Ultrasound Obstet Gynecol 2021; **58**: 509–518
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.23652.

Fetal hydrops and the Incremental yield of Next-generation sequencing over standard prenatal Diagnostic testing (FIND) study: prospective cohort study and meta-analysis

F. MONE[1,2], R. Y. EBERHARDT[3], M. E. HURLES[3], D. J. McMULLAN[4], E. R. MAHER[5,6,7], J. LORD[3], L. S. CHITTY[8,9], E. DEMPSEY[10], T. HOMFRAY[11], J. L. GIORDANO[12,13], R. J. WAPNER[12,13], L. SUN[14], T. N. SPARKS[15], M. E. NORTON[15] and M. D. KILBY[1,2]

[1]*Institute of Metabolism and Systems Research, College of Medical & Dental Sciences, University of Birmingham, Edgbaston, Birmingham, UK;* [2]*Fetal Medicine Centre, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, UK;* [3]*Wellcome Sanger Institute, Hinxton, UK;* [4]*West Midlands Regional Genetics Service, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, UK;* [5]*Department of Medical Genetics, University of Cambridge, Cambridge, UK;* [6]*NIHR Cambridge Biomedical Research Centre, Cambridge, UK;* [7]*Department of Clinical Genetics, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK;* [8]*North Thames Genomic Laboratory Hub, Great Ormond Street NHS Foundation Trust, London, UK;* [9]*UCL Great Ormond Street Institute of Child Health, London, UK;* [10]*Molecular and Clinical Sciences, St George's University of London, London, UK;* [11]*SW Thames Regional Genetics Department, St George's University Hospitals NHS Foundation Trust, London, UK;* [12]*Institute for Genomic Medicine, Columbia University Medical Center, New York, NY, USA;* [13]*Division of Maternal–Fetal Medicine, Department of Obstetrics and Gynecology, Columbia University Vagelos Medical Center, New York, NY, USA;* [14]*Fetal Medicine Unit and Prenatal Diagnosis Center, Shanghai First Maternity and Infant Hospital of Tongji University, Shanghai, China;* [15]*Center for Maternal–Fetal Precision Medicine, Division of Maternal–Fetal Medicine, University of California, San Francisco, CA, USA*

KEYWORDS: exome sequencing; fetus; hydrops; next-generation sequencing; non-immune hydrops fetalis; prenatal diagnosis

CONTRIBUTION

What are the novel findings of this work?
This is the first systematic review assessing the incremental diagnostic yield of prenatal exome sequencing over chromosomal microarray analysis or karyotyping in prenatally diagnosed non-immune hydrops fetalis. An apparent incremental yield of exome sequencing was demonstrated.

What are the clinical implications of this work?
Prenatal exome sequencing should be considered in prenatally diagnosed non-immune hydrops fetalis that is unexplained by standard genetic testing, in both isolated cases and those associated with an additional fetal structural anomaly.

ABSTRACT

Objective *To determine the incremental yield of exome sequencing (ES) over chromosomal microarray analysis (CMA) or karyotyping in prenatally diagnosed non-immune hydrops fetalis (NIHF).*

Methods *A prospective cohort study (comprising an extended group of the Prenatal Assessment of Genomes and Exomes (PAGE) study) was performed which included 28 cases of prenatally diagnosed NIHF undergoing trio ES following negative CMA or karyotyping. These cases were combined with data from a systematic review of the literature. MEDLINE, EMBASE, CINAHL and ClinicalTrials.gov databases were searched electronically (January 2000 to October 2020) for studies reporting on the incremental yield of ES over CMA or karyotyping in fetuses with prenatally detected NIHF. Inclusion criteria for the systematic review were: (i) at least two cases of NIHF undergoing sequencing; (ii) testing initiated based on prenatal ultrasound-based phenotype; and (iii) negative CMA or karyotyping result. The incremental diagnostic yield of ES was assessed in: (i) all cases of NIHF; (ii) isolated NIHF; (iii) NIHF associated with an additional fetal structural anomaly; and (iv) NIHF according to severity (i.e. two* vs *three or more cavities affected).*

Results *In the extended PAGE study cohort, the additional diagnostic yield of ES over CMA or karyotyping was 25.0% (7/28) in all NIHF cases, 21.4% (3/14) in those with isolated NIHF and 28.6% (4/14) in those with*

Correspondence to: Dr F. Mone, Fetal Medicine Centre, Birmingham Women's and Children's NHS Foundation Trust, Mindelsohn Way, Edgbaston, Birmingham B15 2TG, UK (e-mail: f.mone@bham.ac.uk)

Accepted: 29 March 2021

non-isolated NIHF. In the meta-analysis, the pooled incremental yield based on 21 studies (306 cases) was 29% (95% CI, 24–34%; P < 0.00001; $I^2 = 0\%$) in all NIHF, 21% (95% CI, 13–30%; P < 0.00001; $I^2 = 0\%$) in isolated NIHF and 39% (95% CI, 30–49%; P < 0.00001; $I^2 = 1\%$) in NIHF associated with an additional fetal structural anomaly. In the latter group, congenital limb contractures were the most prevalent additional structural anomaly associated with a causative pathogenic variant, occurring in 17.3% (19/110) of cases. The incremental yield did not differ significantly according to hydrops severity. The most common genetic disorders identified were RASopathies, occurring in 30.3% (27/89) of cases with a causative pathogenic variant, most frequently due to a PTPN11 *variant (44.4%; 12/27). The predominant inheritance pattern in causative pathogenic variants was autosomal dominant in monoallelic disease genes (57.3%; 51/89), with most being* de novo *(86.3%; 44/51).*

Conclusions *Use of prenatal next-generation sequencing in both isolated and non-isolated NIHF should be considered in the development of clinical pathways. Given the wide range of potential syndromic diagnoses and heterogeneity in the prenatal phenotype of NIHF, exome or whole-genome sequencing may prove to be a more appropriate testing approach than a targeted gene panel testing strategy.* © *2021 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Non-immune hydrops fetalis (NIHF) is defined traditionally as fluid accumulation in two or more fetal body cavities in cases not secondary to maternal red cell alloimmunization[1]. It affects up to 1 in 1700 pregnancies, with associated high risks of perinatal morbidity and mortality[2]. Excluding cases due to infection, fetal structural anomaly (FSA) or complications of twin pregnancy, aneuploidy may explain one-quarter of cases, with chromosomal microarray analysis (CMA) demonstrating copy-number variants (CNVs) in a further 6–14% of cases[3,4]. Despite this, the definitive diagnostic yield of CMA over standard G-banding karyotyping is moderate and, following exclusion of the aforementioned causes, up to 50% of cases of NIHF remain unexplained, with a significant proportion thought to be secondary to single-gene variants[5]. Over 170 genes have been identified as being associated with NIHF and, until the recent emergence of next-generation sequencing (NGS), testing for such conditions has relied on targeted gene testing and enzyme assays[3,6]. Single-gene causes of NIHF are associated with significant risks of perinatal death or neurodevelopmental sequelae[2]. Establishing the diagnostic etiology of NIHF prenatally is a vital step in facilitating informed decision-making for both parents and clinicians when considering options such as termination of pregnancy, planning neonatal care and addressing recurrence risks[2]. The latter risk could theoretically be mitigated by using novel technologies, such as preimplantation genetic testing[7]. While individual cohort studies have assessed the diagnostic yield of exome sequencing (ES) over quantitative fluorescence polymerase chain reaction (QF-PCR) and CMA or karyotyping in NIHF, they are heterogeneous in relation to the populations assessed and the genetic platforms used[3]. Given this heterogeneity, there is a need to integrate existing data on single-gene disorders underlying NIHF. Hence, the aims of this study were to evaluate the incremental diagnostic yield of prenatal ES over CMA or karyotyping in prenatally diagnosed NIHF for: (i) all cases of NIHF; (ii) isolated NIHF; (iii) NIHF associated with an additional FSA; and (iv) NIHF according to severity (i.e. two cavities *vs* three or more cavities affected).

