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Diagnostic Yield of Exome Sequencing in Prenatal Agenesis of Corpus Callosum:

A Systematic Review and Meta-analysis

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Contribution

What are the novel findings of this work?

Of the 267 included prenatal agenesis of corpus callosum (ACC) cases, 43% had positive pathogenic/likely pathogenic variants. The highest yield was for ACC with extracranial anomalies 55%, then ACC with other cranial anomalies 43%, followed by isolated ACC 32%. We classified 116 genetic variants in 83 genes.

What are the clinical implications of this work?

The use of prenatal exome sequencing in both isolated ACC and ACC with extracranial anomalies should be considered after negative standard genetic testing with chromosomal microarray given the heterogeneity in the prenatal phenotype of vast associated syndromic categories.

ABSTRACT

Objective: To determine the incremental increase in diagnostic yield of exome sequencing (ES) after negative chromosomal microarray analysis (CMA) in prenatally diagnosed ACC and to classify associated genes and variants.

Methods: A systematic search was performed to identify relevant studies published until June 2022 using four databases including PubMed, Scopus, Web of Science, and 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 of which two provided their extended cohorts. The incremental increase in diagnostic yield with ES was assessed for pathogenic/likely pathogenic in: (1) all cases of ACC; (2) isolated ACC; (3) ACC with other cranial anomalies; and (4) non-isolated ACC (ACC with extracranial anomalies). To be able to identify all reported genetic variants, the systematic review portion included all ACC cases, however, for the meta-analysis portion, we included studies with ≥ 3 ACC cases. 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: Twenty-eight studies encompassing 285 prenatal ACC cases that underwent ES following a negative CMA met the inclusion criteria for the systematic review. We classified 116 genetic variants in 83 genes associated with prenatal ACC along with full phenotypic description. Studies reporting on ≥3 ACC cases were total of 15 studies encompassing 267 cases. Of all the included cases, 43% had positive P/LP ES. The highest yield was for ACC with extracranial anomalies 55% (95% CI 35, 73), then ACC with other cranial anomalies 43% (95% CI 30, 57), followed by isolated ACC 32% (95% CI 18, 51).

Conclusion: There is an apparent incremental increase in diagnostic yield of ES following negative CMA in prenatally diagnosed ACC. While the greatest yield is in ACC with extracranial

anomalies and ACC with other CNS anomalies, consideration should also be given to performing ES in the presence of isolated ACC as the only brain anomaly on prenatal imaging.

INTRODUCTION

Agenesis of the corpus callosum (ACC) is defined as an absence of the commissural tract of fibers that connects both hemispheres of the brain and can be classified as partial or complete.[1] The corpus callosum consists of 4 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 could be isolated or associated with other cranial or extracranial anomalies.[1] ACC is the most common commissural malformation with an incidence of 0.05 to 70 per 10,000 live births.[5,6]

ACC is diagnosed prenatally during the second trimester ultrasound, by either an absent cavum septum pellucidum in the axial plane, or by colpocephaly of the lateral ventricles. [1] Color doppler can also be done to visualize the course of the pericallosal artery to pinpoint the portion of dysgenesis from 11 weeks of gestation onwards. [3]

ACC has a heterogenous etiology with associations to different genes and syndromes. CDK5RAP2 and DCC gene are both linked to isolated ACC. ACC is widely associated with Coffin-Siris syndrome and is now also seen in novel congenital syndromes like Vici syndrome and Mowat-Wilson syndrome.[7]

Reported neurodevelopmental outcomes in isolated ACC are normal in 71.2% of cases, while, the remaining patients manifest borderline to severe abnormalities. [4, 8] These unpredictable outcomes make prenatal counseling a challenge. Genetic testing like karyotype, chromosomal microarray analysis (CMA) and exome sequencing (ES) enhance the availability of information necessary for prenatal counseling.[9]

ES has proven to be a powerful tool for evaluating postnatal patients, achieving an average molecular diagnostic rate of 25% of pathogenic/likely pathogenic (P/LP) variants when performed for mendelian disorders.[10] This is in comparison to the currently used CMA which detects clinically significant CNVs in 5.7% of isolated ACC with a normal karyotype.[11] Prenatal diagnostic yield of fetal structural anomalies with ES, is higher in cases of pre-selected cohorts

for monogenic etiology compared to un-selected cohorts (42% vs. 15% respectively).[12] In prenatally detected ACC, ES is estimated to have a higher diagnostic rate of P/LP variants when compared to CMA or karyotype.[13]

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

METHODS

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

Search strategy

A systematic search was performed in four electronic databases including Cochrane Library, Web of Science, Scopus, and MEDLINE by two authors (E.S and J.B), independently, from inception until June 2022. The search strategy included a combination of relevant medical subject heading (MeSH) terms and relevant 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 literature is available in supplementary material The generated articles were transferred to Rayyan software for abstract screening. Duplicates that were identified both by Rayyan software and manually were removed. Abstract screening was performed independently by two authors (E.S. and J.B.) and disagreements were resolved by discussion with a third party (H.J.M.). Included studies' full texts were retrieved for data extraction.

Eligibility criteria

We defined our eligibility criteria based on the PICO framework: (P) Population: pregnancies complicated by ACC whether complete or partial. (I) Intervention: ES. (C) Comparison: microarray/karyotype tests. (O) Outcome: P/LP variants. Inclusion criteria included pregnancies that were prenatally diagnosed with ACC on imaging with or without other anomalies (CNS or multi-system) undergoing ES following a negative CMA. The exclusion criteria were absence of CMA/karyotype or ES, papers of which authors did not provide missing number of cases and individual data information, and non-English papers. To be able to identify all reported

genetic variants, the systematic review portion included all ACC cases, however, for the meta-analysis portion, we included studies with ≥ 3 ACC cases.

Data extraction and outcome measures

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

Four studies had unpublished data regarding associated genes or variants for which authors were contacted and they provided full relevant data,[15-18] and two of which provided extended cohorts as well.[17, 18]

Quality assessment

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

Variant classification or re-classification

Variant reclassification was done 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 to verify correct nomenclature. Alamut is a genome browser that can generate variants and their corresponding 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 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 through searches of 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 American College of Medical Genetics (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 classification for Pathogenic or Likely Pathogenic variants and one for 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 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 5 tier system which is pathogenic (P), likely pathogenic (LP), uncertain significance, likely benign and benign.[21] All variants were classified by our genomic scientist (C.B.) and these classifications were reviewed by an additional study member (J.B.). We

also included ClinGen recommendations regarding evidence type PVS1.[22] Evidence type PP5, report by a reputable source, was used judiciously to avoid double counting in cases where 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 Pathogenic or Likely Pathogenic in both instances or if it was VUS in both instances. In 3 cases of classification for compound heterozygous inheritance of an AR disorder, a pathogenic variant with a VUS was considered a likely pathogenic diagnosis.

