Expansion of Clinical and Genetic Spectrum of DDX3X Neurodevelopmental Disorder in 23 Chinese Patients

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Aim: De novo DDX3X variants account for 1–3% of unexplained intellectual disability cases in females and very rarely in males. Yet, the clinical and genetic features of DDX3X neurodevelopmental disorder in the Chinese cohort have not been characterized.

Method: A total of 23 Chinese patients (i.e., 22 female and 1 male) with 22 de novo DDX3X deleterious variants were detected among 2,317 probands with unexplained intellectual disability (ID) undertaking whole exome sequencing (WES). The age, sex, genetic data, feeding situation, growth, developmental conditions, and auxiliary examinations of the cohort were collected. The Chinese version of the Gesell Development Diagnosis Scale (GDDS-C) was used to evaluate neurodevelopment of DDX3X patients. The Social Communication Questionnaire (SCQ)-Lifetime version was applied as a primary screener to assess risk for autism spectrum disorder (ASD).

Result: A total of 17 DDX3X variants were novel and 22 were de novo. Missense variants overall were only slightly more common than loss-of-function variants and were mainly located in two functional subdomains. The average age of this cohort was 2.67 (±1.42) years old. The overlapping phenotypic spectrum between this cohort and previously described reports includes intellectual disability (23/23, 100%) with varying degrees of severity, muscle tone abnormalities (17/23, 73.9%), feeding difficulties (13/23, 56.5%), ophthalmologic problems (11/23, 47.8%), and seizures (6/23, 26.1%). A total of 15 individuals had notable brain anatomical disruption (15/23, 65.2%), including lateral ventricle enlargement, corpus callosum abnormalities, and delayed myelination. Furthermore, 9 patients showed abnormal electroencephalogram results (9/23, 39.1%). Hypothyroidism was first noted as a novel clinical feature (6/23, 26.1%). The five primary neurodevelopmental domains of GDDS-C in 21 patients were impaired severely, and 13 individuals were above the “at-risk” threshold for ASD.
DDX3X Syndrome in Chinese Population

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INTRODUCTION

DDX3X (OMIM: 300160) locates in Xp11.4 and encodes a conserved ATP-independent DEAD-box RNA helicase, which is involved in transcription, splicing, RNA transport, and translation (Abdelhaleem, 2005; Garbelli et al., 2011). The DDX3X is composed of 622 amino acid residues containing two functional domains, namely, a helicase ATP-binding domain and a helicase C-terminal domain (Snijders Blok et al., 2015). De novo DDX3X variants account for 1–3% of unexplained intellectual disability (ID) or developmental delay (DD) (Deciphering Developmental Disorders Study., 2017; Maulik et al., 2011) and also perform as a highly plausible pathogenic gene for childhood apraxia of speech (CAS) (Hildebrand et al., 2020). Most cases of DDX3X variants have been reported in females but very rarely in males, and three previous large cohort studies have described heterogeneous clinical manifestations of DDX3X neurodevelopmental disorder, including ID or DD, dystonia, movement disorders, microcephaly, behavioral issues, feeding difficulties in infancy, and seizure (Snijders Blok et al., 2015; Wang et al., 2018; Johnson-Kerner et al., 2020; Lennox et al., 2020). However, the clinical and genetic features of DDX3X neurodevelopmental disorder in the Chinese cohort have not been described yet.

In this study, we elaborated on clinical manifestations of pathogenic variants of DDX3X in 23 patients (i.e., 22 female and 1 male) in the Chinese cohort and explore the association between genotypes and phenotypes.

MATERIALS AND METHODS

Patients

With the support of the National Key Research and Development Program regarding the birth defect and developmental disorders screening (No. 2019YFC1005100), we collected whole exome sequencing (WES) data on 2,317 patients (1,622 males, 695 females, 5.33 ± 2.10 years old) with unexplained ID or DD and identified 23 DDX3X heterozygous variants in 23 patients by viewing those initial reports of WES. These patients further visited the Xiangya Hospital, Central South University, Hunan Provincial Maternal and Child Health Care Hospital, and Hunan Children’s Hospital between March 2018 and December 2020. Basic demographic information and detailed clinical data, including perinatal conditions, gender, date at birth, family history, genetic data, feeding situation, growth, and developmental conditions, were recorded clearly.

