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## Monogenic conditions and central nervous system anomalies: A prospective study, systematic review and meta-analysis

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### CONFLICT OF INTEREST STATEMENT

LSC has grant funding from the UK NIHR to evaluate the implementation of fetal sequencing in the English National Health Service. NV received supplies in kind from Illumina for an NIH funded grant on genome sequencing. MDK is a Senior Principal Clinical Scientist in the Medical Genomics Research Group, Illumina, Cambridge, UK.

### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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## Abstract

**Objectives:** Determine the incremental diagnostic yield of prenatal exome sequencing (pES) over chromosome microarray (CMA) or G-banding karyotype in fetuses with central nervous system (CNS) abnormalities.

**Methods:** Data were collected via electronic searches from January 2010 to April 2022 in MEDLINE, Cochrane, Web of Science and EMBASE. The NHS England prenatal exome cohort was also included. Incremental yield was calculated as a pooled value using a random-effects model.

**Results:** Thirty studies were included ( $n = 1583$  cases). The incremental yield with pES for any CNS anomaly was 32% [95% CI 27%–36%;  $I^2 = 72\%$ ]. Subgroup analysis revealed apparent incremental yields in: (a) isolated CNS anomalies; 27% [95% CI 19%–34%;  $I^2 = 74\%$ ]; (b) single CNS anomaly; 16% [95% CI 10%–23%;  $I^2 = 41\%$ ]; (c) more than one CNS anomaly; 31% [95% CI 21%–40%;  $I^2 = 56\%$ ]; and (d) the anatomical subtype with the most optimal yield was Type 1 malformation of cortical development, related to abnormal cell proliferation or apoptosis, incorporating microcephalies, megalencephalies and dysplasia; 40% (22%–57%;  $I^2 = 68\%$ ). The commonest syndromes in isolated cases were Lissencephaly 3 and X-linked hydrocephalus.

**Conclusions:** Prenatal exome sequencing provides a high incremental diagnostic yield in fetuses with CNS abnormalities with optimal yields in cases with multiple CNS anomalies, particularly those affecting the midline, posterior fossa and cortex.

## 1 | INTRODUCTION

Congenital structural anomalies affect 2.2% of births, many of which have an underlying genetic etiology.<sup>1</sup> Anomalies affecting the fetal central nervous system (CNS) contribute

substantially to this figure, occurring in 0.26%–0.31% of all births and 3%–6% of stillbirths.<sup>2,3</sup> CNS anomalies pose a specific challenge related to the need for deep phenotyping using additional imaging modalities such as fetal magnetic resonance imaging (MRI), assessment of the phenotypic evolution as pregnancy progresses and ambiguity related to counseling regarding long-term outcomes.<sup>4</sup> Obtaining a unifying genetic diagnosis can prove invaluable to women and practitioners in guiding pregnancy management, treatment, delivery plans and postnatal management, enabling prognostication and providing information on the index and subsequent pregnancies.<sup>5</sup>

Conventional genetic testing, namely G-banding karyotype and chromosome microarray analysis (CMA), are limited to identifying aneuploidy, structural aberrations and copy number variation (CNV), yielding a unifying diagnosis in one-fifth of fetal CNS anomalies.<sup>6–8</sup> Identification of pathogenic single gene variants using next-generation sequencing (NGS) technologies, namely prenatal exome sequencing (pES) has been demonstrated to increase this yield.<sup>9,10</sup>

The NHS England pES pathway recommends the provision of trio pES in instances of major CNS anomaly, excluding neural tube defects; however, what should be included within this major category has not yet been specified.<sup>11</sup> Hence, the objectives of this prospective study, systematic review and meta-analysis are to determine the incremental yield of pES over and above karyotype and/or CMA for; (i) isolated or multisystem, (ii) single and multiple CNS anomalies, and (iii) CNS anomalies based on anatomical subtype as classified by a pediatric neuroradiologist.

