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Fetal central nervous system anomalies

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Title: Fetal central nervous system anomalies: When should we offer exome sequencing?

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What's already known about this topic?

- In fetuses with a structural abnormality and normal karyotype and chromosomal microarray, exome sequencing can provide additional diagnostic yield.
- The role of exome sequencing in fetuses with anomalies specific to the central nervous system remains unclear.

What does this study add?

- This study supports the importance of including whole exome sequencing in the workup of fetuses affected by CNS anomalies, even if the CNS anomaly is found in isolation.

Data Sharing Statement

- The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abstract

Objective

To investigate the detection of pathogenic variants using exome sequencing in an international cohort of fetuses with central nervous system (CNS) anomalies.

Methods

We reviewed trio exome sequencing (ES) results for two previously reported unselected cohorts (Prenatal Assessment of Genomes and Exomes (PAGE) and CUIMC) to identify fetuses with CNS anomalies with unremarkable karyotypes and chromosomal microarrays. Variants were classified according to ACMG guidelines and association of pathogenic variants with specific types of CNS anomalies explored.

Results

ES was performed in 268 pregnancies with a CNS anomaly identified using prenatal ultrasound. Of those with an isolated, single, CNS anomaly, 7/97 (7.2%) had a likely pathogenic/pathogenic (LP/P) variant. This includes 3/23 (13%) fetuses with isolated mild ventriculomegaly and 3/10 (30%) fetuses with isolated agenesis of the corpus callosum.

Where there were multiple anomalies within the CNS, 12/63 (19%) had LP/P variants. Of the 108 cases with CNS and other organ system anomalies, 18 (16.7%) had LP/P findings.

Conclusion

ES is an important tool in the prenatal evaluation of fetuses with any CNS anomaly. The rate of LP/P variants tends to be highest in fetuses with multiple CNS anomalies and multisystem anomalies, however, ES may also be of benefit for isolated CNS anomalies as well. ~~Compared to those with a single CNS anomaly, the rate of P/LP causative variants is higher with multiple CNS anomalies and those with anomalies in additional organ systems.~~

Introduction

Malformations of the central nervous system (CNS) are commonly diagnosed on prenatal ultrasound but, even with additional imaging to define the abnormality, giving parents an accurate prognosis can be difficult because of the variable association with underlying genetic etiologies and the immaturity of the brain *in utero.*[1] Karyotyping and microarray testing can identify pathogenic chromosomal changes in around 20-40% of fetuses with sonographic anomalies[2,-4] and the advent of next generation sequencing has now enabled rapid diagnosis of underlying monogenic conditions.[5-9] in chromosomally normal fetuses. However, diagnostic rates vary widely across phenotypes[9-11] and prescreening with genetic review to select cases most likely to have a monogenic etiology has been shown to increase diagnostic yield.[12] In two large, prospective studies of unselected fetuses with any structural abnormality and normal chromosomes and microarrays, ES provided a diagnosis in 8 - 10% of cases.[10,11] In fetuses with anomalies in the CNS, between 5 – 22% were found to have diagnostic genetic variants.[10,11] However, these studies did not publish the full details on the CNS anomalies diagnosed.

As costs decrease and availability, as well as ~~and~~ speed of sequencing increase, an evidence-based approach would help manage patients with prenatally diagnosed CNS anomalies. Here we reviewed the extended datasets from the United Kingdom Prenatal Assessment of Genomes and Exomes (PAGE) and U.S. Columbia (CUIMC) exome studies to identify all cases presenting with isolated CNS anomalies, complex CNS anomalies, and CNS findings in the setting of multiple anomalies, aiming to further delineate which fetuses would benefit most from prenatal exome sequencing.

Methods

This is an expanded review of two previously published prospectively collected cohort studies of fetuses presenting with a CNS anomaly diagnosed on ultrasound and recruited to the UK PAGE[10] and US CUIMC[11] fetal exome sequencing studies. In these

studies, both cohorts were sequentially recruited based only on the presence of at least one structural anomaly of any system. Some, but not all, of these cases have been previously reported.[10,11]

PAGE Study

From the PAGE study we reviewed a total of 876 fetuses and 1727 matched parental samples (851 fetus-parent trios and 25 fetus-parent duos), of which 610 cases (596 trios and 14 duos) have previously been reported.[10] Study methodology and eligibility criteria were as previously published. Couples undergoing invasive testing for any ultrasound identified fetal abnormality were consented for exome sequencing when fetal karyotype and chromosomal microarray (CMA) were normal or non-causative. Exome sequencing was performed with analysis targeted to a virtual panel of 1628 genes associated with developmental disorders[10] for all cases discussed here.

