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Research article

A nonsense *CC2D1A* variant is associated with congenital anomalies, motor delay, hypotonia, and slight deformities

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ABSTRACT

Background: Autosomal recessive intellectual developmental disorder-3 is caused by homozygous or compound heterozygous mutations in the *CC2D1A* gene. The disorder is characterized by intellectual disability (ID) and autism spectrum disorder (ASD). To date, 39 patients from 17 families with *CC2D1A* -related disorders have been reported worldwide, in whom only six pathogenic or likely pathogenic loss-of-function variants and three variants of uncertain significance (VUS) in the *CC2D1A* gene have been identified in these patients.
Methods: We described a patient with ID from a non-consanguineous Chinese family and whole-exome sequencing (WES) was used to identify the causative gene.
Results: The patient presented with severe ID and ASD, speech impairment, motor delay, hypotonia, slight facial anomalies, and finger deformities. Threatened abortion and abnormal fetal movements occurred during pregnancy with the proband but not his older healthy sister. WES analysis identified a homozygous nonsense variant, c.736C > T (p.Gln246Ter), in the *CC2D1A* gene. In addition, six novel likely pathogenic *CC2D1A* variants were identified by a retrospective review of the in-house database.
Conclusions: This study expands the genetic and clinical spectra of *CC2D1A*-associated disorders, and may aid in increasing awareness of this rare condition. Our findings have provided new insights into the clinical heterogeneity of the disease and further phenotype-genotype correlation, which could help to offer scope for more accurate genetic testing and counseling to affected families.

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1. Introduction

Autism spectrum disorder (ASD) comprises a heterogeneous group of highly genetic neurodevelopmental disorders characterized by language, cognitive, social, and behavioral abnormalities [1]. Intellectual disability (ID) and ASD are the most common developmental disorders in humans and often show comorbidity [2]. Hundreds of genes have been implicated in non-syndromic ID and ASD. These disorders have been suggested to be caused by mutations in an estimated ≥10% or more of autosomal genes [3].

Autosomal recessive intellectual developmental disorder-3 (MRT3; MIM #608443) is caused by biallelic mutations in the coiled-coil and C2 domains containing protein 1A (*CC2D1A*) gene (MIM #610055). *CC2D1A* encodes a transcriptional repressor that regulates the expression of the 5-hydroxytryptamine receptor 1A (*HTR1A*) gene in neuronal cells. CC2D1A is emerging as a critical regulator of several intracellular signaling pathways which are crucial for neuronal function (e.g., the protein kinase 1/Akt and nuclear factor kappaB signaling pathways) [4,5]. Biallelic loss-of-function mutations in the *CC2D1A* gene lead to a range of non-syndromic neurodevelopmental deficits [6–9]. Moreover, some patients with *CC2D1A* mutations have ocular and renal anomalies [10].

To date, 39 patients from 17 families (including nine families in the same village) with *CC2D1A*-related disorders have been reported worldwide, in whom only six pathogenic or likely pathogenic loss-of-function variants and three VUS (missense) in the *CC2D1A* gene have been identified in these patients (Table 1). Additionally, 10 *CC2D1A* variants are listed as pathogenic or likely pathogenic in the ClinVar database or the Leiden Open Variation Database, and two heterozygous likely pathogenic variants were detected in patients with ASD (Table S1).

Here, we describe the case of a boy from a non-consanguineous Chinese family who was diagnosed with severe ASD and ID, motor delay, hypotonia, slight facial anomalies, and finger deformities. Molecular analyses revealed a nonsense *CC2D1A* variant. Moreover, six novel likely pathogenic variants of *CC2D1A* by a retrospective review of the in-house database. And in our cohort, the total allele frequency (AF) of the pathogenic and likely pathogenic *CC2D1A* variants was estimated to be 5.81×10^{-4}. In addition, on the basis of a review of the literature, we provide a summary of the genotypes and phenotypes of *CC2D1A* alterations. Thus, the present study expands the clinical phenotype and mutation spectra of *CC2D1A*.

