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Original Article

Clinical application of whole exome sequencing technology in small-for-gestational-age children

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Abstract: Aim: To apply whole exome sequencing (WES) for molecular diagnosis of small-for-gestational-age (SGA) children. Methods: We retrospectively analyzed the data of 60 SGA children in our hospital, and performed developmental assessment, laboratory tests, imaging tests, and whole exome sequencing (WES), which were combined with clinical phenotypes to clarify the pathogenicity of the variant genes in the children. Results: Sixty SGA children were tested, and pathogenic SGA was detected at relatively high frequencies on chromosomes 7, 8, and 22. Of these, karyotype analysis clearly suggested developmental disorders in 4 patients. Also, a case of Wiedemann-Steiner syndrome due to a de novo nonsense variant in the KMT2A gene was detected. Conclusions: The use of WES testing technology to increase the diagnosis rate of children with special SGA is conducive to the correct diagnosis and treatment of such children.

Keywords: Whole exome sequencing technology, small-for-gestational-age infant, clinical application

Introduction

Small-for-gestational-age (SGA) infants are defined as babies whose birth weight and/or birth length fall below two standard deviations or more than the third percentile of the same gestational age, with a prevalence of about 5-10% [1]. SGA infants not only have a higher incidence of perinatal complications and mortality, but also have a poorer long-term prognosis, including short stature, mental retardation, developmental delay, and metabolic syndrome in adulthood compared with appropriate for gestational age (AGA) [2]. SGA accounted for 9.19% of the hospitalized infants in neonatal intensive care units, and the morbidity, mortality, and auto-discharge rate accounted for 15.12% of the total hospitalized infants [3]. In order to improve the recognition and management of SGA infants, in 2016 WHO defined SGA as an infant's birth weight below the 10th percentile of the weight of an infant of the same gestational age, a concept that improves the diagnostic sensitivity and decreases the specificity of SGA, and facilitates enhanced interventions in some underdiagnosed SGA infants. Approximately 87% of SGA children begin to catch up in the first month of life, with significant catch-up within 6 months, and most complete catch-up within 2 years of age. Children with preterm SGA take longer to catch up the growth, and 15% of children with SGA are still less than -2 standard deviations taller at two years of age, resulting in short stature. The U.S. Food and Drug Administration recommends that growth hormone (GH) therapy can be started at the earliest from the age of two years, and Europe and China recommend GH therapy to be started at the age of 4 years [4]. The diverse mechanisms of SGA lead to different prognoses and treatments for SGA. For instance, Bloom syndrome can increase incidence of malignant tumors, and for this group GH therapy is not recommended, so the analysis of the etiology of SGA is particularly important. The diverse etiology of SGA is currently thought to be caused mainly by genetic diseases, congenital infections, and multiple pregnancies, of which 5-33.3% of SGA is caused by genetic abnormalities [5].

In recent years, genetic testing for dwarfism has been gradually carried out in clinical work, using molecular diagnostic methods such as

