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SYSTEMATIC REVIEW

A systematic review to assess the utility of genomic autopsy using exome or genome sequencing in cases of congenital anomalies and perinatal death

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ABSTRACT

Purpose: Exome or genome sequencing (ES or GS) can identify genetic causes of otherwise unexplained congenital anomaly and perinatal death (PND) but is not routine practice. The evidence base for "genomic autopsy" after termination of pregnancy for fetal anomaly (TOPFA) and PND has been synthesized to determine the value of this investigation.

Methods: We conducted a systematic review and meta-analysis of studies meeting prespecified inclusion criteria and containing ≥10 cases of TOPFA or PND (with or without major congenital abnormality), in which ES or GS was conducted. We determined test performance, including diagnostic yield, accuracy, and reliability. We also reported outcomes associated with clinical utility and harms, where described.

Results: From 2245 potentially eligible studies, 32 publications were eligible and had data extracted, representing 2120 cases that could be meta-analyzed. No diagnostic accuracy or comparative studies were identified, although some analysis of concordance between different ES/GS methodologies could be performed. Studies reporting parent-related outcomes or long-term follow-up did not do so in a systematic or quantifiable manner.

Conclusion: Evidence suggests that approximately one-fourth to one-third of fetal losses associated with TOPFA or unexplained PND are associated with a genetic cause identifiable on ES or GS—albeit this estimate varies depending on phenotypic and background risk factors. Despite the large body of evidence on ES and GS, little research has attempted to validate the accuracy of testing, nor measure the clinical or societal outcomes in families that follow the diagnostic investigation in this context.

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Introduction

In the last decade, rates of termination of pregnancy for fetal anomaly (TOPFA) and perinatal death (PND) in countries with modern health care systems have each been reported within the range of 2 to 10 deaths per 1000 births.[1,2] Approximately half of PND can be attributed to premature delivery or complications associated with carriage, labor, or infection, but for those not explained by these factors, a major congenital abnormality (MCA) is present in approximately two-thirds of the cases, whereas for the remaining cases there is no obvious anomaly.[3] Such an end to a pregnancy or death of a baby is tragic for parents and society and hence the strong desire to discover "why," to understand the occurrence, and to prevent recurrence.

Investigations are recommended to seek the cause of congenital development error or unexplained death; "first-tier" genetic investigations (karyotyping and/or chromosomal microarray [CMA]) that detect large genetic coding errors (eg, chromosomal or submicroscopic deletions or duplications) are routine. These identify genetic errors in 10% to 20% of cases,[3] but for the majority, the underlying cause is elusive. Full autopsy is considered the "gold standard" method of investigation, but the invasiveness is distressing to parents, and consent is often declined.[1,4]

The increased accessibility of massive parallel gene sequencing technology has opened up a new era of investigative diagnostics at the genetic level. Some pathologies can be explained at the molecular level by a single-nucleotide variant (SNV) in a single gene. A lethal genetic variant in a deceased fetus may provide a biological explanation for MCA or PND. Furthermore, in cases which such variants are identified, Mendelian inheritance patterns are applicable, and if coupled with parental genetic information, the likelihood of recurrence can be determined. Parents can utilize this information to prevent PND recurrence or facilitate early detection using reproductive medicine technologies (preimplantation and/or prenatal genetic testing).

Objective

Many publications describe diagnostic success following molecular DNA sequencing in cases of PND and TOPFA and some also report how diagnoses have influenced future pregnancy outcomes.[5-9] However, untargeted genetic analysis potentially increases risk of error or incidental findings, and these consequences are less reported. To determine whether molecular DNA sequencing after PND and TOPFA should become routine practice, we assessed the technology from a population-level perspective. We sought to (1) determine the diagnostic performance (yield and accuracy) of genomic autopsy (exome or genome sequencing [ES/GS]) for identifying a cause of death and pathogenic variants, with or without conventional autopsy and investigations and compared with current practice and (2) identify the extended clinical, social, or economic impacts (benefits and harms) associated with this investigation.

Materials and Methods

Study eligibility criteria

The population of interest was defined as fetuses and babies who have died between an age greater than 11 weeks' gestation and before 4 weeks of life after birth and also cases of TOPFA. This broad definition of "perinatal death" (extending to relatively early fetal losses) acknowledges that modern clinical obstetric practice allows earlier detection of fetal development concerns and also a society increasingly cognizant of the personal impact on parents associated with earlier pregnancy loss.

