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Neurodevelopmental trajectory and modifiers of 16p11.2 microdeletion: A follow-up study of four Chinese children carriers

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Abstract

Background: Neurodevelopmental disorders (NDDs) are a group of disorders with high genetic and phenotypic heterogeneities. The 16p11.2 microdeletion has been implicated as an important genetic risk factor for NDDs.

Methods: Multiple genetic tests were used to detect the 16p11.2 microdeletion from 918 Chinese children with NDDs. Targeted sequencing of genes in the 16p11.2 interval was performed in all carriers of the 16p11.2 microdeletion, and whole-genome expression profiling analysis was performed for the patient carriers and normal carriers in their intra-family.

Results: Three patients carrying the 16p11.2 microdeletion were screened out, indicating a frequency of 0.33% for the 16p11.2 microdeletion in this cohort. We reviewed the neurodevelopmental trajectories of the 16p11.2 microdeletion carriers from childhood to puberty and confirmed that this microdeletion was associated with abnormal neurodevelopment, with varied neurodevelopmental phenotypes. A differential *PRRT2* genotype (rs10204, T>C) was identified between patients and normal carriers of the 16p11.2 microdeletion. Moreover, the determination of differential

whole-genome expression profiling demonstrated the destruction of the top-ranked network in neurogenesis and accounted for observation of abnormal neurodevelopmental phenotypes in the 16p11.2 microdeletion carriers.

Conclusions: We have provided the frequency of the 16p11.2 microdeletion in a Chinese pediatric NDD cohort with a variable NDD phenotype from childhood to puberty, which is useful for Chinese geneticists/pediatricians to conduct the 16p11.2 microdeletion testing in children with NDDs.

KEYWORDS

16p11.2 microdeletion, frequency, neurodevelopmental disorder, neurodevelopmental trajectory, whole-genome expression profiling

1 | INTRODUCTION

Neurodevelopmental disorders (NDDs) are disorders caused by alterations in early brain development resulting in severe behavioral abnormity, mainly including intellectual disability/developmental delay (ID/DD), autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD). NDDs show high genetic heterogeneity, and their genetic causations may involve single-nucleotide variants (SNVs) in one or multiple genes, copy number variations (CNVs), and chromosome abnormalities as indicated in many studies (Elia et al., 2012; Faraone et al., 2005; Takumi & Tamada, 2018; Wood, Rijsdijk, Saudino, Asherson, & Kuntsi, 2008).

The 16p11.2 microdeletion is defined as a recurrent heterozygous deletion of about 600 kb at around 29.6–30.2 Mb in the reference genome (GRCh37/hg19). The 16p11.2 microdeletion as an important genetic risk factor implicated series of NDDs and some extra-neurological disorders including ID/DD, ASD, obesity, and congenital scoliosis (Bertero et al., 2018; Bijlsma et al., 2009; Knoll, Arnett, & Hertza, 2018; Raca et al., 2013; Shen et al., 2011; Wu et al., 2015). However, the penetrance and severity of NDDs vary among the 16p11.2 microdeletion carriers, ranging from apparently healthy individuals to mildly or severely affected patients with different NDD categories (Ghebranious, Giampietro, Wesbrook, & Rezkalla, 2007; Raca et al., 2013).

In the current study, we identified three familial 16p11.2 microdeletions in a cohort of Chinese children with NDD and performed a follow-up study to examine the neurodevelopmental trajectories of the 16p11.2 carriers from childhood to puberty. Furthermore, we examined modifier SNVs within the 16p11.2 interval and differential genome-wide expression profile in the 16p11.2 carriers to explain the neurodevelopmental phenotypic heterogeneity of the 16p11.2 microdeletion.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

This study was approved by the ethics committee of the Capital Institute of Pediatrics (SHERLL 2015069). Written informed consents were obtained from all the patients' guardians/parents/next of kin for the publication of this clinical information and any accompanying photographs.

2.2 | Subjects

Patients with NDDs (including ID/DD, ASD, and ADHD) were recruited from the affiliated Children's Hospital of the Capital Institute of Pediatrics. Patients with isolated seizure/epilepsy were excluded, but NDD patients accompanied by seizures/epilepsy were included. Patients with a known environmental etiology (perinatal asphyxia, nervous system infections, postnatal brain injury/trauma) or other definite genetic causation were excluded. All children have received the karyotyping, urine/serum metabolic screening, and *FMR1* (MIM:309550) repeat testing for fragile X syndrome.

