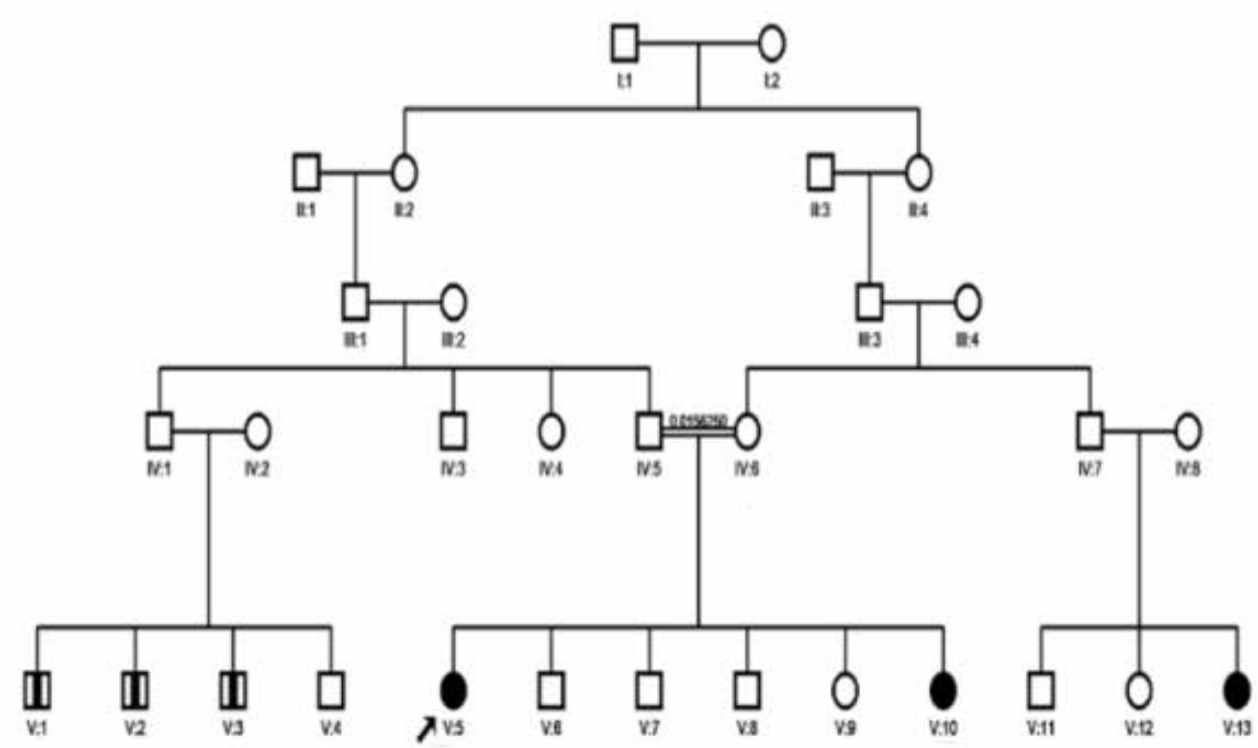


Figure 1. *(a) Results of pathogenicity prediction tools of detected variant. (b) Filtering steps and variants obtained in each level.*

