Received: 23 April 2021 | Revised: 2 July 2021 | Accepted: 29 July 2021

DOI: 10.1002/jcla.23971

RESEARCH ARTICLE

WILEY

Prenatal genetic testing in 19 fetuses with corpus callosum abnormality

Qin She | Erfang Tang | Cui Peng | Li Wang | Dandan Wang | Weihe Tan

Prenatal Diagnostic Center, The Six Affiliated Hospital, Guangzhou Medical University or Qingyuan People's Hospital, Qingyuan, China

Correspondence
Weihe Tan, Prenatal Diagnostic Center, The Six Affiliated Hospital, Guangzhou Medical University, Yinquan Road, Qingyuan 511518, Guangdong Province, China.
Email: tan6806@163.com

Funding information
Medical Scientific Research Foundation of Guangdong Province of China, Grant/Award Number: B2020136; Medical Scientific Research Foundation of Qingyuan people's Hospital, Grant/Award Number: 20190204

Abstract

Background: Corpus callosum abnormality (CCA) can lead to epilepsy, moderate severe neurologic or mental retardation. The prognosis of CCA is closely related to genetic etiology. However, copy number variations (CNVs) associated with fetal CCA are still limited and need to be further identified. Only a few scattered cases have been reported to diagnose CCA by whole exome sequencing (WES).

Methods: Karyotyping analysis, copy number variation sequencing (CNV-seq), chromosomal microarray analysis (CMA) and WES were parallelly performed for prenatal diagnosis of 19 CCA cases.

Results: The total detection rate of karyotyping analysis, CMA (or CNV-seq) and WES were 15.79% (3/19), 21.05% (4/19) and 40.00% (2/5), respectively. Two cases (case 11 and case 15) were diagnosed as aneuploidy (47, XY, + 13 and 47, XX, + 21) by karyotyping analysis and CNV-seq. Karyotyping analysis revealed an unknown origin fragment (46,XY,add(13)(p11.2)) in case 3, which was further confirmed to originate from p13.3p11.2 of chromosome 17 by CNV-seq. CMA revealed arr1q43q44 (238923617–246964774) × 1(8.04 Mb) in case 8 with a negative result of chromosome karyotype. WES revealed that 2 of 5 cases with negative results of karyotyping and CNV-seq or CMA carried pathogenic genes ALDH7A1 and ARID1B.

Conclusion: Parallel genetic tests showed that CNV-seq and CMA are able to identify additional, clinically significant cytogenetic information of CCA compared to karyotyping; WES significantly improves the detection rate of genetic etiology of CCA. For the patients with a negative results of CNV-seq or CMA, further WES test is recommended.

KEYWORDS
chromosomal microarray analysis, copy number variants sequencing, corpus callosum abnormality, karyotyping analysis, whole exome sequencing

1 | INTRODUCTION

The corpus callosum (CC) is the main cerebral commissure in placental mammals and plays a key role in communication between the two brain hemispheres (left and right).[1] Corpus callosum abnormality (CCA) is relatively common brain malformation with estimated prevalence of 9.4 per 10,000 live births,[2] generally resulting in mild to severe intellectual disability, epilepsy, and behavioral difficulties.[3] There is no standard treatment scheme for CCA. Prognosis commonly depends on the extent and severity of malformation and will be much poorer when CCA is accompanied with genetic abnormalities, such as aneuploidies, pathogenic copy number variants (CNVs) and single-gene defects. In 2018, American College of Obstetrics and gynecology (ACOG) and Society for Maternal-Fetal medicine (SMFM) recommend that CMA can replace conventional karyotype analysis as the first-tier detection method when prenatal ultrasound indicates fetal structural abnormalities.[4] However, with further researches in recent years, it is found that the detection rate of CMA in CCA fetuses is low, the highest not over 19.44%.[5] Especially in isolated CCA cases, the detection rate is only 3.57%.[6] In April 2020, ACMG put forward an expert consensus that for fetuses with abnormal ultrasonic structure, we can further perform WES when CMA or karyotype analysis result is negative.[7]

Due to low incidence rate, CCA which is a part of abnormal ultrasound structure, is lack of larger samples, systematic research from cell genetics to molecular genetics. And this brings some trouble to prenatal genetic diagnosis for CCA fetuses.

So here we reported new potential pathogenic genes of 19 CCA fetuses using karyotyping, CNV-seq, CMA, and WES. The findings provide scientific guidance for prenatal diagnosis and consultation, and theoretical fundamental for genetic causes underlying pathogenesis of CCA.

2 | MATERIALS AND METHODS

2.1 | Ethics approval

This study was approved by the Ethics Committee of the Six Affiliated Hospital, Guangzhou Medical University. All patients (pregnant mothers) provided informed consent for the collection of samples and subsequent analysis.

2.2 | Definition

Corpus callosum abnormality is distinguished into three major classes: complete agenesis, partial agenesis, and dysgenesis.[8,9] Complete and partial agenesis of the corpus callosum (ACC) are the complete or partial absence of the CC, whereas dysgenesis is the length or width or the thickness of the CC smaller than the normal CC.