The diagnostic intervention of interest was untargeted DNA molecular sequencing. This included ES (including any described as "whole," clinical, Mendeliome, or medical ES) or GS, for the purpose of identifying a SNV cause of lethal malformation or death. Studies in which molecular analysis was limited to targeted gene sites (a single gene or multiple gene panel) were excluded.

The comparator was diagnosis based on conventional autopsy and genetic investigations in cases of TOPFA and unexplained PND. Primary outcomes of interest related to diagnostic performance (diagnostic yield, accuracy, and test failure rates), clinical utility (subsequent pregnancy outcomes, parent satisfaction, harm, and incidental findings), and economic outcomes (eg, cost-effectiveness) after the use of genomic autopsy to identify single-gene variant causes of PND.

Opinion-based articles were excluded, and non-English publications were set aside for translation only if they provided higher quality evidence than otherwise available. After screening, a protocol amendment to exclude series with fewer than 10 cases was made on the basis that these would not add substantially to total case numbers but would reduce the efficiency of the review process and potentially reduce the reliability, given the low precision associated with small samples.

Search strategy and screening of literature

A search strategy was developed based on the described "population" and "intervention" and the protocol registered with PROSPERO (CRD42022318765). Five databases were searched without filters or limits: Embase, PubMed, the Cochrane Library, the International Network of Agencies for Health Technology Assessment (INAHTA) Health Technology Assessment Database, and the Prospective Register of Systematic Reviews (PROSPERO). The PubMed search strategy is presented in Table A.1 in the Supplemental Materials.

Retrieved bibliographic references were first reviewed using Rayyan web-based citation screening software[10]; citations were excluded in cases which the title/abstract indicated that the publication would not meet eligibility criteria. A blinded second reviewer (J.M.) independently assessed 20% of the initial retrievals (and later, data extractions) to ensure a decision concordance level above a threshold of 80% was met. Full-text copies of the possibly relevant publications that were screened were then retrieved for a final decision about eligibility according to the inclusion criteria. Decision concordance on the independently reviewed sample was 98.9% with consensus subsequently reached on the initial few disagreements. The SpiderCite web-based application[11] was used to conduct forward and backward citation searching. The complex inclusion criteria meant additional search methods contributed a significant quantity of relevant literature. Studies in which fetuses with prenatally identified anomalies had postmortem DNA analysis were rarely explicitly described as postmortem in the abstract or keywords.

There was some inconsistency between publications as to whether case numbers counted fetuses or families (numerous couples had multiple affected pregnancies included in the same study). In this review "cases" refer to families (multiple sibling PNDs are counted as a single case given their related genetic dispositions).

Data on diagnostic yield were meta-analyzed (for all studies collectively and for relevant subgroups) in STATA MP 17 software[12] using the metaprop command with a random effects model. Metaprop is specifically designed for pooling proportions by use of the binomial distribution to model the within-study variability or by allowing Freeman-Tukey double arcsine transformation to stabilize the variances.[13] Subgroup analyses of population characteristics (phenotype selection and severity and family history/consanguinity rates factors) or testing methodology factors (trio or singleton sampling and GS or ES) were examined.

Data were reported according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses conventions.[14]

Results

Included studies

The search of databases (to April 20, 2023) yielded 10,419 references after duplicates were removed, of which 2244 were retrieved as full-text articles (or conference abstracts) to assess for inclusion. After inclusion of additional articles identified by forward/backward citation searching, 296 publications described cases that met the original selection criteria, of which 97 included 10 or more cases. However, some of these did not report results or contained hypothetical data, included cases reported in other publications, or reported data that were ambiguous with regard to eligibility and/or aggregated with cases that did not meet the specified population, intervention, comparator and outcome (PICO) criteria.