Electroencephalography (EEG) and brain MRI were re-reviewed and reanalyzed by two experienced neurological physicians, and they were blind to the genetic results.

Assessment

The Chinese version of the Gesell Development Diagnosis Scale (GDDS-C) was applied to assess the neurodevelopment of infants aged 0–6 years, and each participant calculated separate developmental quotient (DQ) of the five sub-domains, namely, adaptability, gross motor, fine motor, language, and social-emotional response. Based on the full-scale DQ results, the development of infants was classified as follows: normal (DQ ≥ 85), deficient (DQ < 75), and borderline (75 ≤ DQ < 85). DQ in any single domain below 75 was considered deficient in this field (You et al., 2019). The GDDS-C was conducted by medical professionals in child health clinics (Yang, 2016).

The Social Communication Questionnaire (SCQ) Lifetime version was a brief, 40-item, parent-report clinical tool, which had been widely used as a primary screener to assess risk for autism spectrum disorder (ASD). It was based on a semi-structured parent interview conducted by a trained clinician or researcher. Each item in SCQ required a dichotomous “yes”/“no” response, and each item received a value of 1 point for abnormal behavior. Complete developmental history was needed to be the reference. The caregivers would indicate whether behaviors of Questions 2–19 had ever been presented and whether behaviors of Questions 20–40 were presented at the age 4 or evaluated these behaviors in the past half a year if the child was aged less than 4. Scores above the cutoff of 12 suggested individuals were above the “at-risk” threshold for ASD, and further extended evaluations should be undertaken (Marvin et al., 2017).

Differences between average scores on 2 scales of this cohort and respective cutoff value were statistically evaluated using a one-sample t-test, p-values less than 0.05 (*p < 0.05, **p < 0.01, and ***p < 0.001) were considered significant.

Genetic Analysis

We reanalyzed trio- or single WES data of all probands and their biological parents (19 for trio-WES). Sanger sequencing was conducted to validate whether the variant was de novo. The DDX3X transcript was referenced (NM_001193416.2, GRCh37/hg19). Sequenced reads were aligned to GRCh37/hg19 using the Burrows-Wheeler Aligner (BWA) (v.0.7.12) with default parameters. SAMtools (v0.1.12) was used to call the variants and the ReSeq Genomes database. The Genome Analysis Tool Kit (GATK 3.5) was used for local realignment...
and base quality score recalibration. Synonymous changes and single-nucleotide polymorphisms with a minor allele frequency greater than 5% were removed. Variant pathogenicity was interpreted based on the American College of Medical Genetics (ACMG) guidelines published in 2015 [Richards et al., 2015]. The Genome Aggregation Database (GnomAD) was used to annotate the variants. Pathogenicity of the identified variants was predicted using several in silico predictors, including Polymorphism Phenotyping version 2 (Polyphen-2), Protein Variation Effect Analyzer (PROVEAN), Combined Annotation Dependent Depletion (CADD), and Sorting Intolerant From Tolerant (SIFT). Silico analysis data for missense DDX3X variants was shown in Supplementary material. Screening of neonatal genetic and metabolic diseases as a routine procedure was performed on all probands when they were born.

Ethical Issues
This research was approved by the Ethics Committee of XiangYa Hospital, Central South University (Location: Hunan Province, P.R. China, Approval No.: 2019030496). Written consents for inclusion in this study and rights to use portraits of each proband were obtained from parents of all participants.

RESULTS
Genomic Analysis
Among 2,317 individuals studied by WES who had unexplained ID, 23 deleterious variants in DDX3X were detected, 22 females were found to carry de novo variants in DDX3X, and 1 male was identified to carry an inherited variant in DDX3X from his asymptomatic mother. Furthermore, 17 were novel variants, and the remaining 6 variants were reported previously [c.1595C > T (Lennox et al., 2020); c.136C > T (Snijders Blok et al., 2015); c.865-1G > A (Wang et al., 2018); c.1703C > T (Snijders Blok et al., 2015); c.1463G > A (Lennox et al., 2020); c.1678_1680del (Snijders Blok et al., 2015)]. Of the 23 identified variants in DDX3X, 11 were missense variants, 2 were in-frame deletions, 2 were splice site variants, 5 were frameshift variants, and 3 were nonsense variants (Figure 1A). According to the guidelines set out by the ACMG, 22 variants were interpreted as pathogenic or likely pathogenic variants, and 1 variant was of uncertain significance (VUS) (Table 1).

