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H. J. Mustafa

J. P. Barbera
Thomas Jefferson University

E. V. Sambatur

G. Pagani

Y. Yaron

See next page for additional authors

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Diagnostic yield of exome sequencing in prenatal agenesis of corpus callosum: systematic review and meta-analysis

H. J. MUSTAFA[1,2], J. P. BARBERA[3], E. V. SAMBATUR[4], G. PAGANI[5], Y. YARON[6,7], C. D. BAPTISTE[8], R. J. WAPNER[8], C. J. BREWER[9] and A. KHALIL[10,11]

[1]*Department of Obstetrics and Gynecology, Division of Maternal–Fetal Medicine, Indiana University School of Medicine, Indianapolis, IN, USA;* [2]*Riley Children and Indiana University Health Fetal Center, Indianapolis, IN, USA;* [3]*Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, PA, USA;* [4]*Research Division, Houston Center for Maternal Fetal Medicine, Houston, TX, USA;* [5]*Maternal Fetal Medicine Unit, Department of Obstetrics and Gynecology, ASST-Papa Giovanni XXIII, Bergamo, Italy;* [6]*Prenatal Genetic Diagnosis Unit, Genetics Institute, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel;* [7]*Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel;* [8]*Obstetrics and Gynecology, Reproductive Genetics, Columbia University Medical Center, New York, NY, USA;* [9]*Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA;* [10]*Fetal Medicine Unit, St George's University Hospitals NHS Foundation Trust, University of London, London, UK;* [11]*Vascular Biology Research Centre, Molecular and Clinical Sciences Research Institute, St George's University of London, London, UK*

KEYWORDS: corpus callosum; exome; meta-analysis; microarray; prenatal diagnosis; systematic review

CONTRIBUTION

What are the novel findings of this work?
Of the 268 cases of prenatally diagnosed agenesis of the corpus callosum (ACC), 43% had a pathogenic/likely pathogenic variant identified on exome sequencing following negative chromosomal microarray analysis. The highest yield was observed in ACC with extracranial anomalies (55%), followed by ACC with other cranial anomalies (43%) and isolated ACC (32%). We classified 116 pathogenic/likely pathogenic genetic variants in 83 genes associated with ACC.

What are the clinical implications of this work?
The use of prenatal exome sequencing in both isolated ACC and ACC associated with other anomalies should be considered after a negative result on standard genetic testing using chromosomal microarray analysis given the heterogeneity in the prenatal phenotype of the associated syndromic conditions.

ABSTRACT

Objectives *To determine the incremental diagnostic yield of exome sequencing (ES) after negative chromosomal microarray analysis (CMA) in cases of prenatally diagnosed agenesis of the corpus callosum (ACC) and to identify the associated genes and variants.*

Methods *A systematic search was performed to identify relevant studies published up until June 2022 using four databases: PubMed, SCOPUS, Web of Science and The Cochrane Library. Studies in English reporting on the diagnostic yield of ES following negative CMA in prenatally diagnosed partial or complete ACC were included. Authors of cohort studies were contacted for individual participant data and extended cohorts were provided for two of them. The increase in diagnostic yield with ES for pathogenic/likely pathogenic (P/LP) variants was assessed in all cases of ACC, isolated ACC, ACC with other cranial anomalies and ACC with extracranial anomalies. To identify all reported genetic variants, the systematic review included all ACC cases; however, for the meta-analysis, only studies with ≥ three ACC cases were included. Meta-analysis of proportions was employed using a random-effects model. Quality assessment of the included studies was performed using modified Standards for Reporting of Diagnostic Accuracy criteria.*

Results *A total of 28 studies, encompassing 288 prenatally diagnosed ACC cases that underwent ES following negative CMA, met the inclusion criteria of the systematic review. We classified 116 genetic variants in 83 genes associated with prenatal ACC with a full phenotypic description. There were 15 studies, encompassing 268 cases, that reported on ≥ three ACC cases and were included in the meta-analysis. Of all the included cases,*

Correspondence to: Dr H. J. Mustafa, Division of Maternal–Fetal Medicine, 340 West 10th Street, Fairbanks Hall, Suite 6200, Indianapolis, IN 46202-3082, USA; The Fetal Center at Riley Children's and Indiana University Health, 575 Riley Hospital Dr #4300, Indianapolis, IN 46202, USA (e-mail: hmustafa@iu.edu)
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43% had a P/LP variant on ES. The highest yield was for ACC with extracranial anomalies (55% (95% CI, 35–73%)), followed by ACC with other cranial anomalies (43% (95% CI, 30–57%)) and isolated ACC (32% (95% CI, 18–51%)).

Conclusions *ES demonstrated an incremental diagnostic yield in cases of prenatally diagnosed ACC following negative CMA. While the greatest diagnostic yield was observed in ACC with extracranial anomalies and ACC with other central nervous system anomalies, ES should also be considered in cases of isolated ACC. © 2023 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Agenesis of the corpus callosum (ACC) is defined as the absence of the commissural tract of fibers that connects the hemispheres of the brain and can be classified as partial or complete[1]. The corpus callosum consists of four parts: rostrum, genu, body and splenium[2]. As the corpus callosum develops from anterior to posterior, the most affected segment in ACC is the posterior segment, consisting of the body and splenium[1,3,4]. ACC can be isolated or associated with other cranial or extracranial anomalies[1]. ACC is the most common commissural malformation with an incidence of 0.5 to 70 per 10 000 live births[5,6].