## 2 | METHODS

### 2.1 | Protocol and registration

We developed a review protocol aligned to the recommended methods for systematic review and PRISMA guidance, which was prospectively registered with the PROSPERO international systematic review database (PROSPERO No. CRD42022328851 registration 04.05.22).<sup>12–14</sup>

### 2.2 | Eligibility criteria

Inclusion criteria detailed any retrospective or prospective studies which: (i) included 10 or more pregnancies with a prenatal diagnosis of any CNS anomaly based upon ultrasound with or without fetal MRI; (ii) which underwent NGS (whole, clinical, targeted ES or whole genome sequencing (WGS)); (iii) in which CMA and/or karyotype was non-diagnostic; (iv) in which NGS was initiated based upon prenatal phenotype only; and (v) results of genetic testing were known. Where adequate information was not obtainable from the manuscript the corresponding author was contacted and data were requested. In addition to the search, prospectively collected data from the NHS England pES pathway, commencing October 2021 to February 2023, where pES was performed in instances of a ‘major’ CNS phenotype (excluding neural tube defects) were included. The methodology of this pathway has been described previously and data used here was collected as part of a registered audit.<sup>11</sup>

If NGS was initiated postnatally, studies were included only if testing was based solely on prenatal phenotype. Conference abstracts, case series and case reports were also subjected to eligibility criteria assessment. In studies that were not specific to CNS anomalies but detailed such cases, the data was extracted either from the paper or following author correspondence and data sharing. Where available, extended datasets were included. Variants were deemed diagnostic if they were causative of the phenotype and classified as class IV (likely pathogenic) or V (pathogenic) according to the American College of Medical Genetics and Genomics (ACMG) or the Association for Clinical Genomic Science (ACGS).<sup>15</sup> Class III variants of uncertain significance (VUS) and incidental findings (IFs) were also recorded.

### 2.3 | Information sources and search strategy

Databases including MEDLINE, Web of Science, Cochrane Library and EMBASE were electronically searched for relevant citations from 1st January 2010 (inception of pES) to first May 2022. The search strategy consisted of relevant Medical Subject Headings (MeSH) terms, keywords and word variants for ‘prenatal’, ‘exome sequencing’, and ‘abnormality’ were used with alternative terms encompassing ‘fetus’, ‘fetal’, ‘prenatal diagnosis’, ‘antenatal’, ‘whole exome sequencing’, ‘exome’, ‘whole genome sequencing’, ‘genome human’, ‘sequence analysis, DNA’, ‘anomaly’ and ‘defect’. All study abstracts, were reviewed and full manuscripts were subsequently retrieved for further analysis if they met inclusion criteria. Manuscripts were excluded if they were duplicates; did not meet the inclusion criteria; or if there was inadequate phenotypic information of positive and/or negative cases. In the latter scenario, the corresponding authors were contacted and the study included if further information was available.

### 2.4 | Data extraction and assessment of risk of bias

Data on study characteristics and outcomes were independently extracted from each study by two reviewers (G.V.B, and P.A.J.) and any conflicts were resolved by a senior reviewer (F.M.). Categories included prenatal phenotype based on ultrasound and when available, fetal MRI; gestation at testing; the source of DNA; sequencing approach; variants reported including gene, clinical syndrome; inheritance pattern; turnaround time; pregnancy outcome; and type of initial non-diagnostic genetic testing (karyotype or CMA). The categorisation of neurological abnormalities and/or disruptions was reviewed and verified by a pediatric neuroradiologist (E.L.). Study characteristics and outcome data were logged under a generated case number and categorized as isolated and non-isolated and under categories including (1) developmental for example, neural tube defects, (2) posterior fossa anomalies for example, Dandy-Walker variants and Chiari II malformations, (3) ventricular, (4) midline for example, holoprosencephaly, agenesis of corpus callosum, (5) malformations of cortical development; A. Abnormal cell proliferation or apoptosis for example, microcephaly, megalencephaly, dysplasia; B. Abnormal cell migration for example, heterotopia, lissencephaly (heterotopia/cobblestone), schizencephaly, C. Abnormal post-migrational development for example, polymicrogyria or D. Miscellaneous—porencephaly, tumors, intracranial haemorrhage.<sup>17,18</sup>

Quality assessment of the included studies was performed using modified Standards for Reporting of Diagnostic Accuracy (STARD) criteria.<sup>16</sup>