Isolated CCA is defined as CCA without other anomalies, whereas complex CCA is complicated with other malformations.

2.3 | Participants, sample collection, and DNA extraction

Nineteen patients were recruited from January 2015 to November 2020. All the fetuses were clinically diagnosed by the clinicians with ultrasonography and MRI during the second or third trimester of pregnancy at the Six Affiliated Hospital, Guangzhou Medical University.

Blood samples were obtained from 19 father-mother-fetus trios for DNA isolation. Amniotic fluid or cord blood was collected immediately after amniocentesis or cord blood puncture, respectively. Each sample was thoroughly mixed and divided into two tubes: one for karyotyping and another for CNV-seq (or CMA) and WES. DNA was extracted from 200 μl blood and 10 ml amniotic fluid with QIAamp DNA blood mini kits (Qiagen) according to the manufacturer's protocol.

2.4 | Karyotyping analysis

About 20 ml of amniotic fluid or 1 ml cord blood was collected for culture. Conventional karyotyping analysis using G-banding at 320–400 band resolution was performed with cultured cells according to the standard protocol. Amniotic fluid or cord blood samples were inoculated into two culture bottles. They were cultured in 5% CO_2 at 37°C. Before harvest, colchicine was added to harvest cells, then KCl hypotonic was added, and then fixed with fixed solution. G-banding was performed routinely. Twenty mitotic phases were counted, and five karyotypes were analyzed. The karyotypes were described according to ISCN 2016.

2.5 | CNV-seq

Genomic DNA was extracted from the samples for library construction with rapid PCR-free library construction technology. Briefly, 10 ng genomic DNA was randomly sheared to 100–400 bp fragments using a Nebulizer. The gap and double chain end were repaired and a "A-overhangs" was added at the 3′ ends to generate the final sequencing library by connecting with sequencing universal primers according to TA connection method. The library was assessed with quality control testing and sequenced on a NextSeq CN500 gene sequencer to generate more than 2.5 M single-end reads of 36 bp in length. The data were analyzed by the corollary data analysis software (NCBI build37, Hangzhou Berrygenomics Diagnosis Technology).

2.6 | CMA

Each DNA sample was quantitatively examined by NanoDrop spectrophotometry and qualitatively assessed by agarose gel electrophoresis for CMA. CMA of amniotic fluid or cord blood was

performed with Affymetrix CytoScan 750K arrays (Affymetrix). The arrays were processed according to the manufacturer's protocols. The results were analyzed by Chromosome Analysis suite software.

2.7 | Evaluation of the properties of CNVs detected by CNV-seq or CMA

Copy numbers were assessed against publicly available CNV databases (DGV database (http://dgv.tcag.ca/dgv/app/home), DECIPHER database (http://decipher.sanger.ac.uk/), ISCA database (https://www.iscaconsortium.org/), OMIM database (http://www.omim.org) and UCSC database (http://genome.ucsc.edu/).

Copy number variations were classified as pathogenic, likely pathogenic, variants of unknown significance (VOUS), likely benign, and benign according to American College of Medical Genetics' standards and guidelines for the interpretation and reporting of postnatal constitutional CNVs.[10] Disease-associated CNVs and biological analyses were performed to explore possible candidate genes within altered chromosomal regions.

2.8 | WES

A father-mother-fetus trios approach was used for WES. Briefly, exomes were sequenced using DNA isolated from amniotic fluid or blood according to standard procedures. DNA libraries were prepared using a NEXTflex™ Rapid DNA Sequencing Kit (Kit No. 5144-02, Bioo Scientific) according to the manufacturer's protocol. The libraries were tested with qPCR for enrichment, and size distribution and concentration were determined using an Agilent Bioanalyzer 2100 (Agilent Technologies). The libraries were subjected to paired-end sequencing on a HiSeq2500 sequencer according to the manufacturer's protocol (version 3, Illumina). Raw image files were processed using Bcl To Fastq (Illumina) for base calling to generate raw data that were analyzed and filtered. The clean reads were mapped to the reference genome (GRCh37) to identify de novo variants. All the selected variants were then classified as pathogenic, likely pathogenic, VOUS, likely benign or benign according to the American College of Medical Genetics and Genomics (ACMG) guidelines,[11] and validated by standard sanger sequencing of patient's and parental DNAs.

3 | RESULTS

3.1 | Baseline characteristics of patients

From January 2015 to November 2020, 19 pregnant women with fetal CCA were treated at the Prenatal Diagnosis Center of the Six Affiliated Hospital, Guangzhou Medical University, China. For all of the 19 cases invasive prenatal diagnosis was conducted. The baseline data are summarized in Table 1.

The 19 fetuses and 19 matched parental samples were included in the cohort.

Fetal DNA was isolated from either amniotic fluid (8/19, 42.11%) or cord blood (11/19, 57.89%).