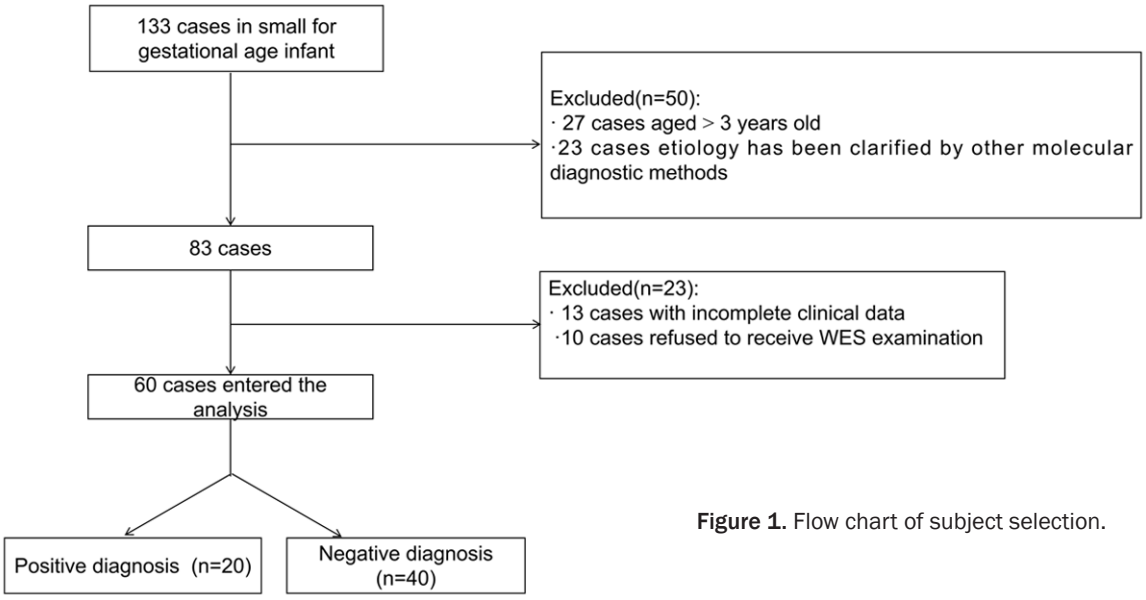

Figure 1. Flow chart of subject selection.

chromosomal microarray analysis (CMA), targeted sequencing, and whole exome sequencing (WES), to find the cause of the disease, but there are few tests for the SGA gene alone. Most SGA patients have intrauterine fetal growth restriction (FGR), and a high correlation between FGR and chromosomal aberrations has been demonstrated [6]. Currently, CMA technology is the optimal test in the prenatal diagnosis of FGR, but there are still some abnormal fetuses for which a diagnosis cannot be made. Since WES can simultaneously detect single-nucleotide variants (SNVs), insertion/deletion variants (Indels), and copy number variants (CNVs), it has been advocated that WES should replace CMA as the first choice for prenatal genetic testing [7-9]. The American College of Medical Genetics and Genomics suggests that WES should be considered if fetal abnormalities remain undiagnosed after karyotyping and CMA testing [10]. WES yields more than 20,000 exonic SNVs per individual, requiring a strategy to narrow the number of variants [11-14]. In pedigrees characterized by potential autosomal dominant inheritance, filtering strategies based on segregation have facilitated identification of causal variants. However, this approach requires large, multigenerational pedigrees with available genetic samples and clinical characterization [15-19]. Furthermore, WES in SGA children has been reported in one study, involving a limited number of candidate gene investigations [20]. Therefore, some of the successful treatment strategies for SGA children may require discovery of novel potential disease-causing genes.

Therefore, the aim of this study was to use WES for molecular diagnosis of SGA children, so as to provide a basis for clinical diagnosis and treatment, reduce misdiagnosis and underdiagnosis of the disease, and give genetic counseling and prenatal guidance.

Methods and materials

Study design and subjects

This study was approved by the Ethics Committee of Jiangxi Provincial Children's Hospital and adopted a retrospective cohort study design. From the electronic medical record system, 60 SGA children diagnosed in our hospital from August 2020 to August 2023 were selected according to the following inclusion criteria: 1) children diagnosed with SGA; 2) children at an age of 1 month-36 months; 3) children with complete clinical data (sex, age, height, body mass index, and results of WES). We excluded 1) children with maternal or placental etiology, including multiple pregnancy, placental insufficiency, preeclampsia/HELLP syndrome, renal insufficiency, autoimmune diseases, or smoking history; 2) children with congenital infection or major congenital anomalies; 3) children with incomplete clinical data. Flow diagram detailing the selection of patients is shown in **Figure 1**.

Data collection

We collected required data of eligible patients from the patient records, including sex, age, BMI, height, abnormal body proportions, facial dysmorphism, intellectual disability/neurodevelopmental delay, microcephaly, major malformation, skeletal dysplasia, short stature parent, consanguinity, birth weight, and birth height. We manually reviewed the printed electronic WES records of the 60 children, and compared the results with those obtained through the electronic data acquisition algorithm for these cases. We found that the data assurance was fulfilled.