Table 1
Clinical characteristics and genetic information of patients with *CC2D1A*-associated disorders have been described in the literature.

Reference	Patients	Ethnicity	Mutations	Zygosity	Clinical characteristics
Basel-Vanagaite et al., 2006 (PMID: 16033914)	9 families (16 patients)	Israeli-Arab	IVS13_IVS16del	Homozygous	non-syndromic mental retardation, psychomotor developmental delay
Manzini et al., 2014 (PMID: 25066123)	Family 1:1	Saudi Arabia	c.748+1G > T(p.Thr172Valfs*51)	Homozygous	cognitive problems and aggressive behavior
	Family 1:2				moderate-to-severe NSID
	Family 1:3				moderate-to-severe NSID
	Family 1:4				ASD and ID
	Family 2 (3 patients)	Saudi Arabia	c.748+1G > T(p.Thr172Valfs*51)	Homozygous	ASD, severe NSID, language impairment, and seizures
	Family 3 (4 patients)	Saudi Arabia	c.748+1G > T(p.Thr172Valfs*51)	Homozygous	severe NSID with language impairment
	Family 4:1	Pakistani	c.346delA(p.Lys116Argfs*81)	Homozygous	moderate NSID
	Family 4:2				moderate NSID
	Family 4:3				language impairment and autistic features
	Family 4:4				moderate NSID
	Family 4:5				moderate ASD/ID
Loviglio et al., 2016 (PMID: 27799067)	BAB2321		c.1739C > T(p.Thr580Ile) [Δ] c.2657G > A(p.Arg886His) [Δ]	Phase unknown	developmental delay, cognitive impairment, facial deformities, behavior abnormities, psoriasis, pectus carinatum, decreased visual acuity
Reuter et al., 2017 (PMID: 28097321)	MR331 (2 patients)	Egypt	c.2693delG(p.Gly898Valfs*45)	Homozygous	mild ID, aggressive behavior
McSherry et al., 2018 (PMID: 30500859)	AU10-II:1	–	c.811delG(p.Ala271Pfs*30)	Homozygous	ID, ASD, seizures
	AU10-II:2				ID, history of global developmental delay with autistic features
	AU10-II:3				Developmental delay especially speech delay with autistic features, seizures
Jauss et al., 2022 (PMID: 36553572)	Family 13	–	c.1620_1623dup(p.Pro542Alafs*38) c.1345G > A(p.Val449Met) [Δ]	Compound heterozygous	Mental retardation, syndromic stigmata and ocular anomalies, GDD, microphthalmia, aniridia, corneal opacity and renal agenesis

Numbering for DNA mutation is based on cDNA sequence (GenBank no. NM_017721.4), with nucleotide +1 corresponding to A of the ATG translation initiation codon.
[Δ], variant of uncertain significance. NSID, Non-syndromic intellectual disability. ASD, Autism spectrum disorder. ID, Intellectual disability. GDD, global developmental delay.