All 23 identified variants were likely to cause changes in the DDX3X protein, 6 of which were in the helicase ATP-binding domain (i.e., p.Ala233del, p.P212L, p.R351G, p.R362G, p.A250Gfs*42, and p.C298*) while 8 were in the helicase C-terminal domain (i.e., p.R488H, p.T532M, p.Leu560del, p.L560R, p.P568L, p.H527fs*9, p.S543*, and p.F545Fs*2) (Figure 1B).

Clinical Features
Table 2 shows the clinical features of 23 participants. The average age of this cohort was 2.67 (±1.42) years old. In the cohort of 23 patients with DDX3X variants, all of them meet the criteria for ID or DD (23/23, 100%), ranging from mild to severe. Muscle tone abnormalities (17/23, 73.9%), including isolated hypotonia, hypertonia, or mixture of hypertonia and hypotonia, microcephaly (9/23, 39.1%), feeding difficulties, or low weight gain (13/23, 56.5%), associated with ophthalmologic problems (11/23, 47.8%), were the most typical clinical characteristics. Movement disorders (9/23, 39.1%), seizures (6/23, 26.1%), behavior issues (5/23, 21.7%), cardiac abnormalities (3/23, 13.0%), and hearing impairment (2/23, 8.7%) were observed in this cohort. Furthermore, six patients presented with hypothyroidism (6/23, 26.1%).

Intellectual Disability or Developmental Delay
Only 2 patients (i.e., Female 14 and Female 18) could raise their heads at the age of 3 months, and 20 patients (except Female 7, Female 10, and Female 21) could not walk independently before the age of 2 years, all of them had poor motor coordination. All parents complained their children showed poor performance in language or speech function. Nearly 50% could express their simple needs in no more than four words and only say several single words. The two eldest individuals above 5 years (i.e., Female 13 and Female 16) had not developed speech ability but could just follow simple instructions.

Seizures and Electroencephalography Monitoring
All of them underwent scalp EEG monitoring, and 9 of them showed abnormal profiles. Slow background activity was observed in 7 out of 23 patients. Focal epileptiform discharges were detected in two patients, and generalized spike waves and sharp waves were detected in four patients. Hyperarrhythmia, associated with multifocal epileptiform discharge, was prevalent in one patient. In 6 individuals with seizures (i.e., Female 1, Female 9, Female 11, Female 16, Female 17, and Female 19), their age at the onset of seizures ranged from 5 to 14 months. Atonic seizures occurred in 1, absence seizures in 3, focal partial seizures in 1, and infantile spasms in 1. Female 17 was diagnosed with infantile spasms induced by fever at the age of 5 months and recurred at the age of 13 months. They had a good response to antiepileptic drugs and no seizures in 6 months. Figure 1C shows the latest EEG of Female 17.

Feeding Difficulties
Among the 13 individuals with feeding difficulties or low weight in our cohort, 8 of their parents reflected that they were intolerant of lactose and allergic to multiple high-protein food sources. Constipation and chewing weakness were common manifestations in 10 cases. Furthermore, 7 of 13 individuals were vitamin B-deficient, but none of them was hyperhomocysteinemia.