Outcome measures of WES

(1) DNA extraction: After fully communicating with the child's family and obtaining the informed consent form and the approval pass from the Ethics Committee of Jiangxi Provincial Children's Hospital, we collected 2 ml of venous blood from the children and the parents (EDTA anticoagulation tubes), and genomic DNA of the three individuals was extracted using the Lab-Aid Nucleic Acid Extractor (Xiamen Zhi-Shan Biologicals) as well as the Nucleic Acid Extraction Magnetic Bead Reagent Kit. The DNA of the samples was quantified using Qubit 3.0. Quantification.

(2) Gene sequencing and data interpretation: The samples were prepared using the Aligent SureSelect method, and the experimental method followed the operation manual. The target region was captured, and the library was constructed using the Agilent Hybridization Capture Reagent. The libraries were analyzed by a 2200 Bioanalyzer (Agilent) for quality control and then sequenced. High-throughput sequencing was performed using Illumina NovaSeq, with a read length of 2×150 bp. Subsequently, the sequencing results were compared with the genome (GRCh37/hg19). SNVs and Indels were detected by using the GATK software and annotated by using the software packages such as Annovar and VEP. Mutations detected included all genome-wide variants (SNVs and Indels) in the coding region and its neighboring ±50 bp intronic region. Additionally, known or potentially clinically relevant CNVs associated with the subject's disease phenotype were interpreted with reference to various disease databases (e.g., OMIM, ClinVar, HGMD) and population-based genetic variation databases (e.g., dbSNP, HGMD). Utilized databases included dbSNP database (SNP150), Thousand Genomes database, ESP6500 database, ExAC database, gnomAD database, etc. The interpretation followed the American College of Medical Genetics and Genomics (ACMG) 2019 edition standards and guidelines. However, certain types of SNVs/Indels were not listed in the official report: (i) those assessed as (probably) benign according to the ACMG Guidelines; (ii) VOUS-like genetic variants inherited from parents without any clinical phenotype (dominant mode of inheritance only); and (iii) variants assessed as (possibly) pathogenic according to the ACMG criteria, but do not explain the clinical phenotype of the subject.

(3) Sanger sequencing validation: Suspected pathogenic SNVs/Indels screened by the above methods were analyzed by designing PCR primers for amplification and Sanger sequencing using Primer3 (v.0.4.0) software. Sequencing data were analyzed using software such as Mutation Surveyor.

Statistical analysis

Statistical data were analyzed using SPSS 23.0. Categorical data were expressed as number of cases and relative frequencies (%), and continuous variables were expressed as mean ± standard deviation (SD). For continuous data with normal distribution and homogeneity of variance, the independent samples t-test was used for between-group comparison. The chi square (χ^2) test or Fisher's exact test was used for comparison of categorical variables. For all the analysis, a *P* value of smaller than 0.05 (two-sided) was considered statistically significant.

Results

Clinical characteristics of SGA children analyzed by WES

The cohort was characterized by a male predominance (n = 36 [60%]). The SGA children presented at a mean chronological age of 1.8 years and marked short stature. The most recurrent phenotypes associated with short stature among these patients were dysmorphic facial features (n = 36 [82%]), developmental delay, intellectual disability (n = 26 [43.3%]),

Table 1. Clinical characteristics of small-for-gestational-age children analyzed by WES