METHODS

Extended PAGE study cohort

The Fetal hydrops and the Incremental yield of Next-generation sequencing over standard prenatal Diagnostic testing (FIND) study included prospectively identified cases of prenatally confirmed NIHF from an extended cohort of the Prenatal Assessment of Genomes and Exomes (PAGE) study[8]. For the purposes of this study, we defined NIHF as pathological fluid accumulation in at least two fetal cavities confirmed prenatally on ultrasound, excluding cases with aneuploidy, congenital infection, alloimmunization and/or twin–twin transfusion syndrome[1,2]. The final extended PAGE cohort comprised 850 fetuses (including 596 of the published cohort) with fetal–parental trio ES performed when an ultrasound-confirmed FSA was detected[8]. These cases were recruited between October 2014 and May 2018 across 34 fetal medicine centers in England and Scotland, with ES performed centrally at the Wellcome Trust Sanger Institute, Hinxton, UK[8]. PAGE eligibility criteria included: (i) prenatal detection of a FSA after 11 weeks' gestation; (ii) availability of proband and parental DNA; and (iii) negative CMA or karyotyping result. The PAGE study methodology has been published previously and utilized a standard ES approach with variant interpretation based on a targeted virtual gene panel for developmental disorders encompassing 1628 genes[8,9]. Phenotypes of all cases were classified using Human Phenotype Ontology (HPO) terms[10], and those defined as hydrops fetalis (HP:0001789) were selected and analyzed further to determine if the criteria for NIHF for the purposes of the FIND study were met. Cases were classified further as isolated or associated with an additional FSA using the HPO approach to coding additional anomalies. The fetal phenotype was described by fetal medicine specialists/sonographers and documented principally on ViewPoint® version 5.6.16 (GE Healthcare, Zipf, Austria). Variants were classified in accordance with the American College of Medical Genetics and Genomics (ACMG) guidelines[11], as agreed by a clinical review panel, and incidental findings (IFs) were not reported. Pathogenic and likely

pathogenic variants explaining the fetal phenotype were confirmed using Sanger sequencing, and the results were returned to the parents after the end of the pregnancy. Ethical approval was obtained from the research ethics committees at West Midlands – South Birmingham (ref: 13/WM/1219) and Harrow (ref: 01/0095). Local research and development offices subsequently approved the study at each participating organization.

Systematic review and meta-analysis

Information sources

This review was performed in a standardized manner in line with recommended methods for systematic reviews and the preferred reporting items for systematic reviews and meta-analyses (PRISMA)[12] and meta-analysis of observational studies in epidemiology (MOOSE)[13] guidance and was registered prospectively (PROSPERO No. CRD42020221427). The following databases were searched electronically for relevant citations, from January 2000 (ES technology was not available prior to this time) until October 2020: MEDLINE, EMBASE, CINAHL and ClinicalTrials.gov. The search strategy consisted of relevant medical subject headings (MeSH) terms, keywords and word variants for 'exome sequencing', 'fetus' and 'abnormality', used with alternative terms encompassing 'genome sequencing', 'exome', 'fetal', 'prenatal', 'antenatal', 'defect' and 'anomaly'. Bibliographies of relevant articles were searched manually and experts in prenatal genomics were contacted to identify further relevant studies. The search strategy is available from the corresponding author on request.

Study selection

The inclusion criteria for study selection were any prospective or retrospective cohort study or case series which: (i) included two or more cases of NIHF undergoing ES; (ii) initiated testing based on prenatal ultrasound-based phenotype; (iii) included cases with a negative CMA or karyotyping result; and (iv) included cases with known genetic testing result. Cases in which ES was initiated postnatally were included if testing was based on the prenatal phenotype. Cases in which sequential Sanger sequencing was utilized were also included. When studies were not specific to NIHF exclusively, data regarding NIHF cases were extracted from the paper or were requested from the corresponding author. All study abstracts were screened by two reviewers (F.M. and M.D.K.) and the full manuscripts were reviewed subsequently when further information was required.

Data extraction and quality assessment

Both reviewers extracted independently data on study characteristics and outcome using a proforma. Data extracted from studies, when obtainable, included: ultrasound phenotype, sequencing approach, reported variants, source of fetal DNA, turnaround time, fetal outcome, maternal age and gestational age at testing. Quality assessment was performed using modified standards for reporting of diagnostic accuracy studies (STARD) criteria[14]. The criteria deemed most important to optimize accuracy were: (i) trio analysis; (ii) use of ACMG criteria for variant interpretation; (iii) Sanger sequencing validation; and (iv) description of the prenatal phenotype.

Statistical analysis

Descriptive tables were produced detailing study characteristics and outcomes. The incremental diagnostic yield for causative Class-IV and Class-V variants, or risk difference, with 95% CI, of ES over CMA or karyotyping was calculated for each study and as a pooled value for: (i) all NIHF; (ii) isolated NIHF; (iii) NIHF associated with an additional FSA; and (iv) NIHF according to severity (i.e. two *vs* three or more cavities affected). When reported, pooled values for variants of uncertain significance (VOUS) and IFs were also determined. Risk differences from each study were pooled using a random-effects model throughout to estimate incremental yield using a previously published method which facilitates calculation with adjustment for zero values from negative CMA or karyotyping[9,15]. Results were displayed in forest plots with corresponding 95% CI. Heterogeneity was assessed graphically using forest plots and statistically using the Higgins I^2 statistic. 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.

RESULTS

Extended PAGE study cohort

Of the 850 cases with a FSA detected prenatally on ultrasound that underwent ES in the extended PAGE cohort, 28 (3.3%) met the definition for NIHF. Of these, 50.0% ($n = 14$) were apparently isolated and 50.0% ($n = 14$) were associated with an additional FSA. In the majority of cases (96.4%; 27/28), the initial genetic test was CMA, while the remainder had karyotyping, and proband DNA most frequently originated from cultured amniocytes (50.0%; $n = 14$). The additional diagnostic yield of ES was 25.0% (7/28) in all NIHF cases, 21.4% (3/14) in isolated NIHF cases and 28.6% (4/14) in NIHF cases associated with an additional FSA. When an additional anomaly associated with a causative pathogenic variant was present, the most common additional anomaly was congenital limb contractures due to arthrogryposis multiplex congenita (HP0002804) (75%; 3/4). In cases with an associated anomaly in which no causative pathogenic variant was obtained, the most common additional anomalies were cardiac, genitourinary or thoracic in nature (50.0%

(5/10) for each). One case of Noonan syndrome was not detected initially as pathogenic as it was filtered out of the bioinformatic pipeline due to inheritance from an apparently unaffected parent. Subsequently, the pipeline was adjusted so that such variants were not filtered out even if they were inherited. The incidence of VOUS was 7.1% (2/28). Causative pathogenic variants (Classes IV and V) and VOUS are described in Tables S1 and S2, respectively.

Systematic review and meta-analysis

For nine studies that were suitable for inclusion but data were incomplete, the corresponding author was contacted to request further data regarding fetal phenotype, of whom two responded and provided full datasets[16,17]. For the study of Petrovski *et al.*[16], based in Columbia University Medical Centre, New York, USA, the authors provided an extended dataset. In total, in addition to the extended PAGE study cohort, a further 20 studies met the inclusion criteria (Figure 1)[2,16–34]. Table 1 shows the characteristics of the included studies and Figure 2 shows the overall quality assessment.