## 2.5 | Data synthesis

The primary outcome of interest was the incremental yield of pES over CMA/karyotype expressed as a risk difference. This was estimated by pooling risk differences from each included study using a random effects model, using a previously published method with adjustment for 'zero' values from negative karyotype/CMA testing.<sup>19–21</sup> Results were displayed as forest plots with corresponding 95% confidence intervals (CIs) and pooled for all studies in a meta-analysis using a random effects model. Both the overall yield and yield for isolated CNS anomalies were calculated. A subgroup analysis for the key neurological categories with greater than or equal to  $n = 5$  cases previously listed were used to investigate the effect on incremental yield of pre-test case selection for higher likelihood of monogenic disease. Between-study heterogeneity was assessed graphically within the forest plot and statistically using 'Higgins'  $I^2$ . Publication bias was assessed graphically using funnel plots. Statistical analysis was performed using RevMan version 5.3.4 (Review Manager®, The Cochrane Collaboration, Copenhagen, Denmark) statistical software.

## 3 | RESULTS

### 3.1 | Study selection and characteristics

The study selection process is demonstrated in the PRISMA flow diagram (Figure 1). Thirty studies fulfilled the eligibility criteria and were suitable for meta-analysis (1583 cases).<sup>4,7,9,10,22–47</sup> This included data from the NHS England prenatal exome sequencing pathway.<sup>39</sup> For studies that met the inclusion criteria but provided inadequate phenotypic information, corresponding authors were contacted to request further data ( $n = 57$ ) of which 15 (26.3%) responded. Eleven studies provided extended data sets.<sup>4,9,10,27,29,37,40,43,46–48</sup> Supplementary Table 1 highlights the characteristics of the included studies and Figure 2 shows the overall quality assessment.

### 3.2 | Synthesis of results

Twenty-three studies were included in the sub-analysis (1264 cases). All cases underwent G-banding karyotype or CMA prior to pES with 52% having both. The median maternal age at testing was 31 years (range 19–49). Ethnicity was known in 21% ( $n = 265$ ) of cases, of which 64% ( $n = 170$ ) were Caucasian. The source of fetal DNA was specified in 41% ( $n = 516$ ) of cases, of which the majority, 53% ( $n = 274$ ), was from amniocentesis. In the cases that documented turnaround time for prenatal sequencing ( $n = 755$ ) the median time was 34 days (range 6–1001 days). Pregnancy outcome was known in 63% ( $n = 792$ ) of cases, of which 65% ( $n = 513$ ) ended in the termination of pregnancy. Multi-system abnormalities (CNS plus at least one other system) occurred in 50% ( $n = 627/1264$ ) of cases. Most frequent extra-CNS anomalies included those affecting the extremities (30%), heart (29%) or face (28%). A single CNS abnormality occurred in 57% ( $n = 717$ ) of cases, with the remainder ( $n = 547$ ) classified as multiple/complex CNS abnormalities. Only four (27.5%) studies and the NHS England pES series clearly documented whether fetal MRI was used as an additional imaging modality, hence sub-analysis of this group was not possible.

### 3.3 | Systematic review of pathogenic variants

In total 454 cases were identified as having a causative pathogenic or likely pathogenic (P/LP) variant with pES with an incremental yield of 32% (95% CI 27–36;  $I^2 = 72\%$ ) for any CNS anomaly; 35% (95% CI 27–44;  $I^2 = 77\%$ ) for cases of CNS anomaly as part of a multisystem anomaly; 27% (95% CI 19–34;  $I^2 = 74\%$ ) for isolated CNS anomaly (Table 1 and Supplementary Figures 1–5). Incremental yields for a single isolated CNS anomaly of 16% (95% CI 10–23;  $I^2 = 41\%$ ) and a multiple isolated CNS anomaly of 31% (95% CI 21–40;  $I^2 = 56\%$ ) are also recorded. Incremental yields from the sub-analysis for specific phenotypes are demonstrated in Table 2 with most optimal yields for anomalies of the posterior fossa; 36% [95% CI 28–43,  $I^2 = 50\%$ ], the midline; 35% [95% CI 27–44,  $I^2 = 77\%$ ] and the cortex; 35% [95% CI 26–44,  $I^2 = 32\%$ ], with the greatest yield in those with Type 1 malformations of cortical development, related to abnormal cell proliferation or apoptosis incorporating microcephaly, megalencephaly and dysplasia; 40% (22%–57%;  $I^2 = 68\%$ ) (Supplementary Figures 6–10).