Group	Total (n = 60)	Positive diagnosis (n = 20)	Negative diagnosis (n = 40)	t	*P*
Sex				1.009	0.102
Male	36	10	26		
Female	24	10	14		
Age (months)	1.8±0.3	1.8±0.9	1.7±0.6	1.123	0.204
Height (cm)	78.1±3.9	73.5±6.9	77.9±5.6	7.128	0.002
Body mass index	12.5±1.1	9.0±2.9	10.7±2.1	5.217	0.015
Abnormal body proportions (%)	19	11	8	7.347	0.007
Facial dysmorphism (%)	26	17	9	13.123	0.004
Intellectual disability/neurodevelopmental delay (%)	21	12	9	2.768	0.134
Microcephaly (%)	20	13	7	1.334	0.219
Major malformation (%)	24	15	9	7.895	0.036
Skeletal dysplasia (%)	28	16	12	13.009	0.002
Short stature parent (%)	27	15	12	4.398	0.055
Consanguinity (%)	24	12	12	1.023	0.104
Birth weight (kg)	4.9±1.7	3.8±1.2	4.7±1.0	5.654	0.043
Birth height (cm)	52.2±7.3	49.6±6.9	50.8±5.9	2.398	0.298

and microcephaly (n = 20 [33.3%]). The results are compatible with skeletal dysplasia (signs of skeletal deformities, severe body disproportion, and/or radiographic abnormalities) (**Table 1**).

WES positive results

Among the pathogenic CNVs detected by the WES technique, 18 children (P9-P11) had chromosome number abnormalities, trisomy 13, 18, and 21, and a large deletion of 19.84 Mb, and 2 children were diagnosed with VOUS (ACMG). WES testing clarified mar as a duplicated segment of WES chromosome 15, 8.92 Mb, which can cause the 15q11-q13 deletion syndrome, and is associated with developmental delay and mental retardation. In addition, there were also carriers of the Roche translocation with a karyotype of 45, XY, rob (13,14) and a chromosomal karyotype that does not explain the developmental delay phenotype (**Table 2**).

Clinical phenotyping

Of the 60 patients, cardiovascular system, specific facial/structural malformations, autism, and other phenotypes were included in the tested patients. Specific facial or other multiple malformations were found to be more common. Among the SGA children with SGA of undetermined significance, 13 cases combined phenotypes other than developmental delay: 2 cases of congenital heart disease (1 case of cardiac hypertrophy and 1 case of ventricular septal defect), 2 cases of autism, 10 cases of congenital anomalies/dysmorphisms (1 case of congenital blindness, 2 cases of muscular anesthesia, 1 case of myotonia, 1 case of entropion, 1 case of valgus, 1 case of sphincter, 1 case of right hand agenesis, 1 case of skin pigmentation, and 1 case with abnormal posture of both hands), and 2 cases with special facial features (**Table 3**). The advent of new genomic WES technologies has provided a genetic diagnosis for many SAG children with short stature of unknown cause.

KMT2A schematic representation of the gene locus

WES detected a case of Wiedemann-Steiner syndrome due to a de novo nonsense variant in the KMT2A gene. The child was diagnosed with global developmental delay with facial dysmorphism, epilepsy, and spastic seizures. WES was performed to screen for phenotypic and potentially pathogenic variants, and Sanger sequencing was applied to validate the variants in the patient and her parents. The results of the WES showed the presence of a heterozygous variant