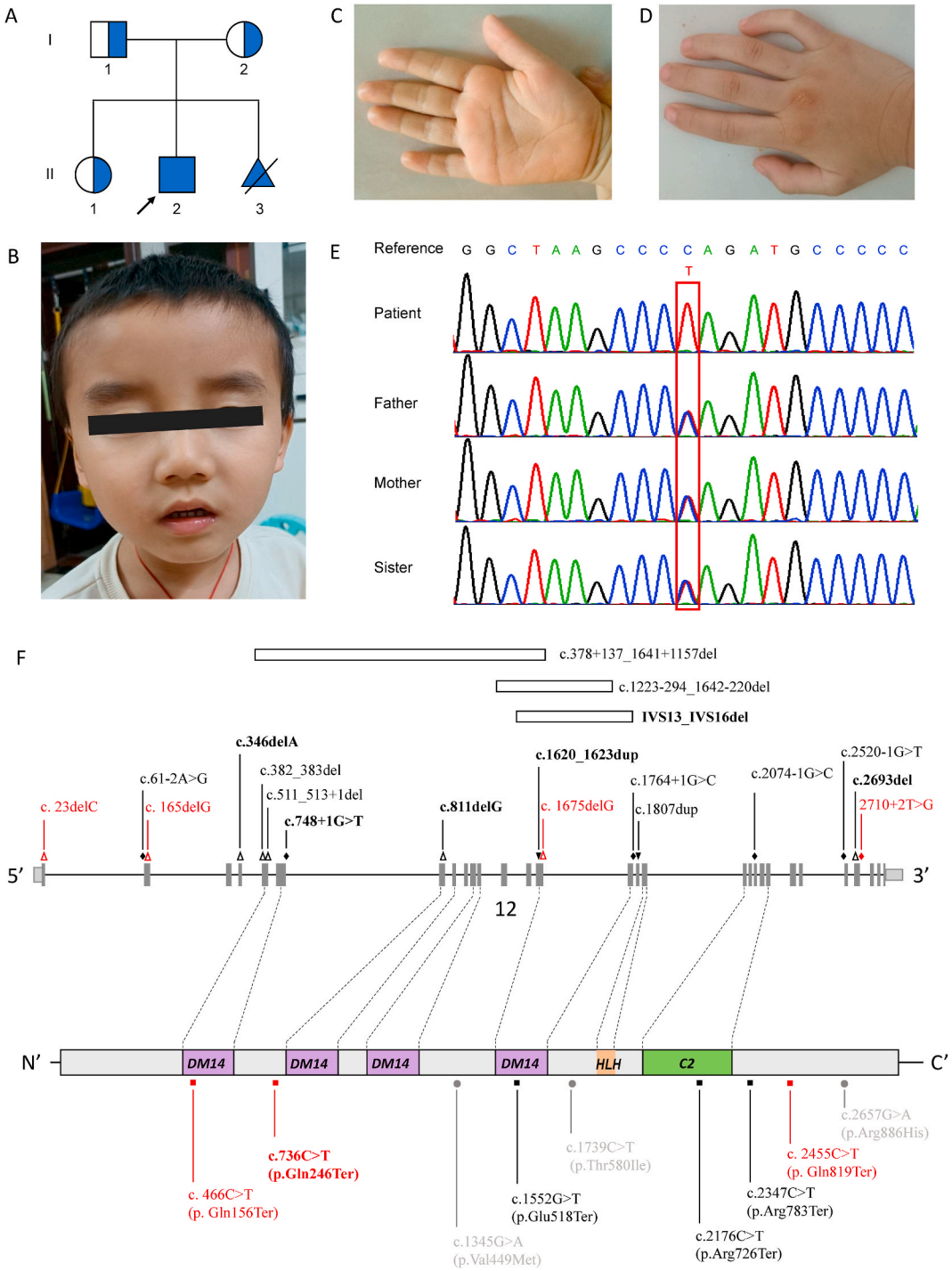

Fig. 1. Clinical features and genetic results. (A) Pedigree of the affected family. Arrow indicates the proband (II2). Unaffected parents and elder sister are carriers. (B–D) narrow forehead, thick eyebrows, long palpebral fissure, deep philtrum, diastema and clinodactyly. (E) DNA sequencing results of *CC2D1A* gene. The variant site (c.736C) is marked with a red frame. The proband was homozygous for a nonsense variant, c.736C > T(p. Gln246Ter), and his parents and elder sister were heterozygous for the transition. (F) The spectrum of *CC2D1A* mutations. Hollow rectangle, multi-exon deletion; diamond, splicing mutation; solid triangle, insertion; hollow triangle, deletion; square, nonsense mutation; gray dot, missense mutations (VUS).Homozygous and compound heterozygous mutations identified in the patients are highlighted in bold. Heterozygous *CC2D1A* variants in the in-house database are highlighted in red. The nonsense variant in our patient is highlighted in red bold. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2. Materials and methods

2.1. Subjects and ethics approval

The participants were from Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region. From January 2016 to July 2022, a total of 6886 participants (2313 patients with unrelated disorders and 4573 controls) were enrolled (see details in Table S2). Written informed consent was signed from parents of the patient. This study was approved by the medical ethics committee of Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region (2020-1/15).