(a)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I:2
II:1
II:2
II:3
II:4
III:1
III:2
III:3
III:4
IV:1
IV:2
IV:3
IV:4
IV:5
IV:6
IV:7
IV:8
V:1
V:2
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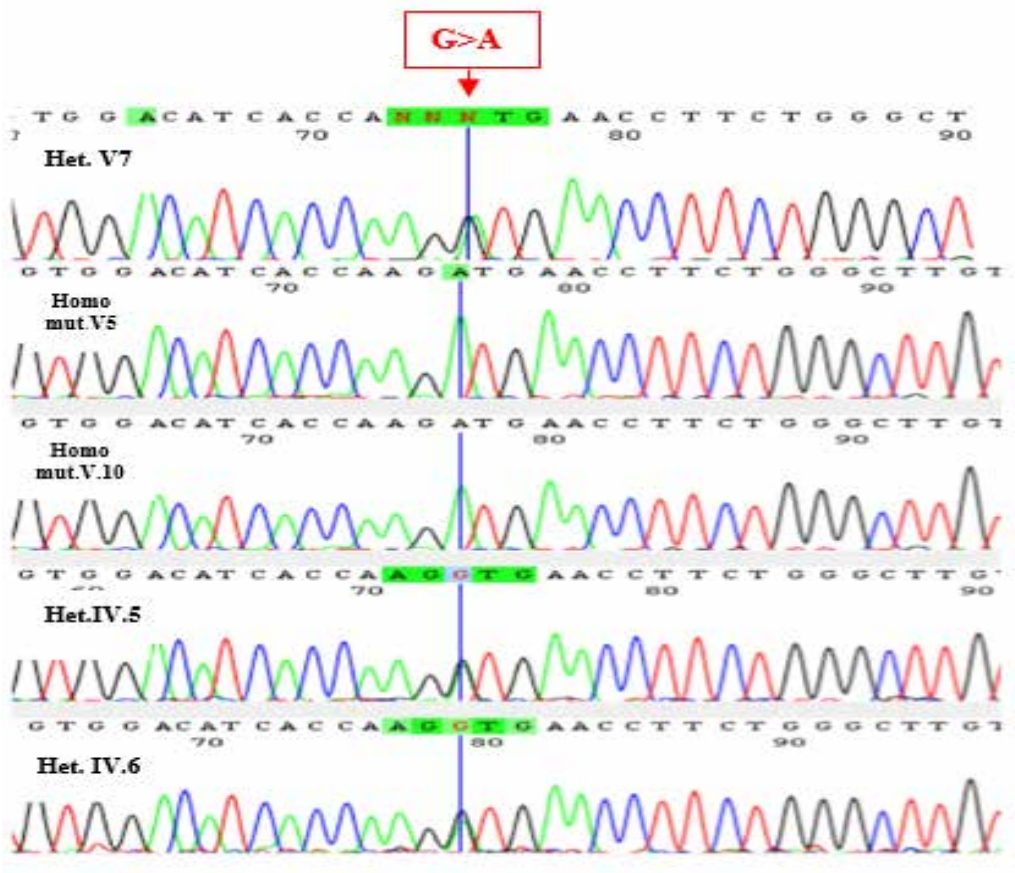
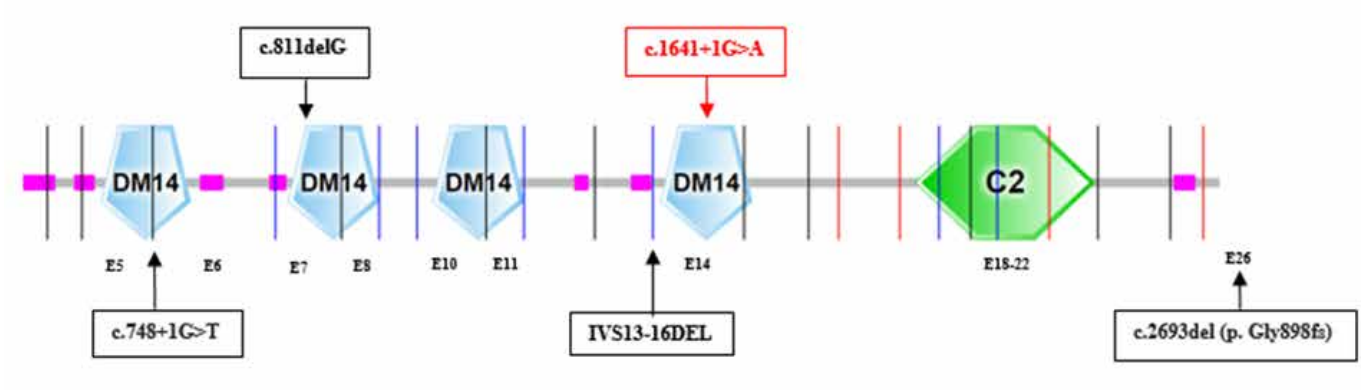
V:6
V:7
V:8
V:9
V:10
V:11
V:12
V:13