ACC is diagnosed prenatally during the second-trimester ultrasound examination, based on the absence of the cavum septi pellucidi in the axial plane or colpocephaly of the lateral ventricles[1]. Color Doppler can also be used to visualize the course of the pericallosal artery to identify the portion of dysgenesis from 11 weeks of gestation onwards[3].

ACC has a heterogeneous etiology and is associated with different genetic variants and syndromes. *CDK5RAP2* and *DCC* genes are both linked to isolated ACC. ACC is widely associated with Coffin–Siris syndrome and has recently been reported in association with other congenital syndromes, such as Vici syndrome and Mowat–Wilson syndrome[7].

Neurodevelopmental outcome in isolated ACC has been reported to be normal in 71.2% of cases, while the remaining patients manifest moderate-to-severe abnormalities[4,8]. The unpredictability of the outcome poses a challenge for prenatal counseling. Genetic testing, including karyotyping, chromosomal microarray analysis (CMA) and exome sequencing (ES), provides valuable information necessary for prenatal counseling[9].

ES has proven to be a powerful tool for evaluating patients postnatally, achieving an average molecular diagnostic rate of 25% for pathogenic/likely pathogenic (P/LP) variants when performed for Mendelian disorders[10]. In comparison, the currently used CMA detects clinically significant copy number variants (CNVs) in 5.7% of isolated ACC with a normal karyotype[11]. Prenatal diagnostic yield of ES for fetal structural anomalies is higher in cohorts preselected for monogenic etiology compared with unselected cohorts (42% *vs* 15%, respectively)[12]. In prenatally detected ACC, ES is estimated to have a higher diagnostic rate of P/LP variants compared with CMA or karyotyping[13].

There is a paucity of studies that have assessed formally the additional diagnostic yield of ES after negative CMA in prenatally diagnosed ACC and there is no evidence to suggest which phenotypic ACC subtypes have the highest diagnostic yield. Hence, the objectives of this systematic review and meta-analysis were to determine the incremental diagnostic yield of ES after normal CMA in prenatally diagnosed ACC and to identify the associated genes and variants.

METHODS

This study was conducted based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 guideline[14]. The study protocol was registered with PROSPERO (CRD42022333562).

Search strategy

A systematic search was performed independently by two authors (E.V.S. and J.P.B.) of four electronic databases, from inception until June 2022: The Cochrane Library, Web of Science, SCOPUS and PubMed. The search strategy included a combination of relevant medical subject heading (MeSH) terms and keywords for ('prenatal diagnosis' or 'antenatal diagnosis' or 'fetal diseases' or 'fetal development') and ('exome sequencing' or 'whole genome sequencing' or 'whole exome sequencing' or 'genome-wide sequencing'). Further details regarding the systematic search of the literature is available in Appendix S1. The identified articles were transferred to Rayyan software (Rayyan; http://rayyan.qcri.org) for abstract screening. Duplicates that were identified by Rayyan software or manually were removed. Abstract screening was performed independently by two authors (E.V.S. and J.P.B.) and any disagreement was resolved by discussion with a third (H.J.M.). The full text of the included studies was retrieved for data extraction.

Eligibility criteria

We defined our eligibility criteria based on the Population, Intervention, Comparison, Outcome (PICO) framework and included studies focusing on pregnancies complicated by complete or partial ACC (population), undergoing ES (intervention) and CMA or karyotyping (comparison) and reporting P/LP variants (outcome). We included pregnancies that were diagnosed prenatally with ACC on imaging, with or without other anomalies (central nervous system (CNS) or multisystem), undergoing ES following negative CMA/karyotyping. The exclusion criteria were studies lacking CMA/karyotyping or ES data, those not specifying the number of missing cases

or not providing individual data information, and manuscripts written in a language other than English. To identify all reported genetic variants, the systematic review included all ACC cases, whereas the meta-analysis included studies with ≥ three ACC cases.

Data extraction and outcome measures

Two independent authors (E.V.S. and J.P.B.) performed data extraction using a standardized spreadsheet. Any disagreement regarding the inclusion, exclusion or data extraction was resolved through a discussion with a third author (H.J.M.). The standardized spreadsheet included the following columns: first author, publication year, study period, country, institute, study design, ES laboratory methodology, total number of cases, number of ACC cases, sequencing method, ES turnaround time (TAT), postmortem or postnatal examination, number of negative CMA/karyotyping results, total number of CMA/karyotyping tests performed, number of positive ES cases, total number of ES tests performed and detailed information on positive ES cases, including prenatal phenotype, gene, variant, inheritance and clinical syndrome or diagnosis, if any.

Four studies did not include all data regarding the associated genes or variants[15–18]. The authors of these studies were contacted and provided full relevant data. Additionally, data on the extended cohort were provided for two of the studies[17,18].

Quality assessment

Quality assessment of the included studies was performed using modified Standards for Reporting of Diagnostic Accuracy (STARD) criteria[19]. The quality criteria deemed most important to optimize accuracy were: (1) whether trio analysis was performed; (2) whether American College of Medical Genetics and Genomics (ACMG) criteria were used for variant interpretation; and (3) whether there was Sanger validation of variants[20]. Quality assessment was performed by two reviewers (E.V.S. and J.P.B.) and any disagreement was resolved through discussion with a third author (H.J.M.).