2.2. Molecular analysis

Genomic DNA was extracted from peripheral blood of the participates using Lab-Aid DNA kit (Zeesan, China) in accordance with the manufacturer's protocol. DNA quality was assessed by agarose gel electrophoresis and DNA concentration was measured with a Nanodrop spectrophotometer (Thermo, USA). The samples were randomly fragmented with a Covaris ultrasonic disruptor, and sequencing libraries were generated using an Agilent SureSelect Human All Exon v6 kit (Agilent, CA). The libraries were pooled and exome sequencing was performed on a HiSeq2500 platform (Illumina, CA). After removing the redundant reads , sequencing reads were aligned to the human reference sequence hg19.Identified variants were evaluated and classified in accordance with American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guidelines [11].

3. Results

3.1. Clinical description

A family was referred to our hospital for genetic evaluation of a child diagnosed with ID, ASD, and motor problems. The proband was a 5-year-old boy who was the second child of non-consanguineous Chinese parents who had no notable family health history (Fig. 1A). During gestation with the proband, the mother had symptoms of threatened abortion in the first trimester. She observed a decrease in fetal movement in the third trimester. She had attended regular antenatal check-ups, and no other abnormalities were noted. According to her description, she did not experience these abnormalities during pregnancy with the proband's healthy sister. The boy was born full-term after 40 weeks of gestation by a normal vaginal delivery, with a birth weight of 3.5 kg and a birth length of 50 cm. He had a history of recurrent respiratory infections and malnutrition during his early infancy. His motor milestones were delayed; he raised his head at the age of 3 months, but he was not able to sit, roll, or crawl. The patient was diagnosed with hypotonia at 6 months. At approximately 1 year of age, he showed global developmental delay and ASD. No clear abnormalities were evident on cardiac color ultrasound, brain magnetic resonance imaging, and electroencephalography. After rehabilitation, he was able to sit alone at 16 months of age and to walk independently at 21 months. At 5 years of age, he was able to run but not jump with both feet off the ground. He was unable to use a pincher grasp. Moreover, he was unable to generate meaningful language or understand even simple instructions. He showed autistic behavioral tendencies, including staring at hands or lights, self-talk, inattentiveness, cold, yelling, inappropriate laughter, and poor eye contact. His height was 110 cm (normal), and his weight was 16.0 kg (−1 SD). Physical examination indicated a narrow forehead, thick eyebrows, a long palpebral fissure, a deep philtrum, diastema, clinodactyly, and flexion contracture of the toes (Fig. 1B–D). His Gesell Developmental Scales scores indicated moderate to severe developmental delay (adaptive ability developmental quotient [DQ] = 32; gross motor DQ = 54; fine motor DQ = 39; verbal ability DQ = 27 and social ability DQ = 26).