Many studies identified included subpopulations within the target population, but the study as a whole did not exactly align with the selection criteria. For example, some studies on fetuses with prenatally identified anomalies pooled data from ongoing pregnancies and deceased fetuses, and some studies looking at postmortem molecular diagnosis included both neonates and older children. Studies that contained mixed and overlapping populations were closely inspected (including Supplemental Material); quantitative findings were reported only in cases which there were sufficient disaggregated or individual data to identify cases wholly meeting the review criteria. Guadagnolo et al[15] (2021) noted that the inclusion of deceased cases in the prenatal ES context may introduce bias, and similarly, we considered the inclusion of cases from ongoing pregnancies to be problematic when examining the value of ES/GS specifically as a PND investigation. Hence, we excluded cases where sequencing was before death and could have influenced pregnancy outcome; cases with prenatal sampling were only relevant if sequence interpretation occurred knowingly after the fact of TOPFA or PND (intrauterine death, stillbirth, or neonatal death). Populations and/or cases that could not be differentiated or were ambiguous with respect to the timing of sequencing were excluded. The studies and extent of data omitted on these grounds is reported in the Supplemental Materials.

A PRISMA flow chart detailing the article selection process is provided in Figure 1. Ultimately, 32 studies with extractable data on 2090 unique cases that met the PICO criteria were identified (summarized in Section 3 and assessed for risk of bias in Section 4 of the Supplemental Materials), but there were no studies that compared ES/GS with current practice (ie, controlled studies).

Diagnostic yield

Diagnostic yield is generally defined as the likelihood that a test or procedure will provide the information needed to establish a diagnosis.[16] Determining the cause of the anomaly (resulting in termination) or PND is the primary goal of the postmortem investigation; therefore, the diagnostic yield is a key indicator of test utility. In view of the data available, the yields reported here reflect the proportion of cases with causative single-gene variants identified of those successfully completing testing (test failures are reported separately under "reliability").

All studies (except Yang et al[17]) report that first-tier genetic testing (karyotyping and CMA) with no findings, is generally confirmed before testing for SNVs. Therefore, the outcomes reported represent incremental outcomes after karyotyping/CMA.

Consistent with accepted standards, variant(s) are only considered causative when they are identified as pathogenic or likely pathogenic variants (P/LP) and have an association