3.2 | Karyotyping analysis

Karyotyping analysis showed that 3 of 19 cases (3/19, 15.79%) were abnormal. Among three abnormal cases, two (case 11 and case 15) were diagnosed as aneuploidy (47, XY, + 13 and 47, XX, + 21), and one (case 3) was diagnosed with an unknown origin fragment (46, XY, add (13)(p11.2)) (Figure 1).

3.3 | CNV-seq and CMA

As shown in Table 2, 4 of 19 cases (21.05%) had pathogenic CNVs (case 3, case 8, case 11 and case 15), among which two cases (case 11 and case 15) were aneuploidy, consistent with the results of karyotype analysis. CNV-seq revealed that the unknown fragment (46, XY, add(13)(p11.2)) in case 3 was originated from p13.3p11.2 of chromosome 17(Figure 2). CMA showed that CNV in case 8 was arr1q43q44 (238923617–246964774) × 1 (8.04 Mb) (Figure 3) after negative result of karyotyping analysis.

Further analysis showed 322 protein coding genes and 72 pathogenic genes (Table S1) in the region of duplication of chromosome 17 in case 3, and 28 protein coding genes and 9 pathogenic genes in the region of microdeletion in case 8 (Table S2), respectively.

3.4 | WES

Six of 19 isolated CCA cases were performed father-mother-fetus trios, among which one case was excluded because of the unqualified fetal DNA. In the remaining five cases, 2 (40.00%, 2/5) had pathogenic genes (Table 3).

In case 1, c.328C>T (p.R110X) and c.1061A>G (p.Y354C) were detected in ALDH7A1 gene (NM001182). The c.328C>T (R110X) mutation (r12192708) was De novo (Figure 4), with very low frequency of occurrence in the population (0.00003 TOPMED), and annotated as a pathogenic variation (CM060818) by ClinVar, thereby leading to a termination codon to affect the structure and function of protein. The mutation of 1061A>G (p.Y354C) (rs1471249688) was inherited from the mother (Figure 4), with very low frequency (0.00001GnomAD), and detected in patients with pyridoxine-dependent epilepsy (CM121731). In silico pathogenicity prediction tools (PROVEAN, SIFT, PolyPhen-2, Mutation Taster, and mutation assessor software) predicted 1061A>G (p.Y354C) mutation as pathogenic variation. However, FATHMM showed 1061A>G (p.Y354C) as benign. Therefore, this mutation was defined as a likely pathogenic variation.

In case 4, c.1601_1605 delACCCT (p.N534TfsX117) was detected in ARID1B gene (NM020732) (Figure 5). This mutation was

TABLE 1 Summary of clinical features of patients with CCA

Case No.	Age (year)	Gestational age (week)	Gender of the fetus	Type of ACC	Additional sonographic findings	Outcome
Case 1	25	31	Female	cACC	-	TOP
Case 2	26	28	Male	cACC	-	TOP
Case 3	37	32^{+4}	Male	pACC	Dysplasia of right kidney	TOP
Case 4	27	23	Male	pACC	-	TOP
Case 5	31	25	Male	cACC	-	TOP
Case 6	23	27^{+3}	Male	cACC	-	TOP
Case 7	25	28^{+2}	Male	cACC	Increased of cardiothoracic ratio; Pleural effusion; enhanced of the echo of renal parenchyma	TOP
Case 8	38	26^{+3}	Female	pACC	-	TOP
Case 9	17	26^{+1}	Female	cACC	-	TOP
Case 10	25	30^{+2}	Female	cACC	-	TOP
Case 11	30	27^{+1}	Male	cACC	Cerebellar dysplasia; Dandy Walker syndrome; Tetralogy of Fallot; Cleft lip and palate	TOP
Case 12	39	23^{+5}	Male	cACC	-	TOP
Case 13	31	25^{+2}	Male	cACC	-	TOP
Case 14	44	22^{+3}	Male	cACC	-	Live birth
Case 15	36	18^{+4}	Female	cACC	-	TOP
Case 16	30	35^{+4}	Female	cACC	-	TOP
Case 17	35	26^{+1}	Female	cACC	-	TOP
Case 18	28	23^{+1}	Male	cACC	-	Live birth, impaired neuromotor skills
Case 19	40	24^{+3}	Male	cACC	-	TOP

Abbreviations: cACC, complete agenesis and partial agenesis of the corpus callosum; CCA, corpus callosum abnormality; pACC, partial agenesis and partial agenesis of the corpus callosum; TOP, termination of pregnancy.

not detected in the parents, without previous results of the frequency and pathogenicity in population. The mutation caused a shift, thereby leading to a termination codon, which might affect the structure and function of the protein. The protein function was predicted as a pathogenic variation by mutation taster software.

3.5 | Outcome

Among the 19 cases included, two babies with negative results of karyotype analysis and CMA were born, and the other 17 pregnancies were terminated after prenatal diagnosis. Of the two babies born, case 14 had a normal prognosis, while case 18 was diagnosed by psychomotor retardation.