CUIMC Study

CUIMC recruited a total of 494 fetuses with matched parental samples, of which 234 trios have been previously reported.[11] Pregnancies complicated by any fetal abnormality were offered participation in the study following invasive testing or collection of a cord sample after birth. Untargeted trio WES was performed when karyotype/CMA was non-causative of the anomaly. The bioinformatic analysis is described in a previous publication.[11]

For both cohorts, LP/P variants considered causative of the phenotype were disclosed to the families and providers. Secondary findings were disclosed according to ACMG guidelines.

Variant interpretation

In the PAGE study whole exome sequencing was performed and sequencing data was analyzed for candidate P/LP variants from a modified list or panel of genes that are likely to be associated with developmental disorders.[30] The Columbia group performed

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untargeted exome sequencing rather than using panel of genes. In both studies, a multidisciplinary clinical review panel (MCRP) consisting of relevant clinicians and scientists reviewed candidate pathogenic variants. Pathogenic variants or likely pathogenic variants that explained the fetal phenotype were classified according to American College of Medical Genetics and Genomics (ACMG) guidelines,[7] orthogonally confirmed using Sanger sequencing, and reported to parents.[10,11]

Procedures

Review of the study databases was undertaken to identify cases presenting with any CNS anomaly whether in isolation or in combination with other anomalies. Clinical information was manually reviewed, including the phenotypes recorded in the study databases and ultrasound scan reports at presentation if available. If an MRI was performed and the report was available, this was also reviewed.

Due to the multifactorial inheritance pattern of non-syndromic neural tube defects, and the contribution of environmental factors, cases with isolated open neural tube defects were excluded.[13] Recognized CNS sequelae of open neural tube defects were not counted as separate anomalies. For example, if a fetus had ventriculomegaly, a Chiari malformation and a myelomeningocele, this was categorized as an isolated neural tube defect and excluded.

Following manual review of the dataset, each case was categorized into 1) a single, isolated CNS anomaly (e.g isolated ventriculomegaly), 2) multiple CNS anomalies (e.g ventriculomegaly, an interhemispheric cyst and parenchymal defects) or 3) a CNS anomaly with extra-CNS findings (e.g. ventriculomegaly and a cardiac defect). If there was a discrepancy between the MRI and ultrasound, the MRI findings were used for classification. Ventriculomegaly was classified based on the lateral ventricular diameter as mild (10–12 mm), moderate (12–15 mm), or severe (> 15 mm).

Cases with multiple CNS anomalies were then reviewed by a pediatric neurologist to ensure that our categorization was accurate. For example, a case initially categorized as multiple CNS anomalies where the fetus had mild ventriculomegaly and agenesis of the corpus callosum (ACC) was re-categorized as an isolated, single finding of ACC as

the ventriculomegaly represents colpocephaly, part of the ACC anomaly. When possible, images were reviewed to clarify classification.

For all cases, further ultrasound reports and clinical information from later in pregnancy were reviewed, however the findings of the initial referral were used to categorize cases. Pregnancy outcomes, and postnatal clinical information or post-mortem findings were ascertained when this information was available. However, the majority of pregnancies were ultimately managed by the local, referring providers and thus outcome data was not available.

Outcomes

All variants were classified according to ACMG guidelines and the rate of pathogenic (P) or likely pathogenic (LP) genetic variants in the different categories was assessed. We calculated rates of LP/P variants that were considered causative of the phenotype for fetuses with, 1) a single, isolated CNS anomaly including isolated ventriculomegaly, 2) multiple CNS anomalies, 3) a CNS anomaly as well as an anomaly in another organ system.

Results

In total, 268 fetuses with anomalies of the central nervous system were identified; 97 were classified as single, isolated findings, while 63 had multiple anomalies within the CNS, and 108 also had multiple organ system anomalies. The average gestational age at the time of enrollment was 22 weeks. MRI was performed on 56 fetuses, of which 24/56 (43%) cases had discrepant or additional findings compared to ultrasound. Pregnancy outcome data is missing for 62 cases (23.1%). Of the 206 with known outcomes, 112 pregnancies were terminated, 86 delivered a liveborn baby, and eight were stillborn or died in the neonatal period.