Clinical evaluations, including physical examinations and psychiatric assessments, were carried out by a neurologist, an orthopedic surgeon, and a developmental specialist. Intelligence quotient (IQ) was evaluated using the Wechsler Intelligence Scale for Children and ASD was evaluated according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV).

2.3 | Detection of the 16p11.2 microdeletion

Genomic DNA was isolated using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's

instructions. Array comparative genomic hybridization (array-CGH), multiplex ligand-dependent probe amplification (MLPA) or whole-exome sequencing (WES) were used to detect 16p11.2 microdeletion.

Array-CGH was performed using an Agilent 244K, 4 × 180K SNP + CNV or 6 × 80K customer oligonucleotide microarray, as described previously (Shen & Wu, 2009). Raw data were analyzed using DNA CytoGenomics software (Agilent Technologies, Palo Alto, CA, USA).

MLPA analysis was carried out using a commercial kit (MLPA probemix P343 and P297; MRC-Holland, Amsterdam, Holland) whose probes were target-designed on the 16p11.2 region. Patients not carrying the 16p11.2 microdeletion, as confirmed by 244K chip analysis, were chosen as MLPA controls. The data were analyzed using Genemarker V5.0.14 (SoftGenetics, State College, PA, USA).

For WES, the 16p11.2 microdeletion was screened for using the CNV tool in NextGENe software (version 2.4.1.2; SoftGenetics, PA, USA), comparing the coverage of the 16p11.2 region in tested samples and control samples. These probabilities were then entered into a Hidden Markov Model (Wang, Nettleton, & Ying, 2014) to produce CNV classifications (duplication, deletion, normal, or uncalled), calculating the coverage ratios and the amount of dispersion (noise) for each region. Further MLPA experiments were used to verify the 16p11.2 microdeletion in WES experiments.

2.4 | Targeted sequencing of genes in the 16p11.2 interval

The amplified DNA was captured using a GenCap capture kit (MyGenostics GenCap Enrichment Technologies, Beijing, China). DNA probes were designed to tile along the exon regions of the 16p11.2 interval genes (Table S1) and capture was conducted according to the manufacturer's protocol. The enrichment libraries were sequenced using an Illumina HiSeq X Ten sequencer (Illumina, San Diego, CA, USA) for paired reads of 150 bp. After sequencing, the raw data were saved in FASTQ format and then subjected to bioinformatics analysis. Illumina sequencing adapters and low-quality reads (<80 bp) were filtered out and the clean reads were mapped to the UCSC hg19 human reference genome. The mapped sequences were then processed using GATK software (https://software.broadinstitute.org/gatk), and single-nucleotide polymorphism (SNP) and insertion/deletion variants were detected by GATK HaplotypeCaller. The data were then transformed to variant call format (VCF) and variants were further annotated by ANNOVAR and associated with multiple databases, including 1000 Genomes, ESP6500, dbSNP, and ExAC. The

average coverage ranged from 224 to 1216X, and 90.37%–97.22% of the targeted areas were covered at a minimum of 20X.

2.5 | Whole-genome expression profiling analysis

Total RNA was extracted from peripheral blood using an RNeasy Mini Kit (Qiagen, Hilden, USA), and RNA quantity and quality were estimated with an Agilent 2100 Bioanalyzer (Agilent Technologies). The GeneChip Human Gene 1.0 ST Array (Affymetrix, Santa Clara, CA, USA) was applied according to the manufacturer's instructions. Double-stranded cDNA was synthesized, labeled, hybridized, washed, and scanned according to Affymetrix protocols. Quality control was included at each step to ensure acceptable standards. Normalization and comparison of the probe values were conducted using Expression Console software (Affymetrix).

Raw expression data from the chip were uploaded for online iReport analysis (Ingenuity Systems, Redwood City, CA, USA) and differentially expressed genes (DEGs) (fold change [FC] >1.5, $p < 0.05$) were extracted. These DEGs were further uploaded to the online Ingenuity Pathway Analysis (IPA) tool (Ingenuity Systems).

3 | CASE REPORTS AND RESULTS

3.1 | Frequency of the 16p11.2 microdeletion in a Chinese pediatric NDD cohort

A total of 918 Chinese children with NDD of unknown etiology (boy: girl = 2.87; age: 4 months to 14 years and 5 months) were recruited. The 16p11.2 microdeletion was identified in three independent patients, indicating a frequency of 0.33% (3/918) for the 16p11.2 microdeletion in this cohort. All the 16p11.2 microdeletions in the probands were inherited from either the paternal or maternal chromosomes. Analysis of the grandparents confirmed that two (Family 1&3) of the parental microdeletions were *de novo* deletions (Figure 1).