4 | DISCUSSION

4.1 | Aneuploidy and CCA

Chromosome karyotyping analysis has been used as a standard diagnostic method for prenatal testing of CCA fetuses since the late 1960s. Aneuploidy is one of the abnormal karyotypes associated with CCA cases, mainly including Patau syndrome (trisomy 13), Edward syndrome (trisomy 18), Down syndrome (trisomy 21), Klinefelter (XXY), and Turner syndrome (45,X).[12] In this study, karyotyping analysis revealed 2 cases of aneuploidy (one is trisomy 21 and another is trisomy 13) with 10.53% of detection rate, which was basically consistent with the previous report.[12] Up to now, 35 (35/172) patients with trisomy 13 have been reported to be associated with CCA.[12–18] Only 20 cases of trisomy 21 have been reported either in detailed or partial case reports, suggesting rare association of trisomy 21 with CCA. Jacob et al.[19] presented the first report of a monozygotic twin pregnancy with trisomy 21 and partial ACC in both fetuses. Our finding provides strong evidence for the relationship between CCA and trisomy 21 syndrome. The mechanism underlying the genetic causes of trisomy 13 or trisomy 21 in CCA remains unknown. However, additional studies are needed to further clarify the mechanism.

4.2 | 17p13.3p11.2 duplication and CCA

In this study, we discovered an unknown origin fragment on p11.2 of chromosome 13 in case 3 by karyotype analysis. Further CNV-seq examination revealed that the unknown fragment was originated

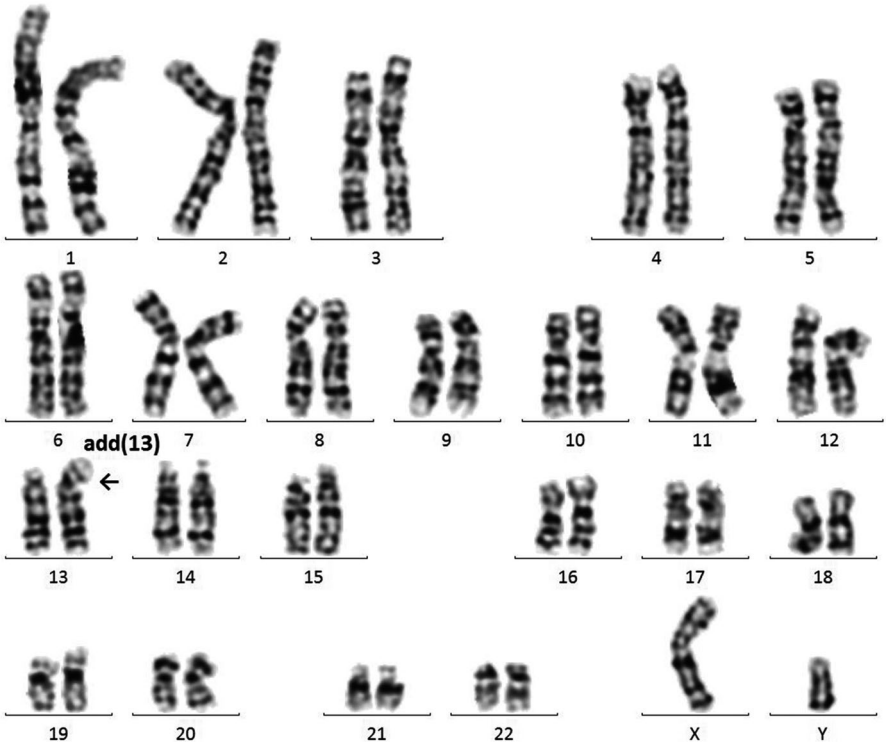

FIGURE 1 Karyotyping result of case 3. The karyotyping result of case 3 was 46, XY, add (13)(p11.2). Karyotyping analysis revealed addition of an unknown origin fragment to p11.2 of chromosome 13 (black arrow)

TABLE 2 Summary of pathogenic or likely pathogenic CNVs detected by CNV-seq or CMA

Case no.	Result	CNV type	Size of CNV
Case 3	17p13.3p11.2 (1–21400000) × 3	Gain	21.4 Mb
Case 8	arr1q43q44 (238923617–246964774) × 1	Loss	8.04 Mb
Case 11	13q12.11q34 (19500000–115169878) × 3	Gain	-
Case 15	21q11.2q22.3 (14300000–48129895) × 3	Gain	-

Abbreviations: CNV, copy number variation; CMA, chromosomal microarray analysis.