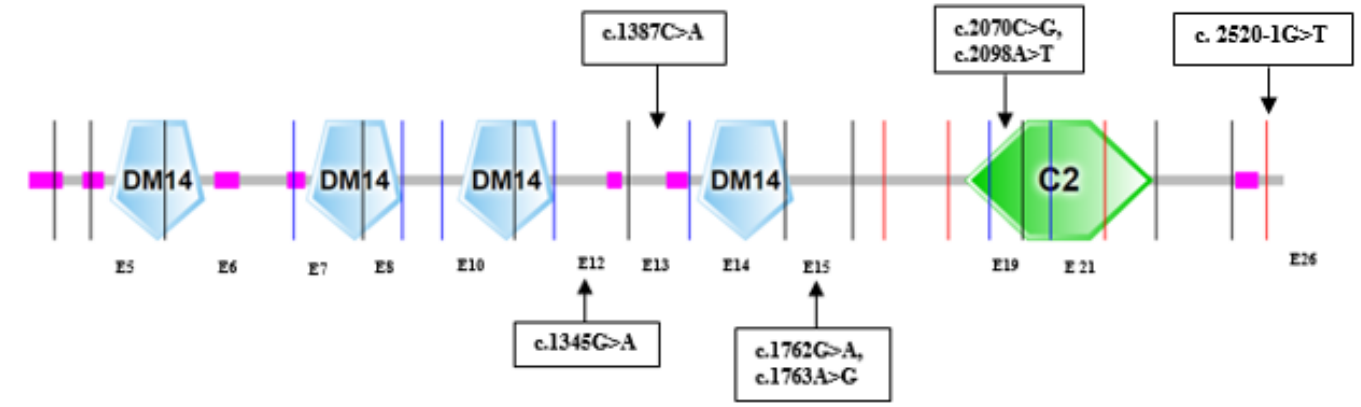
(b)


G>A
Het. V7
Homo mut.V5
Homo mut.V.10
Het.IV.5
Het. IV.6

(C)



(d)



(e)

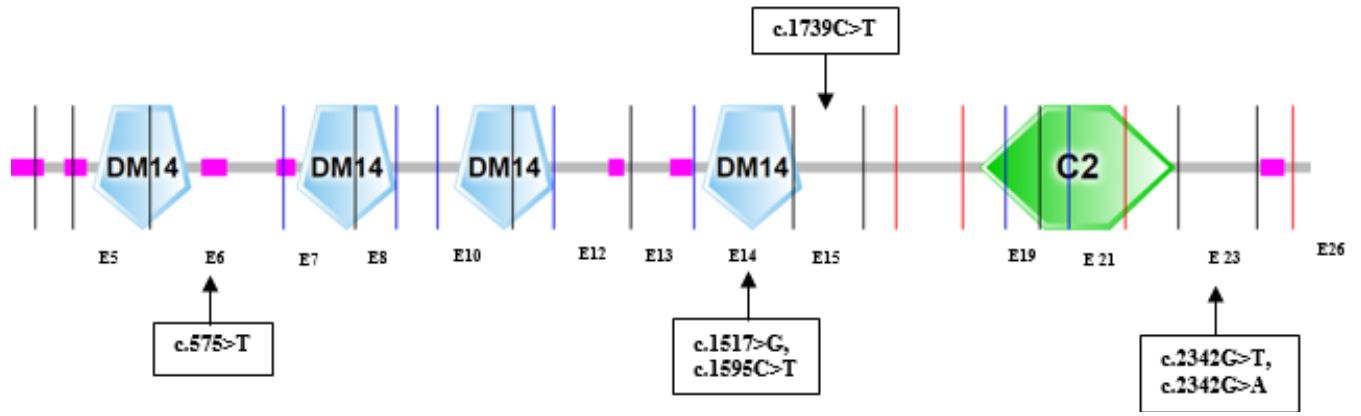


Figure 2. *(a) The pedigree of the family. (b) Sanger sequencing results of CC2D1A gene in the family. (c) Schematics structure of the CC2D1A gene with loss of function mutations found in ARID families. The variant in the red frame is detected in the current study. (d) Schematics structure of the CC2D1A gene with missense mutations found in ASD patients. (e) Schematics structure of the CC2D1A gene with missense mutations found in ciliopathy patients.*

causes of intellectual disability

Conflict of Interest

The authors have no conflicts of interest to declare.