3.2 | Neurodevelopmental trajectories of the four 16p11.2 microdeletion carriers from three families

For these three families, four child carriers with the 16p11.2 microdeletion (III:1 and III:2 of family 1, III:1 of family 2, III:1 of family 3) were available for follow-up study till puberty. The neurodevelopmental trajectories and dynamic
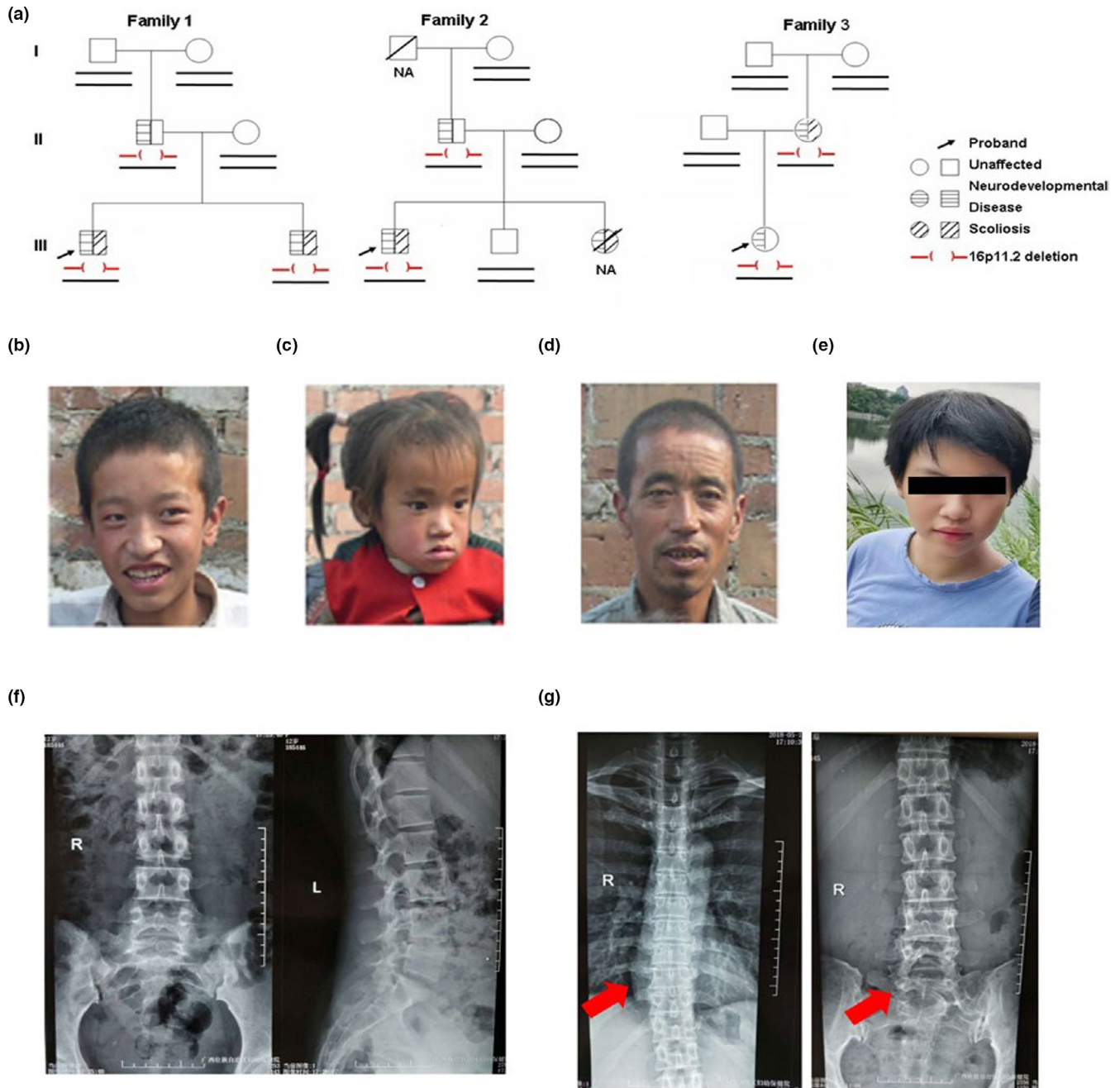
FIGURE 1 Pedigrees and clinical features of three families with familial 16p11.2 microdeletions. (a) Pedigrees of the three families. (b–d) Facial features of the proband, their sister, and father from Family 2. (e) Facial features of the proband from Family 3. (f and g) Spinal X-ray features for Family 3. (f) Lumbar and sacral vertebrae in the proband without malformation; (g) Malformation in sacral vertebrae of the mother. Red arrows indicate mild scoliosis and lumbarization of S1

clinical information (Table 1) regarding these carriers are described in detail below.