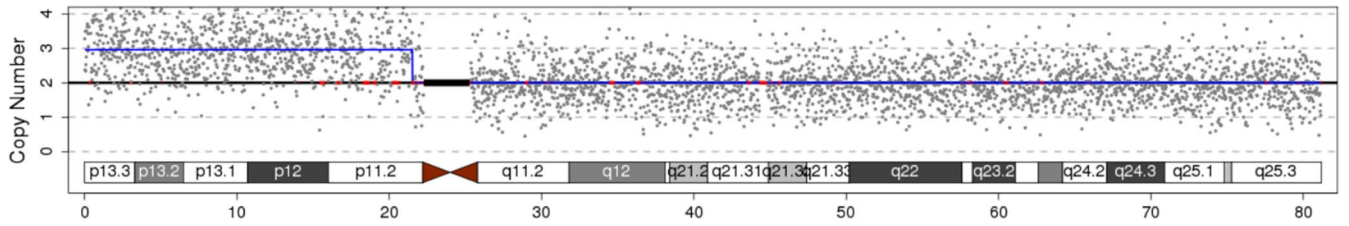

FIGURE 2 CNV-seq result of case 3. CNV-seq revealed a 21.40-Mb duplication extending genomic position 1–21400000 (hg19) at 17p13.3-p11.2 region

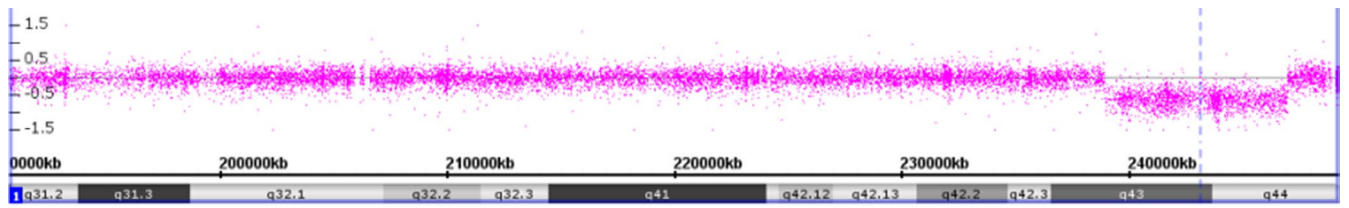

FIGURE 3 CMA result of case 8. CMA revealed a 8.04-Mb deletion extending genomic position 238923617–24696 4774 (hg19) at 1q43q44 region

TABLE 3 Summary of pathogenic or likely pathogenic mutations revealed by WES

Case no.	Gene	Chr[a]	Inheritance mode	Mutation and het/hom[b]	Consequence	P/LP	Inheritance confirmation
Case 1	ALDH7A1	5	AR	NM_001182: c.328C>T(p.R110X) het	Stop_gained	P	De novo
			AR	NM_001182: c.1061A>G(p.Y354C) het	Missense	LP	Mat[c]
Case 4	ARID1B	6	AD	NM_020732:c.1601_1605 delACCCT(p.N534TfsX117)het	Frameshift	P	De novo

Abbreviations: P/LP, Pathogenetic/likely pathogenetic; WES, whole exome sequencing.

[a]Chr: chromosome.

[b]het refer to heterozygous mutation or homozygous.

[c]Mat refer to maternally inherited.

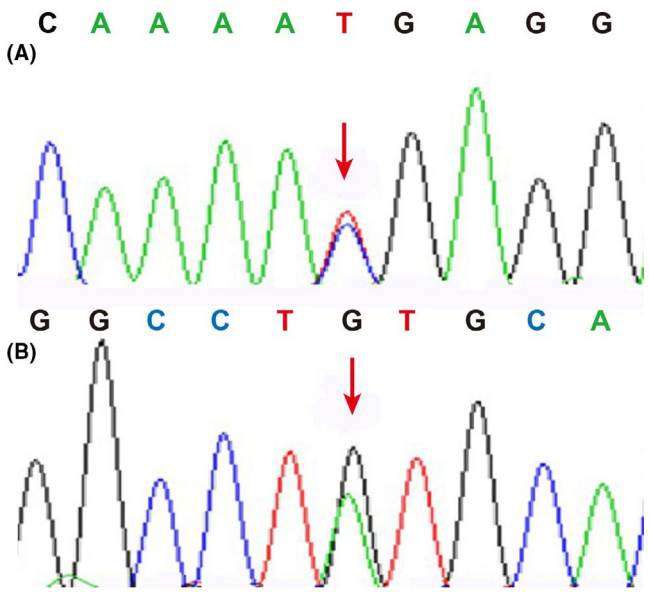

FIGURE 4 Sanger sequencing result of the mutation of ALDH7A1 in case 1. (A) A substitution C to T at position 328 of cDNA (c.328C>T; p.R110X) of ALDH7A1 in case 1. (B) A substitution A to G at position 1061 of cDNA (c.1061A>G; p.Y354C) of ALDH7A1 in case 1. Red arrow indicated base change in Sanger sequencing

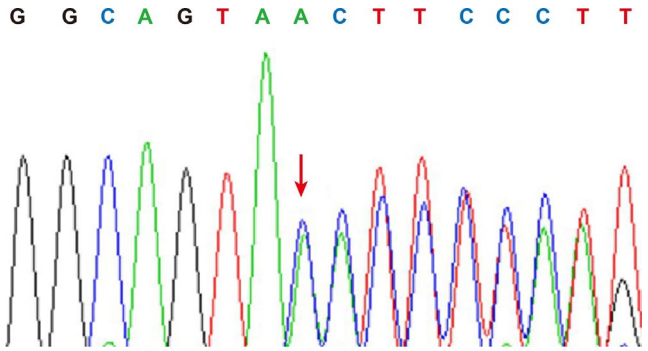

FIGURE 5 Sanger sequencing result of the mutation of ARID1B in case 4, showing a deletion ACCCT at position 1601–1605 of cDNA (c.1601_1605 delACCCT; p.N534TfsX117) of ARID1B in case 4. Red arrow indicated deletions in Sanger sequencing