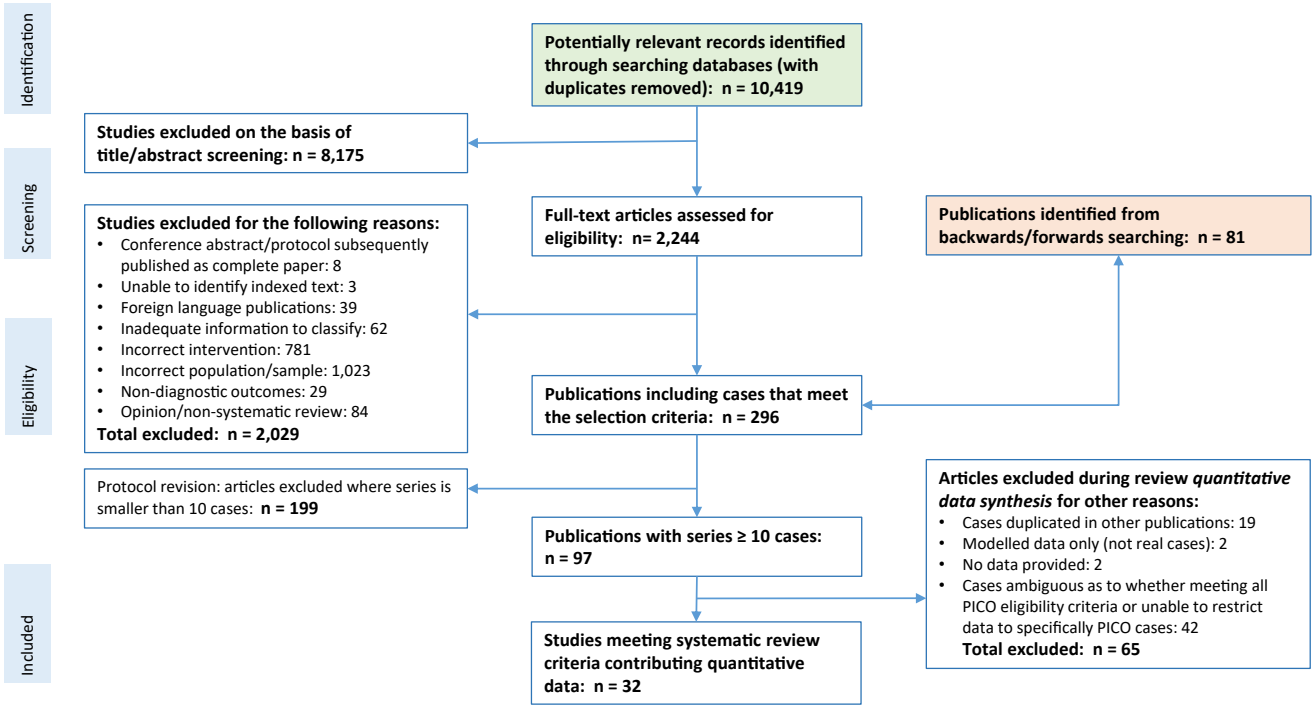

Figure 1 PRISMA flow chart identifying publication selection for inclusion in the systematic review.

with the phenotype. Findings of variants of uncertain significance (VUS) and candidate variants are not included in this review. Forest plots for all reported analyses not presented in this article are presented in the Supplemental Materials (Section 5, Supplementary Figures 1-6).

A random effects model estimated overall diagnostic yield for P/LP variants across all studies as 32% (95% CI: 25%-39%); however, the I^2 value was >90%, showing substantial heterogeneity. Excluding the 2 studies[18,19] that were conducted specifically in the context of reanalysis (cases previously identified as negative on molecular sequencing) increased the yield by only 2%; however, the I^2 value remained high, suggesting other causes of substantial interstudy heterogeneity. Subgroup meta-analysis by study design and publication year did not indicate these were meaningful causes of heterogeneity (although a trend of steadily increasing yield was observed for publications between 2014-2021), therefore the differences in clinical characteristics of the cases and variation in sequencing methods were also examined.

Consanguinity and/or family history

The proportion of cases involving consanguineous parents and/or a history of affected pregnancies is one explanation for some heterogeneity. Multiple studies observed consanguinity and familial history as factors associated with the increased likelihood of diagnosis.[6,7,9,20-23] Across studies, parental consanguinity ranged from 0%[24] to 93%[25]; with respective yields of 11% and 58%. Similarly, parental history of fetal abnormality ranged from 6%[26] to 58%,[27] with respective yields of 12.5% and 52%. The meta-analysis presented in Figure 2 shows the increased yield rates associated with consanguinity and/or family history vs neither. Heterogeneity within the subgroups still persisted and was moderate to high.

Phenotype-related factors

Many of the studies required at least 1 physical anomaly to meet the inclusion criteria, and some required multiple anomalies. Meta-analysis of diagnostic yield by the extent of phenotype manifestation is presented in Figure 3, showing a distinctly lower yield in cases without a physical phenotype (5%, 95% CI: 2%-7%) and the highest yield in complex or multiorgan manifestations (32%, 95% CI: 27%-38%).

Further meta-analysis grouped phenotypes by the organ system affected and estimated the following diagnostic yields across the studies (highest to lowest): skeletal 68% (95% CI: 52%-85%), lymphatic (including nonimmune hydrops fetalis) 47% (95% CI: 31%-64%), muscular/neuromuscular 41% (95% CI: 15%-67%), genitourinary 30% (95% CI: 2%-58%), cardiac/cardiovascular 26% (95% CI: 14%-38%), and central nervous system (CNS) 17% (95% CI: 12%-23%).

Sequencing approach

Only 2 of the studies[17,26] routinely sequenced the genome, and 1 of them[17] was selective for cases with CNS