Statistical Analysis and Data synthesis

For studies with 3 or more fetal ACC cases undergoing ES following negative CMA, we calculated the pooled proportions and their 95% confidence intervals in 4 different groups of ACC cases: (1) all ACC cases; (2) isolated ACC (ACC is the only finding); (3) ACC with other cranial anomalies; and (4) non-isolated ACC (ACC with extracranial anomalies).

Heterogeneity of the included studies was assessed graphically and statistically by 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 effect model was used for pooling the effect sizes and their 95% CI was consequently calculated. To test the overall significance of the random model, *z*-test was performed. Potential publication bias was graphically assessed by creating funnel plots for each of the groups. RStudio[23] (RStudio, Inc., Boston, MA) was used for the statistical analysis and creating forest and funnel plots.

RESULTS

Study characteristics

As shown in PRISMA flow chart (Figure 1), our search strategy generated 13,102 abstracts. There were 5,011 removed for duplication. Following abstract screening, a total of 168 studies underwent full-text assessment of which 28 studies met our criteria.

Table 1 shows characteristics of the included studies in the systematic review. 15 studies reported on ≥3 ACC cases and 13 studies had less than 3 cases. Publication years ranged between 2014 and 2022. 17 studies were retrospective and 11 were prospective. Full exome methodology for each study is outlined in Table 1. Twenty-one studies performed trio ES, 5 studies had a combination of proband, duo and trio ES, and in two studies methodology was not reported.

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

Systematic review

The systematic review portion included a total of 285 ACC cases that had ES performed after negative CMA. We also aimed to include cases undergoing karyotype, all included studies performed CMA. There were 115 variants including 82 genes that were P/LP per the original articles. Upon further re-analysis, one variant was downgraded to a benign, and 2 VUS cases were upgraded to P/LP resulting in total of 116 P/LP variants in 83 genes. The rest of the VUS remained as VUS.

Pregnancy outcomes were reported for 84 positive cases of which 69 had pregnancy termination (69/84, 82.1%), two stillbirths (2/84, 2.4%), three neonatal demise (3/84, 3.6%), and ten live birth (10/84, 11.9%). 113 specified the type of ES performed. Maternal-paternal-fetal trio

testing was done in most cases (108/113, 95.6%). Duo ES was performed in one case (1/113, 0.9%). Proband only ES was performed in four cases (4/113, 3.5%).

Table 2 shows the genes with the highest overall frequency which include TUBA1A (7 cases, 6.0%), L1CAM (6 cases, 5.2%), FGFR2 (5 cases, 4.3%), ARID1B (4 cases, 3.4%), ARX (3 cases, 2.6%), COL4A1 (3 cases, 2.6%), EPG5 (3 cases, 2.6%), PEX1 (3 cases, 2.6%), TUBB (3 cases, 2.6%), and ZEB2 (3 cases, 2.6%) and two cases (1.7%) each of KANSL1, NFIA, and TUBB3 genes. 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 3A). The genes included ARID1B (3 cases, 12%), L1CAM (3 cases, 12%), EPG5 (2 cases, 8%), NFIA (2 cases, 8%), 1 case (4%) each of AP4M1, ALDH7A1, EXOSC3, KANSL1, KCNQ2, PPP2R1A, PTCH1, PTDSS1, PTPN11, SCN2A, SHH, SON, TUBB2B, ZBTB20, and ZEB2. The most common genetic syndromes were Coffin-Siris Syndrome, X-linked hydrocephaly, and Vici Syndrome.

Inheritance pattern was documented in 24 of these cases (24/25, 96%). Out of these 24 isolated single ACC cases, inheritance patterns were autosomal dominant (17/24, 70.8%), autosomal recessive (4/24, 16.7%), and X-linked (3/24, 12.5%). Among the autosomal dominant cases, 16/17 (94.1%) were *de novo*. Among the X-linked cases, 2/3 (66.6%) were *de novo.* Also 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 31 genes associated with 42 cases (Table 3B). The genes included TUBA1A (6 cases, 14.3%), COL4A1 (3 cases, 7.1%), TUBB (3 cases, 7.1%), ARX (2 cases, 4.8%), L1CAM

(2 cases, 4.8%), and OFD1 (2 cases, 4.8%). There was one case (2.4%) each of the remaining 25 genes, of which one case had two different genes. The most common genetic syndromes were Tubulinopathy, X-linked hydrocephalus, Brain small vessel disease, X-linked lissencephaly, and Orofaciodigital syndrome.

Inheritance pattern was documented in 38 of these cases (38/42, 90.5%). Inheritance patterns were autosomal dominant (21/38, 55.3%), autosomal recessive (6/38, 15.8%), and X-linked (11/38, 28.9%). 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 3C). The genes included FGFR2 (5 cases, 11.4%) and ZEB2 (2 cases, 4.5%), and one case (2.3%) each of 38 remaining genes, of which one case had two different genes. The most common genetic syndromes were Apert Syndrome and Mowat Wilson Syndrome.

Inheritance pattern was documented in 38 of these cases (38/44, 86.4%). Inheritance patterns were autosomal dominant (24/38, 63.2%), autosomal recessive (9/38, 23.7%), and X-linked (5/38, 13.2%). Among the autosomal dominant cases, 18/24 (75%) were *de novo*. Among the X-linked cases, 1/5 (20%) was *de novo.*

Meta-analysis of pooled proportions for exome sequencing diagnostic yield

As mentioned previously in the methods section, synthetic analysis was performed on studies reporting ≥3 ACC cases which included a total of 15 studies encompassing 267 positive P/LP cases and negative CMA. Of the total included cases, 43% (95% 31, 56) had positive P/LP ES. The highest yield was for ACC with extracranial anomalies 55% (95% CI 35, 73), then ACC with cranial anomalies 43% (95% CI 30, 57), followed by isolated ACC 32% (95% CI 18, 51) (Table 4, Supplemental Figures 1-4).

DISCUSSION

Summary of the main findings

Our review reports 267 cases with prenatal ACC that underwent ES following negative CMA. Of the included cases, positive P/LP yield was 43%. The highest yield was for ACC with extracranial anomalies 55%, then ACC with other cranial anomalies 43%, followed by isolated ACC 32%. We also classified 116 genetic variants in 83 genes associated with prenatal ACC along with full phenotypic description.