from p13.3p11.2 of chromosome 17. The fine delineation of the extent of genomic aberration by CNV-seq could help us better understand the molecular mechanism and genotype–phenotype correlations in complete trisomy of the short arm of chromosome 17. Our study demonstrated the association of chromosome duplication of 17p13.3p11.2 with CCA, supporting the previous results.[20] Human chromosome 17 is a small, gene-rich chromosome associated with several well-known duplication syndromes. So far the duplication region of 17p13.3p11.2 includes chromosome 17p13.3 duplication syndrome[OMIM:613215],[20]Charcot Marie Tooth Syndrome Type 1A (CMT1A),[21] and Potocki Lupski syndrome (17p11.2 duplication syndrome).[22] Among these, 17p13.3 duplication syndrome[23] and Potocki-Lupski syndrome[22] were reported to be related with CCA.

We further analyzed the functions of 322 coding genes in the17p13.3p11.2 fragment with Gene Ontology (GO) analysis. In the molecular function (MF) category, the coding genes were mainly assigned to microfilament motor activity. In the cell components (CC) category, the coding genes were mostly enriched in the muscle myosin complex. In the biological processes category, the coding genes were mainly involved in the hepoxilin biosynthetic process. In the future study, we can screen the pathological mechanism of CCA through these MFs, cell composition, and the biological process.

4.3 | 1q43q44 microdeletion and CCA

In this study, 1q43q44 microdeletion was firstly reported to be associated with the phenotype of CCA. We detected a 8.04 Mb microdeletion in 1q43q44 by CMA in case 8 that was negative with karyotyping analysis. The microdeletion was too tiny to be discerned on traditional chromosome karyotyping analysis. Our result demonstrated the capacity of CMA in detection of the minimal pathogenic CNVs that are lower than the resolution of chromosome karyotype. In addition, further analysis of the coding genes in the 1q43q44 microdeletion fragment showed associations of AKT3, FMN2, and ZBTB18 with the central nervous system. GO analysis assigned main involvement of 28 coding genes in N-methyltransferase activity in the MF category, and microtubule cytoskeleton and microtubule in the CC category. In the future study, we can screen the pathological mechanism of CCA through the MF, cell composition, and biological process.

4.4 | ALDH7A1 and CCA

We reported for the first time that two mutations of c.328C>T (p.R110X) and c.1061A>G (Y354C) in ALDH7A1 were associated with fetal dysplasia of the CC. ALDH7A1 in 5q23.2 with 18 exons encodes α-aminoadipic semialdehyde dehydrogenase, thereby playing a critical role in the lysine degradation pathway. Homozygous and compound heterozygous mutations in ALDH7A1 cause decline of α -aminoadipic semialdehyde dehydrogenase to block the lysine metabolism in patients, thereby leading to pyridoxine (vitamin B6)-dependent epilepsy. A total of 108 mutations had been reported in HGMD database (http://www.hgmd.cf.ac.uk/ac/index.php) until April 2019. Recently, several different cerebral malformations associated with ALDH7A1 mutations have been described, including agenesia/hypoplasia of the CC, nonspecific white matter aberrations, large cisterna magna, ventriculomegaly, hemorrhage, cerebellar hypoplasia /dysplasia, and, more rarely, dysplasia of the brainstem and hydrocephalus.[24] In this study, the heterozygous mutations of c.328C>T (p.R110X) and c.1061A>G (Y354C) in ALDH7A1 gene (NM001182) were detected in case 1. Although both mutations were previously reported in patients with pyridoxine-dependent epilepsy,[25,26] no abnormal CC was detected with MRI in the reported cases. Further studies are needed to address the possible association of ALDH7A1 mutations with dysplasia of the CC.

4.5 | ARID1B and CCA

In this study, we firstly reported a frameshift mutation in ARID1B. ARID1B gene is located in 6q25.3 with 25 exons, and encodes a 250 kDa protein with two nuclear localization domains: the AT-rich ARID domain and the DUF3518 domain with unknown function. The protein is one of the largest subunits of SWI/SNF complex[27] belonging to chromatin remodeling complex with the active site of ATPase. Chromatin remodeling is carried out by hydrolyzing ATP to generate energy, thereby playing a key role in cell growth, differentiation, and gene activity regulation. Sim et al.[28] demonstrated the key role of ARID1B in brain development. Recent studies have revealed that ARID1B is the pathogenic gene of Coffin-Siris syndrome type I.[29–32] The clinical manifestations of ARID1B mainly include special facial features (hairy, low ear position, wide nose, big mouth, etc.), feeding difficulty, fifth finger/toe bone hypoplasia, psychomotor retardation, mental retardation, and corpus callosum dysplasia, which is autosomal dominant inheritance. Pirola et al.[33] firstly reported a possible association of insufficient haploid dose of ARID1B gene with CCA, supported by Halgren et al.[34] who also demonstrated a relationship between CCA and insufficient dose of ARID1B. Celen et al.[35] discovered CCA in Arid1b$^{+/-}$ mice. In the present study, we firstly discovered frameshift mutation in exon 1 of ARID1B. This point mutation affects the translation of all subsequent proteins and their functions, resulting in fetal CCA.