Of the 268 sequenced fetuses, a total of 37 (13.8%), had a pathogenic or likely pathogenic genetic variant that was considered causative of the fetal phenotype (Figure 1, Table 1). One of these variants was diagnosed in a monochorionic, diamniotic twin gestation. This was considered as a single case. In addition, there were 10 other LP/P

variants identified but their contribution to the phenotype was uncertain and thus they were not reported.

Of the 268 cases in the cohort, 173 (65%) came from the PAGE cases overall, and 95 (35%) came from the Columbia group. The overall diagnostic rate for the PAGE group was 10.4% (18/173) and for the Columbia group was 20% (19/95). Although the diagnostic rates are different, every gene diagnosed in the Columbia cohort was included on the PAGE panel and thus the difference in detection is due to the difference in cases themselves rather than the approach to sequencing.

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Fetuses with an isolated, single CNS anomaly

Ninety-seven fetuses had an isolated, single anomaly in the CNS. Causative pathogenic or likely pathogenic variants were found in 7 (7.2%) (Figure 2). The most common isolated finding was isolated mild ventriculomegaly, seen in 23 fetuses, of which three (13.0%) had pathogenic or likely pathogenic findings on ES. The isolated finding with the highest likelihood of having a finding on ES was agenesis of the corpus callosum where 30% (3/10) had pathogenic or likely pathogenic variants. Table 1 details the pathogenic findings found in fetuses with isolated CNS anomalies.

The three pathogenic variants in cases of isolated mild ventriculomegaly were in the *CHD7*, *B3GLCT* and *ARID1A* genes. The *CHD7* variant, associated with CHARGE syndrome was a *de novo* variant. In the original PAGE study this was initially reported as 'potentially clinically relevant' because the contribution to the phenotype which commonly includes choanal atresia, malformations of the heart, inner ear and retina,[14] was uncertain based on prenatal imaging. Follow-up at 7 months of age revealed bilateral colobomas and left renal agenesis. A post-natal MRI confirmed bilateral, mild ventriculomegaly (11-12mm) and the clinical review panel thus reclassified the variant as clinically relevant.

The *B3GLCT* gene variants causes Peters plus syndrome which is characterized by eye abnormalities, short stature, intellectual disability, ventriculomegaly and distinctive facies.[15] This case had compound heterozygous, autosomal recessive inheritance and the family had terminated a prior pregnancy due to ventriculomegaly.

The third case of isolated ventriculomegaly which occurred in a MC/DA gestation had a *de novo* pathogenic variant in the *ARID1A* gene, consistent with Coffin -Siris syndrome. [16] Prenatal ultrasounds demonstrated mild, bilateral ventriculomegaly in both twins which remained stable throughout pregnancy.

Thirty percent of fetuses with isolated agenesis of the corpus callosum had a pathogenic variant (*L1CAM, SHH,* and *PTCH1)*. The *L1CAM* variant was inherited from an unaffected mother who had previous unexplained, pregnancies with CNS anomalies. The *SHH* variant arose *de novo and* the *PTCH1* gene variant was inherited from an affected father whose disease status was not known to the clinical or research teams at the time. Postnatally, the diagnosis of Gorlin syndrome was confirmed clinically. The father had been diagnosed with Gorlin syndrome in childhood but had not disclosed this.

Fetuses with multiple CNS anomalies:

There were 63 fetuses with multiple CNS anomalies, 12 of whom (19.0%), had pathogenic or likely pathogenic findings (Table 1).

Fetuses with anomalies in multiple organ systems

Of the 108 cases with anomalies in multiple organ systems, 18 (16.7%) had causative pathogenic or likely pathogenic findings. There were 53 cases where the CNS and one other organ system was involved. Fetuses with an anomaly in the CNS and renal or genitourinary system were most likely to have pathogenic findings.

Pathogenic/likely pathogenic genetic variants and their inheritance pattern

Fifty four percent (20/37) of variants were inherited from one or both parents. Of these, 17 were autosomal recessive, one was X-linked recessive and one was autosomal dominant. Forty six percent (17/37) of variants were *de novo*, 15 of which were autosomal dominant and 2 of which were X-linked dominant.