Table 2. WES positive results

Patient ID	Sex/Age	Karyotype analysis	Result annotations or reference cases
SGA1	Male/8 months	47, XY+mar	15q11-q13 deficiency syndrome
SGA2	Female/17 months	47, XX+13	13 trisomy/Patau syndrome
SGA3	Male/17 months	47, XX+21	21 trisomy/Down's syndrome
SGA6	Female/7 months	47, XX+18	18 trisomy/Eiwrds syndrome
SGA8	Female/one year old	46, XX del (11)(q13.5q21)	Multiple literature reports have reported that the relevant symptoms
SGA9	Male/one year old	45, XY, rob (13,14)	VOUS (ACMG)
SGA11	Female/one year old	46, X, t (X, 11Xq112: p15.1)	21 trisomy/Down's syndrome
SGA13	Female/12 months	47, XY+mar	18 trisomy/Eiwrds syndrome
SGA14	Male/16 months	47, XX+13	21 trisomy/Down's syndrome
SGA16	Female/13 months	47, XX+21	18 trisomy/Eiwrds syndrome
SGA31	Female/17 months	47, XX+18	Multiple literature reports have reported that the relevant symptoms
SGA32	Male/27 months	46, XX del (11)(q13.5q21)	VOUS (ACMG)
SGA33	Female/16 months	46, XX del (11)(q13.5q21)	21 trisomy/Down's syndrome
SGA36	Male/22 months	45, XY, rob (13,14)	21 trisomy/Down's syndrome
SGA48	Female/27 months	46, X, t (X, 11Xq112: p15.1)	18 trisomy/Eiwrds syndrome
SGA49	Male/26 months	47, XX+21	21 trisomy/Down's syndrome
SGA51	Female/19 months	47, XX+18	18 trisomy/Eiwrds syndrome
SGA53	Male/30 months	46, XX del (11)(q13.5q21)	21 trisomy/Down's syndrome
SGA54	Male/23 months	46, X, t (X, 11Xq112: p15.1)	18 trisomy/Eiwrds syndrome
SGA56	Male/20 months	47, XX+21	21 trisomy/Down's syndrome

in exon 15, c.4906C>T (pArgI636Ter), in the KMT2A gene (NM001197104) of the child. This variant was not detected in either of the parents of the child and was not reported in the relevant databases and literature, making it a new variant that has not been reported. The c.4906C>T variant in the KMT2A gene was determined to be a pathogenic variant (PVS1+PS2+PM2+PP3) according to the criteria and guidelines for the classification of genetic variants of ACMG (**Figure 2**).

Discussion

In this study, WES was applied to detect genomic CNVs in 60 SGA children. Relatively high frequencies were detected on chromosomes 7, 8, and 22, and most of the microdeletions/duplications detected were known to be associated with SGA disease, suggesting that these high-frequency pathogens have a global epidemiological profile, including Williams syndrome, 8q21.11 microdeletion syndrome, and 22q11 deletion syndrome, among others. The diagnosis of Wiliams-Beuren syndrome (WBS) was made in 2 cases (P7 and P15). The length of the P15 deletion sequence in the 2 children with WBS was 1.40 Mb, less than the 1.70 Mb microdeletion in P7, but the CNVs in both cases contained a deletion of the key WBS gene ELN (OMIM:*130160).

P8 is characterized by an 8q21.11-q21.13 deletion spanning a length of 7.6 Mb. This deletion contains the full length of known 8q21.11 microdeletion syndrome (OMM:#61-4230) CNVs and aligns with the phenotypes typically associated with this syndrome, including mental retardation and distinctive facial features. Thus, the diagnosis was confirmed; 8q24.22-q24.3 of P13 had a 10.64 Mb long repeat. By 2 years of age, it showed isolated speech delay. Although there are no clear cases of pathogenicity reported for this CNV, the presence of a 2.3 Mb inverted repeat at 8q24.3 and microdeletions at 8q24.3 have been reported to cause a variety of developmental delays [21-24], including severe psychomotor delays, mental retardation, facial dysmorphisms, and epilepsy. It is thus clear that the loci for the phenotypes associated with