In this study, 89.47% of pregnancies were terminated, which was higher than that reported in the literature.[36,37] It was considered that the parents did not get proper counseling based on the information regarding the good prognosis for isolated ACC. In the two live babies, one had a normal prognosis, which suggested that the prognosis of CCA fetus might be good when the karyotype and CMA results were negative. However, the other case had psychomotor retardation. We have repeatedly communicated with the parents and suggested further WES to exclude single gene diseases, but the parents refused.

5 | LIMITATIONS

The findings of the present study were limited by the relatively small size of cases. From January 2015 to November 2020, only 19 CCA fetuses were recruited for interventional prenatal diagnosis. Further studies with larger size of samples are needed for validation.

6 | SUMMARY

In this study, we genetically diagnosed 19 cases of fetal CCA with karyotyping analysis and discovered 3 of 19 cases (15.79%) with chromosomal abnormalities. Parallel tests of karyotyping analysis, CNV-seq, CMA, and WES showed that CNV-seq and CMA are able to identify additional, clinically significant cytogenetic information of CCA and WES significantly improves the detection rate of genetic etiology of CCA. For the patients with a negative results of CNV-seq or CMA, further WES test is recommended.

ACKNOWLEDGMENTS

We thank the patients at the Prenatal Diagnostic Center of the Six Affiliated Hospital, Guangzhou Medical University, China for their support and participation. This study was supported by the Medical Scientific Research Foundation of Guangdong Province of China (Grant No. B2020136) and the Medical Scientific Research Foundation of Qingyuan people's Hospital (Grant No. 20190204)

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.

DATA AVAILABILITY STATEMENT

Data was available within the article or its supplementary materials.

ORCID

Qin She https://orcid.org/0000-0003-0216-8950

REFERENCES

1. Alby C, Boutaud L, Bessieres B, et al. Novel de novo ZBTB20 mutations in three cases with Primrose syndrome and constant corpus callosum anomalies. *Am J Med Genet A*. 2018;176(5):1091-1098.
2. Bayram AK, Kutuk MS, Doganay S, et al. An analysis of 109 fetuses with prenatal diagnosis of complete agenesis of corpus callosum. *Neurol Sci*. 2020;41(6):1521-1529.