Variant classification or reclassification

Variant reclassification was performed to reflect newly available data using the same techniques that were employed in the original studies to prevent any bias. All variants were generated in Alamut™ Visual Plus v1.6.1 (SOPHiA GENETICS SA, Rolle, Switzerland) to verify correct nomenclature. Alamut is a genome browser that can generate variants and their corresponding Human Genome Variation Society (HGVS) nomenclature, facilitating variant classification by genomic scientists. All variants were reported in genome build GRCh37 (hg19). Variants from all papers were matched to the same Matched Annotation from the National Center for Biotechnology Information (NCBI) and European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) (MANE) select transcripts for each gene. In six cases, variants could not be reclassified because the reported nomenclature could not be verified or incomplete variant information was provided in the original report, making it impossible to know for certain where the variant was in the genome. Thus, the primary variant classification assigned for these six cases in the original publication was used for the variant analysis. Phenotypic information for reanalysis was gathered by searching several databases (ClinVar, DECIPHER, HGMD, gnomAD) with the assistance of advanced search tools (Genomenon, Alamut Visual, UCSC Genome Browser, PubMed, Google).

Because variant classification guidelines have evolved over the past few years and different groups may apply ACMG guidelines differently, we harmonized all reported variant classifications with current ACMG guidelines[20]. Current ACMG classification of genetic sequence variants includes two parts: one focusing on P or LP variants and one focusing on classification of benign or likely benign variants. Each pathogenic criterion is weighted as very strong (PSV1), strong (PS1–4), moderate (PM1–6) or supporting (PP1–5), and each benign criterion is weighted as stand-alone (BA1), strong (BS1–4) or supporting (BP1–6). The criteria are then combined according to the ACMG scoring rules to choose a classification from the five-tier system: P, LP, variant of uncertain significance (VUS), likely benign and benign[21]. All variants were classified by our genomic scientist (C.J.B.) and the classification was reviewed by an additional author (J.P.B.). We also included ClinGen recommendations regarding the PVS1 criterion[22]. The PP5 criterion (reported by a reputable source) was used judiciously to avoid double counting in cases in which ClinVar entries were from the original case report. Additionally, some reported variant classifications were outdated and were therefore reclassified using current evidence. We considered our variant classification to be concordant with the original report if the variant was P, LP or VUS in both instances. In three cases of classification for compound heterozygous inheritance of an autosomal recessive disorder, a pathogenic variant with VUS was considered a LP diagnosis.

Statistical analysis

For studies with ≥ three fetal ACC cases undergoing ES following negative CMA, we calculated the pooled proportions and their 95% CI in four groups of ACC cases: (1) all ACC cases; (2) isolated ACC (ACC was the only finding); (3) ACC with other cranial anomalies; and (4) ACC with extracranial anomalies.

Heterogeneity of the included studies was assessed graphically and statistically using the Higgins I^2 test. The weight given to each study was decided according to the inverse variance method in order to minimize the imprecision of the pooled effect estimate. The random-effects model was used for pooling the effect sizes and their 95% CI was consequently calculated. To test

the overall significance of the random model, the *Z*-test was performed. Potential publication bias was assessed graphically by creating funnel plots for each of the groups. RStudio (RStudio Inc., Boston, MA, USA) was used for statistical analysis and creating forest and funnel plots[23].

RESULTS

Study characteristics

The literature search strategy generated 13 102 abstracts, of which 5011 were removed as duplicates (Figure 1). Following abstract screening, a total of 168 studies underwent full-text assessment, of which 28 studies met our inclusion criteria.

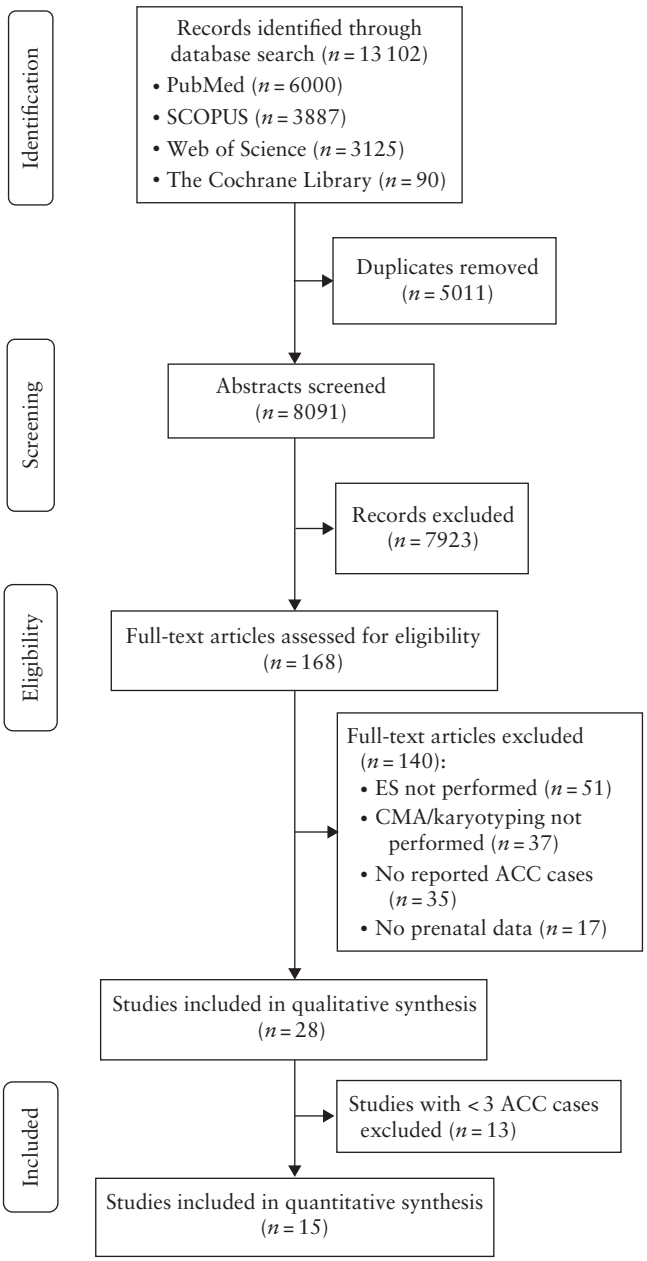

Figure 1 PRISMA flowchart summarizing literature search and inclusion of studies in systematic review and meta-analysis. ACC, agenesis of corpus callosum; CMA, chromosomal microarray analysis; ES, exome sequencing.