Discussion

Principal findings

In a prospective cohort of pregnancies with unselected fetal central nervous system anomalies, in which karyotype and CMA were normal or non-causative, exome sequencing revealed a likely pathogenic / pathogenic variant that was considered causative of the fetal phenotype in 13.8% (37 / 268) of cases. A diagnosis was more than twice as likely in fetuses with multiple CNS anomalies (ES diagnostic rate of 19.0%) or additional extra-CNS anomalies (ES diagnostic rate of 16.7%) compared to fetuses with a single isolated anomaly in the CNS (ES diagnostic rate 7.2%), which is in keeping with other studies showing higher rates of pathogenic variants where there are multisystem abnormalities.[10,11] Over half (54%) of the genetic variants detected were inherited, one of which was autosomal dominant, 17 were recessive and one x-linked. *De novo* P/LP variants accounted for just under half (46%).

Three cases with isolated, mild ventriculomegaly had pathogenic findings on ES. Current professional bodies suggest providing families with reassurance in the setting of isolated mild ventriculomegaly if they have had a normal karyotype and microarray.[18] Under the current guidelines, these three families would have likely received somewhat inaccurate prenatal counseling. The three pathogenic variants were in the *CHD7*, *B3GLCT* and *ARID1A* genes. It is of note that additional, subtle abnormalities not particularly amenable to sonographic diagnosis were diagnosed after birth in the case with CHARGE syndrome demonstrating the limitations of prenatal phenotyping.[19] Detailed phenotyping of neurological changes is challenging prenatally as CNS development continues throughout pregnancy and into the postnatal period. Whilst MRI can refine the diagnosis of some anomalies[1] even when performed, the natural history of many conditions is such that changes may not be detected until late in gestation.

Clinical implications

The rate of pathogenic findings on ES in 13.8% of cases with CNS anomalies falls within the wide range published in the existing literature from 3-55%.[17, 20-22] Unsurprisingly, the diagnostic yield of ES in our cohort of unselected fetuses is lower than cohort of selected fetuses with severe anomalies or in children with postnatal referrals to medical genetics where 24-25% have genetic diagnoses.[23,24]

Our findings highlight the importance of considering ES even when a minor CNS anomaly, such as mild ventriculomegaly or ACC, is found in isolation. Current guidelines from the Society of Maternal Fetal Medicine recommend providing reassurance in the setting of isolated ventriculomegaly if genetic testing is unremarkable.[18] Our finding that 13% of fetuses with isolated, mild VM had a finding on ES highlights the importance of offering ES in the genetic workup of these fetuses prior to providing reassurance.

Our finding that 30% of cases with isolated agenesis of the corpus callosum had a pathogenic, causative variant on ES is higher than one previous literature report[22], but in keeping with the report from Lei and colleagues who reported pathogenic variants in 29% of cases with isolated ACC.[25] In the series looking at 65 fetuses with agenesis of the corpus callosum, 15% of fetuses with isolated ACC had pathogenic variants on ES compared to 42% of fetuses with non-isolated ACC.[22] Of note, in this series, almost 15% of fetuses that were initially diagnosed with isolated ACC subsequently had additional anomalies diagnosed. In our series, 2 out of the 3 cases of isolated ACC terminated the pregnancy during the second trimester. It is possible that other anomalies may been picked up later in pregnancy or postnatally which could have contributed to our higher rate of ES findings. However, ours is a small series and further studies are required to confirm the rate of LP/P variants associated with isolated ACC.

Our data support the use of ES if a fetal CNS anomaly is diagnosed prenatally and traditional genetic testing is not informative since identifying a genetic etiology can provide families and care givers improved insight into the long- and short-term course of the child as well as its risk of recurrence. In 2020, the ACMG stated that one can consider ES in a fetus with one or more significant anomaly(ies) when routine prenatal methods such as karyotype and chromosomal microarray are negative. In 2021, ACMG further recommended that in the pediatric population, exome or genome sequencing be considered as a first- or second-tier test for patients with congenital anomalies.[26]. The International Society of Prenatal Diagnosis and the Royal College of Obstetricians in the UK suggest that sequencing can be useful in the presence of fetal abnormalities when other genetic tests are normal yet The American College of Obstetrician Gynecologists

and the Society for Maternal Fetal Medicine still do not recommend ES in routine prenatal diagnosis.