The 21 included studies encompassed a total of 306 NIHF cases. When stated ($n = 218$), there were 109 (50.0%) cases of apparently isolated NIHF (on detailed prenatal ultrasound) and 109 (50.0%) cases associated with an additional FSA. Mean maternal age and gestational age at testing were 30.9 ± 3.5 years and 21.9 ± 5.4 weeks, respectively. Fetal DNA was obtained via amniocentesis in the majority of cases (50.6%; 121/239). The initial test prior to ES was CMA in 84.0% (257/306) of cases and G-banding karyotyping in the remainder. When documented (eight studies), the median turnaround time for ES was 40 (range, 7–140) days. Pregnancy outcome was available for 83.7% (256/306) of cases (termination of pregnancy (35.2%; 90/256), *in-utero* demise (34.4%; 88/256), neonatal survival (22.3%; 57/256) and neonatal death (8.2%; 21/256)). When reported (60.8%; 186/306), the pooled incremental yield for VOUS and IFs was 19% (95% CI, 6–22%; $P = 0.003$; $I^2 = 62\%$) and 4% (95% CI, −1 to 9%; $P = 0.09$; $I^2 = 0\%$), respectively. All documented pathogenic variants and VOUS are outlined in Tables S1 and S2, respectively.

The pooled incremental yield of ES in all NIHF cases, those with isolated NIHF and those with NIHF associated with an additional FSA is demonstrated in forest plots, with respective values of 29% (95% CI, 24–34%; $P < 0.00001$; $I^2 = 0\%$ (Figure 3)), 21% (95% CI, 13–30%; $P < 0.00001$; $I^2 = 0\%$ (Figure 4)) and 39% (95% CI, 30–49%; $P < 0.00001$; $I^2 = 1\%$ (Figure 5)). The corresponding funnel plots are displayed in Figure S1. In cases with an additional FSA, the most common additional anomalies associated with a causative pathogenic variant were those affecting the upper and/or lower limbs due to congenital contractures (HP:0002803) (17.3%;

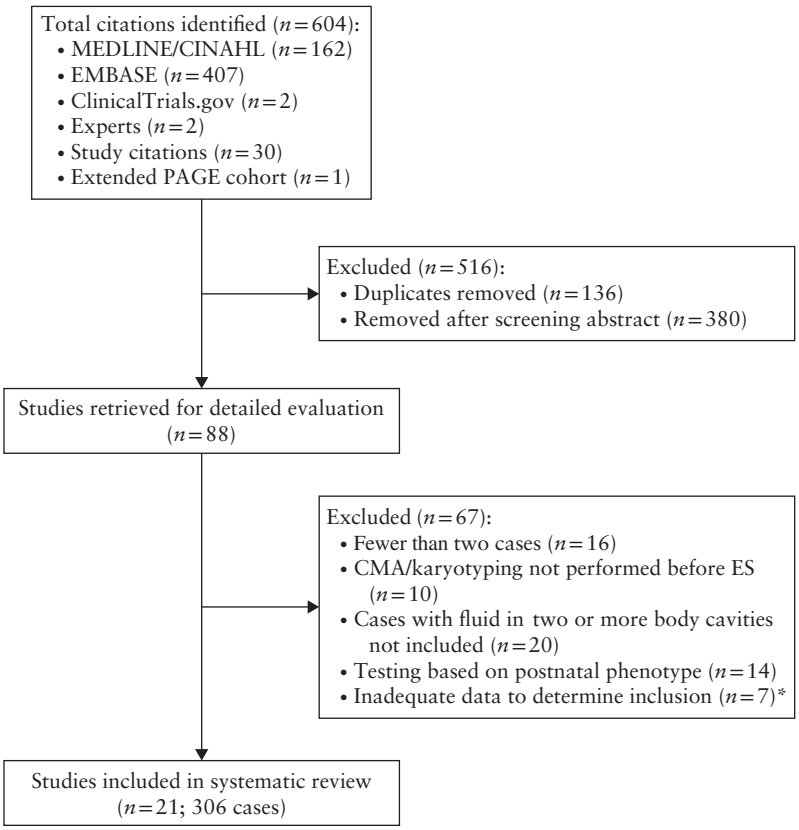

Figure 1 Flowchart summarizing inclusion in the systematic review of studies on the incremental yield of exome sequencing (ES) over chromosomal microarray analysis (CMA) or karyotyping in fetuses with prenatally detected non-immune hydrops fetalis. *Corresponding author was contacted to request additional information but did not respond. PAGE, Prenatal Assessment of Genomes and Exomes study.

19/110). When the NIHF phenotype was described ($n = 156$), the incremental yield for causative pathogenic variants did not differ significantly according to the severity of hydrops (two cavities affected (34%; 95% CI, 23–45%; $P < 0.00001$; $I^2 = 0\%$) *vs* three or more cavities affected (30%; 95% CI, 19–40%; $P < 0.00001$; $I^2 = 0\%$); $P = 0.26$) (Figure S2). When a causative pathogenic variant was documented ($n = 89$) (Table S1), the most common genetic disorders were: (i) RASopathies (30.3%; 27/89), primarily due to *PTPN11* variants (44.4%; 12/27); (ii) musculoskeletal disorders (14.6%; 13/89), primarily due to *RYR1* variants (38.5%; 5/13); and (iii) inborn errors of metabolism (12.4%; 11/89), primarily due to *GUSB* variants (45.5%; 5/11). The predominant inheritance pattern of causative pathogenic variants was autosomal dominant in monoallelic disease genes (57.3%; 51/89), with most being *de novo* (86.3%; 44/51). When the type of ES performed was stated (20 studies; Table 1), the overall incremental yield did not differ significantly according to whether a panel or whole-exome approach was used (26% (95% CI, 16–36%; $I^2 = 0\%$) and 27% (95% CI, 19–36%; $I^2 = 25\%$), respectively).

DISCUSSION

The findings of this systematic review demonstrate a substantial incremental yield of 29% of ES over CMA or karyotyping in cases with prenatally diagnosed NIHF. This yield was higher among cases with an additional FSA, but severity of NIHF did not demonstrate a significant effect on the incremental yield. The majority of causative pathogenic variants were *de novo* in autosomal dominant disease genes, predominantly in those causative of RASopathies.

Table 1 Characteristics of included studies reporting on the incremental yield of exome sequencing over chromosomal microarray analysis or karyotyping in fetuses with prenatally detected non-immune hydrops fetalis (NIHF)

		Number of NIHF cases		
Study	*Next-generation sequencing approach*	*All*	*Isolated*	*With additional FSA*
Becher (2020)[26]	WES, trio, 103× coverage, Roche SeqCap EZ MedExome Plus capture + Illumina NextSeq 500	4	4	0
Boissel (2018)[18]	WES, trio, 110× coverage, Agilent capture + Illumina HiSeq 2000 or 2500	2	0	2
Corsten-Janssen (2020)[32]	WES, trio, 20× coverage, Agilent capture + Illumina NextSeq500	6	2	4
Croonen (2013)[33]*	Clinical exome, Noonan panel, Illustra amplification, sequencer not stated	15	N/S	N/S
Deden (2020)[27]	WES, trio, 200–300× coverage, Agilent capture + Illumina NextSeq500	4	1	3
Deng (2020)[19]	WES, trio, 120× coverage, Agilent capture + Illumina HiSeq X Ten or Novaseq 6000	21	16	5
Greenbaum (2019)[28]	WES, trio, 100× coverage, capture kit unknown + Illumina sequencing	3	2	1
Jelin (2020)[20]	WES, trio, depth of coverage < 10 removed, Agilent capture + Illumina HiSeq 2500	5	3	2
Lord (2020)[8]†	WES, trio, 1628 genes, Agilent capture + Illumina HiSeq 2500, 98.3% of bait regions covered at minimum depth of 5×	28	14	14
Mone (2020)[34]	WES, trio, 1628 genes, Agilent capture + Illumina HiSeq 2500, 98.3% of bait regions covered at minimum depth of 5×	6	3	3
Normand (2018)[21]	WES, trio, 150× coverage, Roche NimbleGen capture, Illumina Genome Analyzer IIx platform/HiSeq 2000	10	N/S	N/S
Petrovski (2019)[16]	WES, trio, Nimblegen SeqCap EZ capture + Illumina Hiseq 2500, average read coverage 89.3 reads, bioinformatic signatures	23	14	9
Sparks (2019)[29]*	WES ($n = 1$), clinical exome ($n = 7$), other details not specified	8	N/S	N/S
Sparks (2020)[2]*	WES, trio, Illumina HiSeq 2500 or Illumina NovaSeq 6000	78	32	46
Stals (2018)[23]	WES, parents only, 80× coverage, Agilent capture + Illumina HiSeq 2500 or NextSeq500, included only heterozygous rare (MAF < 0.001) variants in same gene in both parents	4	0	4
Vora (2017)[22]*	Clinical exome and WES, trio, Illumina HiSeq 2500	2	2	0
Westerfield (2015)[30]	WES, trio, 130× coverage, Roche NimbleGen capture + Illumina Genome Analyzer IIx or HiSeq 2000	2	0	2
Westphal (2019)[24]	WES, trio, 20 000 genes, 150× coverage	2	0	2
Yang (2012)[31]*	Clinical exome, lymphedema panel, Oligo 6.1 PCR amplification + ABI, PRISM 3000 DNA sequencer	27	N/S	N/S
Yates (2017)[25]	WES, trio, 140× coverage, Agilent capture + Illumina HiSeq 2000 or 2500	28	N/S	N/S
Zhou (2020)[17]*	WES, trio in recurrent NIHF, Agilent capture + Illumina HiSeq X Ten	28	16	12