Endocrine Abnormalities
ThorOUGH endocrine hormone examinations were conducted in 23 patients because of poor growth and neurocognitive
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**FIGURE 1** | (A) Schematic view of the DDX3X exon structure based on NM_001193416. Red blocks represent exons, and the exon number is listed on each exon. cDNA change is listed for each variant. Splice site mutations are shown in red font. (B) Location of amino acid substitutions in DDX3X (NM_001193416.2). Missense and in-frame deletions (top, 13), frameshift, and non-sense variants (bottom, 8). DDX3X contains two subdomains, a helicase ATP-binding domain (orange bar) and a helicase C-terminal domain (blue bar). (C) The latest electroencephalogram (EEG) and of Female 17. Abnormal EEG presentation, multiple slow waves in bilateral occipital lobes. (D) Brain magnetic resonance imaging (MRI) of Female 19 at the age of 1 year and 1 month. Axial position T1-weighted images show normal sulci and gyri, Axial position diffusion tensor imaging (DTI) images show delayed of white matter of frontal lobe and centrum semiovale myelination. Numbers 1–8 represent genu of the corpus callosum, white matter of the frontal lobe, anterior limb of the internal capsule, posterior limb of the internal capsule, splenium of the corpus callosum, occipital lobe, and centrum semiovale (7 and 8), respectively. (E) Sagittal image shows diffuse thinning of the corpus callosum of Female 13 at the age of 1 year and 3 months. MRI T1 and T2 axial slices showed widened bilateral lateral ventricles of Female 6 at the age of 3 years and 10 months.

**DISCUSSION**

In this report, we described a Chinese cohort of patients with DDX3X variants (n = 23, 22 confirmed de novo), and we are the first study to pinpoint clinical and genetic characteristics in the Chinese population. Among the 23 DDX3X variants in our cohort, 17 were novel variants and 14 variants were located in two functional domains of DDX3X (9 were amino acid variants and 5 were truncating variants). Significant differences in sex composition of DDX3X neurodevelopmental disorder have been noted (22 female and 1 male). ID was considered as a universal feature of DDX3X neurodevelopmental disorder in our study, followed by tone abnormalities, microcephaly, feeding difficulties, and seizures. Major aspects of neural development were assessed and quantified using the GDDS-C, and mean scores of five domains were significantly lower than the critical value of 75 (all p-value < 0.05), and language domain was impaired strikingly. Hypothyroidism was reported in 6 patients with DDX3X variants for the first time. Altogether, our study expanded the clinical and genetic spectrum associated with DDX3X neurodevelopmental disorder and evaluated the degree of developmental delay by a standardized scale. It highlighted that WES was necessary for those unexplained ID individuals.

DDX3X, an RNA-binding protein of the DEAD-box family encoded by the DDX3X gene (Abdelhaleem, 2005), acts as a translational regulator (Lai et al., 2008; Lee et al., 2008), particularly for mRNAs with highly structured 5' untranslated regions (UTRs) (Chen et al., 2018) and for...
TABLE 1 | Clinical interpretation of variants detected in DDX3X by the ACMG guideline.