Characteristics of the studies included in the systematic review[13,15,16–18,24–46] are shown in Table S1. There were 15 studies that reported on at least three ACC cases and 13 studies included fewer than three cases. The publication year ranged between 2014 and 2022, 17 studies were retrospective and 11 were prospective. Full ES methodology for each study is outlined in Table S1. Trio ES was performed in 21 studies, five studies performed proband, duo or trio ES, and in two studies, methodology was not reported.

Figure 2 summarizes the overall quality assessment of the included studies, using modified STARD as described in the methods section. Most studies used trio ES and Sanger validation for variants, and all studies used ACMG classification criteria. All studies provided CNS phenotypic description.

Systematic review

The systematic review included a total of 288 ACC cases that underwent ES after negative CMA. Although we planned to include cases that underwent CMA and/or karyotyping, all included studies performed CMA. There were 115 variants in 82 genes that were P/LP according to the original articles. Upon further reanalysis, one variant was downgraded to a benign, and two VUS cases were upgraded to P/LP, resulting in a total of 116 ACC cases with a P/LP variant in 83 genes. The rest of the VUS remained classified as VUS.

Pregnancy outcome was reported for 84 cases with a P/LP variant, of which 69 (82.1%) had termination of pregnancy, two (2.4%) had a stillbirth, three (3.6%) had neonatal demise and 10 (11.9%) had a live birth. The type of ES performed was specified for 113 cases. Maternal-paternal-fetal trio testing was done in most cases (108/113 (95.6%)), duo ES was performed in 1/113 (0.9%) case and proband-only ES was performed in 4/113 (3.5%) cases.

The genes affected with the highest overall frequency included *TUBA1A* (7/116 (6.0%)), *L1CAM* (6/116 (5.2%)), *FGFR2* (5/116 (4.3%)), *ARID1B* (4/116 (3.4%)), *ARX*, *COL4A1*, *EPG5*, *PEX1*, *TUBB* and *ZEB2* (3/116 (2.6%) each) (Table 1), and *KANSL1*, *NFIA* and *TUBB3* (2/116 (1.7%) each). The remaining 70 genes were involved in only one case each.

Phenotype association by gene

Isolated ACC

There were 19 genes associated with 25 cases in which ACC was the only finding (Table S2). The genes included *ARID1B* (3/25 (12.0%)), *L1CAM* (3/25 (12.0%)), *EPG5* (2/25 (8.0%)), *NFIA* (2/25 (8.0%)), and *AP4M1*, *ALDH7A1*, *EXOSC3*, *KANSL1*, *KCNQ2*, *PPP2R1A*, *PTCH1*, *PTDSS1*, *PTPN11*, *SCN2A*, *SHH*, *SON*, *TUBB2B*, *ZBTB20* and *ZEB2* (1/25 (4.0%) each). The most common genetic syndromes were Coffin–Siris syndrome, X-linked hydrocephaly and Vici syndrome.

Inheritance pattern was documented in 24/25 (96.0%) of these cases. Among these isolated ACC cases,