Interpretation of the key findings

In cases of fetal ultrasound anomalies, ACOG recommends investigation by CMA for prenatal genetic diagnosis.[24] CMA detects additional pathogenic copy number variants (CNVs) in 0.4-1.7% of fetuses with both normal karyotype and absent structural anomalies, thus is offered to all patients who opt for prenatal genetic diagnosis.[25, 26] The ACMG recommends trio ES for patients with ultrasound anomalies in an index pregnancy, only if CMA and karyotype are both negative.[27, 28]

Currently available knowledge on ES is that it has an incremental yield in identifying diagnostic genetic variants where aneuploidy and CNVs are ruled out with karyotype and CMA, allowing for differentiation between genetic syndromes and isolated congenital anomalies.[18] Its greatest yield is with multi-system anomalies. [29] The isolated CNS finding reported with the highest likelihood of having a P/LP variant diagnosed on ES is ACC, further solidifying the efficacy of ES in identifying causative genetic variants in ACC as also seen in our results.[30]

A limitation of ES, that diminished its use as a prenatal genetic test is its high turn-around time (TAT). In 2014 ES was reported to have an average TAT of 18 weeks.[31] 46 of our cases had TATs reported ranging from 7-107 days with an average of 24. With decreasing TATs, it can be said that ES should now be done at the same time as CMA to lead to a higher genetic diagnosis.

In our analysis of genes associated with ACC, TUBA1A was the most prevalent and associated with phenotypes Lissencephaly type 3 and Tubilinopathy. L1CAM and ARID1B had the greatest number of genetic variants associated with isolated ACC. FGFR2 gene in Apert syndrome had the greatest number of ACC cases with extracranial anomalies.

Knowledge of P/LP genetic variants and their syndromic associations prenatally can allow for paramount decisions to be made on management of the pregnancy. ES is helpful when making decisions on delivery plans, 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. [32, 33]

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 the data, that's standardized and reproducible. International collaboration between two largest series on prenatal congenital anomalies and ES who provided their data, and their extended cohorts increased the number of included cases. All studies used ACMG classification for genetic variant interpretation and most also used trio-analysis for ES and Sanger sequencing for validation. Studies with less than 3 cases were excluded from the meta-analysis, decreasing the chance of bias in our results.

Limitations are that only a few ES studies were done 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. Prenatal findings are phenotypes as described on ultrasound and/or MRI which could limit the classification scheme of ACC used in this review. Intrauterine MRI can detect associated anomalies that are otherwise not picked up on ultrasound, but not all of the 15 studies in our data analysis reported using MRI.[34] 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 evolves and presents with more anomalies in a later gestation or early in the neonatal period. Not all studies provided confirmatory postnatal examinations or autopsy findings that could've allowed us to reach a more accurate classification.

A general limitation of ES is that it has higher diagnostic yield in case of pre-selected cohorts, such as terminated pregnancies or severe cases, for monogenic disorders than it does for unselected cohorts.[12] Although the studies in this review include a wide range of cohorts, both selected and un-selected, it's possible the diagnostic yield would be lower if all studies used unselected cohorts. As seen in Table 1., different sequencers were used in each study ranging between 2000 to 6000 genes and we postulate that this variation has also resulted in a higher diagnostic yield in our results.

Few of the genes are not reported in scientific literature as having a prior known association with ACC or its syndromes. Some genetic variants were also reported as being novel mutations when the study was conducted. Further research must be done regarding the strength and association between these novel genetic variants and ACC.

Conclusions and future clinical and research implications

In conclusion, our results highlight a key finding in the use of ES for prenatal genetic diagnosis. While the highest yield was for cases with extracranial anomalies (55%), consideration for performing ES should also be given for isolated ACC given the yield of 32% for positive P/LP findings.

Use of ES in both 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 also will require following pregnancies with unknown or uncertain variants or those with discordant phenotypes from the prenatal period through childhood to elucidate the causality of the genetic variants and the full expansion of their

phenotypes. It will also be worthwhile to investigate further the implications of the genes catalogued in this review on 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 outcomes, and the impact on decision making for future pregnancies and family planning.

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FIGURE LEGENDS

Figure 1: PRISMA flow chart of search and selection process

Figure 2: Quality assessment of 28 studies included in systematic review, using modified Standards for Reporting of Diagnostic Accuracy criteria.

Table 1. Characteristics of the studies included in the systematic review

Author	Study period	Country	Institute	Study design	Inclusion criteria	Exclusion criteria	Exome Methodology	Total number of cases	Agenesis of Corpus Callosum number
Boissel 2017[35]	2013-2016	Canada	CHU Sainte-Justine's, (Mount Sinai Hospital, Toronto, Canada; Hôpitaux Universitaires de Strasbourg, France, and the Children's Hospital of East Ontario, Ottawa, Canada)	Prospective	Terminated pregnancies or stillborns with: "(i) at least two major malformations, (ii) severe ventriculomegaly (atria >15 mm bilaterally) and/or structural brain malformations, or (iii) an anomaly associated with a high risk of perinatal lethality.	Not Reported	ES, trio, 110× coverage, Agilent capture + Illumina HiSeq 2000 or 2500	36	14
De Wit 2017[36]	2008-2015	Netherlands	Erasmus Medical Center and Sophia Children's Hospital	Retrospective	All patients diagnosed with isolated complete agenesis of the fetal corpus callosum (cACC) on EUE at any moment in pregnancy" "Fetuses with coexisting midline cysts and colpocephaly were included…because these anomalies are considered to be a part of the ACC sequence.	Patients with presumed partial ACC were excluded." Fetuses with other fetal anomalies or "sonomarkers"	Not reported	19	4
Fu 2017[37]	2011-2015	China	Prenatal Diagnostic Center, Guangzhou Women and	Retrospective	Fetal structural malformations on prenatal ultrasound examination	Isolated sonographic soft markers such as choroid plexus cysts,	Agilent Bioanalyzer 2100	196	8

			Children's Medical Center, Guangzhou Medical University		and/or magnetic resonance imaging. The anomalies included structural malformation, nuchal translucency thickness ≥ 3.5 mm and cystic hygroma.	echogenic foci in the heart or bowel, thickened nuchal fold, absent nasal bone, single umbilical artery or persistent right umbilical vein."	(Agilent Technologies, Santa Clara, CA, USA). A HiSeq2500 sequencer was used for sample sequencing according to the manufacturer's protocol (version 3; Illumina, Inc., San Diego, CA, USA). Paired-end sequencing was performed for each sample.		
Normand 2018[15]	2012-2017	USA	Baylor college of medicine	Retrospective	The fetus had at least one structural anomaly detected by fetal imaging or autopsy"	Not reported	Illumina HumanOmni1-Quad or HumanExome-12 v1 SNP array for quality control of the exome data and to detect large CNVs, absence of heterozygosity (AOH), and	146	12

							uniparental disomy." Next generation sequencing Sanger method for confirmation		
Aggarwal 2019[38]	NR	India	Nizam's Institute of Medical Sciences, Punjagutta, Hyderabad, Telangana	Retrospective	Fetuses with a phenotype and/or family pedigree suggestive of genetic etiology but without a specific clinical or laboratory diagnosis.	Cases where the first and second tier evaluation established a specific genetic etiology or indicated an acquired or possible nongenetic basis were excluded from the study.	Trio ES, DNA isolated from amniotic fluid/skin/umbilical cord or cord blood (approximately 1 µg) was used to perform exome capture (n = 28) using Nextera Rapid Capture Exome v1.2 kit (Illumina, San Diego, CA) or SureSelect kit (Agilent Technologies, Santa Clara, USA) and targeted exome capture(8500 genes, n = 4)	32	5