| Patient | Genotype | Inheritance | Variant (NM_001193416) | Evidence of pathogenicity based on ACMG guideline | Category |
|---------|----------|-------------|-------------------------|---------------------------------------------------|----------|
| Female 1 | Het      | De novo     | c.1084C > G             | /                                                  | PS1      |
|          |          |             | p.(R362G)               |                                                   |          |
| Female 2 | Het      | De novo     | c.635C > T              | /                                                  | PS2      |
|          |          |             | p.(P212L)               |                                                   |          |
| Female 3 | Het      | De novo     | c.1579delC              | PSV1                                              |          |
|          |          |             | p.(G527fs*9)            |                                                   |          |
| Female 4 | Het      | De novo     | c.1171-2A > C           | PSV1                                              |          |
|          |          |             | ?                      |                                                   |          |
| Female 5 | Het      | De novo     | c.363delA               | PSV1                                              |          |
|          |          |             | p.(N1247fs*97)          |                                                   |          |
| Female 6 | Het      | De novo     | c.1051C > G             | /                                                  | PS2      |
|          |          |             | p.(R351G)               |                                                   |          |
| Female 7 | Het      | De novo     | c.611C > T              | /                                                  | PS2      |
|          |          |             | p.(T532I)               |                                                   |          |
| Female 8 | Het      | De novo     | c.1595C > T             | PSV1 + PS1                                         |          |
|          |          |             | p.(T532M)               |                                                   |          |
| Female 9 | Het      | De novo     | c.749_756del            | PSV1                                              |          |
|          |          |             | CTGTAGAGG;             |                                                   |          |
|          |          |             | p(A2505G-Y242)          |                                                   |          |
| Female 10| Het      | De novo     | c.136C > T              | PSV1                                              |          |
|          |          |             | p.(R461*)              |                                                   |          |
| Female 11| Het      | De novo     | c.865-1G > A            | PSV1                                              |          |
|          |          |             | ?                      |                                                   |          |
| Female 12| Het      | De novo     | c.690_695del            | /                                                  | PS2      |
|          |          |             | p.(A623del)             |                                                   |          |
| Female 13| Het      | De novo     | c.894C > A              | PSV1                                              |          |
|          |          |             | p.(C298?)              |                                                   |          |
| Female 14| Het      | De novo     | c.1633insT              | PSV1                                              |          |
|          |          |             | p.(F527insT)            |                                                   |          |
| Female 15| Het      | De novo     | c.1671_1680del          | /                                                  | PS2      |
|          |          |             | p.(G566del)             |                                                   |          |
| Female 16| Het      | De novo     | c.1703C > T             | PS1 + PS2                                          |          |
|          |          |             | p.(P568L)              |                                                   |          |
| Female 17| Het      | De novo     | c.1671T > G             | PS1 + PS2                                          |          |
|          |          |             | p.(G566R)              |                                                   |          |
| Female 18| Het      | De novo     | c.1463G > A             | PS1 + PS2                                          |          |
|          |          |             | p.(R488H)              |                                                   |          |
| Female 19| Het      | De novo     | c.620_626               | PSV1                                              |          |
|          |          |             | dup(AAGCA;A)           |                                                   |          |
|          |          |             | p.(A4222dupAAGCA)       |                                                   |          |
| Female 20| Het      | De novo     | c.3G > A                | PSV1                                              |          |
|          |          |             | p.(M1I)                |                                                   |          |
| Female 21| Het      | De novo     | c.1628C > G             | PSV1                                              |          |
|          |          |             | p.(S543T)              |                                                   |          |
| Female 22| Het      | De novo     | c.605G > C              | PSV1                                              |          |
|          |          |             | p.(P202P)              |                                                   |          |
| Male 1   | Het      | Inherited from his | c.329G > A          | /                                                  | PS2      |
|          |          | mother      | p.(R111H)              |                                                   |          |

ACMG, American College of Medical Genetics; PSV, pathogenic very strong; PS, pathogenic strong; PM, pathogenic moderate; PP, pathogenic supporting.

repeat-associated non-AUG translation (Cheng et al., 2019; Linsalata et al., 2019). DDX3X is also the key component of ribonucleoprotein (RNP) granules composed of mRNA and protein (Huang et al., 2019), a pathological hallmark of many neurodegenerative diseases (Ramaswami et al., 2013). Dd3x plays an indispensable role in mouse embryogenesis, synaptogenesis, and brain development (Chen et al., 2016; Lennox et al., 2020). Dd3x neural stem cells knockout at embryonic day (E) 9.5 in mice hampered brain growth, accompanied by seizures and ataxia (Patmore et al., 2020). Boitnott et al. (2021) generated a Ddx3x haploinsufficient mouse (Ddx3x± female) with construct validity for DDX3X loss-of-function mutations. The Ddx3x± mice showed global development delay and evolved into behavioral anomalies in adulthood (Boitnott et al., 2021).