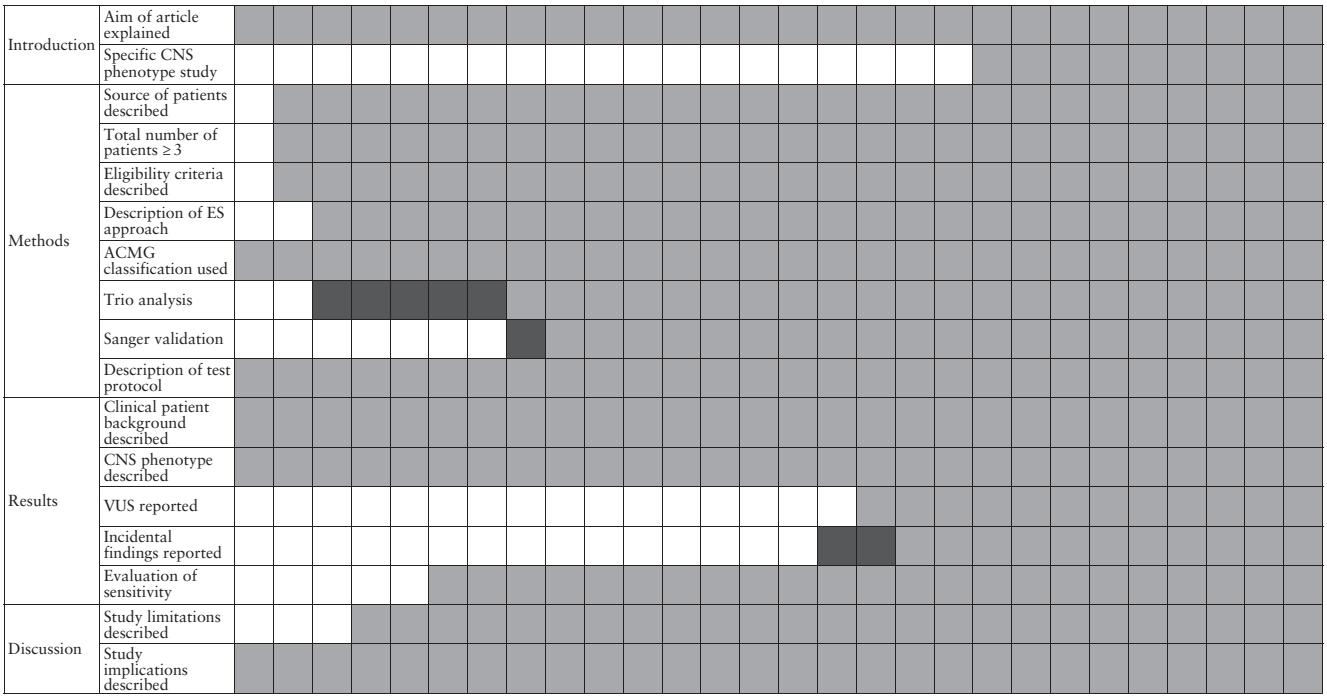

Figure 2 Quality assessment of 28 studies included in systematic review using modified Standards for Reporting of Diagnostic Accuracy criteria. □, No/not specified; ■, yes; ■, partially yes. ACMG, American College of Medical Genetics and Genomics; CNS, central nervous system; ES, exome sequencing; VUS, variant of uncertain significance.

inheritance pattern was autosomal dominant in 17/24 (70.8%), autosomal recessive in 4/24 (16.7%) and X-linked in 3/24 (12.5%) cases. Among the autosomal dominant cases, 16/17 (94.1%) were *de novo*. Among the X-linked cases, 2/3 (66.7%) were *de novo*. Of note, among the autosomal recessive cases, one case had two variants in the *ALDH7A1* gene, with one being *de novo* and the other maternally inherited.

ACC with other cranial anomalies

There were 30 genes associated with 41 cases (Table S3). The genes included *TUBA1A* (6/41 (14.6%)), *COL4A1* (3/41 (7.3%)), *TUBB* (3/41 (7.3%)), *ARX* (2/41 (4.9%)), *L1CAM* (2/41 (4.9%)) and *OFD1* (2/41 (4.9%)). The remaining 24 genes were each affected in 1/41 (2.4%) cases, and one case had two different genes affected. The most common genetic syndromes were tubulinopathy, X-linked hydrocephalus, brain small-vessel disease, X-linked lissencephaly and oral-facial-digital syndrome.

Inheritance pattern was documented in 38/41 (92.7%) of these cases. Inheritance pattern was autosomal dominant in 21/38 (55.3%), autosomal recessive in 6/38 (15.8%) and X-linked in 11/38 (28.9%) cases. Among the autosomal dominant cases, 20/21 (95.2%) were *de novo*. Among the X-linked cases, 6/11 (54.5%) were *de novo*.

ACC with extracranial anomalies

There were 40 genes associated with 44 cases in which ACC occurred with extracranial anomalies (Table S4). The genes included *FGFR2* (5/44 (11.4%)) and *ZEB2* (2/44 (4.5%)), while the remaining 38 genes were each affected in 1/44 (2.3%) cases, and one case had two different genes affected. The most common genetic syndromes were Apert syndrome and Mowat–Wilson syndrome.

Inheritance pattern was documented in 38/44 (86.4%) cases. Inheritance pattern was autosomal dominant in 24/38 (63.2%), autosomal recessive in 9/38 (23.7%) and X-linked in 5/38 (13.2%) cases. Among the autosomal dominant cases, 18/24 (75.0%) were *de novo*. Among the X-linked cases, 1/5 (20.0%) was *de novo*.

Meta-analysis of pooled proportions for ES diagnostic yield

A meta-analysis was performed on studies reporting ≥ three ACC cases, which included a total of 15 studies encompassing 268 ACC cases. Of the total included cases, 43% (95% CI, 31–56%) tested positive for a P/LP variant on ES. The highest yield was for ACC with extracranial anomalies (55% (95% CI, 35–73%)), followed by ACC with other cranial anomalies (43% (95% CI, 30–57%)) and isolated ACC (32% (95% CI, 18–51%)) (Table 2; Figures S1–S4).

DISCUSSION

Summary of main findings

This review reports on 288 cases with prenatal ACC that underwent ES following negative CMA. Among the 268 cases included in the meta-analysis, the diagnostic yield for positive P/LP variants was 43%. The highest yield was

Table 1 Most commonly involved genes and associated phenotype in cases with prenatally diagnosed agenesis of corpus callosum