References

1. Safran M, Dalah I, Alexander J, Rosen N, Iny Stein T, Shmoish M, et al . GeneCards Version 3: the human gene integrator. Database (Oxford). 2010; 2010:baq020. doi: 10.1093/database/baq020.
2. Matsuda A, Suzuki Y, Honda G, Muramatsu S, Matsuzaki O, Nagano Y, et al. Large- scale identification and characterization of human genes that activate NF- κB and MAPK signaling pathways. Oncogene. 2003; 22: 3307-18. doi: 10.1038/sj.onc.1206406.
3. Shi ZY, Li YJ, Zhang KJ, Gao XC, Zheng ZJ, Han N, et al . Positive association of CC2D1A and CC2D2A gene haplotypes with mental retardation in a han chinese population. DNA Cell Biol. 2012; 31:80-7. doi: 10.1089/dna.2011.1253. Epub 2011 Oct 24.
4. Ou XM, Lemonde S, Jafar- Nejad H, Bown CD, Goto A, Rogaeva A, et al . Freud- 1: A neuronal calciumregulated repressor of the 5- HT1A receptor gene. J Neurosci. 2003; 23: 7415-25. doi: 10.1523/JNEUROSCI.23-19-07415.2003.
5. Gallagher CM, Knoblich J. A. The Conserved C2 Domain Protein Lethal (2) Giant Discs Regulates Protein Trafficking in Drosophila. Dev Cell. 2006; 11: 641-53. doi: 10.1016/j.devcel.2006.09.014.
6. Jaekel R, Klein T. The Drosophila Notch Inhibitor and Tumor Suppressor Gene lethal (2) giant discs Encodes a Conserved Regulator of Endosomal TraffickingDev Cell. 2006; 11: 655-69. doi: 10.1016/j.devcel.2006.09.019.
7. Troost T, Jaeckel S, Ohlenhard N, Klein T. The tumour suppressor Lethal (2) giant discs is required for the function of the ESCRT- III component Shrub/ CHMP4. J Cell Sci. 2012; 125:763-76. doi: 10.1242/jcs.097261.
8. Nakamura A, Naito M, Tsuruo T, Fujita N. Freud- 1/ Aki1, a Novel PDK1-Interacting Protein, Functions as a Scaffold To Activate the PDK1/ Akt Pathway in Epidermal Growth Factor Signaling. Mol Cell Biol. 2008; 28: 5996-6009. doi: 10.1128/MCB.00114-08.
9. Chang CH, Lai LC, Cheng HC, Chen KR, Syue YZ, Lu HC, et al. TBK1- associated Protein in Endolysosomes (TAPE) is an innate immune regulator modulating the TLR3 and TLR4 signaling pathways. J Biol Chem. 2011; 286: 7043-51. doi: 10.1074/jbc.M110.164632.
10. Zhao M, Raingo J, Chen ZJ, Kavalali ET. Cc2d1a, a C2 domain containing protein linked to nonsyndromic mental retardation, controls functional maturation of central synapses. J Neurophysiol. 2011;105:1506-15. doi: 10.1152/jn.00950.2010.
11. Chiara Manzini M, Xiong L, Shaheen R, Tambunan DE, Di Costanzo S, Mitisalis V, et al. CC2D1A regulates human intellectual and social function as well as NF-κB signaling homeostasis. Cell Rep. 2014; 8: 647-55. doi: 10.1016/j.celrep.2014.06.039.
12. Al- Tawashi A, Jung SY, Liu D, Su B, Qin J. Protein implicated in nonsyndromic mental retardation regulates protein kinase A (PKA) activity. J Biol Chem. 2012; 287: 14644-58. doi: 10.1074/jbc.M111.261875.