~~Our findings highlight the importance of considering ES even when a minor CNS anomaly, such as mild ventriculomegaly or ACC, is found in isolation.~~ Recently in the UK, prenatal ES was introduced by the NHS into clinical practice and is indicated for fetuses with multiple major structural abnormalities where a monogenic cause is considered likely. This would include major CNS anomalies, but exclude isolated mild ventriculomegaly.[27,28]

Regardless of governing body recommendations, in the prenatal period the time and cost associated with ES presents challenges in choosing appropriate patients for testing. This is further complicated by the incomplete phenotyping available prenatally which could exclude appropriate candidates.[29]

Research implications

Further use of ES in both the prenatal and postnatal setting with assimilation of both genotypes and phenotypes into large data repositories is required to expand the experience of single centers and 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. Further research may also focus on the patient experience of undergoing ES during pregnancy, the impact on provider healthcare utilization and patient outcomes, and the impact on decision making for future pregnancies and family planning.

Strengths and limitations

This is the largest cohort of fetuses with unselected CNS anomalies that have undergone ES in the literature to date. The prospectively collected nature of the study allowed the pregnancies to follow their natural histories making this study relevant to clinical practice where rapid ES may be considered in an ongoing pregnancy.

Although the overall cohort is the largest in the literature to date, the sample size of each specific anomaly remains small which limits the generalizability of our findings. The varied interpretations of whether a constellation of CNS anomalies is actually representative of one anomaly or multiple CNS anomalies may also limit comparison of our results to other cohorts. And, as always, working within the confines of the prenatal phenotype limits the interpretation of prenatal ES.

Conclusion

ES for prenatally detected CNS anomalies yields a genetic diagnosis in almost 14% of pregnancies that have had a negative genetic workup through traditional karyotype and chromosomal microarray. When a CNS anomaly is found in isolation, exome sequencing reveals an overall genetic diagnosis in approximately 7% of fetuses with higher rates in some isolated findings such as mild ventriculomegaly or ACC similar to rates found by others.[22,25]

Securing a genetic diagnosis helps families understand the current pathology and prognosis as well as facilitates planning for future pregnancies. Although limitations remain, including cumbersome interpretation of results and time and cost restraints, ES adds utility to the workup of anomalous pregnancies. With time, these limitations will diminish. Given its utility, we envision that ES will become an important part of the armamentarium of maternal fetal medicine specialists, reproductive geneticists, and genetic counselors in the near future. However, for this to become reality in many health services, costs will need to fall further and health professional and patient education will be required. Similarly, this will require access to laboratories accredited and prepared to deliver prenatal exome sequencing.[29]

References

1. Van den Veyver IB. Prenatally diagnosed developmental abnormalities of the central nervous system and genetic syndromes: A practical review. Prenat Diagn. 2019;39:666-678.

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While the sequencing approaches were different, the interpretation and reporting protocols were the same in both studies (ie only reporting P/LP variants in genes that explain the fetal phenotype). We do not feel that the different sequencing approaches have influenced the overall results.

2. van Zalen-Sprock MM, van Vugt JM, Karsdorp VH, et al. Ultrasound diagnosis of fetal abnormalities and cytogenetic evaluation. Prenat Diagn. 1991;11:655-60.
3. Nicolaides KH, Snijders RJ, Gosden CM, et al. Ultrasonographically detectable markers of fetal chromosomal abnormalities. Lancet. 1992;340(8821):704-7.
4. Wapner RJ, Martin CL, Levy B, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med. 2012;367:2175-84.
5. Best S, Wou K, Vora N, et al. Promises, pitfalls and practicalities of prenatal whole exome sequencing. Prenat Diagn. 2018;38:10-19.
6. Vora NL, Powell B, Brandt A, et al. Prenatal exome sequencing in anomalous fetuses: new opportunities and challenges. Genet Med. 2017;19:1207-1216.
7. 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;17:405-24.
8. Chandler N, Best S, Hayward J, et al. Rapid prenatal diagnosis using targeted exome sequencing: a cohort study to assess feasibility and potential impact on prenatal counseling and pregnancy management. Genet Med. 2018;20:1430-1437.
9. Yang Y, Muzny DM, Xia F, et al. Molecular findings among patients referred for clinical whole-exome sequencing. JAMA. 2014;312:1870-9.
10. Lord J, McMullan DJ, Eberhardt RY, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. Lancet. 2019;393(10173):747–57.
11. Petrovski S, Aggarwal V, Giordano JL, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet. 2019;393(10173):758–67.
12. Normand EA, Braxton A, Nassef S, et al. Clinical exome sequencing for fetuses with ultrasound abnormalities and a suspected Mendelian disorder. Genome Med. 2018;10:74.