A list of clinical syndromes caused by Class IV or V causative variants included within the final meta-analysis is outlined in Tables 1 and 2. Where documented, the most common genetic syndromes in isolated CNS anomalies were Lissencephaly 3 (*TUBA1A*), Coffin-Siris syndrome (*ARID1A/B*) and congenital X-linked hydrocephalus (*LICAM*). In cases in which the inheritance pattern was clearly documented ( $n = 159$ ); (i) 102 (64.2%) were autosomal dominant, (ii) 34 (21.4%) were autosomal recessive, and (iii) 23 (14.4%) were X-linked. Women with causative class IV and V variants identified on pES were more likely to terminate their pregnancy (70%;  $n = 181/259$  of known outcomes) than those in which a causative variant was not identified (61.6%;  $n = 332/539$  of known outcomes)  $p = 0.02$ . The pooled incremental yield for VUS was 4% (95% CI, 2–6;  $I^2 = 57\%$ ) with the number of incidental findings reported too small to derive a pooled value.

## 4 | DISCUSSION

We report a systemic review and meta-analysis assessing the incremental yield of pES over standard genomic testing strategies for fetuses with isolated CNS anomalies with an overall incremental diagnostic yield of 27%. Unlike previous studies, we have categorised cases as isolated single CNS anomaly, multiple CNS anomalies and CNS with multisystem anomalies, thereby allowing us to review specific CNS anomaly sub-categories. For isolated CNS anomalies, the commonest syndromes and causative genes were Lissencephaly 3 (*TUBA1A*), Coffin-Siris syndrome (*ARID1A/B*), both having an autosomal dominant inheritance pattern, and congenital X-linked hydrocephalus (*LICAM*).

Previous studies report variable diagnostic rates of pES in fetal CNS abnormalities ranging from 13% to 71%.<sup>9,25,31,35,44,48,49</sup> A recent systematic review by Mellis *et al.* reported an incremental yield of 17% (95% CI, 12%–22%).<sup>21</sup> Variation in yield may be due to differences in sample size or depth of fetal phenotyping. Notably, our research details a large dataset with high diagnostic rates. This may be due to the selected nature of cases, strict review inclusion criteria and the majority of cases adopting a trio exome approach.

The incremental yield increases when there is more than one CNS anomaly. However, we demonstrate an apparent incremental yield even in less severe single CNS anomalies such as isolated mild ventriculomegaly. We report a 20% incremental yield with isolated severe ventriculomegaly, which is lower than a recent systematic review by Mustafa *et al.*, who reported a yield of 35% although our incremental yield with isolated ACC (36%) was similar to that of a further study (30%) by the same group.<sup>50,51</sup> Of note, these reviews included fewer case numbers and studies with >3 cases compared to our limitation of including >10 cases in an attempt to minimise selection bias. Furthermore, the definition of CNS anomalies ideally requires fetal MRI or advanced neurosonography as many CNS anomalies are not readily detectable by ultrasound alone. Thus, cases of mild ventriculomegaly and ACC, for example, may have underlying cortical abnormalities only detectable by MRI, thus inflating the “isolated” CNS anomaly category.

This systematic review reports Joubert syndrome, Meckel syndrome, Noonan syndrome, Lissencephaly and Coffin-Siris syndrome as the commonest syndromes identified when a causative pathogenic variant was recorded with any CNS anomaly. The most common genes included *CEP290*, *TUBA1A*, *LICAM* and *ARID1A*. *ARID1A*, *LICAM* and *CEP290* were all reported by Mustafa *et al.*, in cases of bilateral severe ventriculomegaly, both isolated and with extracranial anomalies or other brain malformations.<sup>50</sup> *TUBA1A* and *LICAM* were also the genes with the overall highest frequency in cases of ACC.<sup>51</sup> Of note, many of the syndromes identified, which would typically present prenatally with a multisystem phenotype, were reported as an isolated CNS anomaly in 42% ( $n = 5$ ) of Coffin-Siris Syndrome; 33% ( $n = 9$ ) of Joubert or Meckel Syndrome and 15% ( $n = 2$ ) of Noonan’s Syndrome. This demonstrates the incomplete prenatal phenotyping, or poor reporting, offered from prenatal imaging and highlights the need for a low threshold to perform pES in cases of apparently isolated CNS anomaly.