3.3 | Family 1

Family 1 included two boys with a paternally inherited the 16p11.2 microdeletion that was *de novo* in the father (Figure 1a). Their clinical information has been described previously

(Shen et al., 2011). The proband's father died at the age of 50 years as a result of long-term alcohol abuse.

The latest review ended when the male proband was 21 years old (III:1 of family 1, Figure 1a). He has dropped out of school after primary school and his IQ evaluation indicated cognitive disability. Physical examination showed café-au-lait macules over his body. Echocardiography detected pulmonary arterial hypertension and an atrial septal defect. He had no psychiatric disorder with a normal electroencephalogram.

TABLE 1 Clinical characteristics of 16p11.2 microdeletion carriers in three families

	Family 1			Family 2			Family 3	
	III:1	III:2	II:1	III:1	III:3	II:1	III:1	II:2
Gender	M	M	M	M	F	M	F	F
ID/DD	√	–	√	√	–	–	√	–
ASD	√	–	√	–	–	–	√	–
Seizure	√	–	NA	NA	NA	NA	√	√
Learning difficulty	√	–	√	√	NA	–	√	–
Language delay	√	–	√	√	√	–	√	–
Schizophrenia/bipolar disorder[a]	–	√	–	–	–	–	–	–
Spinal deformities	√	–	–	√	√	–	–	√

[a]Showed the new appearing neurodevelopmental phenotype after puberty.

The proband's younger brother (III:2 of family 1, Figure 1a) was an interactive, happy, and well-developed boy and DMS-IV evaluation aged 7 years had excluded ASD (Shen et al., 2011). However, his mother noted that he had begun to appear depressed and shown decreased interests, self-abasement, lack of concentration, and irritability since prepuberty. He was unable to keep up with schoolwork and often stayed home. Notably, he often felt someone calling his name or monitoring his actions, and the local psychiatric hospital diagnosed him as a suspected childhood psychiatric disorder. His latest follow-up was completed at the Affiliated Hospital of Capital Institute of Pediatrics and the Beijing AnDing Hospital (a psychiatric hospital) when he was 15 years old. He was confirmed with bipolar disorder and schizophrenia following a professional psychiatric evaluation. Drugs including risperidone dispersion, alicepate, agomelatine, and escitalopram were prescribed, which reduced his psychiatric symptoms. Physical checkup, electroencephalogram, and electrocardiogram examinations were all normal. Biochemical tests showed abnormal serum lipids (high triglycerides and low high-density lipoprotein) and high uric acid levels, similar to his father (Table S2).

3.4 | Family 2

Family 2 had two children with a paternally inherited the 16p11.2 microdeletion (Figure 1a). The origin of the microdeletion was unknown because the grandfather had died. The parents were non-consanguineous and the proband's pregnancy, delivery, and early developmental history were generally normal. The neurodevelopmental history of the proband's father was unknown because he declined adult intellectual and psychiatric evaluation (Figure 1d).

The boy proband (III:1 of family 2, Figure 1a) was diagnosed with ID at the local hospital at the age of 7 years old and left primary school because of learning difficulties. He was first referred to the Capital Institute of Pediatrics in Beijing at the age of 11 years old due to his ID and short stature. His height was 133 cm (<1th centile) and head circumference was 55 cm (<50th centile). Dysmorphic features included a slightly triangular face, broad nasal root, beaked nose, bilateral epicanthic eye folds, largemouth, thick lower lips, and short neck (Figure 1b). His IQ was 50. ASD was excluded based on the DMS-IV criteria.

The proband's latest review was completed at Beijing Friendship Hospital when he was 21 years old. His height was then 134 cm, head circumference was 56 cm, and weight was 44 kg. He had no psychiatric disorders except for ID. Cardiac ultrasound and electroencephalogram examinations were normal. Biochemical tests showed that the proband and his father both had abnormal serum lipid levels (low high-density lipoprotein, Table S2).