13. ACOG Committee on Practice Bulletins-Obstetrics. ACOG practice bulletin. Neural tube defects. Number 187, December 2017. (Replaces committee opinion number 44, July 2003). Int J Gynaecol Obstet. 2017 Vol 130, No 6, Dec 2017.
14. Hsu P, Ma A, Wilson M, et al. CHARGE syndrome: a review. J Paediatr Child Health. 2014;50:504-11.
15. Demir GÜ, Lafcı NG, Doğan ÖA, et al. Peters Plus syndrome: a recognizable clinical entity. Turk J Pediatr. 2020;62:136-140.
16. Schrier Vergano S, Santen G, et al. Coffin-Siris Syndrome. 2013 Apr 4 [updated 2018 Feb 8]. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2020. PMID: 23556151.
17. Fu F, Li R, Li Y, et al. Whole exome sequencing as a diagnostic adjunct to clinical testing in fetuses with structural abnormalities. Ultrasound Obstet Gynecol. 2018;51:493-502.
18. Fox NS, Monteagudo A, Kuller JA, et al. Mild fetal ventriculomegaly: diagnosis, evaluation, and management. Am J Obstet Gynecol. 2018;219:B2-B9.
19. Busa T, Legendre M, Bauge M, et al. Prenatal findings in children with early postnatal diagnosis of CHARGE syndrome. Prenat Diagn. 2016;36:561-7.
20. Reches A, Hiersch L, Simchoni S, et al. Whole-exome sequencing in fetuses with central nervous system abnormalities. J Perinatol. 2018;38:1301-1308.
21. Tan H, Xie Y, Chen F, et al. Novel and recurrent variants identified in fetuses with central nervous system abnormalities by trios-medical exome sequencing. Clin Chim Acta. 2020;510:599-604.
22. Heide S, Spentchian M, Valence S, et al. Prenatal exome sequencing in 65 fetuses with abnormality of the corpus callosum: contribution to further diagnostic delineation. Genet Med. 2020;22:1887-1891.
23. Zhu X, Petrovski S, Xie P, et al. Whole-exome sequencing in undiagnosed genetic diseases: interpreting 119 trios. Genet Med. 2015;17:774-81.
24. Kin Chau MH, Choy KW. The role of chromosomal microarray and exome sequencing in prenatal diagnosis. Curr Opin Obstet Gynecol. 2021;33:148-155.

25. Lei T-Y, She Q, Fu F et al. Prenatal exome sequencing in fetuses with callosal anomalies. Prenat Diagn 2022. 28 January published on-line.
26. Manickam K, McClain MR, Demmer LA, et al. Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021;23:2029-2037.
27. https://www.england.nhs.uk/wp-content/uploads/2018/08/Rare-and-Inherited-Disease-Eligibility-Criteria-November-2020-21.pdf
28. NHS England and NHS Improvement. Guidance document: Rapid Exome Sequencing Service for fetal anomalies testing [Internet]. Available from: http://www.labs.gosh.nhs.uk/media/1396328/guidance_document_rapid_exome_sequencing_service_for_fetal_anomalies_v3.pdf
29. International Society for Prenatal Diagnosis; Society for Maternal and Fetal Medicine; Perinatal Quality Foundation. Joint Position Statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis. Prenat Diagn. 2018;38:6-9.
30. Wright CF, Fitzgerald TW, Jones WD, et al. Genetic diagnosis of developmental disorders in the DDD study: a scalable analysis of genome-wide research data. *Lancet* 2015; **385:** 1305–14.
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Figure and Table Legends

Figure 1. Flowchart of all cases in the cohort and the rates of pathogenic and likely pathogenic variants on exome sequencing that were considered causative of the phenotype.

Figure 2. Flowchart of isolated CNS anomalies and the rates of pathogenic and likely pathogenic variants on exome sequencing that were considered causative of the phenotype.

Table 1. Rates of likely pathogenic variants on exome sequenicng (ES) in fetuses with central nervous system (CNS) anomalies and the genes involved.