							using Kapa HTP library preparation kit (Illumina, San Diego, CA)" "libraries were sequenced to more than 100× coverage on Illumina HiSeq2000 platform.		
Greenbaum 2019[39]	2015-2018	Israel	Danek Gertner Institute of Human Genetics at Sheba Medical Center	Retrospective	Fetal structural anomalies of terminated or ongoing pregnancies	Not reported	Sequencing was performed on Ilumina platform to obtain an average coverage depth of approximately 100×.	44	5
Lord 2019[18]	2014-1018	UK	34 fetal medicine units in England and Scotland	Prospective	Undergoing invasive testing for identified nuchal translucency or structural anomalies in their fetus, as detected by ultrasound after 11 weeks of gestation.	If abnormal aneuploidy considered to have caused structural abnormality was detected, if one or both parents were younger than 16 years, or if one or both parents did not or could not provide informed consent.	ES, trio, 1628 genes, Agilent capture + Illumina Hi-Seq 2500, 98.3% of bait regions covered at minimum depth of 5×	610	28

Petrovski 2019[17]	2015-2017	USA	Columbia University Carmen and John Thain Center for Prenatal Pediatrics	Prospective	Singleton pregnancies: "all fetal structural anomalies, including nuchal translucency of more than 3.5 mm, were included."	Fetuses with a known infection or exposure to a known teratogenic drug, families with a known diagnosis of a genetic disorder, and cases in which a parental DNA sample was not available were excluded. Fetuses with ultrasound soft markers that were suggestive of Down syndrome but that showed no other anomalies, those with an isolated nuchal translucency of less than 3.5 mm, and those with abnormal karyotype or CMA results that were considered causative of the anomaly were also excluded.	Trio ES of the fetuses and parents (parent–fetus trios)+Illumina HiSeq 2500 platform	234	18
Heide 2020[29]	2018-2020	France	Multiple fetal centers	Prospective	Pregnant women with fetal isolated or nonisolated abnormal corpus callosum who opted for invasive testing (amniocentesis) and consented for participation in the study were included.	Not reported	Trio ES on a NextSeq 500 Sequencing System (Illumina, San Diego, CA), with a 2 × 150 bp high output sequencing kit after a 12-plex enrichment with SeqCap EZ MedExome kit	65	65

							(Roche, Basel, Switzerland)		
Lefebvre 2020[40]	2015-2019	France	Santé, INSERM Université de Bourgogne	Retrospective	The fetuses had to present at least two independent congenital malformations and normal standard chromosomal analysis and array-comparative genomic hybridization(CGH) results. Fetal examinations and investigations should not have identified an etiological clinical diagnosis.	Not reported	Trio ES, Libraries of genomic DNA samples were prepared using the Agilent Sureselect Human All Exon v5 kit (Agilent Technologies, Santa Clara, CA), and were sequenced on a HiSeq instrument (Illumina, San Diego, CA) for paired-end 76-bp reads.	95	8
Tan 2020[41]	2017-2018	China	Department of Fetal Medicine and Prenatal Diagnosis of the Third Affiliated Hospital of Guangzhou Medical University	Retrospective	Fetuses with CNS abnormalities at the routine prenatal ultrasound scan were enrolled, including widen ventriculomegaly, agenesis of corpus callosum, and meningocele." "All cases had a negative result of karyotyping and chromosomal microarray analysis.	Not reported	Trio ES, "NextSeq platform (Illumina) and paired-end reads generated were aligned to the human genome (hg19). Variants were called and annotated using the Biomedical	11	3

							Genomics Workbench (CLC bio-Qiagen, Aarhus, Denmark).		
De Koning 2021[42]	2017-2020	Netherlands	Leiden University Medical Centre	Retrospective	Parents of fetuses with CNS malformations, either isolated or in combination with other structural anomalies as detected by prenatal US	Not reported	WES, trio, 1128 genes, 80× coverage, Agilent capture + NextSeq 500	19	12
She 2021[13]	2015-2020	China	Prenatal Diagnosis Center of the Six Affiliated Hospital, Guangzhou Medical University	Retrospective	Prenatally detected corpus callosum abnormality on imaging	Not reported	Trio ES, 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	19	5

							according to the manufacturer's protocol (version 3, Illumina).		
Lei 2022[43]	2015-2019	China	The six affiliated hospital, Guangzhou medical center	Prospective	Fetuses with callosal anomalies with or without other structural anomalies, but normal findings by karyotyping and chromosomal microarray analysis (CMA).	Fetuses with abnormal karyotyping or CMA results were excluded."	Trio ES, Agilent capture + Illumina HiSeq 6000	50	50
Yaron 2022[44]	2014-2021	Israel	Sourasky medical center, prenatal genetic diagnosis unit, Genetics Institute	Retrospective	All cases referred to our institution for genetic evaluation following termination of pregnancy due to a major fetal CNS anomaly	Mild isolated findings, such as mild ventriculomegaly, were not included in this study.	Trio ES, NovaSeq 6000 sequencer AQ19 (Illumina, San Diego, CA, USA) with 100-bp paired-end reads.	86	34
Characteristics of studies with less than 3 ACC case numbers									
Carss 2014[45]	NR	UK	The Fetal Medicine Centre Birmingham Women's Foundation Trust, UK	Retrospective	Women who had a fetus with a structural anomaly suspected at their routine ultrasound scan at 11–14 weeks or 18 – 20 weeks gestation.	Not Reported	Trio ES, 103× coverage, Agilent capture + Illumina HiSeq	28	2
Shamseldin 2017[46]	NR	Saudi Arabia	Department of Genetics, King Faisal Specialist Hospital and Research Center	Prospective	Pregnancies diagnosed with unexplained intrauterine fetal demise or terminated due to major unexplained fetal malformations	Not reported	For exome analysis, samples were prepared according to the preparation guide of Agilent SureSelect Target	44	2