Gene/Study	*Variant*	*Presence of associated anomaly*	*Phenotype/syndrome*
TUBA1A			
Boissel (2018)[26]	c.55G > A (p.A19T)	Other cranial	Severe microlissencephaly with absence of commissures, basal ganglia and thalami
Deden (2020)[42]	c.1285G > A (p.Glu429Lys)	Other cranial	Lissencephaly Type 3
Heide (2020)[24]	c.832G > C (p.Ala278Pro)	Other cranial	Lissencephaly Type 3
Lei (2021)[46]	c.1169G > C chr12-49 578 980 (p.R390P)	Extracranial	Lissencephaly Type 3
Petrovski (2019)[17]	Not available	Other cranial	Severe bilateral ventriculomegaly, kinking of brainstem, absent cerebellum
Yaron (2022)[16]	c.878A > G (p.Asn293Ser)	Other cranial	Tubulinopathy
	c.1105G > A (p.Ala369Thr)	Other cranial	Tubulinopathy
L1CAM			
Lei (2022)[34]	c.2254G > A (p.Val752Met)	Isolated	X-linked hydrocephaly
	c.176C > T (p.Ala59Val)	Isolated	X-linked hydrocephaly
Petrovski (2019)[17]	c.1417C > T (p.Arg473Cys)	Isolated	L1 syndrome
Tan (2020)[32]	c.1322delG (p.G441Afs*72)	Other cranial	Bilateral hydrocephalus, third ventricular dilatation
	c.551G > A (p.R184Q)	Other cranial	MASA syndrome
Yaron (2022)[16]	c.3581C > T (p.Ser1194Leu)	Extracranial	L1 syndrome
FGFR2			
He (2021)[45]	c.755C > G (p.Ser252Trp)	Extracranial	Apert syndrome
	c.755C > G (p.Ser252Trp)	Extracranial	Apert syndrome
Lei (2022)[34]	c.755C > G (p.Ser252Trp)	Extracranial	Apert syndrome
	c.755C > G (p.Ser252Trp)	Extracranial	Apert syndrome
Meier (2019)[40]	c.755C > G (p.s252W)	Extracranial	Apert syndrome
ARID1B			
Heide (2020)[24]	c.4129C > T (p.Arg1377*)	Isolated	Coffin–Siris syndrome
Lei (2022)[34]	c.316_317insTGTA (p.Gln107TyrfsTer126)	Isolated	Coffin–Siris syndrome
She (2021)[13]	c.1601_1605delACCCT (p.N534TfsX117)	Isolated	Coffin–Siris syndrome
Yaron (2022)[16]	c.1636_1637	Extracranial	Coffin–Siris syndrome
ARX			
Lefebvre (2021)[31]	c.1374_1383del (p.Pro459*)	Extracranial	Hydranencephaly with abnormal genitalia, X-linked lissencephaly 2
Lei (2022)[34]	c.994C > G (p.Arg332Gly)	Other cranial	Proud syndrome, hydranencephaly with abnormal genitalia, X-linked lissencephaly 2
Reches (2018)[38]	c.994C > T (p.Arg332Cys)	Other cranial	Heterotopia, interhemispheric cyst
COL4A1			
Yaron (2022)[16]	c.1186C > T (p.Arg396*)	Other cranial	*COL4A1*-related
	c.2086G > A (p.Gly696Ser)	Other cranial	*COL4A1*-related
	c.388-1G > C	Other cranial	Brain small vessel disease 1 with or without ocular anomalies
EPG5			
Aggarwal (2020)[29]	c.4665del (p.Glu1555Asp fs*12)	Isolated	Vici syndrome
de Koning (2022)[33]	c.5631del (p. Ser1879Alafs*12)	Extracranial	Vici syndrome
Qi (2020)[43]	c.2461C > T (p.R821*) het; c.88C > T (p.Q30*) het	Isolated	Vici syndrome
PEX1			
Aggarwal (2020)[29]	c.1670 + 1G > A	Extracranial	Zellweger syndrome
Boissel (2018)[26]	c.3205C > T (p.Gln1069*); c.2097dup (p.Ile700Tyrfs*42)	Other cranial	Thin corpus callosum, microcephaly, ventriculomegaly, polymicrogyria and heterotopia in both cerebral and cerebellar hemispheres
Normand (2018)[15]	c.2097dupT (p.I700fs); c.3205C > T (p.Q1069X)	Not specified	Not specified
TUBB			
Boissel (2018)[26]	c.920C > T (p.P307L)	Other cranial	Microlissencephaly, dysmorphic basal ganglia, cerebellar hypoplasia, circumferential skin creases, glomerular structures and voluminous germinal area in cortex
Lord (2019)[18]	c.860C > T (p.Pro287Leu)	Other cranial	Lissencephaly
Yaron (2022)[16]	c.947T > C (p.Val316Ala)	Other cranial	Tubulinopathy
ZEB2			
de Koning (2022)[33]	c.786dup (p.His263Thrfs*17)	Isolated	Mowat–Wilson syndrome
de Wit (2017)[27]	c.2403C > G (p.Tyr801*)	Extracranial	Mowat–Wilson syndrome
Heide (2020)[24]	2q22.2q22.3	Extracranial	Mowat–Wilson syndrome

Only first author given for each study. Genes are ordered by descending frequency of involvement.

Table 2 Diagnostic yield of exome sequencing following negative chromosomal microarray analysis in prenatally diagnosed agenesis of corpus callosum (ACC), overall and according to type of ACC

Group	*Studies* (n)	*P/LP* (n)	*ACC cases* (n)	*PP (95% CI) (%)*	I^2 *(%)*
All ACC*	15	100	268	43 (31–56)	64
Isolated ACC†	9	24	102	32 (18–51)	37
ACC with other cranial anomalies	10	36	88	43 (30–57)	29
ACC with extracranial anomalies	12	35	66	55 (35–73)	41

*Data on type of ACC (isolated *vs* non-isolated) was not available in all cases. †ACC was only brain finding. P/LP, pathogenic/likely pathogenic variant; PP, pooled proportion.

for ACC with extracranial anomalies (55%), followed by ACC with other cranial anomalies (43%) and isolated ACC (32%). We also classified 116 genetic variants in 83 genes associated with prenatal ACC with a full phenotypic description.