Comparing with three previously reported cohort studies of DDX3X neurodevelopmental disorder, we found a certain degree of phenotypic overlap but some special points (Snijders Blok et al., 2015; Wang et al., 2018; Lennox et al., 2020; Table 3). ID was still a general phenotypic feature of patients with DDX3X variants in both our study and previous reports. Similarly, compared with healthy controls, the mean score in GDDS-C of the cohort could reflect global DD. The worst performance in the language subdomain of GDDS-C consolidated language impairments as the most prominent clinical feature of DDX3X neurodevelopmental disorder (Johnson-Kerner et al., 2020). Ophthalmologic problems including refractive errors, nystagmus, strabismus, and amblyopia were presented in 11/23 (47.8%) patients. Previous studies have shown that a variety of eye phenotypes including hypoplasia of the eye or absence of...
| Patient | Female 1 | Female 2 | Female 3 | Female 4 | Female 5 | Female 6 | Female 7 | Female 8 | Female 9 | Female 10 | Female 11 |
|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|----------|
| Current age (years, months) | 2y3m | 1y7m | 1y9m | 1y | 4y | 4y3m | 1y | 3y5m | 3y6m | 3y | 2y4m |
| Perinatal conditions | Normal | Normal | Normal | Normal | MLBW | Normal | Normal | Normal | Normal | Normal | Normal |
| ID/DD | + | + | + | + | + | + | + | + | + | + | + |
| Weight | −2SD | −3SD | −3SD | −2SD | Normal | −SD | −2SD | −2SD | −SD | + SD | −SD |
| Height | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| Speech | Single words | Minimally verbal | Single words | Single words | Single words | Single words | Single words | Single words | Minimally verbal | Single words | Minimally verbal |
| Age at walking | 2y2m (with rollator) | No | No | No | 3 | 1y5m (wide base gait) | No | 2y9m (wide base gait) | 1y8m | No | No |
| Tone abnormalities | Hypertonia | Normal | Hypotonia | Mixture | Hypotonia | Mixture | Hypotonia | Hypotonia | Normal | Normal | Normal |
| Movement disorders | Normal | No | Hypotonia | No | Hypotonia | No | Hypotonia | No | Normal | Normal | Normal |
| Seizures | Absence seizures | No | No | No | No | No | No | No | No | No | Absence seizures |
| Microcephaly | No | No | No | No | No | No | No | No | +, −2SD | +, −2SD | Refractive errors |
| Ophthalmologic problems | Refractive errors | No | No | No | No | No | No | No | Refractive errors | Refractive errors | Refractive errors |
| Behavior issues | No | No | No | No | No | No | No | No | No | No | ASD |
| SCQ Lifetime | 20 | NA | NA | 13 | 24 | 13 | NA | 22 | 17 | 10 | 21 |
| Others | PFO | Feeding difficulties | Constipation | Constipation, Inability to chew; Hearing impairment | Hypothyroidism (8.97 mIU/L) | No | No | No | No | No | Hypothyroidism (9.21 mIU/L) |
| Endocrine abnormalities (TSH level) | Hypothyroidism | No (Normal) | No (Normal) | No | No | No | No | No | No | No | Hypothyroidism |
| MRI findings | Ventricular enlargement | Ventricular enlargement | Ventricular enlargement | Normal | Normal | Ventricular enlargement | Ventricular enlargement | White matter volume reduction; subdural effusion | Ventricular enlargement | Normal | Normal |

(Continued)
| Patient | Female 12 | Female 13 | Female 14 | Female 15 | Female 16 | Female 17 | Female 18 | Female 19 | Female 20 | Female 21 | Female 22 | Male 1 |
|---------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--------|
| Current age (years, months) | 1y4m | 6y10m | 1y4m | 2y | 5y | 2y10m | 3y | 3y | 1y8m | 1y10m | 1y10m | 1y4m |
| Perinatal conditions | Normal | Normal | Normal | MLBW | MLBW | Normal | Normal | Neonatal jaundice | Normal | Normal | Normal | Neonatal jaundice |
| ID/DD | + | + | + | + | + | + | + | + | + | + | + | + |
| Weight | −SD | Normal | −SD | −3SD | −2SD | −3SD | −SD | −SD | −2SD | Normal | Normal | −2SD |
| Height | Normal | Normal | Normal | −SD | Normal | −2SD | Normal | Normal | Normal | + 2SD | −SD | −SD |
| Speech | Single words | Single words | Single words | Minimally verbal | Minimally verbal | Minimally verbal | Minimally verbal | Single words | Minimally verbal | Single words | Minimally verbal | Single words |
| Age at walking | No | No | No | No | No | No | No | No | No | 1y8m | No | No |
| Tone abnormalities | Hypertonia | Hypotonia | Normal | Hypotonia | Mixture | Hypotonia | Hypotonia | Normal | Hypotonia | Normal | Hypotonia | Hypotonia |
| Movement disorders | + dyskinesia | No | + ataxia | No | + dyskinesia | No | No | No | No | No | No | No |
| Seizures | Absence seizures | No | No | No | No | Focal partial seizure | Infantile spasms | +, −2SD | +, −2SD | +, −2SD | No | No |
| Microcephaly | No | No | No | +, −2SD | Nystagmus | No | No | No | No | No | No | No |
| Ophthalmologic problems | No | No | No | No | No | No | No | No | No | No | No | Amblyopia |
| Behavior issues | No | No | No | No | No | No | No | No | Hyperactivity | No | No | ASD |
| SCQ lifetime | NA | 11 | NA | 13 | 17 | 20 | 14 | NA | 10 | 12 | 19 | 19 |
| Others | No | Constipation | Constipation | PFO; hearing impairment; feeding difficulties, constipation | Inability to chew | Inability to chew | Normal | Normal | Normal | Normal | Normal | Normal |
| Endocrine abnormalities (TSH level) | No | No | No | Hypothyroidism (7.74 mIU/L) | Hypothyroidism (5.8 mIU/L) | No | No | Hypothyroidism (8.35 mIU/L) | No | No | No |
| MRI findings | Delayed myelination | Delayed myelination | Normal | Ventricular callosal abnormalities | Corpus callosal abnormalities | Ventricular enlargement | Ventricular enlargement | Delayed myelination | Normal | Normal | Normal | 