							Enrichment Kit (Santa Clara, CA, USA) and the resulting libraries were sequenced using the Illumina HiSeq2000 sequencer (Santa Clara, CA, USA)." Sanger sequencing for confirmation		
Aarabi 2018[47]	NR	USA	Medical Genetics and Genomics Laboratories, Magee-Womens Hospital of UPMC, Pittsburgh, PA	Retrospective	Prenatal cases with congenital anomalies detected by ultrasound…at least one major structural birth defect" "All participants had normal fetal karyotype and microarray studies prior to enrollment.	Not reported	Trio ES, 20,000 gene panel, 60 – 140 × coverage	20	1
Reches 2018[48]	2014-2017	Israel	The Obstetrics and Gynecology Ultrasound Division at the Lis Maternity Hospital	Retrospective	Cases with prenatally diagnosed CNS abnormality, whose chromosomal microarray analysis was negative	Not reported	Trio ES, approximately 37 Mb (214,405 exons) of the Consensus Coding Sequences (CCS) were enriched from fragmented	7	2

							genomic DNA by >340,000 probes designed against the human genome (Nextera Rapid Capture Exome, Illumina) and the generated library sequenced on an Illumina NextSeq or HiSeq 4000 platform (Illumina) to an average coverage depth ×100–130.		
Jiang 2019[49]	2019	China	Department of Obstetrics, Women's Hospital, School of Medicine, Zhejiang University	Retrospective	Not reported	Not reported	Trio ES; Target enrichment of target region sequences by Agilent SureSelect Human Exon Sequence Capture Kit, Illumina DNA Standards and Primer Premix Kit (Kapa Biosystems,	Jiang 2019	2019

							Boston, MA, USA), Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA)		
Meier 2019[50]	NR	Switzerland	Medical Genetics, Institute of Medical Genetics and Pathology, University Hospital Basel	Prospective	Families were included in the study if (i) the fetus showed a pattern of two or more anomalies associated with a high risk for fetal or perinatal lethality that suggested a genetic disorder or (ii) there was familial recurrence of the fetal anomaly phenotype and if (iii) there were detailed clinical fetal ultrasound and/or autopsy data available and (iv) high-resolution chromosomal microarray did not show a causal chromosomal anomaly or copy number variant.	Not reported	Trio ES, Library preparation (Agilent SureSelectXT Library Prep Kit) and exome capture using the Agilent SureSelectXT Human All Exon V6 (Agilent, Santa Clara, CA) was followed by paired-end read sequencing (2 × 100 bp read length) on a HiSeq 2500 or HiSeq 4000 platform (Illumina, San Diego, CA) with an	12	2

							average coverage of ×100.		
Corsten-Janssen 2020[51]	2018-2019	Netherlands	University Medical Centre Groningen	Prospective	(a) Two or more independent major fetal anomalies, (b) Hydrops fetalis or bilateral renal cysts alone, or (c) One major fetal anomaly and a first-degree relative with the same anomaly.	Excluded fetuses diagnosed prenatally of having an anomaly for which no underlying genetic defect is known	Trio ES, Fetal and parental DNA were prepared for rES using SureSelect Human All Exon V6 (Agilent, USA) target enrichment, according to standard procedures, on Bravo automated liquid handling robots (Agilent), and then sequenced on an Illumina NextSeq500 sequencer aiming for 20× coverage for 95% of the target genes.	55	1
Deden 2020[52]	2016-2020	Netherlands	Radboud University Medical Center, Radboud Institute for Health Sciences	Prospective	Fetal structural anomalies suspicious for genetic cause detected by ultrasound	Fetal materials derived from a pregnancy that had ended in fetal death, or from a termination of	Trio ES, DNA library preparation was performed using SureSelect	54	1

						pregnancy (TOP) were not included in this study.	QXT in combination with the Sure Select All Human Exon Kit (v5, Agilent), followed by 2x150bp paired-end sequencing on a NextSeq500 (Illumina). Sequence coverage was 200 to 300×.		
Qi 2020[53]	2016-2019	China	Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences	Prospective	(1) Singleton pregnancy and a fetus with at least one ultrasonic structural anomaly; (2) fetal sample was obtained through an invasive procedure, including chorionic villus sampling (CVS), amniocentesis or cordocentesis; (3) prenatal genetic diagnosis including karyotyping, CMA and trio-based CES was performed in parallel; (4) all of the above-mentioned testing were performed on each prenatal sample successfully; and (5) karyotyping results were normal.	(1) Parents that refused to accept the procedure of genetic analysis simultaneously; and (2) abnormal karyotype results.	Trio ES, libraries of genomic DNA samples were prepared using the Agilent Sureselect Human All Exon v5 kit (Agilent Technologies, Santa Clara, CA, USA), and were sequenced on a HiSeq instrument (Illumina, San Diego, CA, USA. The average	83	1

							coverage depth was about 80–100×.		
Rinaldi 2020[54]	2016-2018	Belgium	University Hospitals Leuven	Retrospective	Fetal malformation or a severe condition on US (e.g., growth restriction, absence of spontaneous movements), negative 1st-tier genetic testing during the pregnancy, couples planning a new pregnancy.	Not reported	Trio ES. "Library preparation was performed using TruSeq DNA Sample Preparation Kit (Illumina, CA, USA) whereas for library amplification and paired-end sequencing we used a Hiseq2500 (Illumina, CA, USA)."	29	1
He 2021[55]	2017-2019	China	The First Affiliated Hospital of Sun Yat-sen University	Prospective	Singleton pregnancies: "Fetuses with structural anomalies detected by prenatal ultrasound	Cases with anomalies in the first trimester and fetuses with aneuploidies or CNVs were excluded. Fetuses with a known family history of genetic mutation or a known infection or exposure to a known teratogenic drug were excluded.	Trio and proband ES. "The DNA libraries, after enrichment and purification, were sequenced on a NovaSeq 6000 sequencer according to the manufacturer's	94	2

							instructions (Illumina, San Diego, CA, United States)		
Lei 2021[56]	2017-2019	China	Chong Qing Health Center for Women and Children	Retrospective	Fetuses with ultrasound scanning anomalies	Fetuses with skeletal anomalies	Trio ES, Sample dilution and flow-cell loading sequencing were performed according to Illumina specifications. DNA libraries were sequenced on the Novaseq (Illumina, San Diego, CA, USA) with 150-bp paired ends.	85	2
Tolusso 2021[32]	2015-2019	USA	Cincinnati Children's Hospital Medical Center	Retrospective	Patients evaluated in our fetal care center who had ES ordered during pregnancy or after a fetal demise or termination of pregnancy" "fetus had congenital anomalies thought to be caused by an underlying genetic disorder but for which multigene panels were not felt to be suitable	Patients pregnant at the time of chart review	Not reported	20	1

Abbreviations: ACC, agenesis of corpus callosum; CES, clinical exome sequencing; CNS, central nervous system; CNV, copy number variation; CMA, chromosomal microarray analysis; DNA, deoxyribonucleic acid; ES, exome sequencing; EUE, expert ultrasound examination; NR, not reported; rES, rapid exome sequencing; SNP, single nucleotide polymorphisms; US, ultrasound; WES, whole exome sequencing.