Only first author of each study is given. *Coverage not stated. †Including cases identified in the current study. FSA, fetal structural anomaly; MAF, minor allele freuency; N/S, not stated; PCR, polymerase chain reaction; WES, whole-exome sequencing.

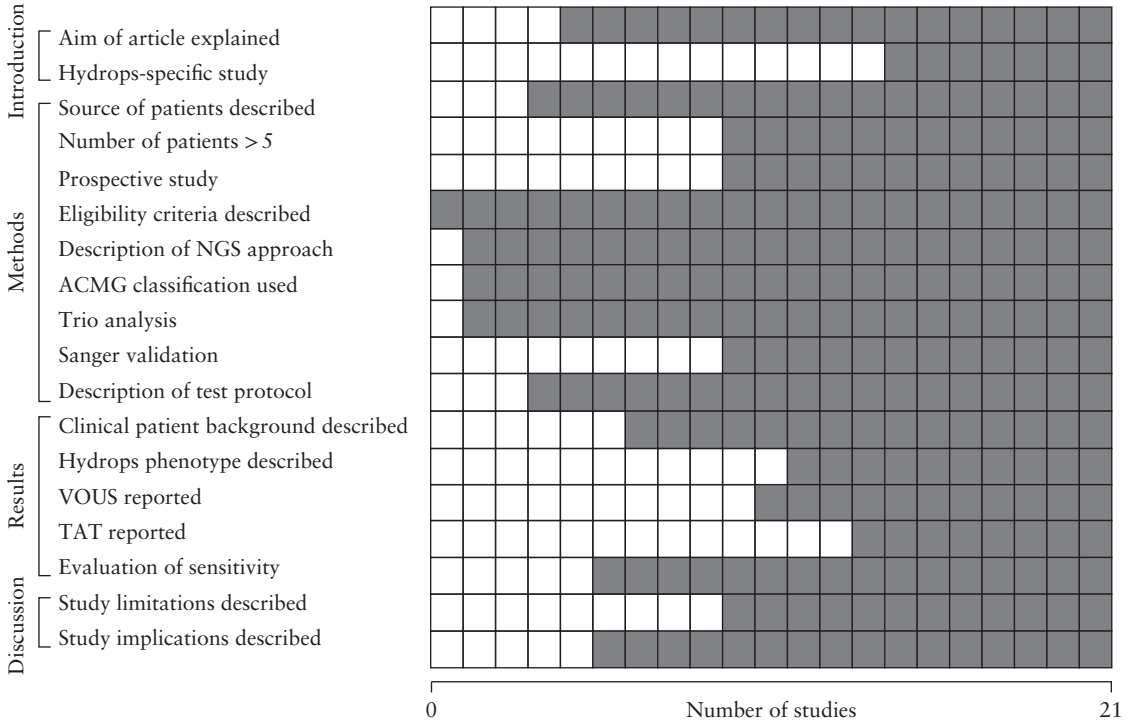

Figure 2 Quality assessment of 21 studies included in systematic review, using modified standards for reporting of diagnostic accuracy studies (STARD) criteria. ACMG, American College of Medical Genetics and Genomics; NGS, next-generation sequencing; TAT, turnaround time; VOUS, variants of uncertain significance. □, no; ■, yes.

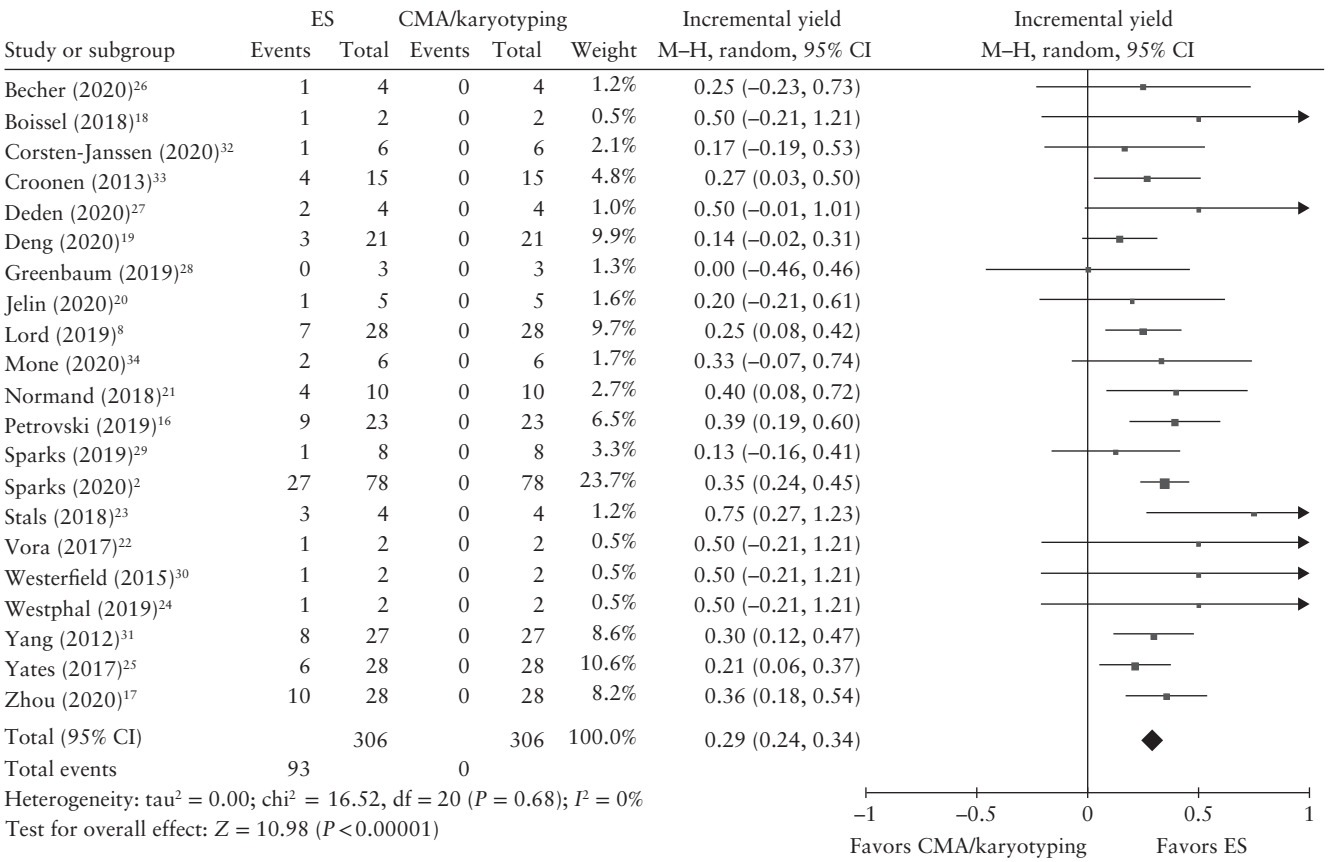

Figure 3 Forest plot showing incremental yield of exome sequencing (ES) over chromosomal microarray analysis (CMA) or karyotyping in all fetuses with prenatally detected non-immune hydrops fetalis. Only first author of each study is given. M–H, Mantel–Haenszel.