Interpretation of key findings

In cases of fetal ultrasound anomalies, the American College of Obstetricians and Gynecologists (ACOG) recommends investigation by CMA for prenatal genetic diagnosis[47]. CMA detects additional pathogenic CNVs in 0.4–1.7% of fetuses with a normal karyotype and absent structural anomalies, and is therefore offered to all patients who opt for prenatal genetic diagnosis[48,49]. ACMG recommends trio ES for patients with ultrasound anomalies in an index pregnancy only if CMA and karyotype are both negative[50,51].

Current evidence suggests that ES has an incremental yield in identifying diagnostic genetic variants in cases in which aneuploidy and CNVs are ruled out by karyotyping and CMA, allowing for differentiation between genetic syndromes and isolated congenital anomalies[18]. ES demonstrates the greatest yield in cases with multisystem anomalies[24]. ACC has also been reported to be the isolated CNS finding with the highest likelihood of having a P/LP variant diagnosed on ES[52], supporting further the efficacy of ES in identifying causative genetic variants in ACC, as seen in this study.

A limitation of ES that diminishes its use as a prenatal genetic test is its long TAT. ES has been reported to have an average TAT of 18 weeks[53]. In this study, TAT was reported for 46 cases and ranged between 7 and 107 days, with an average of 24 days. Given the decreasing TAT, clinicians should consider performing ES at the same time as CMA to lead to a higher rate of genetic diagnosis.

In our analysis of genes associated with ACC, *TUBA1A* was the most frequently affected and was associated with phenotypes such as lissencephaly Type 3 and tubulinopathy. Isolated ACC was most frequently associated with variants in *L1CAM* and *ARID1B*, while ACC with extracranial anomalies was most frequently associated with variants in *FGFR2* (Apert syndrome).

Knowledge of P/LP genetic variants and their syndromic associations prenatally may facilitate important decision-making regarding pregnancy management. ES is helpful when making decisions regarding delivery planning, intrapartum fetal monitoring, evaluation with additional imaging and procedures, referral to pediatric specialists and tertiary-care centers for delivery, and an overall earlier intervention in the pathogenic process[25,54].

Clinical and research implications

The addition of ES data in the prenatal and postnatal setting, with characterization of both genotypes and phenotypes into large data repositories, is required to improve our understanding of phenotype–genotype relationships. This will also require following pregnancies with unknown or uncertain variants, or those with discordant phenotypes, from the prenatal period to childhood to elucidate the causality of the genetic variants and their phenotype. It may also be worthwhile to investigate further the implications of the genes catalogued in this review in the development of the corpus callosum. Further research may also focus on the patient experience of undergoing ES during pregnancy, the impact on provider healthcare utilization, patient outcome and decision-making for future pregnancies and family planning.

Strengths and limitations

The strengths of this review are the thorough search strategy in four large databases and the methodology used to collect and interpret data, which was standardized and reproducible. International collaboration between two largest series on prenatal congenital anomalies and ES and their extended cohorts increased the number of included cases[17,18]. All studies used ACMG classification for genetic variant interpretation, and most also used trio ES analysis and Sanger sequencing for validation. Studies with fewer than three cases were excluded from the meta-analysis, decreasing the chance of bias in our results.

A limitation of this review is that only a few ES studies focused specifically on ACC, with high heterogeneity in the included studies. Most studies did not specify whether ACC was complete or partial, limiting our ability to determine the yield of ES in these subgroups. Description of phenotypes was based on ultrasound and/or magnetic resonance imaging (MRI) findings, which could limit the classification of ACC in this review. Intrauterine MRI can detect associated anomalies that are otherwise not picked up on ultrasound, but not all of the 15 studies

included in the meta-analysis reported using MRI[55]. This is a limiting factor that may have led to misclassification of cases as isolated ACC. Although cases were classified as isolated, it is possible that their disease process would evolve and present with more anomalies later in gestation or early in the neonatal period. Not all studies provided confirmatory postnatal examinations or autopsy findings that would have allowed us to reach a more accurate classification.

A general limitation of ES is that it has a higher diagnostic yield for monogenic disorders in preselected cohorts, such as terminated pregnancies or severe cases, than in unselected cohorts[12]. Although the studies in this review include a wide range of cohorts, both preselected and unselected, it is possible that the diagnostic yield would be lower if all studies used unselected cohorts. Different sequencing platforms were used in each study, targeting between 2000 and 6000 genes (Table S1), and we postulate that this variation has also resulted in a higher diagnostic yield in our results.

Some of the genes identified in this systematic review have not been reported in previous literature as being related to ACC or the associated syndromes. Some genetic variants were also reported as being novel mutations when the study was conducted. Further research should be conducted on the strength of the association between these novel genetic variants and ACC.

Conclusions

Our findings highlight the value of ES in prenatal genetic diagnosis. While the highest diagnostic yield was observed in ACC cases with extracranial (55%) or additional cranial (43%) anomalies, ES should also be considered in cases of isolated ACC, given the yield of 32% for P/LP variants.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figures S1–S4 Forest plots of pooled proportions of positive exome sequencing (ES) for pathogenic/likely pathogenic genetic variants in all prenatally diagnosed agenesis of corpus callosum cases (Figure S1), cases in which agenesis of corpus callosum was the only finding (Figure S2), cases of agenesis of corpus callosum with other cranial anomalies (Figure S3) and cases of agenesis of corpus callosum with extracranial anomalies (Figure S4). Only first author given for each study.