It is important to highlight that the natural history of many neurological condition abnormalities is such that changes may not be detected until later in gestation. In this review, 200 cases (16%) were identified after 24 weeks of gestation and 106 (8%) after 30 weeks. This indicates the evolving nature of a CNS phenotype as pregnancy progresses and the need for deep phenotyping. Within the limitations of reporting, advanced neurosonography was not specifically reported and fetal MRI was documented only as having been performed in under a third of cases, which is likely a significant under-representation.<sup>52,53</sup> Interestingly, Type 1 cortical anomalies demonstrated the greatest yield, highlighting the importance of investigating a primary or secondary microcephaly (defined prenatally as a head circumference (HC) more than 3 standard deviations (SDs) below the mean for gestational age) which may only manifest in the third trimester and the challenges regarding megalencephaly (HC more than 2SDs above the mean for gestational age) which rarely present prenatally but have a strong association with genes within the P13L-AKT-mTOR pathway and often lead to developmental delay, intellectual disability and early onset seizures.<sup>54</sup> It is important to consider that the cause may also be benign/familial in nature hence it is always useful to measure the parental occipital frontal circumference.<sup>55</sup> Furthermore, whilst prenatal diagnosis may not guide management in the index pregnancy in cases of serious CNS abnormality, such as Lissencephaly or small cerebellum, it can be extremely useful for guiding future pregnancies.



Key strengths of this study include the global-scale contribution depicted through the included studies which have contributed to this large-scale review of over 1500 cases of fetal CNS anomaly and the subsequent classification of the CNS phenotype by a neuroradiologist. Limitations include the fact that the phenotype was limited to what was provided by the authors and the phenotypic information detailed within the included studies, including the lack of fetal MRI results. Additionally, heterogeneity was high although we attempted to minimise this by applying a random effects model and sub-analysis limited to studies with  $n \geq 5$  cases.

## 5 | CONCLUSION

The findings of this review reveal a high incremental yield for fetal CNS anomalies with pES over and above standard genomic testing strategies, most notably where there are multiple CNS anomalies, particularly those affecting the midline, posterior fossa and cortex. Prenatal exome sequencing in CNS anomalies can assist with prenatal genetic counseling, providing parents with more information on prognosis and inheritance and assisting clinicians in developing targeted management plans. Although one should always strive to obtain a deep phenotype, pES can facilitate in establishing a diagnosis where this is not feasible or where the CNS phenotype appears mild.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGMENTS

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## DATA AVAILABILITY STATEMENT

The anonymized dataset is available from the corresponding author on reasonable request.