The proband's young sister died at the age of 12 years old and her neurodevelopmental status was not evaluated. However, based on family photographs and medical records, she had experienced severe language delay, short stature, congenital scoliosis, and facial features including a flat face, prominent forehead, hypotelorism, and low posterior hairline (Figure 1c).

3.5 | Family 3

Family 3 had one girl with maternally inherited the 16p11.2 microdeletion, which was *de novo* in the mother (Figure 1a). The mother had experienced febrile convulsions and early language delay during childhood, but appeared generally normal in neurodevelopment status as an adult.

The proband was born following the first pregnancy of two unrelated parents. The whole pregnancy and vaginal delivery at 39 weeks of gestation were uneventful. Her Apgar score was 10 after childbirth, her birth weight was 3300 g, and there were no records of her birth length or head circumference, but her mother recalled her as a normal baby. The proband suffered from febrile seizures at the age of 6 months,

with up to 12 episodes per day, but these seizures resolved spontaneously at her age of 10 months old. However, she was unable to sit, stand, or walk at 18 months old, suggesting moderate developmental delay, and was unable to say "mum" or "dad" at the age of 2 years. She was frequently referred to the hospital due to developmental delay, severe language deficiency, and social deficiency throughout her childhood. Physical examination identified no malformations. She showed no eye-contact during social communication, but demonstrated repetitive motor mannerisms including drumming on the table. Her mother reported her repetitive actions of a flushing toilet and fascinated music at the age of 2 years old. Routine electroencephalogram and 24-hour electroencephalogram were normal. No facial dysmorphism was noted (Figure 1e). She was diagnosed with severe language deficiency and ASD based upon the DMS-IV criteria by a pediatric psychiatrist at the Sixth Hospital of Peking University.

The proband's latest review was completed when she was 12 years old. She had started menstruation and entered puberty at the age of twelve. She remained fascinated by music and demonstrated stereotypic behaviors such as stamping, and drumming on the table. However, she was only able to say single words such as "baba" or "mama" in simple orders. Intellectual evaluation showed an IQ equivalent to a 2-year-old. Both the proband and her mother had abnormal serum lipid levels; the proband had high low-density lipoprotein and her mother had high triglycerides, cholesterol, and low-density lipoprotein (Table S2). X-ray examinations showed normal spine development in her daughter (Figure 1f) but mild scoliosis and sacral lumbarization in the mother (Figure 1g).

3.6 | SNVs within the 16p11.2 microdeletion interval

The different phenotypes observed in carriers with the same 16p11.2 microdeletion suggest that genetic factors other than CNVs are involved in the development of the phenotype. Deletion can influence gene function via molecular mechanisms such as unmasking a pathogenic/modifier mutation in a recessive allele or extending the modifier effect of a common SNP on the hemizygous allele (Lupski & Stankiewicz, 2005). Targeted sequencing of the 16p11.2 microdeletion region identified only one common SNP of *PRRT2* (MIM:614386, rs10204, T>C) that was consistently segregated in patient carriers with neurodevelopmental abnormalities ("C" genotype, II:1, III:1, III:2 of Family 1; III:1 of Family 2; II:1, III:1 of Family 3) and normal carrier without neurodevelopmental abnormalities ("T" genotype, II:1 of Family 2) among the three families, suggesting that *PRRT2* may modify the neurodevelopmental phenotype. We previously showed that the *TBX6* (MIM:602427) haplotype T-C-A (defined by the three common SNPs-rs2289292, rs3809624, and rs3809627)

compound with the 16p11.2 microdeletion was associated with congenital scoliosis in Families 1 and 2 (Wu et al., 2015), while the same haplotype was identified in the mother but not in the girl proband in Family 3.

3.7 | Differential whole-genome transcript profiles in 16p11.2 microdeletion carriers

We analyzed the haploinsufficiency of dosage-sensitive genes due to the 16p11.2 microdeletion by whole-genome mRNA expression profiling of peripheral blood in eight core members of two families (Family 1 and Family 2). We divided the eight members into two groups: five 16p11.2 microdeletion carriers (II:1, III:1, and III:2 of Family 1; II:1 and III:1 of Family 2; Figure 1a) and three non-deletion controls (II:2 of Family 1; II:2 and III:2 of Family 2; Figure 1a). A total of 57 DEGs (FC >1.5, $p < 0.05$) were identified from the iReport analysis. Among these, seven genes (*SPN* [MIM:182160], *ALDOA* [MIM:103850], *PPP4C* [MIM:602035], *MVP* [MIM:605088], *TAOK2* [MIM:613199], *KIF22* [MIM:603213], and *PAGR1* [MIM:612033]) in the 16p11.2 interval were expressed at consistently lower levels in the 16p11.2 carriers compared with their family controls without the 16p11.2 microdeletion.