Appendix S1 Search strategy

Table S1 Characteristics of studies included in systematic review

Tables S2–S4 Phenotypic expression of genetic variants in cases of isolated agenesis of corpus callosum (Table S2), agenesis of corpus callosum with other cranial anomalies (Table S3) and agenesis of corpus callosum with extracranial anomalies (Table S4)

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Rendimiento diagnóstico de la secuenciación del exoma en la agenesia prenatal del cuerpo calloso: revisión sistemática y metaanálisis

RESUMEN

Objetivos. Determinar el rendimiento diagnóstico incremental de la secuenciación del exoma (SE) tras un análisis de microarrays cromosómicos (AMC) negativo en casos de agenesia del cuerpo calloso (ACC) diagnosticada prenatalmente e identificar los genes y variantes asociados.

Métodos. Se realizó una búsqueda sistemática para identificar estudios relevantes publicados hasta junio de 2022 en la que se utilizaron cuatro bases de datos: PubMed, SCOPUS, Web of Science y The Cochrane Library. Se incluyeron estudios en inglés que informaban sobre el rendimiento diagnóstico de la SE tras una AMC negativa en la ACC parcial o completa diagnosticada prenatalmente. Se contactó a los autores de los estudios de cohortes para solicitar datos individuales de los participantes y se facilitaron cohortes ampliadas de dos de ellos. Se evaluó el aumento del rendimiento diagnóstico mediante la SE para variantes patogénicas/probablemente patogénicas (P/PP) en todos los casos de ACC, ACC aislada, ACC con otro tipo de anomalías craneales y ACC con anomalías extracraneales. Para identificar todas las variantes genéticas reportadas, la revisión sistemática incluyó todos los casos de ACC; sin embargo, para el metaanálisis, solo se incluyeron estudios con ≥ tres casos de ACC. Se empleó un metaanálisis de proporciones utilizando un modelo de efectos aleatorios. La evaluación de la calidad de los estudios incluidos se realizó utilizando criterios modificados de los Estándares para Informes sobre Precisión Diagnóstica.

Resultados. Un total de 28 estudios, que abarcaban 288 casos de ACC diagnosticados prenatalmente que se sometieron a SE tras un AMC negativo, cumplieron los criterios de inclusión de la revisión sistemática. Se clasificaron 116 variantes genéticas en 83 genes asociados a la ACC prenatal con una descripción fenotípica completa. Se identificaron 15 estudios, que abarcaban 268 casos, que reportaron sobre ≥ tres casos de ACC y que se incluyeron en el metaanálisis. De todos los casos incluidos, el 43% presentaba una variante P/PP en la SE. El mayor rendimiento correspondió a la ACC con anomalías extracraneales (55% [IC 95%, 35–73%]), seguido de la ACC con otro tipo de anomalías craneales (43% [IC 95%, 30–57%]) y la ACC aislada (32% [IC 95%, 18–51%]).

Conclusiones. La SE demostró un mayor rendimiento diagnóstico en los casos de ACC diagnosticada prenatalmente después de un AMC negativo. Aunque el mayor rendimiento diagnóstico se observó en la ACC con anomalías extracraneales y en la ACC con otras anomalías del sistema nervioso central, la SE también debe considerarse en casos de ACC aislada.

外显子组测序对产前胼胝体发育不全的诊断率：系统综述和荟萃分析

摘要

目的 确定在产前确诊胼胝体发育不全（ACC）的病例经染色体微阵列分析（CMA）阴性后，外显子组测序（ES）的诊断率增量并确定相关基因和变异。

方法 使用以下四个数据库对截至 2022 年 6 月发表的相关研究进行系统检索：PubMed、SCOPUS、Web of Science 和 Cochrane Library。纳入的研究为报告产前确诊部分性或完全性 ACC 的 CMA 阴性后 ES 诊断率的英文研究。我们联系了队列研究的作者以获得个体参与者的数据，其中两项研究提供了扩展队列。在所有 ACC 病例、孤立 ACC 病例、伴有其他头颅异常的 ACC 病例和伴有颅外异常的 ACC 病例中，对 ES 对致病性/可能致病性（P/LP）变异的诊断率增量进行了评估。为了确定所有已报道的基因变异，系统综述纳入了所有 ACC 病例；但在荟萃分析中，只纳入了 ACC 病例≥ 3 例的研究。采用随机效应模型对比例进行了荟萃分析。采用修改后的《诊断准确性报告标准》对纳入的研究进行了质量评估。

结果 共有 28 项研究符合系统综述的纳入标准，包括 288 例产前确诊的 ACC 病例，这些病例在 CMA 阴性后接受了 ES。我们对与产前 ACC 相关的 83 个基因中的 116 个遗传变异进行了分类，并进行了完整的表型描述。共有 15 项研究（268 个病例）报告了≥ 3 个 ACC 病例并纳入了荟萃分析。在所有纳入的病例中，43% 经 ES 发现有 P/LP 变异。伴有颅外异常的 ACC 发病率最高（55%（95% CI，35-73%）），其次是伴有其他头颅异常的 ACC（43%（95% CI，30 - 57%））和孤立的 ACC（32%（95% CI，18 - 51%））。

结论 ES 在产前确诊且 CMA 阴性的 ACC 病例中展现出了诊断率增量。虽然在伴有颅外异常的 ACC 和伴有其他中枢神经系统异常的 ACC 中，ES 的诊断率最高，但孤立的 ACC 病例也应考虑 ES。