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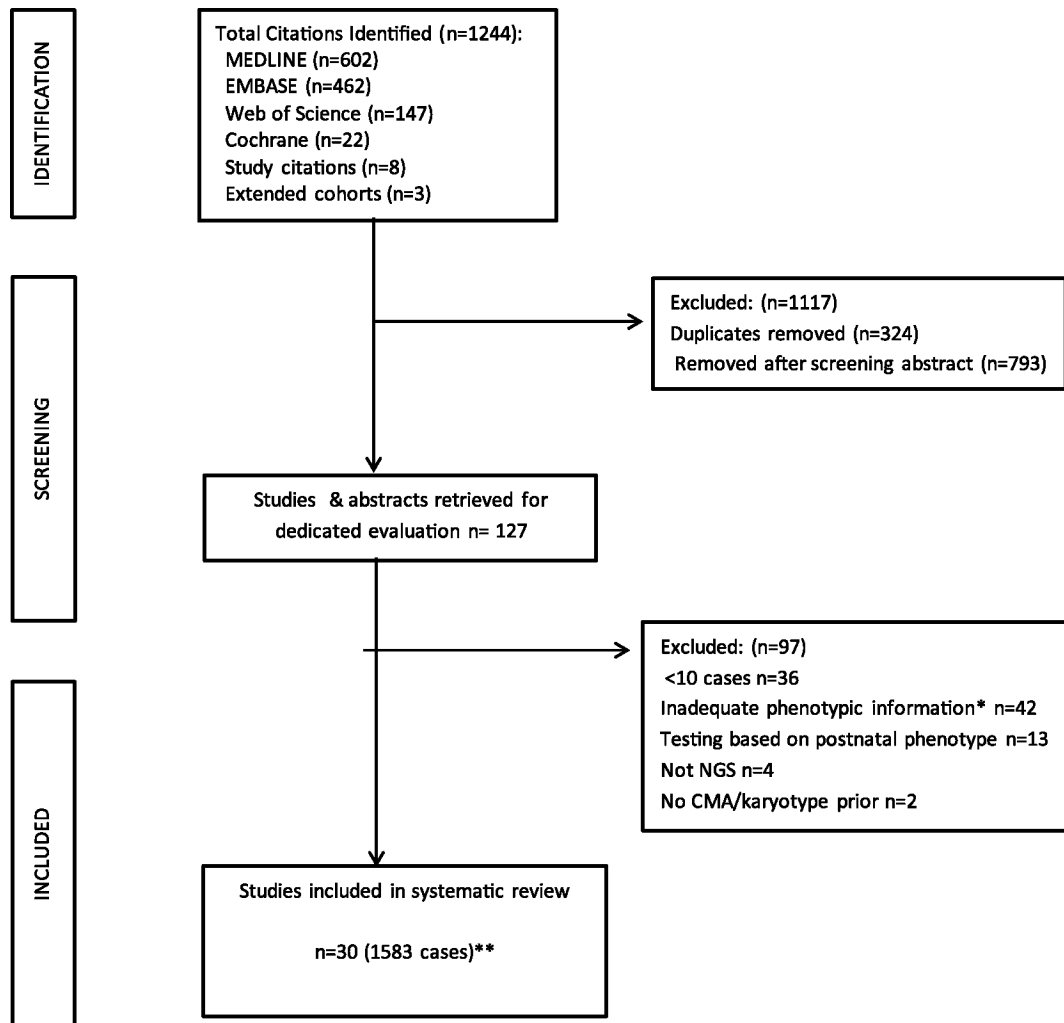
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**key points****What is already known about this topic?**

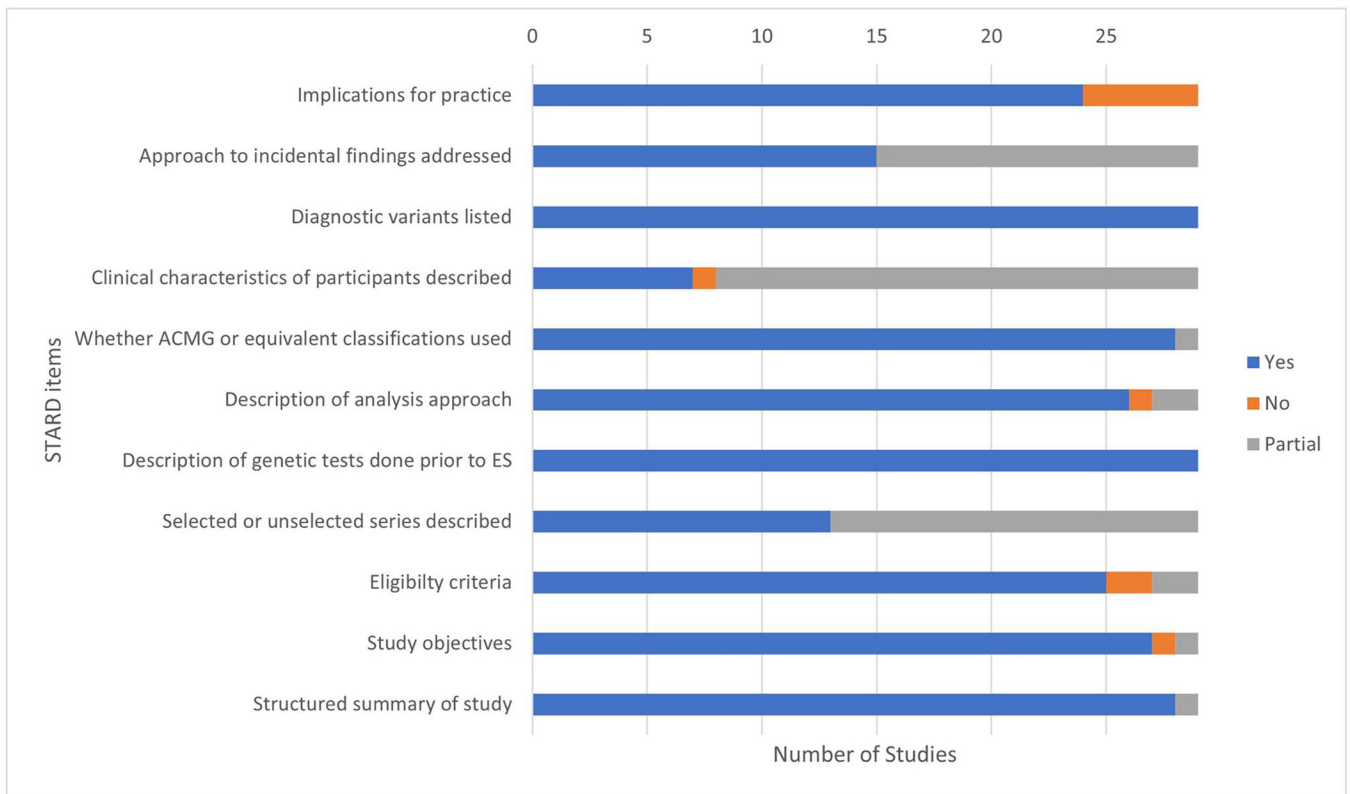
- Prenatal next-generation sequencing increases the incremental diagnostic yield in fetuses with sonographic structural abnormalities and a normal G-banding karyotype and/or chromosome microarray.
- Published diagnostic yields specific to central nervous system abnormalities are variable, highlighting the need for a systematic review.