Table 2. Phenotype associations by gene

Gene	Paper	Variant	Isolated ACC, ACC with other cranial anomalies, ACC with extracranial anomalies, or Non-specified	Phenotype /Syndrome
TUBA1A	Lei 2021	c.1169G>C chr12-49578980 p.R390P	**ACC with extracranial anomalies**	Lissencephaly type 3
	Heide 2020	c.832G>C, p.(Ala278Pro)	**ACC with extracranial anomalies**	Lissencephaly type 3
	Deden 2020	c.1285G>A; p.(Glu429Lys)	**ACC with other cranial anomalies**	Lissencephaly type 3
	Yaron 2022	c.878A>G (p.Asn293Ser)	**ACC with other cranial anomalies**	Tubilinopathy
		c.1105G>A (p.Ala369Thr)	**ACC with other cranial anomalies**	Tubilinopathy
	Boissel 2017	c.55G>A (p.A19T)	**ACC with other cranial anomalies**	Severe microlissencephaly with absence of commissures, basal ganglia and thalami

	Petrovski 2019	Not available	**ACC with other cranial anomalies**	Agenesis of corpus callosum, severe bilateral ventriculomegaly, kinking of brainstem, absent cerebellum
L1CAM	Yaron 2022	c.3581C>T (p.Ser1194Leu)	**ACC with extracranial anomalies**	L1 Syndrome
	Petrovski 2019	c.1417C>T p.(Arg473Cys)	**Isolated ACC**	L1 Syndrome
	Lei 2022	c.2254G>A p.(Val752Met)	**Isolated ACC**	X-linked Hydrocephaly
		c.176C>T p. (Ala59Val)	**Isolated ACC**	X-linked Hydrocephaly
	Tan 2020	c.1322delG p.G441Afs*72	**ACC with other cranial anomalies**	Agenesis of corpus callosum, bilateral hydrocephalus, and third ventricular dilatation
		c.551G > A p.R184Q	**ACC with other cranial anomalies**	MASA Syndrome
FGFR2	He 2021	c.755C>G, p.Ser252Trp	**ACC with extracranial anomalies**	Apert Syndrome
		c.755C>G, p.Ser252Trp	**ACC with extracranial anomalies**	Apert Syndrome
	Lei 2022	c.755C>G p. (Ser252Trp)	**ACC with extracranial anomalies**	Apert Syndrome

		c.755C>G p. (Ser252Trp)	**ACC with extracranial anomalies**	Apert Syndrome
	Meier 2019	c.[755C>G], p.(s252W)	**ACC with extracranial anomalies**	Apert Syndrome
ARID1B	Heide 2020	c.4129C>T, p.(Arg1377*)	**Isolated ACC**	Coffin-Siris Syndrome
	She 2021	c.1601_1605delACCCT (p.N534TfsX117)	**Isolated ACC**	Coffin-Siris Syndrome
	Yaron 2022	c.1636_1637	**ACC with extracranial anomalies**	Coffin-Siris Syndrome
	Lei 2022	c.316_317insTGTA p.(Gln107TyrfsTer126)	**Isolated ACC**	Coffin-Siris Syndrome
ARX	Lei 2022	c.994C>G p. (Arg332Gly)	**ACC with other cranial anomalies**	Proud Syndrome, Hydranencephaly with abnormal genitalia, Lissencephaly, X-linked 2
	Reches 2018	c.994C>T; p.Arg332Cys	**ACC with other cranial anomalies**	Agenesis of corpus callosum, heterotopia and an interhemispheric cyst
	Lefebvre 2020	c.1374_1383del p.(Pro459*)	**ACC with extracranial anomalies**	Hydranencephaly with abnormal genitalia, Lissencephaly, X-linked 2
COL4A1	Yaron 2022	c.1186C>T (p.Arg396*)	**ACC with other cranial anomalies**	COL4A1-related

		c.2086G>A (p.Gly696Ser)	**ACC with other cranial anomalies**	COL4A1-related
		c.388-1G>C	**ACC with other cranial anomalies**	Brain small vessel disease 1 with or without ocular anomalies
EPG5	De Koning 2021	c.5631del: p.(Ser1879Alafs*12)	**ACC with extracranial anomalies**	Vici Syndrome
	Aggarwal 2019	c.4665del; p.Glu1555Aspfs*12	**Isolated ACC**	Vici Syndrome
	Qi 2020	c.2461C>T(p.R821*);Het, c.88C>T(p.Q30*); Het	**Isolated ACC**	Vici Syndrome
PEX1	Boissel 2017	c.3205C>T;p.(Gln1069*) c.2097dup; p.(Ile700Tyrfs*42)	**ACC with other cranial anomalies**	Thin corpus callosum, microcephaly, ventriculomegaly, polymicrogyria and heterotopia in both cerebral and cerebellar hemispheres
	Normand 2018	c.2097dupT;(p.I700fs) c.3205C>T;(p.Q1069X)	Non-specified	Non-specified
	Aggrawal 2019	c.1670+1G>A	**ACC with extracranial anomalies**	Zellweger Syndrome

TUBB	Yaron 2022	c.947T>C (p.Val316Ala)	**ACC with other cranial anomalies**	Tubulinopathy
	Boissel 2017	c.920C>T (p.P307L)	**ACC with other cranial anomalies**	Microlissencephaly, agenesis of the corpus callosum, dysmorphic basal ganglia, cerebellar hypoplasia, and circumferential skin creases. Glomerular structures and a voluminous germinal area in cortex.
	Lord 2019	c.860C>T, p.(Pro287Leu)	**ACC with other cranial anomalies**	Dysgenesis of the corpus callosum and lissencephaly
ZEB2	De Wit 2017	c.2403C>G (p.(Tyr801*)	**ACC with extracranial anomalies**	Mowat Wilson Syndrome
	Heide 2020	2q22.2q22.3	**ACC with extracranial anomalies**	Mowat Wilson Syndrome
	De Koning 2021	c.786dup: p. (His263Thrfs*17)	**Isolated ACC**	Mowat Wilson Syndrome

Table 3a. Phenotypic Expression of Genetic Variants in isolated ACC cases

Gene	Number of Cases	Phenotype/Syndrome
ARID1B	3	Coffin-Siris Syndrome
L1CAM	3	L1 Syndrome, X-linked Hydrocephaly
EPG5	2	Vici Syndrome
NFIA	2	Brain Malformations with or without urinary defects
ALDH7A1	1	Non-specified (She)
AP4M1	1	Spastic Paraplegia 50, autosomal recessive
EXOSC3	1	Pontocerebellar hypoplasia, type 1B
KANSL1	1	Koolen de Vries syndrome
KCNQ2	1	Non-specified (Petrovski)
PPP2R1A	1	Mental Retardation, Autosomal Dominant 36
PTCH1	1	Non-specified (Petrovski)
PTDSS1	1	Lenz-Majewski Hyperostotic Dwarfism
PTPN11	1	Noonan syndrome
SCN2A	1	Seizures, benign familial infantile 3, developmental and epileptic encephalopathy 11
SHH	1	Non-specified (Petrovski)
SON	1	ZTTK Syndrome
TUBB2B	1	Cortical Dysplasia, complex, with other brain malformations 7