3.2. Genetic analysis

On the basis of WES analysis of the proband, we identified 1003 variants in protein-coding exons and predicted splice sites, after filtering out minor AF >3% from our local database and commercial databases including the Genome Aggregation Database, dbSNP, and 1000 Genomes. Through clinical phenotype analysis, four candidate heterozygous variants in four autosomal dominant genes (*HSD11B1*, *RIMS1*, *SMARCA2*, and *SETBP1*) were of special interest and subsequently validated them by Sanger sequencing of samples obtained from the patient's parents. He was found to have inherited the c.573T > A *HSD11B1* (endocrine disease) variant and c.675_676insCAA *SMARCA2* (neurological disorder) variants from his unaffected father, and the c.2644C > G *RIMS1* (oculopathy and neurological disorder) and c.1596G > T *SETBP1* (neurological disorder) variants from his unaffected mother. Compound heterozygous variants of genes known to be involved in neurological disorders were concurrently excluded. A homozygous variant in the *CC2D1A* gene (NM_017721.5) was identified (c.736C > T, p.Gln246Ter). His parents and elder sister were asymptomatic and were heterozygous for this variant (Fig. 1E). The nonsense variant is listed as rs564008874 in the dbSNP database, but, has not been reported in patients to date. Its AF in the gnomAD database is very low (MAF = 0.00000836), and the substitution can be classified as pathogenic with supporting evidence for pathogenicity criteria PVS1, PM2_supporting and PM3_supporting, according to the ACMG/AMP guidelines. Subsequently, genetic counseling was provided to this family, and prenatal diagnosis for his mother's third pregnancy was performed on the basis of amniotic fluid. Unfortunately, the results indicated that the fetus had the same genotype as the proband.

3.3. Local frequency analysis

In order to reveal the local variant frequency of *CC2D1A*, we reviewed all detected *CC2D1A* variants of our in-house database, which were detected by WES (n = 6886). After excluding intronic variants (except variants located at exon-intron junctions ranging from −5 to +5) and common variants (AF ≥1%), 112 variants remained. According to the ACMG/AMP guidelines, seven variants were classified as likely pathogenic and 97 were as being of VUS (Table S2). The data predict that the local mutation frequency was approximately 5.81×10^{-4}. Among these variants, 72 were present in the gnomAD database, which included one likely pathogenic variant, eight benign or likely benign variants, and 63 VUS. The c.2347C > T(p.Arg783Ter) variant had a MAF of 0.00001206 in gnomAD, but it was absent in the East Asian population. The local frequency was higher (MAF = 0.0000726) and this difference may be due to the sample size (1 in 13,732 vs 3 in 248,766). The other likely pathogenic variants were all novel, and it was difficult to compare the total mutation frequency in our local database with the other ethnicities. The vast majority of the VUS (55 out of 63) were present at higher frequencies in our cohort than in gnomAD, and the mean values were 3.1 × 10-4 and 8.3 × 10-5, respectively (p = 0.0004). Interestingly, the local frequencies were similar to those of the East Asian population (3.1 × 10-4 vs 2.5 × 10-4, p = 0.55).

4. Discussion

Here, we described a patient with severe ID, ASD, speech impairment, motor delay, hypotonia, facial abnormalities, and finger and toe abnormalities. WES analysis identified a homozygous nonsense variant (c.736C > T) in the *CC2D1A* gene. Threatened abortion and abnormal fetal movements occurred during pregnancy with the proband but not his older sister. Threatened abortion was associated with placenta previa, pregnancy induced hypertension/preeclampsia, increased risk of preterm delivery, low birth weight and neonatal intensive care unit admission. It can be attributed to various causes, including embryonic factors, several maternal factors (infections, genital tract abnormalities and endocrine factors, etc.), probably paternal factors and environmental factors [12,13]. Abnormal fetal movements were associated with poor fetal conditions, such as preterm delivery and low birth weight, as well as fetal death [14]. Interestingly, according to the pregnant mother, her third pregnancy (with affected fetus) also had symptoms of threatened abortion. After prenatal diagnosis and detailed genetic counseling, the family decided to terminate the pregnancy. This may suggest a potential association between *CC2D1A*-related disease and threatened abortion or fetal abnormalities. Nonetheless, additional research is needed to clarify our speculation.