13. Morawa KS, Schneider M, Klein T. Lgd regulates the activity of the BMP/Dpp signalling pathway during Drosophila oogenesis. Development. 2015; 142: 1325-35. doi: 10.1242/dev.112961.
14. Gaugler T, Klei L, Sanders SJ, Bodea CA, Goldberg AP, Lee AB, et al. Most genetic risk for autism resides with common variation. Nat Genet. 2014; 46: 881-5. doi: 10.1038/ng.3039.
15. Rogaeva A, Ou XM, Jafar- Nejad H, Lemonde S, Albert PR. Differential repression by Freud- 1/ CC2D1A at a polymorphic site in the dopamine- D2 receptor gene. J Biol Chem. 2007; 282: 20897-905. doi: 10.1074/jbc. M610038200.
16. Szewczyk B, Albert PR, Rogaeva A, Fitzgibbon H, May WL, Rajkowska G, et al . Decreased expression of Freud- 1/ CC2D1A, a transcriptional repressor of the 5- HT1A receptor, in the prefrontal cortex of subjects with major depression. Int J Neuropsychopharmacol. 2010; 13:1089-101 doi: 10.1017/S1461145710000301.
17. Oaks AW, Zamarbide M, Tambunan DE, Santini E, Di Costanzo S, Pond HL, Johnson MW, Lin J, Gonzalez DM, Boehler JF, Wu GK, Klann E, Walsh CA, Manzini MC. Cc2d1a Loss of Function Disrupts Functional and Morphological Development in Forebrain Neurons Leading to Cognitive and Social Deficits. Cereb Cortex. 2017 Feb 1;27(2):1670-1685. doi: 10.1093/cercor/bhw009.
18. Hamosh A, Scott AF, Amberger J, Valle D, McKusick VA. Online Mendelian Inheritance in Man (OMIM). Hum Mutat. 2000; 15:57-61. doi: 10.1002/(SICI)1098-1004(200001)15:1<57::AID-HUMU12>3.0.CO;2-G.
19. Basel-Vanagaite L, Muncher L, Straussberg R, Pasmanik-Chor M, Yahav M, Rainshtein L, et al. Mutated nup62 causes autosomal recessive infantile bilateral striatal necrosis. Ann Neurol. 2006; 60: 214-22. doi: 10.1002/ana.20902.
20- Rashvand Z, Kahrizi K, Najmabadi H, Najafipour R, Omrani MD. Clinical and genetic characteristics of splicing variant in cyp27a1 in an iranian family with cerebrotendinous xanthomatosis. Iran Biomed J. 2021; 25: 132-9. doi: 10.29252/ibj.25.2.132.
21. Tuncel G, Kaymakamzade B, Engindereli Y, Temel SG, Ergoren MC. A homozygous synonymous variant likely cause of severe ciliopathy phenotype. Genes (Basel). 2021; 12:945. doi: 10.3390/genes12060945.
22. Ma ACH, Mak CCY, Yeung KS, Pei SLC, Ying D, Yu MHC, et al . Monoallelic Mutations in CC2D1A Suggest a Novel Role in Human Heterotaxy and Ciliary Dysfunction.Circ Genom Precis Med. 2020; 13:e003000. doi: 10.1161/CIRCGEN.120.003000.
23. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988 Feb 11;16(3):1215. doi: 10.1093/nar/16.3.1215.
24. Najmabadi H, Hu H, Garshasbi M, Zemojtel T, Abedini SS, Chen W, et al . Deep Sequencing reveals 50 novel genes for recessive cognitive disorders. Nature. 2011; 478: 57-63. doi: 10.1038/nature10423.
25. Hu H, Kahrizi K, Musante L, Fattahi Z, Herwig R, Hosseini M, et al. Genetics of intellectual disability in consanguineous families. Mol

Psychiatry. 2019; 24: 1027-1039. doi: 10.1038/ s41380-017-0012-2.

26. Tian Y, Pesaran T, Chamberlin A, Fenwick RB, Li S, Gau CL, et al. REVEL and BayesDel outperform other in silico meta-predictors for clinical variant classification. Sci Rep. 2019; 9:12752. doi: 10.1038/s41598-019-49224-8.
27. Quang D, Chen Y, Xie X. DANN: A deep learning approach for annotating the pathogenicity of genetic variants. Bioinformatics. 2015; 31: 761-3. doi: 10.1093/bioinformatics/btu703.
28. Bateman A, Martin MJ, Orchard S, Magrane M, Agivetova R, Ahmad S, et al. UniProt: the universal protein knowledgebase in 2021. UniProt Consortium.Nucleic Acids Res. 2021; 49: 480-489. doi: 10.1093/nar/gkaa1100.
29. Davletov BA, Sudhof TC. A single C2 domain from synaptotagmin I is sufficient for high affinity Ca2+/ phospholipid binding. J Biol Chem. 1993; 268: 26386-90. doi.org/ 10.1016/ s0021- 9258(19) 74326- 9.
30. Zhao M, Li XD, Chen Z. CC2D1A, a DM14 and C2 domain protein, activates NF- κB through the canonical pathway. J Biol Chem. 2010; 285:24372-80. doi: 10.1074/jbc.M109.100057.
31. Al- Tawashi A, Gehring C. Phosphodiesterase activity is regulated by CC2D1A that is implicated in non-syndromic intellectual disability.Cell Commun Signal . 2013; 11: 47. doi: 10.1186/1478-811X-11-47.
32. Landrum MJ, Chitipiralla S, Brown GR, Chen C, Gu B, Hart J, et al. ClinVar: Improvements to accessing data. Nucleic Acids Res. 2020 48: 835-844. doi: 10.1093/nar/gkz972.
33. McSherry M, Masih KE, Elcioglu NH, Celik P, Balci O, Cengiz FB, et al. Identification of candidate gene FAM183A and novel pathogenic variants in known genes: High genetic heterogeneity for autosomal recessive intellectual disability. PLoS One. 2018; 13:e0208324. doi: 10.1371/journal. pone.0208324.
34. Sener EF, Onal MG, Dal F, Nalbantoglu U, Ozkul Y, Canatan H, et al . Novel alterations of CC2D1A as a candidate gene in a Turkish sample of patients with autism spectrum disorder. Int J Neurosci. 2022; 132:1072-1079. doi: 10.1080/00207454.2020.1860968.

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