ZBTB20	1	Primrose Syndrome
ZEB2	1	Mowat–Wilson syndrome
Genes are arranged by the number of cases and then alphabetically		

Table 3b. Phenotypic Expression of Genetic Variants in cases of ACC with other cranial anomalies

Gene	Number of Cases	Phenotype/Syndrome
TUBA1A	6	Lissencephaly Type 3, Tubulinopathy,
COL4A1	3	COL4A1-related (2), Brain small vessel disease 1
TUBB	3	Tubulinopathy, lissencephaly
ARX	2	Proud Syndrome, Hydranencephaly with abnormal genitalia, Lissencephaly, X-linked 2
L1CAM	2	MASA syndrome, hydrocephalus due to aqueductal stenosis
OFD1	2	X-linked Dominant Orofacial Digital Syndrome Type 1, orofaciodigital syndrome 2
ADCY5	1	Dyskinesia with orofacial involvement, autosomal dominant
ASPM	1	Microcephaly 5
ATRX	1	Alpha-thalassemia/mental retardation syndrome
BRPF1, RTTN	1 case with 2 mutations	Intellectual Developmental Disorder with dysmorphic facies and ptosis; microcephaly, short stature and polymicrogyria with seizures
CLTC	1	Mental retardation, AD 56
COL4A2	1	Brain small vessel disease 2
EBP	1	MEND syndrome
EFNB1	1	Complete Agenesis of Corpus Callosum
FOXG1	1	Non-specified (Yaron)
GFAP	1	Alexander Disease

GPSM2(CHET)	1	Non-specified (Petrovski)
GRIN2B	1	Non-specified (Tan)
KIAA0586	1	Joubert Syndrome type 23
LAMA1	1	Poretti–Boltshauser syndrome
MED12	1	Complete Agenesis of Corpus Callosum
NBN	1	Nijmegen Breakage syndrome
PDHA1	1	Non-specified (Boissel)
PEX1	1	Non-specified (Boissel)
POMGNT2	1	Muscle-eye-brain (yaron)
POMT1	1	Walker Warburg syndrome
RAC1	1	Dandy-Walker malformation, Intrauterine growth restriction
TMEM67	1	Joubert type 6/ Meckel type 3
TUBB3	1	Non-specified (Boissel)
Genes are arranged by the number of cases and then alphabetically		

Table 3c. Phenotypic Expression of Genetic Variants in cases of ACC with extracranial anomalies

Gene	Number of Cases	Phenotype/Syndrome
FGFR2	5	Apert Syndrome
ZEB2	2	Mowat Wilson Syndrome
ACTG1	1	Baraitser-Winter Syndrome
ACVR1	1	Firbodysplasia Ossificans Progressiva
AHI1	1	Joubert syndrome-3
ALDH18A1	1	Cutis laxa, autosomal recessive, type IIIA
AMPD2	1	Pontocerebellar Hypoplasia Type 9
ARID1A	1	Coffin-Siris Syndrome 2
ARID1B	1	Coffin-Siris Syndrome
ARX	1	Hydranencephaly with abnormal genitalia, Lissencephaly, X-linked 2
ASXL3	1	Bainbridge-Ropers Syndrome
B3GLCT	1	Peters-plus syndrome
BRAT1	1	Rigidity and Multifocal Seizure Syndrome
CPT2	1	CPT II Deficiency
Dcorpus callosum	1	Mirror movements 1
EPG5	1	Vici Syndrome

ERcorpus callosum2	1	cerebro-oculo-facio-skeletal syndrome 2 (COFS2)
GLI3, EPHB4	1 case with 2 mutations	Greig cephalopolysyndactyly syndrome, Capillary Malformation - Arteriovenous Malformation Type 2
KANSL1	1	Koolen de Vries syndrome
KIF1A	1	Mental retardation, autosomal dominant 9
KIF14	1	Non-specified (Meier)
L1CAM	1	L1 Syndrome
MED12	1	Opitz-Kaveggia Syndrome, Ohdo syndrome
MRPS16	1	Non-specified (Shamseldin)
MYBPC3	1	Hypertrophic Cardiomyopathy
MYCN	1	Non-specified (Lord)
NOTCH3	1	Lateral Meningocele syndrome
PEX1	1	Zellweger Syndrome
RXYLT1	1	Congenital Muscular Dystrophy-dystroglycanopathy with brain and eye anomalies type A10
SHROOM4	1	Stocco Dos Santos X-linked Mental Retardation Syndrome
SMC3	1	Cornelia de Lange Syndrome
SMARCE1	1	Coffin-Siris Syndrome 5
STAG2	1	X-linked neurodevelopmental disorder with craniofacial abnormalities (NEDXCF)
TAPT1	1	Osteochondrodysplasia
TCF12	1	Craniosynostosis 3

TCF4	1	Pitt-Hopkins Syndrome
TCTN2	1	Meckel–Gruber type 8 syndrome
TUBA1A	1	Lissencephaly Type 3
TUBB3	1	Non-specified (Reches)
Genes are arranged by the number of cases and then alphabetically		

Table 4: Aggregate types of prenatal ACC and incremental increase in diagnostic yield with exome sequencing

Variable	Studies (n)	ES Positive (n)	Total ES (n)	Pooled Proportion % (95% CI)	I^2 (%)
Total ACC	15	100	267	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

ES, exome sequencing; CMA, chromosomal microarray analysis; CI, confidence interval; ACC, agenesis of corpus callosum; * ACC is the only brain finding