3.8 | Impact of transcriptional alterations on neurodevelopmental phenotype

We confirmed the modifying effect of *PRRT2* (rs10204, T>C) on the neurodevelopmental phenotype by comparing RNA expression levels of the *PRRT2* gene between a patient carrier (III:1) and a normal carrier (II:1) from the same family (Family 2). However, there was no significant difference in RNA levels of *PRRT2* between the two carriers. We also generated a list of the main DEGs between the patient carrier and normal carrier using R software, but none of these DEGs were located in the 16p11.2 interval. The top-ranked network of DEGs revealed by IPA was involved in neurological Disease (Table S3). Notably, the zinc-finger protein EGR1 and leucine zipper protein FOS were the key nodes in the top-ranked network (Figure S1). One recent study showed that the 16p11.2 CNV strongly affected the expression of many genes outside the 16p11.2 region, based on results from the cerebral cortex in mouse models and lymphoblast lines from ASD-affected patients (Blumenthal et al., 2014). To explore the influence of the 16p11.2 interval on the whole-genomic expression profile, we extracted functional genes from the 16p11.2 interval and examined their interactions with the top DEGs. There were significantly direct interactions between genes within the 16p11.2 interval and the key nodes of the top-ranked network, such as interactions

between *MVP* [MIM:605088] /*MAZ* [MIM:600999] /*MAPK3* [MIM:601795] and FOS, and *MAPK3/HIRIP3* [MIM:603365] and DUSP1/DUSP4 (Figure 2).

4 | DISCUSSION

The 16p11.2 microdeletion is a typical example of a recurrent CNV, harboring several genes associated with neurodevelopment (Weiss et al., 2008). 16p11.2 microdeletion was first reported in a patient with Asperger's syndrome (Sebat et al., 2007), since when large microarray databases from patients with sporadic and familial ASD have highlighted 16p11.2 microdeletion as a risk factor for ASD, with high frequencies of 0.6%–1% (Bertero et al., 2018; Knoll et al., 2018; Kumar et al., 2008; Marshall et al., 2008; Weiss et al., 2008). The microdeletion is also associated with other NDDs, such as ID/DD, language delay, learning difficulties, and epilepsy (Bijlsma et al., 2009; Knoll et al., 2018; Raca et al., 2013; Shen et al., 2011; Tardivo et al., 2017; Xiang et al., 2010). Based on a review of the available literature, Rosenfeld et al. reported that the frequency of the 16p11.2 microdeletion among clinically tested samples was approximately 0.5% (Rosenfeld et al., 2010). Weiss et al. identified five 16p11.2 microdeletions among 512 western children referred for clinical genetic testing (frequency of 0.98%) (Weiss et al., 2008). So far, previous studies were performed for patient populations of European ancestry. The frequency of the 16p11.2

microdeletion in the Chinese general or patient population is unknown. We previously retrieved the raw array dataset from five Chinese cohorts including 3671 normal Chinese individuals (97% Han) (Li et al., 2009; Lou et al., 2011; Xu et al., 2011; Zhang, Li, Zhang, Wang, & Yu, 2012). No 16p11.2 carriers were identified in that dataset, confirming that the 16p11.2 is a rare microdeletion in the Chinese population. Our study demonstrated a frequency of 0.33% for the 16p11.2 microdeletion in a Chinese pediatric NDD cohort, which was slightly lower than those in Western studies (Rosenfeld et al., 2010; Weiss et al., 2008). This information is useful for Chinese geneticists/pediatricians before conducting the 16p11.2 microdeletion testing in children with NDDs.