Table 3. Clinical phenotyping

Number	Sex	Age	Karyotype analysis	CNV-seq test results (hg19)	Developmental delay	Congenital heart disease	Leukorrhea	Congenital abnormalities/ deformities	Special facial features
P19	Male	1 year	45, XY, rob (13,14)	dup(3)(p11)(0.48 Mb)	+	-	-	-	-
P20	Male	1 year	46, XY, 15pstk+	dup(2)(q24.2q24.3)(2.24 Mb)	+	+	-	+	-
P21	Male	4 years	/	dup(16)(p13.2)(0.22 Mb)	+	-	+	-	-
P22	Male	3 years	/	del(1)(p22.11)(0.22 Mb); del(3)(q25.2)(0.3 Mb)	+	-	-	+	-
P23	Female	1 year	/	del(1)(p31.2p31.1)(7.86 Mb)	+	-	-	-	-
P24	Male	0 year	/	del(3)(q27.1)(0.8 Mb); dup(1)(131.3)(0.3 Mb); dup(6)(p21.32)(0.3 Mb)	+	-	-	-	+
P25	Male	0 year	/	del(5)(p15.33)(2.18 Mb); dup(5)(p15.33p14.1)(25.98 Mb)	+	-	-	-	-
P26	Female	0 year	/	dup(7)(p11.2)(0.5 Mb)	+	+	-	+	-
P27	Female	4 years	/	dup(4)(q35.2)(0.98 Mb)	+	-	-	-	-
P28	Male	5 years	/	del(22)(q13.32)(0.1 Mb)	+	-	-	-	-
P29	Female	0 year	/	dup(S)(p23.3)(0.16 Mb)	+	-	-	+	-
P30	Male	2 years	/	dup(1)(q43)(1.16 Mb); dup(11)(q11.12)(0.6 Mb)	+	-	-	-	-
P31	Male	5 years	/	del(1)(q31.2)(0.18 Mb); dup(12)(q21.3)(0.12 Mb)	+	-	-	-	-
P32	Female	1 year	/	dup(20)(q13.2)(0.20 Mb)	+	-	-	-	-
P33	Male	0 year	/	dup(X)(p22.11p213)(2.664 Mb)	+	-	-	-	-
P34	Male	2 years	/	dup(2)(p23)(1.06 Mb)	+	-	-	-	-
P35	Male	2 years	/	dup(2)(p21)(0.18 Mb)	+	-	-	-	-
P36	Male	1 year	/	dup(3)(p26.1)(0.48 Mb)	+	-	-	-	-
P37	Male	5 years	/	dup(14)(q12q13.1) (2.20 Mb)	+	-	-	-	-
P38	Male	2 years	/	dup(14)(p32.33)(0.48 Mb)	+	-	-	-	-
P39	Male	2 years	/	dup(3)(p26.1)(0.12 Mb)	+	-	-	-	-
P40	Male	2 years	/	dup(16)(p13.2)(0.38 Mb)	+	-	-	-	-
P41	Female	0 year	/	dup(X)(p22.11)(0.52 Mb)	+	-	-	+	-
P42	Female	1 year	/	dup(5)(p12)(0.54 Mb)	+	-	-	+	-
P43	Male	0 year	/	dup(4)(q28.1)(0.34 Mb)	+	-	-	-	-

P44	Female	3 years	/	dup(1)(p13.2)(0.12 Mb); dup(14)(q32.3lq32 32)(1.08 Mb)	+	-	-	-	-
P45	Male	5 years	/	del(2)(q35)(0.54 Mb)	+	-	+	-	-
P46	Female	0 year	/	dup(4)(q32.3)(0.22 Mb)	+	-	-	+	-
P47	Female	1 year	/	dup(4)(q31.3)(0.34 Mb)	+	-	-	+	+
P48	Male	5 years	/	del(4)(p16.1)(0.22 Mb)	+	-	-	+	-
P49	Female	3 years	/	dup(1)(p36.2)(0.2 Mb); dup(3)(q13.32)(0.32 Mb); dup(12)(q24.33)(0.72 Mb); del(14)(q11.2)(0.34 Mb)	+	-	-	+	-
P50	Male	1 year	/	dup(2)(p24.3)(0.76 Mb)	+	-	-	-	-