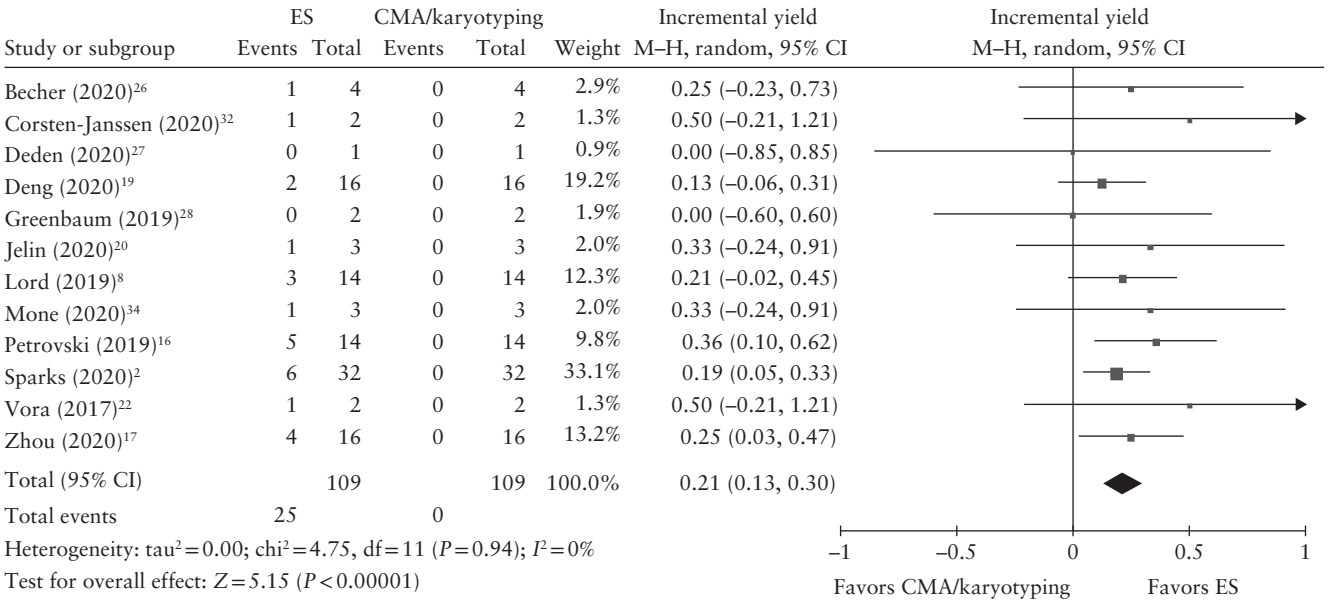

Figure 4 Forest plot showing incremental yield of exome sequencing (ES) over chromosomal microarray analysis (CMA) or karyotyping in fetuses with prenatally detected isolated non-immune hydrops fetalis. Only first author of each study is given. M–H, Mantel–Haenszel.

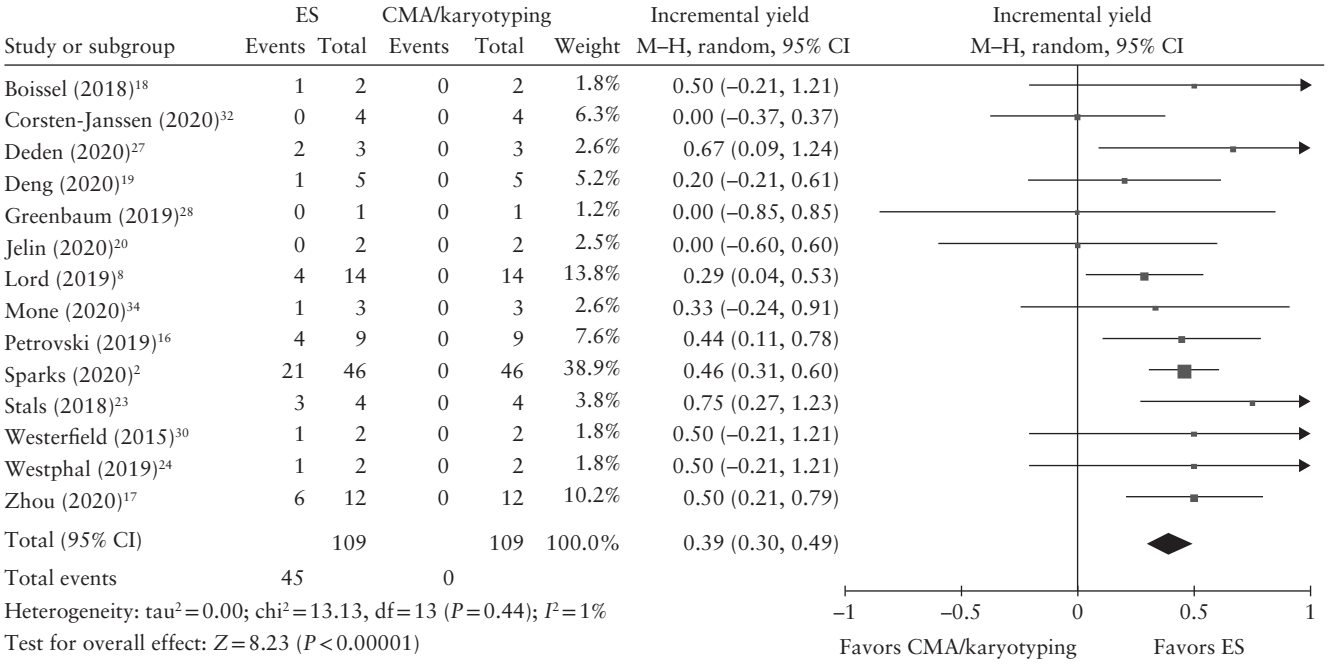

Figure 5 Forest plot showing incremental yield of exome sequencing (ES) over chromosomal microarray analysis (CMA) or karyotyping in fetuses with prenatally detected non-immune hydrops fetalis with an additional fetal structural anomaly. Only first author of each study is given. M–H, Mantel–Haenszel.

The findings of the extended PAGE cohort and the systematic review were broadly concordant, but with a lower incremental yield of ES in the cohort study, which may be explained by the smaller number of cases as well as the unselected approach to case selection. The high incidence of RASopathies and of *de-novo* variants in autosomal dominant disease genes is expected and not mutually exclusive[2]. The incremental yield of ES was higher in NIHF cases in which an additional FSA was present, particularly in cases of congenital arthrogryposis, which is intuitive given that contractures are a common musculoskeletal phenotype known to have a higher diagnostic yield on sequencing[35]. In contrast, isolated NIHF was seen commonly within the RASopathies (40.7%; 11/27). This is in keeping with the variable phenotype reported in RASopathies and supports the use of prenatal ES in cases of isolated NIHF[36]. There is phenotypic variability in cases with a pathogenic variant in other types of genetic disease in addition to in those with a known RASopathy pathogenic variant.