The *CC2D1A* gene is located on chromosome 19p13.12, spans approximately 24kp and comprises of 29 coding exons encoding a 951-amino acid protein. The encoded protein contains four drosophila melanogaster 14 (DM14) domains, one helix-loop-helix (HLH) domain, and one protein kinase C conserved region 2 (C2) in the human sequence [15]. The CC2D1A protein regulates the expression of *HTR1A* in neuronal cells via binding to a conserved 14-bp 5′-repressor element upstream of the *HTR1A* promoter [16].

CC2D1A is abundantly expressed in the brain, and biallelic *CC2D1A* mutations cause a broad range of neurological disorders, including ID, ASD, and seizures, thus indicating critical roles of this gene in cognitive and social development [17]. A homozygous multi-exon deletion in the *CC2D1A* gene was firstly reported by Basel-Vanagaite et al. as the cause of non-syndromic ID in affected members of nine consanguineous Israeli-Arab families [6]. The initial clinical presentation in all 16 affected individuals (who had the same surname and were from the same village) was psychomotor developmental delay. All affected individuals had severe ID and language impairment, but none had autistic features, seizures, or dysmorphic features [6]. In 2014, Manzini et al. identified a splicing mutation and a frameshift mutation in the *CC2D1A* gene from four consanguineous families, which included 16 affected individuals. In addition to non-syndromic ID, the patients presented with a wide range of social phenotypes and seizures [7]. Manzini et al. also observed clinical heterogeneity, even within the same family. Among the multiple intracellular signaling pathways regulated by CC2D1A, the strongest effect was on the transcription factor nuclear factor kappaB (NF-κB). *Cc2d1a* loss and gain of function have both been found to activate NF-κB in developing neurons. Moreover, knockdown of *Cc2d1a* in mouse hippocampal neurons has been reported to decrease complexity in vitro, whereas this effect is rescued by NF-κB inhibitors [7]. In 2017, Reuter et al. reported the cases of two cousins with mild ID and aggressive behavior [18]. In 2018, McSherry et al. reported the cases of three sisters with ID, ASD, developmental delay, and seizures [8]. To data, all five homozygous *CC2D1A* mutations have been identified in consanguineous families. The patients' clinical manifestations involved only neurological dysfunction. Our patient presented with severe hypotonia and mild dysmorphic features, in addition to severe ID, ASD and speech impairment. His parents denied that they were close relatives and confirmed that they were from different cities. Beyond the limited number of identified *CC2D1A* mutations, several heterozygous pathogenic variants in the *CC2D1A* gene have been identified in patients with ASD [9,19]. The relationship between *CC2D1A* and ASD has also been validated in functional studies and animal models [20–22].

In addition to being associated with neurological abnormalities, the *CC2D1A* gene is associated with human heterotaxy, ciliary dysfunction, and chemotherapy resistance in ovarian cancer [23,24]. Jauss et al. have reported the case of a girl with compound heterozygous variants (a frameshift and a missense) in the *CC2D1A* gene, who showed global developmental delay, renal agenesis, microphthalmia, aniridia and corneal opacity. Consequently, the authors have proposed that the phenotypic spectrum of *CC2D1A*-associated diseases should be expanded to eliminate the non-syndromic restriction [10]. Loviglio et al. have described the case of a boy with developmental delay, cognitive impairment, facial deformities, behavior abnormalities, and skin and vision problems [25]. Interestingly, the patient was originally suspected to have Smith-Magenis Syndrome, which is a multiple congenital anomaly disease characterized by distinctive facial features, brachydactyly, developmental delay, and a distinct behavioral phenotype. However, the identified missense variants were classified as VUS, because the phase of the two variants had not been determined, and the REVEL scores were fairly low (0.025 and 0.033, respectively). Additionally, Tuncel et al. have reported the case of a boy whose main clinical findings were Joubert syndrome (MIM #213300, classified as neurodevelopmental disorder and ciliopathy disease),