3. Vasudevan C, McKechnie L, Levene M. Long-term outcome of antenatally diagnosed agenesis of corpus callosum and cerebellar malformations. *Semin Fetal Neonatal Med*. 2012;17(5):295-300.
4. Hay SB, Sahoo T, Travis MK, et al. ACOG and SMFM guidelines for prenatal diagnosis: is karyotyping really sufficient? *Prenat Diagn*. 2018;38(3):184-189.
5. Al-Hashim AH, Blaser S, Raybaud C, et al. Corpus callosum abnormalities: neuroradiological and clinical correlations. *Dev Med Child Neurol*. 2016;58(5):475-484.
6. D'Antonio F, Pagani G, Familiari A, et al. Outcomes associated with isolated agenesis of the corpus callosum: a meta-analysis. *Pediatrics*. 2016;138(3):e20160445.
7. Monaghan KG, Leach NT, Pekarek D, et al. The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2020;22(4):675-680.
8. Heide S, Keren B, Billette de Villemeur T, et al. Copy number variations found in patients with a corpus callosum abnormality and intellectual disability. *J Pediatr*. 2017;185:160-166.e161.
9. Palmer EE, Mowat D. Agenesis of the corpus callosum: a clinical approach to diagnosis. *Am J Med Genet C Semin Med Genet*. 2014;166C (2):184-197.
10. Riggs ER, Andersen EF, Cherry AM, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). *Genet Med*. 2020;22(2):245-257.
11. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424.
12. Glass HC, Shaw GM, Ma C, et al. Agenesis of the corpus callosum in California 1983–2003: a population-based study. *Am J Med Genet A*. 2008;146A(19):2495-2500.
13. Vergani P, Ghidini A, Strobelt N, et al. Prognostic indicators in the prenatal diagnosis of agenesis of corpus callosum. *Am J Obstet Gynecol*. 1994;170(3):753-758.
14. Kobori JA, Herrick MK, Arhinencephaly UH. The spectrum of associated malformations. *Brain*. 1987;110(Pt 1):237-260.
15. Battin J. Congenital malformations and chromosomal aberrations. *Fetal Ther*. 1986;1(2–3):68-72.
16. Goetzinger KR, Stamilio DM, Dicke JM, et al. Evaluating the incidence and likelihood ratios for chromosomal abnormalities in fetuses with common central nervous system malformations. *Am J Obstet Gynecol*. 2008;199(3):285.e281-286.
17. Wainwright H, Bowen R, Radcliffe M. Lipoma of corpus callosum associated with dysraphic lesions and trisomy 13. *Am J Med Genet Part A*. 1995;57(1):10-13.
18. Kitova TT, Kitov B, Milkov D, et al. Postnatally diagnosed agenesis of corpus callosum in fetuses. *Fetal Pediatr Pathol*. 2014;33(4):239-243.
19. Jacob FD, Dobson LJ, Estroff JA, et al. Monozygotic twins with trisomy 21 and partial agenesis of the corpus callosum. *Pediatr Neurol*. 2013;48(4):314-316.
20. Blazejewski SM, Bennison SA, Smith TH, et al. Neurodevelopmental genetic diseases associated with microdeletions and microduplications of chromosome 17p13.3. *Front Genet*. 2018;9:80.
21. Bjelica B, Peric S, Basta I, et al. Neuropathic pain in patients with Charcot-Marie-Tooth type 1A. *Neurol Sci*. 2020;41(3):625-630.
22. Franciskovich R, Soler-Alfonso C, Neira-Fresneda J, et al. Short stature and growth hormone deficiency in a subset of patients with Potocki-Lupski syndrome: expanding the phenotype of PTLS. *Am J Med Genet A*. 2020;182(9):2077-2084.
23. Curry CJ, Rosenfeld JA, Grant E, et al. The duplication 17p13.3 phenotype: analysis of 21 families delineates developmental, behavioral and brain abnormalities, and rare variant phenotypes. *Am J Med Genet A*. 2013;161A(8):1833-1852.
24. Toldo I, Bonardi CM, Bettella E, et al. Brain malformations associated to Aldh7a1 gene mutations: report of a novel homozygous mutation and literature review. *Eur J Paediatr Neurol*. 2018;22(6):1042-1053.
25. Mills PB, Struys E, Jakobs C, et al. Mutations in antiquitin in individuals with pyridoxine-dependent seizures. *Nat Med*. 2006;12(3):307-309.
26. Nam SH, Kwon MJ, Lee J, et al. Clinical and genetic analysis of three Korean children with pyridoxine-dependent epilepsy. *Ann Clin Lab Sci*. 2012;42(1):65-72.
27. Pascolini G, Valiante M, Bottillo I, et al. Striking phenotypic overlap between Nicolaides-Baraitser and Coffin-Siris syndromes in monozygotic twins with ARID1B intragenic deletion. *Eur J Med Genet*. 2020;63(3):103739.
28. Sim JC, White SM, Lockhart PJ. ARID1B-mediated disorders: mutations and possible mechanisms. *Intractable Rare Dis Res*. 2015;4(1):17-23.
29. Demily C, Duwime C, Lopez C, et al. Corpus callosum metrics predict severity of visuospatial and neuromotor dysfunctions in ARID1B mutations with Coffin-Siris syndrome. *Psychiatr Genet*. 2019;29(6):237-242.
30. Kalmbach A, Schroder C, Klein-Hitpass L, et al. Genome-wide analysis of the nucleosome landscape in individuals with Coffin-Siris syndrome. *Cytogenetic Genome Res*. 2019;159(1):1-11.
31. Pranckeniene L, Siavriene E, Gueneau L, et al. De novo splice site variant of ARID1B associated with pathogenesis of Coffin-Siris syndrome. *Mol Genet Genomic Med*. 2019;7(12):e1006.
32. Sekiguchi F, Tsurusaki Y, Okamoto N, et al. Genetic abnormalities in a large cohort of Coffin-Siris syndrome patients. *J Hum Genet*. 2019;64(12):1173-1186.
33. Pirola B, Bortotto L, Giglio S, et al. Agenesis of the corpus callosum with Probst bundles owing to haploinsufficiency for a gene in an 8 cM region of 6q25. *J Med Genet*. 1998;35(12):1031-1033.
34. Halgren C, Kjaergaard S, Bak M, et al. Corpus callosum abnormalities, intellectual disability, speech impairment, and autism in patients with haploinsufficiency of ARID1B. *Clin Genet*. 2012;82(3):248-255.
35. Celen C, Chuang JC, Luo X, et al. Arid1b haploinsufficient mice reveal neuropsychiatric phenotypes and reversible causes of growth impairment. *eLife*. 2017;6:e25730.
36. Sotiriadis A, Makrydimas G. Neurodevelopment after prenatal diagnosis of isolated agenesis of the corpus callosum: an integrative review. *Am J Obstet Gynecol*. 2012;206(4):337.e331-335.
37. Fratelli N, Papageorghiou AT, Prefumo F, et al. Outcome of prenatally diagnosed agenesis of the corpus callosum. *Prenat Diagn*. 2007;27(6):512-517.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: She Q, Tang E, Peng C, Wang L, Wang D, Tan W. Prenatal genetic testing in 19 fetuses with corpus callosum abnormality. *J Clin Lab Anal*. 2021;35:e23971. https://doi.org/10.1002/jcla.23971