PRISMA flowchart of the search and selection process

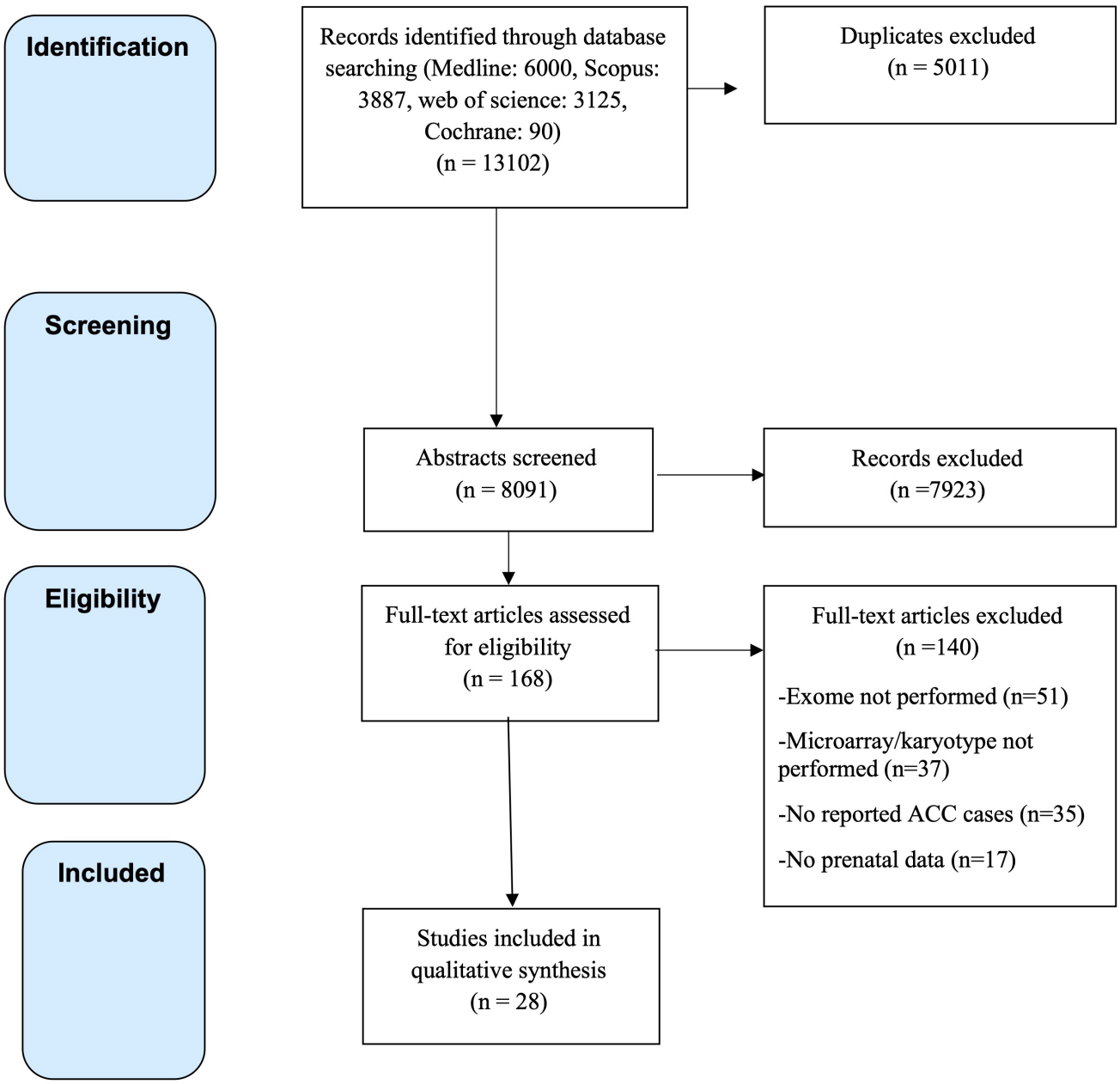
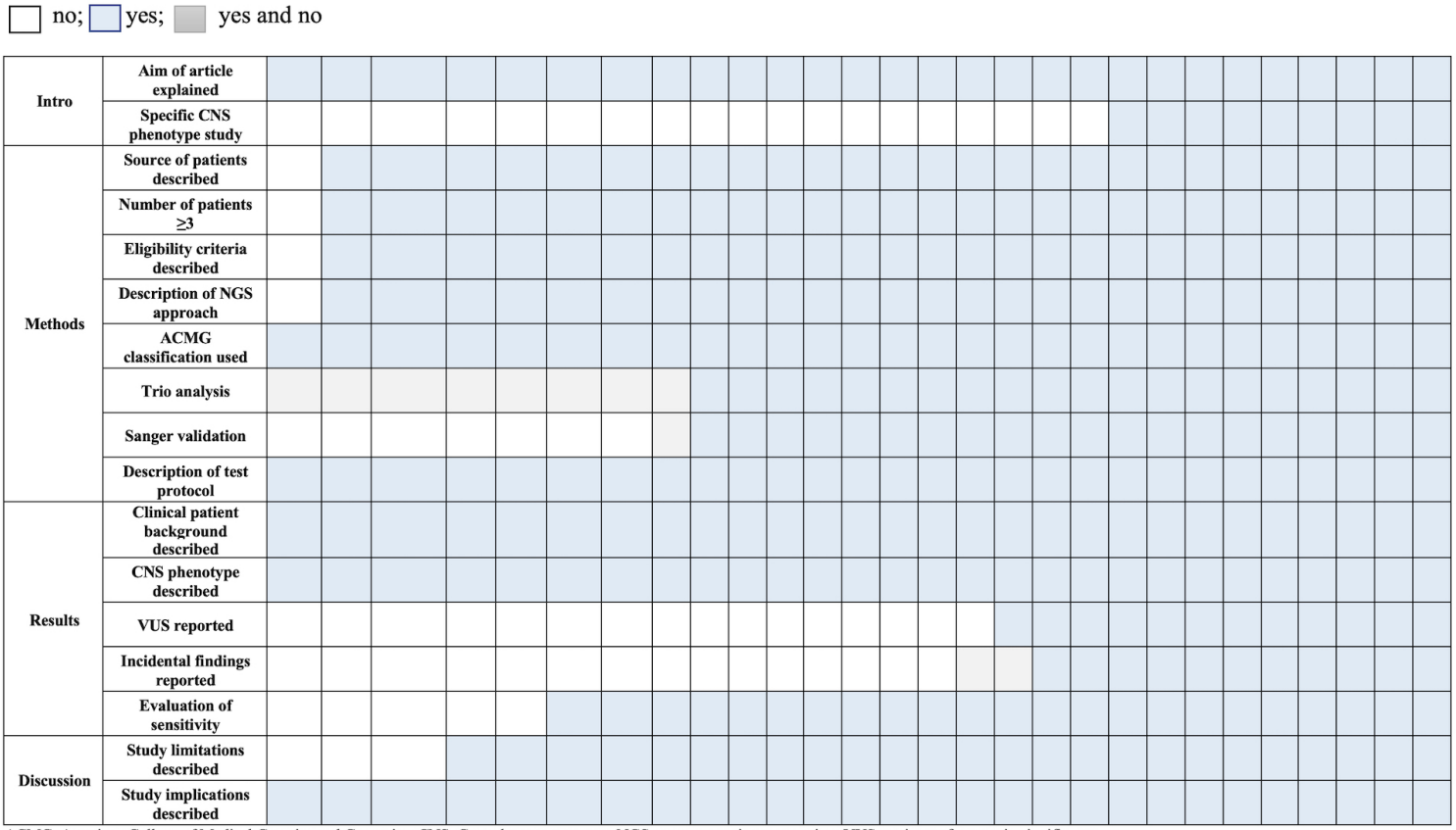


Fig 1_PRISMA flowchart of the search and selection process.jpg



ACMG, American College of Medical Genetics and Genomics; CNS, Central nervous system; NGS, next-generation sequencing; VUS, variants of uncertain significance.

Fig 2_STARD.jpg