**What does this study add?**

- This is the first systematic review and meta-analysis of the literature available to date in this area with sub-classification by a pediatric neuroradiologist
- A subgroup analysis provides the incremental diagnostic yield for specific anatomical CNS anomalies



**FIGURE 1.** PRISMA flow diagram (\*Authors contacted for further information \*\*Includes unpublished audit of NHS England cases).



**FIGURE 2.** Quality assessment of 29 studies included in systematic review, using modified Standards for Reporting of Diagnostic Accuracy criteria (ACMG, American College of Medical Genetics and Genomics; ES, exome sequencing).

TABLE 1

The incremental yield of prenatal exome sequencing over chromosome microarray and/or G-banding karyotype in prenatally identified CNS anomalies.

CNS anomaly	Incremental yield (%) [95% CI]	Most common affected genes	Corresponding syndromes	VUS (%) [95% CI]
All CNS anomalies	32% [27%–36%] $\hat{P} = 72\%$	<i>TUBA1A</i> 4% ( $n = 15/375$ ) <sup>a</sup> <i>ARID1A/B</i> 3.2% ( $n = 12/375$ ) <i>TUBB2</i> 2.7% ( $n = 10/375$ )	Lissencephaly 3 Coffin-Siris Cortical dysplasia, complex, with other brain malformations	4% [2%–6%] $\hat{P} = 57\%$
Cases of CNS anomaly with multisystem anomaly	35% [27%–44%] $\hat{P} = 77\%$	<i>CEP290</i> 2.7% ( $n = 10/375$ ) <i>CC2D2A</i> or <i>CEP290</i> or <i>TCTN2</i> or <i>TMEM22</i> 10% ( $n = 22/222$ ) <i>BRAF</i> or <i>PTPN11</i> 5% ( $n = 11/222$ ) <i>ARID1A/B</i> 3.2% ( $n = 7/222$ )	Joubert or Meckel Joubert or Meckel Noonan Coffin-Siris	5% [2%–8%] $\hat{P} = 43\%$
Cases of isolated CNS anomaly	27% [19%–34%] $\hat{P} = 74\%$	<i>TUBA1A</i> 10.1% ( $N = 14/139$ ) <i>ARID1A/B</i> 3.6% ( $N = 5/139$ ) <i>LICAM</i> 4.3% ( $N = 6/139$ )	Lissencephaly 3 Coffin Siris Hydrocephalus, congenital, X-linked	3% [1%–4%] $\hat{P} = 0\%$

Abbreviations: CNS, central nervous system; VUS, variant of uncertain significance.