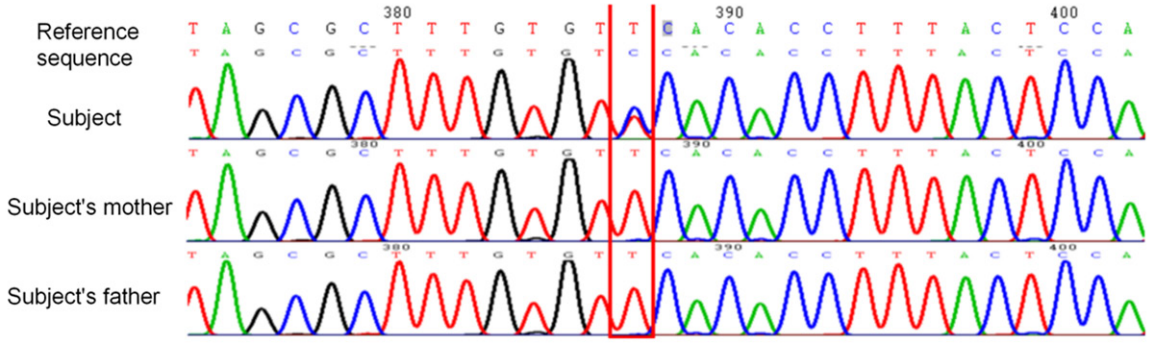

Figure 2. KMT2A schematic representation of the gene locus.

8q24.22-q24.3, especially 8q24.3, need to be further pinpointed.

In the present study, we followed up with the use of WES technology to identify the causative gene in SGA children. That is, a nonsense variant in the de novo KMT2A gene causes Wiedemann-Steiner syndrome, a very rare autosomal dominant congenital growth disorder with a global prevalence of less than 1/100,000,000. Clinical manifestations are diverse, with developmental delay, mental retardation, hirsutism, and characteristic facial deformities being the most common. The pathological basis of the clinical phenotype of Wiedemann-Steiner syndrome and the genotypic phenotypic association are still unclear. A previous study reviewed 71 patients with WDSTS [25] and found that mental retardation (70/71) with peculiar facial features (70/71) was present in almost all of the patients, while those with epilepsy accounted for only 8.5% (6/71). In the present case, the child was younger than 2 years old, and the main phenotypes were severe GDD (prognosis of mental retardation), peculiar facial features, and recurrent epilepsy, while the common hirsutism was not seen among the other concomitant phenotypes, which may be related to the difference in genetic background between the Chinese and Western populations. In a study including 33 French cases of WDSTS, it was hypothesized that defects in this structural domain may primarily cause neurodevelopmental disorders. The latter leads to a wide range of developmental brain dysfunctions, including developmental delay/intellectual disability, autism spectrum disorders, epilepsy, and attention deficit hyperactivity disorder [26-29].

The KMT2A gene is located on chromosome 1lq23 and contains 36 exons encoding a protein of 3972 amino acid residues. More than 70 KMT2A variants associated with Wiedemann-Steiner syndrome have been reported (ClinVar database), of which more than 50 are pathogenic, including missense variants, shifted variants, nonsense variants, and splice variants. Summarizing the WDSTS-associated variants c.7071delC, c10051delA and c97629765deGATT reported in China [30, 31], along with the variant loci we examined, reveals that the gene lacks pathogenic variant hotspots. This contrasts with previous reports suggesting that although more than 50% of the pathogenic variants occur in the two longest of KMT2A (and thus the probability of random variants), there is also a higher probability of random variants. This is consistent with previous reports indicating that although more than 50% of disease-causing variants occur in the 2 longest exons, namely 3 and 27, of KMT2A (resulting higher probability of random variants), no specific hotspots of variants were found. Additionally, the c.4906C>T (pArg1636Ter) variant of the KMT2A gene represents a truncating mutation, leading to the synthesis of a shortened KMT2A protein through transcription and translation. This results in a complete loss of function of the downstream protein chain, elucidating the heterozygous variant's role in causing KMT2A haplotype deficiency and consequently triggering Wiedemann-Steiner syndrome due to defective KMT2A protein function. In addition, the detection of the new variant enriches the spectrum of KMT2A gene variants.

We conclude that KMT2A haplotype deficiency identified by WES might contribute to SGA, and

WES is helpful in establishing an early diagnosis in SGA children. Further studies are required to explore the genetic causes of SGA and whether such genetic evaluation can become an effective diagnostic approach.

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Disclosure of conflict of interest

None.

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