The 16p11.2 microdeletion carriers presented with mild to severe NDDs, or even with no medical problems (Bijlsma et al., 2009; Ghebranious et al., 2007). Bijlsma et al. reported on a family with three 16p11.2 microdeletion carriers, all of whom had a severe speech delay, while the proband had ID and ASD and his older brother was mildly retarded (Bijlsma et al., 2009). However, previous phenotypic studies of the 16p11.2 carriers were cross-sectional and thus had limited ability to reveal the neurodevelopmental trajectories of the 16p11.2 deletion carriers. The current study was thus designed as a preliminary follow-up study in four children with familial 16p11.2 deletion. We demonstrated the discordant neurodevelopmental phenotypes among the carriers, which confirmed the phenotypic complexity and heterogeneity of the 16p11.2 microdeletion. We also carried
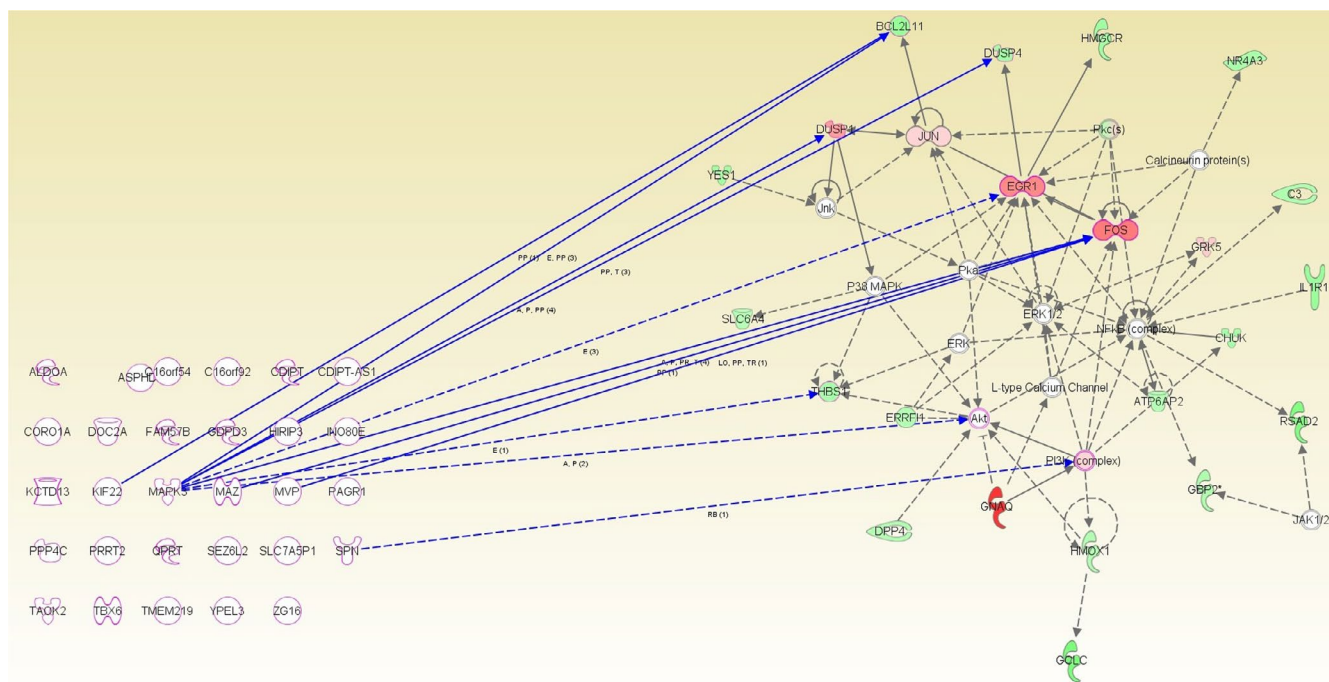
FIGURE 2 Network showing interactions of genes in 16p11.2 microdeletion interval with DEGs. Genes are represented as nodes and the biological relationships between nodes are represented as lines. Genes in the left part of the figure are within the 16p11.2 interval; genes in the right part of the figure in the network built by the DEGs

out neurodevelopmental evaluations and clinical reviews at different age stages, and revealed inconsistent neurodevelopment in one 16p11.2 microdeletion carrier after puberty, suggesting the need for a prospective follow-up study of the 16p11.2 microdeletion carriers to clarify their neurodevelopmental trajectories.

The 16p11.2 microdeletion in conjunction with the *TBX6* T-C-A haplotype was an important genetic factor associated with congenital scoliosis. Herein, we provided evidence of the 16p11.2 microdeletion combined with the T-C-A *TBX6* haplotype related to mild scoliosis and sacral lumbarization. The 16p11.2 microdeletion has also been associated with obesity (Loviglio et al., 2017; Schuh, Taylor, Font-Montgomery, & Tosh, 2016; Yu et al., 2011). Notably, most 16p11.2 microdeletion carriers in the present study had different abnormal serum lipid levels, suggesting that the 16p11.2 microdeletion is a risk factor for abnormal serum lipids. Further follow-up study during adulthood with more samples is required in the future.