<sup>a</sup>Denominator is where a pathogenic variant genotype was recorded.



The incremental yield of prenatal exome sequencing over chromosome microarray and/or G-banding karyotype in prenatally identified CNS anomalies according to sub-analysis for specific phenotypes.

**TABLE 2**

CNS anomaly	Incremental yield (%) [95% CI]	Most common affected genes	Corresponding syndromes
Developmental	19% [7%–31%] $P = 38\%$	<i>CC2D2A</i> 21.1% ( $n = 4/19$ ), <sup>a</sup> <i>CEP290</i> 21.1% ( $n = 4/19$ )	Joubert or Meckel
Ventricular	32% [25%–40%] $P = 68\%$	<i>TUBA1A</i> 4.4% ( $n = 7/158$ ) <i>ARID1A/B</i> 3.8% ( $n = 6/158$ ) <i>POMT1/2</i> 3.8% ( $n = 6/158$ )	Lissencephaly 3 Coffin-Siris Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies)
Mild VM	20% [4%–36%] $P = 60\%$	N/S	Hydrocephalus, congenital, X-linked
Moderate VM	22% [5%–39%] $P = 49\%$	N/S	Joubert or Meckel
Severe VM	20% [13%–27%] $P = 0\%$	<i>LICAM</i> 20% ( $n = 2/10$ )	Lissencephaly 3 CHARGE
Posterior fossa	36% [28%–43%] $P = 50\%$	<i>CEP290</i> or <i>TMEM67</i> 138 11.9% ( $n = 16/135$ ) <i>TUBA1A</i> 8.1% ( $n = 11/135$ ) <i>CHD7</i> 4.4% ( $n = 6/135$ )	Lissencephaly 3 Cortical dysplasia, complex, with other brain malformations Coffin-Siris
Midline	35% [27%–44%] $P = 77\%$	<i>TUBA1A</i> 6.8% ( $n = 11/161$ ) <i>TUBB</i> 5.6% ( $n = 9/161$ ) <i>ARID1A/B</i> 4.3% ( $n = 7/161$ )	Lissencephaly 3 Cortical dysplasia, complex, with other brain malformations Coffin-Siris
Absent corpus callosum	36% [28%–44%] $P = 46\%$	<i>TUBA1A</i> 7.5% ( $n = 8/107$ ) <i>ARID1A/B</i> 6.5% ( $n = 7/107$ )	Lissencephaly 3 Coffin-Siris
Holoprosencephaly	34% [15%–53%] $P = 28\%$	<i>TUBB</i> 4.7% ( $n = 5/107$ )	Cortical dysplasia, complex, with other brain malformations
Malformation of cortical development	35% [26%–44%] $P = 55\%$	<i>SHH</i> 38.5% ( $n = 5/13$ ) <i>TUBA1A</i> 9.8% ( $n = 9/92$ ) <i>TUBB</i> 6.5% ( $n = 6/92$ )	Holoprosencephaly 3 Lissencephaly 3
Type 1 cortical	40% [22%–57%] $P = 68\%$	<i>COL4A1</i> 7.9% ( $n = 3/38$ )	Cortical dysplasia, complex, with other brain malformations Microangiopathy and leukoencephalopathy, pontine, autosomal dominant
(Abnormal cell proliferation and apoptosis)			
Type 2 cortical	31% [15%–49%] $P = 63\%$	<i>TUBB</i> 15.4% ( $N = 4/26$ ) <i>TUBA1A</i> 11.5% ( $N = 3/26$ )	Cortical dysplasia, complex, with other brain malformations Lissencephaly 3
(Abnormal cell migration)			
Type 3 cortical	32% [21%–44%] $P = 4\%$	<i>TUBB</i> 13% ( $N = 3/23$ ) <i>TUBA1A</i> 8.7% ( $N = 2/23$ )	Cortical dysplasia, complex, with other brain malformations Lissencephaly 3
(Abnormal post-migrational development)			

Abbreviations: CNS, central nervous system; N/S, not specified; VM, ventriculomegaly; VUS, variant of uncertain significance.

Denominator is where a pathogenic variant genotype was recorded.

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