oculomotor apraxia, truncal ataxia, and obsessive-compulsive disorder. The patient had two homozygous variants, one in the Abelson helper integration 1 (*AHI1*) gene (c.2106G > A, p.Thr702 =), and the other in the *CC2D1A* gene (c.1739C > T, p.Thr580Ile) [26]. A recent study by Ma et al. has revealed that loss of function of *CC2D1A* leads to heterotaxy and ciliary dysfunction in a zebrafish model [23]. Ciliary dysfunction can manifest as a variety of clinically features including renal disease, retinal degeneration, cerebral anomalies, and nervous and skeletal system disorders, thus potentially explaining why the *CC2D1A* gene is associated with mental retardation and ocular and renal anomalies [27]. Further studies should be conducted to validate whether the *CC2D1A* gene has any association with ciliopathy phenotypes.

Our patient had no ocular or renal anomalies, which are the most common ciliopathy phenotypes, although he showed facial abnormalities and finger and toe abnormalities. Moreover, he presented with severe hypotonia, which has not been observed in other patients with *CC2D1A*-associated disorders so far. These findings were highly suggestive of the involvement of *CC2D1A* in other biological processes in addition to regulating neuropsychology-related behaviors. *CC2D1A* mutations might be implicated in a much broader phenotypic spectrum; however, data from more patients and additional studies would be necessary to confirm this possibility. The novel variants in four autosomal dominant genes (*HSD11B1*, *RIMS1*, *SMARCA2* and *SETBP1*) were inherited from the patient's healthy parents, and they had a limited probability of being the primary cause of his symptoms. But the potential effects of these variants on the patient's clinical phenotype should not be ignored.

Currently, 241 *CC2D1A* variants have been deposited in the ClinVar database (Table S3). 11 variants are categorized as pathogenic or likely pathogenic, including 10 LOF variants and one missense for which there was no specific information. In addition, three variants on canonical splice-sites were annotated as 'Conflicting interpretations of pathogenicity' or 'not provided'. At least half of the variations (128 of 241) have been designated as VUS, which included 112 missense,10 splicing variants, three synonymous, one nonsense and two in-frame duplication/deletion. REVEL scores for the missense variants are relatively low, with an average of 0.139 (0–0.667). Moreover, the *CC2D1A* gene is tolerant to missense variants (overall z-score in gnomAD: 0.94; the PP2 evidence was not satisfied), and missense variants were all classified as VUS or benign according to the most recent ACMG/AMP guidelines. A similar situation existed for the missense variants of our local databases, and the average value of REVEL scores was 0.134 (0.009–0.491). However, as more patients are identified and functional studies are conducted, some VUS may be reclassified. Accordingly, the estimated mutation frequency of *CC2D1A* was likely to be underestimated. The Guangxi Zhuang Autonomous Region, with a population of 50 million, is a multi-ethnic region in Southwest China. The results of our in-house data should partially reflect the frequencies in the Southern Chinese population. Of note, as data accumulate, the local frequency of some rare variants may change.

5. Conclusion

We reported the case of a boy with severe ID from a non-consanguineous Chinese family. Beyond the common features of *CC2D1A*-associated ASD, he had motor delay, severe hypotonia, and slight deformities. In addition, six additional novel likely pathogenic variants in the *CC2D1A* gene were identified. Therefore, the present study expands the genetic and clinical spectra of *CC2D1A*-associated disorders, and may aid in increasing awareness of this rare condition (Fig. 1F).

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Sheng Yi: Writing – review & editing, Writing – original draft, Validation, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Xianglian Tang:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Qiang Zhang:** Formal analysis. **Yu Liang:** Formal analysis, Data curation. **Jing Huang:** Investigation, Formal analysis. **Shujie Zhang:** Formal analysis. **Limei Huang:** Validation. **Shang Yi:** Software, Formal analysis. **Minpan Huang:** Validation. **Zailong Qin:** Formal analysis, Conceptualization. **Jingsi Luo:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27946.

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