ID, intellectual disability; DD, developmental disability; MLBW, mature low birth weight; Mixture, Mixed hypo and hypertonia; PFO, patent foramen ovale; ASD, atrial septal defect; SD, standard deviation; +, positive; NO, negative; NA, not available; TSH, thyroid-stimulating hormone.
Defective RNA metabolism was considered as the potential mechanism through which DDX3X missense variants hamper fetal brain cortical development (Kumar et al., 2015; Lennox et al., 2020). We speculated that TH deficiency may intensify the adverse effect on RNA metabolism caused by DDX3X missense variants. Appropriate TH supplementation in DDX3X patients with hypothyroidism could be worth trying, but overtreatment or prophylactic hormonal therapy should be avoided because the higher dose of TH supplementation could worsen the outcome (Tuhan et al., 2016). Therefore, it is crucial to have a close follow-up in DDX3X patients with hypothyroidism. Hypothyroidism has not been verified in animal models with in vivo depletion of Ddx3x.

Many study reports have tried to establish the connection between the severity of clinical phenotypes and the location and type of variants and obtained two main findings (Lennox et al., 2020). First, the same recurrent de novo variants were more likely to have similar phenotypes. Recurrent amino acids changes, including R326, I415, and T532, all could cause polymicrogyria (PMG) (Abdelhaleem, 2005; Tuhan et al., 2016; Lennox et al., 2020). Besides the 11 individuals with PMG, 10 were missense variants and one was in-frame deletion, underscoring a striking association between missense variants and severe cerebral anatomical phenotypes, like PMG or dysgyria (Lennox et al., 2020).

Regrettably, no PMG or dysgyria was observed in our cohort, nor the previously reported DDX3X variants relating with PMG. However, we found that females with missense or in-frame deletion DDX3X variants (10/11, 90.9%) were more likely to have abnormal brain structural MRI compared with those with LOF variants (4/11, 36.3%). This could be interpreted by different pathogenic mechanisms. Many aberrant truncating mRNAs (i.e., frameshift or non-sense variants) might undergo non-sense-mediated RNA decay (NMD) and resulted in a haploinsufficiency effect, while a subset of missense variants could function in a dominant-negative manner (Chen et al., 2020; Lennox et al., 2020). Further investigations about gain-of-function mechanism behind certain missense mutations will be carried out by modeling missense mutations in mice. Multiple malignancies have a solid association with somatic DDX3X variants, like malignant melanoma and medulloblastoma (Phung et al., 2019; Patmore et al., 2020). Even though no malignancy was reported in our cohort yet, regular cancer screening is still quite necessary. Finally, a small sample size and a relatively small number of novel phenotypes, such as hypothyroidism, were the main limitations of this study.

In summary, we identified 23 unrelated Chinese patients with causal variants in DDX3X and expanded the knowledge of these increasingly recognized ID disorders. Our study delineated many clinical characteristics of the Chinese cohort with DDX3X variants, largely overlapping with phenotypic spectrum in previously reported studies, but hypothyroidism was first noted as a novel clinical feature. Overall, missense variants were only slightly more common than loss-of-function variants and were mainly located in two functional subdomains. The DDX3X missense variants may have a certain association with abnormal brain anatomical structures. Given the heterogeneous clinical
manifestations and involvement of the nervous system and non-nervous systems, unexplained ID in both males and females should take the use of multigene panels that include DDX3X or WES into consideration.