This supports the use of ES or whole-genome sequencing (WGS), rather than a targeted or stepwise approach, in the investigation of NIHF[37]. The role of QF-PCR or conventional karyotyping in NIHF should always be respected, given the high incidence of aneuploidy[38]. However, given the limited additional yield of CMA over karyotyping, and considering the ability of WGS to detect both structural variants and aneuploidy, it may be reasonable in the future to consider WGS as the second-line test after QF-PCR[5]. The list of novel causative genes in NIHF is constantly expanding and, over time, the yield of prenatal NGS will likely improve as more genes are discovered and our understanding of the prenatal phenotype develops[2,37]. This is supported by the high number of Class-III variants (VOUS) identified within candidate genes in this systematic review and highlighted by the largest included series[2]. Reanalysis and potential reclassification of VOUS is currently underway for the PAGE cohort, which may increase the diagnostic yield.

Due to the relatively high yield of ES evident in isolated NIHF in this study (and in individual papers in the literature), it was decided to add NIHF (from March 2021) as an indication for inclusion in the R21 pathway of the National Health Service (NHS) England National Genomic Test Directory for Rare and Inherited Disease[36,39]. The R21 pathway is a nationally (England presently) commissioned rapid prenatal ES service for fetuses with multiple, multisystemic, major and selected isolated FSAs, performed by two genomic laboratory hubs, in line with a set protocol[40]. Furthermore, the Fetal Oedema and Lymphatic Disorder (FOLD) study is ongoing in the UK[41].

The selection criteria for this study were based on the routine definition of NIHF[1]. It has been proposed that this definition should be expanded to include pathological fluid accumulation in one or more fetal body cavity, inclusive of increased (> 3.5 mm) nuchal translucency thickness (NT) or cystic hygroma[2]. This is being explored further, but appears to be a reasonable argument given the large variability in NIHF phenotypes as well as their complex evolution, and sometimes resolution, seen in causative syndromes such as RASopathies. This notion is also supported by our finding that the mere presence of NIHF, as opposed to its severity, influences the diagnostic yield of ES. Hydropic phenotypes can change during pregnancy and findings that may be evident in the first trimester may regress by the third trimester, hence pleural effusion or cystic hygroma in the first trimester may be the only opportunity to detect an anomaly and offer testing. There is a need for studies documenting the evolution of the different phenotypes of NIHF and the respective diagnostic yields of NGS. Despite this, prenatal ES in cases of isolated elevated NT appears to offer a modest increase in diagnostic yield over CMA[2,42–44], of around 5–7%. The severity of increased (≥ 5 mm) NT, its persistence and its association with additional anomalies also appear to influence the diagnostic yield of NGS[2,37,44].

The strengths of this systematic review lie in its novelty with regard to concept, the robust methodology utilized, as well as collaboration between experts of some of the largest contemporary series in this area[2,8,16,17]. Despite the relatively small number of cases ($n = 306$), the present systematic review represents the largest review of prenatally diagnosed NIHF cases, and heterogeneity did not appear to be affected. None of the included studies used a WGS approach, and therefore the difference in yield between WGS and ES could not be assessed. The lack of studies utilizing WGS is likely to change in the coming years and it will likely prove to be more beneficial than ES due to its all-in-one ability to detect most chromosomal and genetic differences[7,39].

In conclusion, the use of prenatal NGS in both isolated NIHF and NIHF associated with an additional FSA should be considered in the development of clinical pathways. Given the vast syndromic categories and heterogeneity in the prenatal phenotype of NIHF, a whole-exome or WGS approach in combination with accurate prenatal phenotyping is likely to be a more appropriate tool than a targeted or stepwise single-gene testing strategy in achieving an optimum diagnostic yield. The existing definition of NIHF appears to be appropriate for assessing the diagnostic yield of ES, although further studies assessing expansion of this definition are required to support this.

ACKNOWLEDGMENTS

The PAGE study was supported by a Health Innovation Challenge from the UK Department of Health and Wellcome Trust (no. HICF-R7-396). We are grateful to Jane Fisher from Antenatal Results and Choices and to Michael Parker of The Ethox Centre, Nuffield Department of Population Health and Wellcome Centre for Ethics and Humanities, University of Oxford, Oxford, UK, for their valuable input to the study. We are also grateful to the members of the PAGE study clinical review panel. L.S.C., a NIHR Senior Investigator, is funded partially by the National Institute for Health Research (NIHR) Biomedical Research Centre at Great Ormond Street Hospital, London, UK. E.R.M. acknowledges support from NIHR Cambridge Biomedical Research Centre, Cambridge, UK (NIHR Senior Investigator Award). The University of Cambridge, Cambridge, UK, has received salary support with regard to E.R.M. from the UK National Health Service (NHS) in the East of England through the Clinical Academic Reserve. The views expressed herein are those of the authors and not necessarily those of the NIHR, NHS or Department of Health.

Disclosures

R.Y.E. and J.L. report grants from the Health Innovation Challenge Fund during the conduct of the PAGE study. D.J.M. reports grants for travel expenses from Congenica to attend educational symposia during the conduct of the

PAGE study. M.E.H. reports grants from the Wellcome Trust and the UK Government Department of Health during the conduct of the study and personal fees from Congenica, outside of the submitted work. M.D.K. is a member of Illumina's International Perinatal Advisory Group (but receives no payment for this) and is the Fetal Medicine Representative for the Central and South GLH. He is also the Royal College of Obstetricians and Gynaecologists' (RCOG) representative on the Joint Colleges Committee of Genomic and Genetic Medicine and the RCOG Genomic Taskforce. E.R.M. has received travel expenses, accommodation and consultant fees for participating in an Illumina International Advisory Group after completion of the PAGE study. M.D.K. is funded through the Department of Health, Wellcome Trust and Health Innovation Challenge Fund (award number HICF-R7-396) for the PAGE and PAGE2 research studies completed in August 2019. L.S.C. was funded partially by the same group in relation to the PAGE study. R.J.W. receives funding from Illumina and NIH for research. M.E.N. has been funded from Ultragenyx and the NIH for research relevant to included cases.