CNVs affect genome expression, and a positive correlation between gene expression levels and the 16p11.2 CNV has previously been demonstrated (Henrichsen et al., 2009; Kusenda et al., 2015; Luo et al., 2012; Stranger et al., 2007). We observed that the 16p11.2 carriers had decreased expression levels in seven genes within the 16p11.2 interval, indicating that some genes within this interval (7/29, 24%) were dosage-sensitive. Among those genes, *TAOK2*, *ALDOA*, and *MVP* are known neurodevelopmental genes, supporting that the decreased expressions of key genes in the 16p11.2 region are the physical basis of neurodevelopmental disorders. *TAOK2* is considered as an autism-susceptibility gene, which affects basal dendrite formation in the neocortex (de Anda et al., 2012; Richter et al., 2019). Moore et al. reported that TAOK2 interacted with the JNK mitogen-activated protein kinase pathway, which played an important role in survival, proliferation, and differentiation of central and peripheral nervous system cells (Moore et al., 2000). ALDOA is involved in glycolysis and energy balance, which is important for synaptic metabolism and neurotransmitter release (Pellerin, 2010). MVP can activate Elk-1 by inhibiting YPEL4 in the MAPK signaling pathway (Liang et al., 2010), and MVP protein was shown to be increased in the frontal cortex in patients with refractory epilepsy (Liu et al., 2011).

DEGs from the whole-genome level demonstrated that FOS and EGR1 were the key nodes in the top-ranked network involved in neurological disease. Furthermore, there were multiple direct interactions between the key nodes of the top-ranked network and genes within the 16p11.2 interval, such as FOS and *MVP/MAZ/MAPK3*. All the above results suggest that the disturbed gene expression in the 16p11.2 region can affect the key expression nodes and networks involved in nervous system development from the whole-genome profile,

and finally increase the risk of a neurodevelopmental phenotype in the 16p11.2 microdeletion carriers.

In this study, only one common SNP of *PRRT2* was segregated between patient carriers and normal carriers, implying that this *PRRT2* SNP could modify the risk of an NDD phenotype in the 16p11.2 microdeletion carriers. PRRT2 was widely expressed in the mouse brain, with high expression in the cerebral cortex during embryonic and postnatal periods (Chen et al., 2011). *PRRT2* has been considered to be the major pathogenic gene in paroxysmal kinesigenic dyskinesia and other paroxysmal disorders (Chen et al., 2011; Ebrahimi-Fakhari, Saffari, Westenberger, & Klein, 2015; Lee et al., 2012; Tan et al., 2018; Zhao, Liu, Zhang, & Wang, 2018). However, the differences in *PRRT2* expression between patients and normal carriers in the current study did not support that the common *PRRT2* SNP is the modified variant of the 16p11.2 microdeletion. Notably, the current study had several possibly relevant limitations, including the use of peripheral blood rather than the brain or spinal cord tissues, which may not have reflected the changes in gene expression in nervous tissue. Moreover, the control carrier in our study was an adult, and age-difference was a possible factor influencing gene expression.

In summary, this study has provided the first evidence for the frequency of the 16p11.2 microdeletion in a Chinese pediatric NDD cohort. Our follow-up study revealed the diverse neurodevelopmental trajectories of children with the 16p11.2 microdeletion through puberty. A differential *PRRT2* genotype was identified between the patients and normal carriers of the 16p11.2 microdeletion. Destruction of the top-ranked network in nervous system development may have caused the neurodevelopmental phenotype in the 16p11.2 microdeletion carriers. In addition, we reported mild scoliosis and sacral lumbarization in an adult carrier of the *TBX6* hypomorphic risk allele and abnormal serum lipid levels in carriers with the 16p11.2 microdeletion.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Xiaoli Chen designed the study and revised the manuscript. Hua Xie is responsible for target gene design, whole-genome transcript profiles experiment and analysis, and manuscript drafting. Fang Liu is responsible for target sequencing and MLPA and variant interpretation. Yu Zhang performed most of the bench works including WES and aCGH experiment, and paternity testing. Qian Chen, Zhijie Gao, Nan Wu, and Lin Wang were responsible for clinical works including

patient recruitment/diagnosis/review and clinical data collection. Jian Wang performed most of the dry works for 16p11.2 target sequencing including bioinformatics analysis and primer design. Lin Wang and Xiaodai Cui gave key suggestions for study design and manuscript writing. All authors read and approved the final manuscript.

CONSENT FOR PUBLICATION

We confirm that the patients' parents have given their written consent for publication of their medical data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Table S1-S3-Fig S1