DATA AVAILABILITY STATEMENT

Sequencing data involved in the study are available through the Genbank repository (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA795095). There are restrictions to the full availability of sequencing data of the research participants due to privacy and ethical/legal issues. The data that support the findings of this study are available from the corresponding author, upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of XiangYa Hospital, Central South University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

YD, ZY, and LL: study design, analysis and revision of the manuscript. YD, ZY, and JGu: follow-up of patient's information. HL, JGo, and YX: reanalysis of WES data and original draft preparation. YD, BX, HW, and LL: collection of clinical and WES data. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants from the National Key Research and Development Program of China (2021YFC1005300 and 2019YFC1005100), the National Natural Science Foundation of China (82171454 and 81671300), the Key Research and Development Program of Hunan Province (2022SK2042), the Natural Science Foundation of Hunan Province Project (2020JJ5914 and 2021JJ70389), the NHC Key Laboratory of Birth Defect for Research and Prevention (Hunan Provincial Maternal and Child Health Care Hospital) (KF2020001), and the Scientific Research Project of Hunan Provincial Health Commission (20200475).

ACKNOWLEDGMENTS

We thank all the patients and their family members involved in this study for their participation, and the Berry Genomics Co., for their technical support.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnmol.2022.793001/full#supplementary-material

| TABLE 3 | Comparison of clinical characteristics in our cohort and three previously published cohorts. |
| --- | --- | --- | --- | --- |
| **Clinical findings** | **Numbers (percentage, %)[our study]** | **Numbers (percentage, %)[Snijders Blok et al., 2015]** | **Numbers (percentage, %)[Wang et al., 2018]** | **Numbers (percentage, %)[Lennox et al., 2020]** |
| **Neurological** | | | | |
| Intellectual disability (ID) or developmental delay (DD) | 23/23 (100%) | 38/38 (100%) | 28/28 (100%) | 106/106 (100%) |
| Hypotonia | 11/23 (47.8%) | 29/38 (76%) | 19/28 (68%) | 54/93 (58%) |
| Hypertonia | 3/23 (10.7%) | N/A | 2/12 (17%) | 5/93 (5%) |
| Mixed hypo and hypertonia | 3/23 (10.7%) | N/A | N/A | 31/93 (33%) |
| Movement disorders | 9/23 (39.1%) | 17/38 (45%) | 17/28 (61%) | 18/93 (22%) |
| Seizures | 6/23 (26.1%) | 6/38 (16%) | N/A | 17/93 (18%) |
| Ophthalmologic problems | 11/23 (47.8%) | 13/38 (34%) | 9/28 (32%) | 29/92 (31.5%) |
| Microcephaly | 9/23 (39.1%) | 12/38 (32%) | 7/28 (25%) | 34/90 (38%) |
| Behavior issues | 5/23 (21.7%) | 20/38 (53%) | 6/28 (21%) | N/A |
| **Others** | | | | |
| Cardiac abnormalities | 3/23 (13.0%) | N/A | 5/27 (11%) | 13/90 (14%) |
| Feeding difficulties or low weight | 13/23 (56.5%) | 12/38 (32%) | N/A | N/A |
| Hearing impairment | 2/23 (8.7%) | 3/38 (3%) | N/A | 4/78 (5%) |
| **Imaging findings** | | | | |
| Corpus callosum abnormalities | 2/23 (8.7%) | 13/37 (35%) | 18/20 (90%) | 77/89 (87%) |
| Ventricular enlargement | 10/23 (43.5%) | 13/37 (35%) | N/A | 61/89 (68%) |
| Cortical malformation | 3/23 (13.1%) | 4/37 (11%) | N/A | 50/89 (56%) |
| Delayed myelination | 2/23 (8.7%) | N/A | N/A | N/A |

<sup>a</sup>In Snijders Blok et al. (2015), movement disorders include spasticity.  
<sup>b</sup>In Wang et al. (2018), imaging findings refer to structural brain abnormalities.
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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