REFRENCES

1. Norton ME, Chauhan SP, Dashe JS. Society for Maternal–Fetal Medicine (SMFM) clinical guideline #7: nonimmune hydrops fetalis. *Am J Obstet Gynecol* 2015; **212**: 127–139.
2. Sparks TN, Lianoglou BR, Adami RR, Pluym ID, Holliman K, Duffy J, Downum SL, Patel S, Faubel A, Boe NM, Field NT, Murphy A, Laurent LC, Jolley J, Uy C, Slavotinek AM, Devine P, Hodoglugil U, Van Ziffle J, Sanders SJ, MacKenzie TC, Norton ME, University of California Fetal–Maternal Consortium; University of California, San Francisco Center for Maternal–Fetal Precision Medicine. Exome Sequencing for Prenatal Diagnosis in Nonimmune Hydrops Fetalis. *N Engl J Med* 2020; **383**: 1746–1756.
3. Mardy AH, Chetty SP, Norton ME, Sparks TN. A system-based approach to the genetic etiologies of non-immune hydrops fetalis. *Prenat Diagn* 2019; **39**: 732–750.
4. Ota S, Sahara J, Mabuchi A, Yamamoto R, Ishii K, Mitsuda N. Perinatal and one-year outcomes of non-immune hydrops fetalis by etiology and age at diagnosis. *J Obstet Gynaecol Res* 2016; **42**: 385–391.
5. Mardy AH, Rangwala N, Yessenia Hernandez-Cruz Y, Gosnell KA, Gonzalez JM, Norton ME, Sparks TN. Utility of chromosomal microarray for diagnosis in cases of nonimmune hydrops fetalis. *Prenat Diagn* 2020; **40**: 492–496.
6. Quinn AM, Valcarcel BN, Makhamreh MM, Al-Kouatly HB, Berger SI. A systematic review of monogenic etiologies of nonimmune hydrops fetalis. *Genet Med* 2021; **23**: 3–12.
7. Mone F, Quinlan-Jones E, Ewer AK, Kilby MD. Exome sequencing in the assessment of congenital malformations in the fetus and neonate. *Arch Dis Child Fetal Neonatal Ed* 2019; **104**: F452–456.
8. Lord J, McMullan DJ, Eberhardt RY, Rinck G, Hamilton SJ, Quinlan-Jones E, Prigmore E, Keelagher R, Best SK, Carey GK, Mellis R, Robart S, Berry IR, Chandler KE, Cilliers D, Cresswell L, Edwards SL, Gardiner C, Henderson A, Holden ST, Homfray T, Lester T, Lewis RA, Newbury-Ecob R, Prescott K, Quarrell OW, Ramsden SC, Roberts E, Tapon D, Tooley MJ, Vasudevan PC, Weber AP, Wellesley DG, Westwood P, White H, Parker M, Williams D, Jenkins L, Scott RH, Kilby MD, Chitty LS, Hurles ME, Maher ER; Prenatal Assessment of Genomes and Exomes Consortium. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. *Lancet* 2019; **393**: 747–757.
9. Mone F, Eberhardt RY, Morris RK, Hurles ME, McMullan DJ, Maher ER, Lord J, Chitty LS, Giordano JL, Wapner RJ, Kilby MD; CODE Study Collaborators. COngenital heart disease and the Diagnostic yield with Exome sequencing (CODE) study: prospective cohort study and systematic review. *Ultrasound Obstet Gynecol* 2021; **57**: 43–51.
10. Köhler S, Gargano M, Matentzoglu N, Carmody LC, Lewis-Smith D, Vasilevsky NA, Danis D, Balagura G, Baynam G, Brower AM, Callahan TJ, Chute CG, Est JL, Galer PD, Ganesan S, Griese M, Haimel M, Pazmandi J, Hanauer M, Harris NL, Hartnett MJ, Hastreiter M, Hauck F, He Y, Jeske T, Kearney H, Kindle G, Klein C, Knoflach K, Krause R, Lagorce D, McMurry JA, Miller JA, Munoz-Torres MC, Peters RL, Rapp CK, Rath AM, Rind SA, Rosenberg AZ, Segal MM, Seidel MG, Smedley D, Talmy T, Thomas Y, Wiafe SA, Xian J, Yüksel Z, Helbig I, Mungall CJ, Haendel MA, Robinson PN. The Human Phenotype Ontology in 2021. *Nucleic Acids Res* 2021; **49**: D1207–1217.
11. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. 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**: 405–524.
12. Liberati A, Altman DG, Tetzlaff J, Mulrow C. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Plos Med* 2009; **6**: e1000100.
13. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008–2012.
14. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, Lijmer JG, Moher D, Rennie D, de Vet HC; Standards for Reporting of Diagnostic Accuracy. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Standards for Reporting of Diagnostic Accuracy. *Clin Chem* 2003; **49**: 1–6.
15. Jansen FA, Blumenfeld YJ, Fisher A, Cobben JM, Odibo AO, Borrell A, Haak MC. Array comparative genomic hybridization and fetal congenital heart defects; a systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2015; **45**: 27–35.
16. Petrovski S, Aggarwal V, Giordano JL, Stosic M, Wou K, Bier L, Spiegel E, Brennan K, Stong N, Jobanputra V, Ren Z, Zhu X, Mebane C, Nahum O, Wang Q, Kamalakaran S, Malone C, Anyane-Yeboa K, Miller R, Levy B, Goldstein DB, Wapner RJ. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. *Lancet* 2019; **393**: 758–767.
17. Zhou X, Zhou J, Wei X, Yang Y, Guo M, Deng L, Wang J, Sun L. Whole-exome sequencing for prenatal diagnosis of fetuses with recurrent nonimmune hydrops fetalis (NIHF). *Prenat Diagn* 2020; **40**: 3–21.
18. Boissel S, Fallet-Bianco C, Chitayat D, Kremer V, Nassif C, Rypens F, Delrue MA, Dal Soglio D, Oligny LL, Patey N, Flori E, Cloutier M, Dyment D, Campeau P, Karalis A, Nizard S, Fraser WD, Audibert F, Lemyre E, Rouleau GA, Hamdan FF, Kibar Z, Michaud JL. Genomic study of severe fetal anomalies and discovery of GREB1L mutations in renal agenesis. *Genet Med* 2018; **20**: 745–753.
19. Deng Q, Fu F, Yu Q, Li R, Li F, Wang D, Lei T, Yang X, Liao C. Nonimmune hydrops fetalis: genetic analysis and clinical outcome. *Prenat Diagn* 2020; **40**: 803–812.
20. Jelin AC, Sobreira N, Wohler E, Solomon B, Sparks T, Sagaser KG, Forster KR, Miller J, Witmer PD, Hamosh A, Valle D, Blakemore K. The utility of exome sequencing for fetal pleural effusions. *Prenat Diagn* 2020; **40**: 590–595.
21. Normand EA, Braxton A, Nassef S, Ward PA, Vetrini F, He W, Patel V, Qu C, Westerfield LE, Stover S, Dharmadhikari AV, Muzny DM, Gibbs RA, Dai H, Meng L, Wang X, Xiao R, Liu P, Bi W, Xia F, Walkiewicz M, Van den Veyver IB, Eng CM, Yang Y. Clinical exome sequencing for fetuses with ultrasound abnormalities and a suspected Mendelian disorder. *Genome Med* 2018; **10**: 74.
22. Vora NL, Powell B, Brandt A, Strande N, Hardisty E, Gilmore K, Foreman AKM, Wilhelmsen K, Bizon C, Reilly J, Owen P, Powell CM, Skinner D, Rini C, Lyerly AD, Boggess KA, Weck K, Berg JS, Evans JP. Prenatal exome sequencing in anomalous fetuses: new opportunities and challenges. *Genet Med* 2017; **19**: 1207–1216.
23. Stals KL, Wakeling M, Baptista J, Caswell R, Parrish A, Rankin J, Tysoe C, Jones G, Gunning AC, Lango Allen H, Bradley L, Brady AF, Carley H, Carmichael J, Castle B, Cilliers D, Cox H, Deshpande C, Dixit A, Eason J, Elmslie F, Fry AE, Fryer A, Holder M, Homfray T, Kivuva E, McKay V, Newbury-Ecob R, Parker M, Savarirayan R, Searle C, Shannon N, Shears D, Smithson S, Thomas E, Turnpenny PD, Varghese V, Vasudevan P, Wakeling E, Baple EL, Ellard S. Diagnosis of lethal or prenatal-onset autosomal recessive disorders by parental exome sequencing. *Prenat Diagn* 2018; **38**: 33–43.
24. Westphal DS, Leszinksi GS, Rieger-Fackeldey E, Graf E, Weirich G, Meitinger T, Ostermayer E, Oberhoffeer R, Wagner M. Lessons from exome sequencing in prenatally diagnosed heart defects: A basis for prenatal testing. *Clin Genet* 2019; **95**: 582–589.
25. Yates CL, Monaghan KG, Copenheaver D, Retterer K, Scuffins J, Kucera CR, Friedman B, Richard G, Juusola J. Whole-exome sequencing on deceased fetuses with ultrasound anomalies: expanding our knowledge of genetic disease during fetal development. *Genet Med* 2017; **19**: 1171–1178.
26. Becher N, Andreasen L, Sandager P, Lou S, Petersen OB, Christensen R, Vogel I. Implementation of exome sequencing in fetal diagnostics-Data and experiences from a tertiary center in Denmark. *Acta Obstet Gynecol Scand* 2020; **99**: 783–790.
27. Deden C, Neveling K, Zafeiropopoulou D, Gilissen C, Fundt R, Rinne T, de Leeuw N, Faas B, Gardeitchik T, Sallevelt SCEH, Paulussen A, Stevens SJC, Sikkel E, Elting MW, van Maarle MC, Diderich KEM, Corsten-Janssen N, Lichtenbelt KD, Lachmeijer G, Vissers LELM, Yntema HG, Nelen M, Feenstra I, van Zelst-Stams WAG. Rapid whole exome sequencing in pregnancies to identify the underlying genetic cause in fetuses with congenital anomalies detected by ultrasound imaging. *Prenat Diagn* 2020; **40**: 972–983.
28. Greenbaum L, Pode-Shakked B, Eisenberg-Barzilai S, Dicastro-Keidar M, Bar-Ziv A, Goldstein N, Reznik-Wolf H, Poran H, Rigbi A, Barel O, Bertoli-Avella AM, Bauer P, Regev M, Raas-Rothschild A, Pras E, Berkenstadt M. Evaluation of Diagnostic Yield in Fetal Whole-Exome Sequencing: A Report on 45 Consecutive Families. *Front Genet* 2019; **10**: 425.
29. Sparks TN, Thao K, Lianoglou BR, Boe NM, Bruce KG, Datkhaeva I, Field NT, Fratto VM, Jolley J, Laurent LC, Mardy AH, Murphy AM, Ngan E, Rangwala N, Rottkamp CAM, Wilson L, Wu E, Uy CC, Lopez PV, Norton ME, University of California Fetal–Maternal Consortium (UCfC). Nonimmune hydrops fetalis: identifying the underlying genetic etiology. *Genet Med* 2019; **21**: 1339–1344.
30. Westerfield LE, Stover SR, Mathur VS, Nassef SA, Carter TG, Yang Y, Eng CM, Van den Veyver IB. Reproductive genetic counseling challenges associated with diagnostic exome sequencing in a large academic private reproductive genetic counseling practice. *Prenat Diagn* 2015; **35**: 1022–1029.

31. Yang YS, Ma GC, Shih JC, Chen CP, Chou CH, Yeh KT, Kuo SJ, Chen TH, Hwu WL, Lee TH, Chen M. Experimental treatment of bilateral fetal chylothorax using in-utero pleurodesis. *Ultrasound Obstet Gynecol* 2012; **39**: 56–62.
32. Corsten-Janssen N, Bouman K, Diphoorn JCD, Scheper AJ, Kinds R, El Mecky, Breet H, Verheij JBGM, Suijkerbuijk R, Duin LK, Manten GTR, van Langen IM, Sijmons RH, Sikkema-Raddatz B, Westers H, van Diemen CC. A prospective study on rapid exome sequencing as a diagnostic test for multiple congenital anomalies on fetal ultrasound. *Prenat Diagn* 2020; **40**: 1300–1309.
33. Croonen EA, Nillesen WM, Stuurman KE, Oudesluijs G, van de Laar IMBM, Martens L, Ockeloen C, Mathijssen IB, Schepens M, Ruiterkamp-Versteeg M, Scheffer H, Faas BHW, van der Burgt I, Yntema HG. Prenatal diagnostic testing of the Noonan syndrome genes in fetuses with abnormal ultrasound findings. *Eur J Hum Genet* 2013; **21**: 936–942.
34. Mone F, Doyle S, Hamilton S, Allen S, Williams D, Kilby MD. VP33.06: Non-immune hydrops fetalis and diagnostic yield with prenatal-exome sequencing: a case series. *Ultrasound Obstet Gynecol* 2020; **56**: 195.
35. Pehlivan D, Bayram Y, Gunes N, Akdemir ZC, Shukla A, Bierhals T, Tabakci B, Sahin Y, Gezdirici A, Fatih JM, Gulec EY, Yesil G, Punetha J, Ocak Z, Grochowski CM, Karaca E, Albayrak HM, Radhakrishnan P, Erdem HB, Sahin I, Yildirim T, Bayhan IA, Bursali A, Muhsin Elmas M, Yuksel Z, Ozdemir O, Silan F, Yildiz O, Yesilbas O, Isikay S, Balta B, Gu S, Jhangiani SN, Doddapaneni H, Hu J, Muzny DM, Baylor-Hopkins Center for Mendelian Genomics; Boerwinkle E, Gibbs RA, Tsiakas K, Hempel M, Girisha KM, Gul D, Posey JE, Elcioglu NH, Tuysuz B, Lupski JR. The Genomics of Arthrogryposis, a Complex Trait: Candidate Genes and Further Evidence for Oligogenic Inheritance. *Am J Hum Genet* 2019; **105**: 132–150.
36. National Genomic Test Directory. NHS England 2020/2021. https://www.england.nhs.uk/publication/national-genomic-test-directories/
37. Stuurman KE, Joosten M, van der Burgt I, Elting M, Yntema HG, Meijers-Heijboer H, Rinne T. Prenatal ultrasound findings of rasopathies in a cohort of 424 fetuses: update on genetic testing in the NGS era. *J Med Genet* 2019; **56**: 654–661.
38. Sileo FG, Kulkarni A, Branescu I, Homfray T, Dempsey E, Mansour S, B Thilaganathan B, Bhide A, Khalil A. Non-immune fetal hydrops: etiology and outcome according to gestational age at diagnosis. *Ultrasound Obstet Gynecol* 2020; **56**: 416–421.
39. Mone F, McMullan DJ, Williams D, Chitty LS, Maher ER, Kilby MD; Fetal Genomics Steering Group of the British Society for Genetic Medicine; the Royal College of Obstetricians and Gynaecologists. Evidence to Support the Clinical Utility of Prenatal Exome Sequencing in Evaluation of the Fetus with Congenital Malformations. Scientific Impact Paper No. 64. *BJOG* 2021; **28**: e39–50.
40. NHS England. *Rapid Exome Sequencing Service for Fetal Anomalies Testing*. 2021. https://www.england.nhs.uk/wp-content/uploads/2021/07/B0179_Guidance-rapid-exome-sequencing-service-for-fetal-anomalies_July21.pdf
41. Dempsey E. ISRCTN22076461. A study to improve our understanding of the genetic causes of swelling in babies before birth. https://doi.org/10.1186/ISRCTN22076461.
42. Achiron R, Heggesh J, Grisaru D, Goldman B, Lipitz S, Yagel S, Frydman M. Noonan syndrome: a cryptic condition in early gestation. *Am J Med Genet* 2000; **92**: 159–165.
43. Yang X, Huang LY, Pan M, Xu L, Zhen L, Han J, Li D. Exome sequencing improves genetic diagnosis of fetal increased nuchal translucency. *Prenat Diagn* 2020; **40**: 1426–1431.
44. Mellis R, Eberhardt RY, Hamilton SJ, PAGE Consortium, McMullan DJ, Kilby MD, Maher ER, Hurles ME, Giordano JL, Aggarwal V, Goldstein DB, Wapner RJ, Chitty LS. Fetal exome sequencing for isolated increased nuchal translucency: Should we be doing it? *BJOG* 2021. DOI: 10.1111/1471-0528.16869.

SUPPORTING INFORMATION ON THE INTERNET

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

Table S1 Documented diagnostic (Class-IV or -V) variants in studies reporting on the incremental yield of exome sequencing over chromosomal microarray analysis or karyotyping in fetuses with prenatally detected non-immune hydrops fetalis

Table S2 Documented variants of uncertain significance (Class III) in studies reporting on the incremental yield of exome sequencing over chromosomal microarray analysis or karyotyping in fetuses with prenatally detected non-immune hydrops fetalis

Figure S1 Funnel plots for studies reporting on the incremental yield of exome sequencing over chromosomal microarray analysis or karyotyping in fetuses with prenatally detected non-immune hydrops fetalis (NIHF), overall (a) and in those with isolated NIHF (b) or NIHF with an additional fetal structural anomaly (c).

Figure S2 Forest plots showing incremental yield of exome sequencing over chromosomal microarray analysis or karyotyping in fetuses with prenatally detected non-immune hydrops fetalis (NIHF), according to whether two (a) or three or more (b) body cavities were affected.