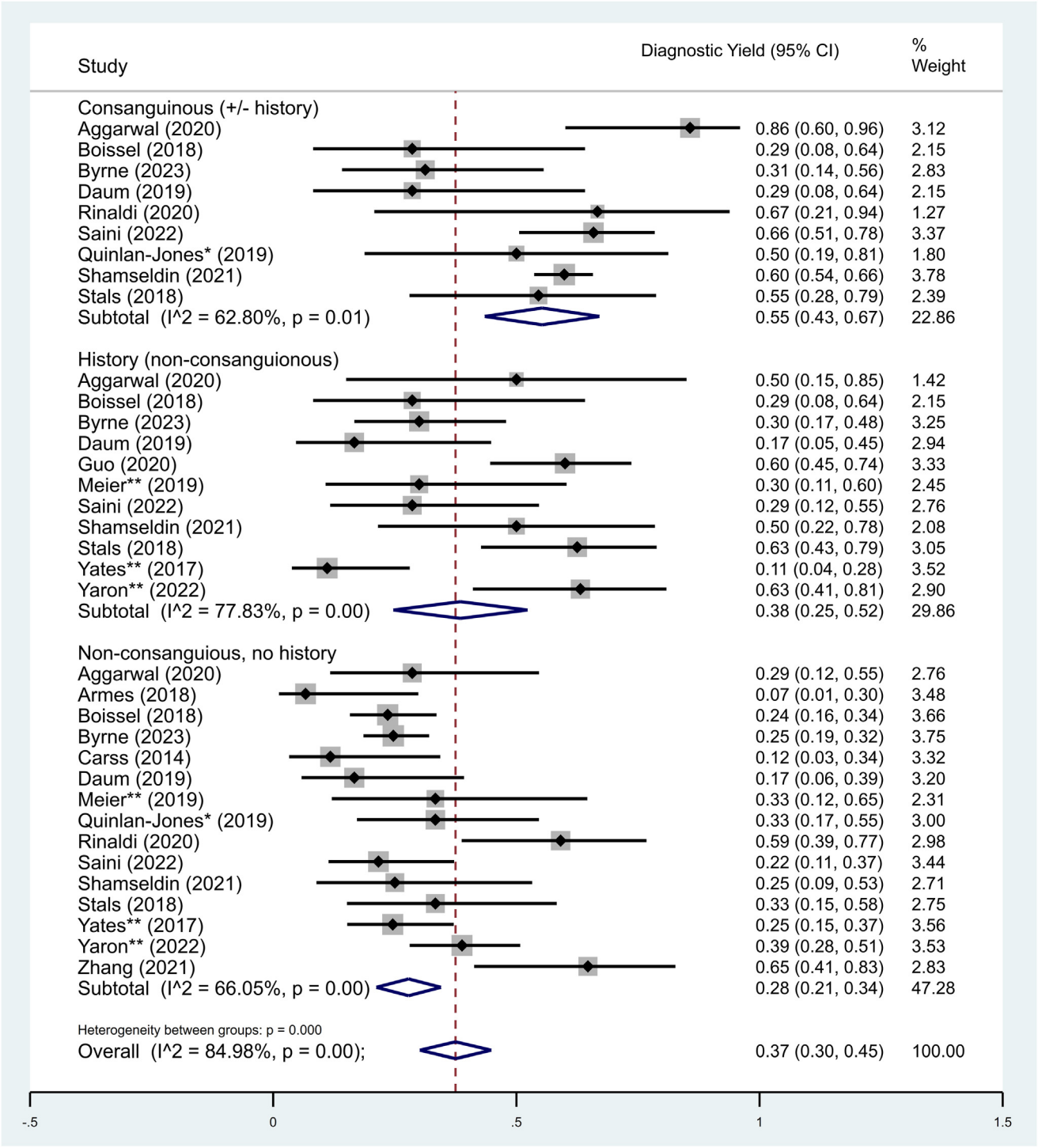

Figure 2 Forest plot showing diagnostic yield with studies grouped by parental consanguinity (with or without history), parental history of fetal anomaly or PND, or neither (ie, nonconsanguineous parents with no relevant familial history). The meta-analysis was originally run including all available data. The analysis excluded cases with History (non-consanguineous) from Armes et al[26], Carss et al,[24] and Rinaldi et al[28] because of the small sample sizes in these subgroups. The meta-analysis was rerun manually excluding these data to improve readability of graphical representation. No extractable data on yield by consanguinity/history status for Aarabi et al,[18] Becher et al,[5] Boughalem ,[29] Correa et al,[20] Coste et al,[19] Greenbaum et al,[30] Lefebvre et al,[31] Ranganath, Perala, and Rasheed,[32] Stanley et al,[33] Sun et al, 2020,[34] Sun et al, 2021,[35] Vora et al,[36] Westphal et al,[37] Yang et al, 2014,[38] Yang et al, 2022,[17] or Zhao.[39] *Publication reports by consanguinity status only, history unclear. **Publication reports by family history/recurrence status only, consanguinity unclear.

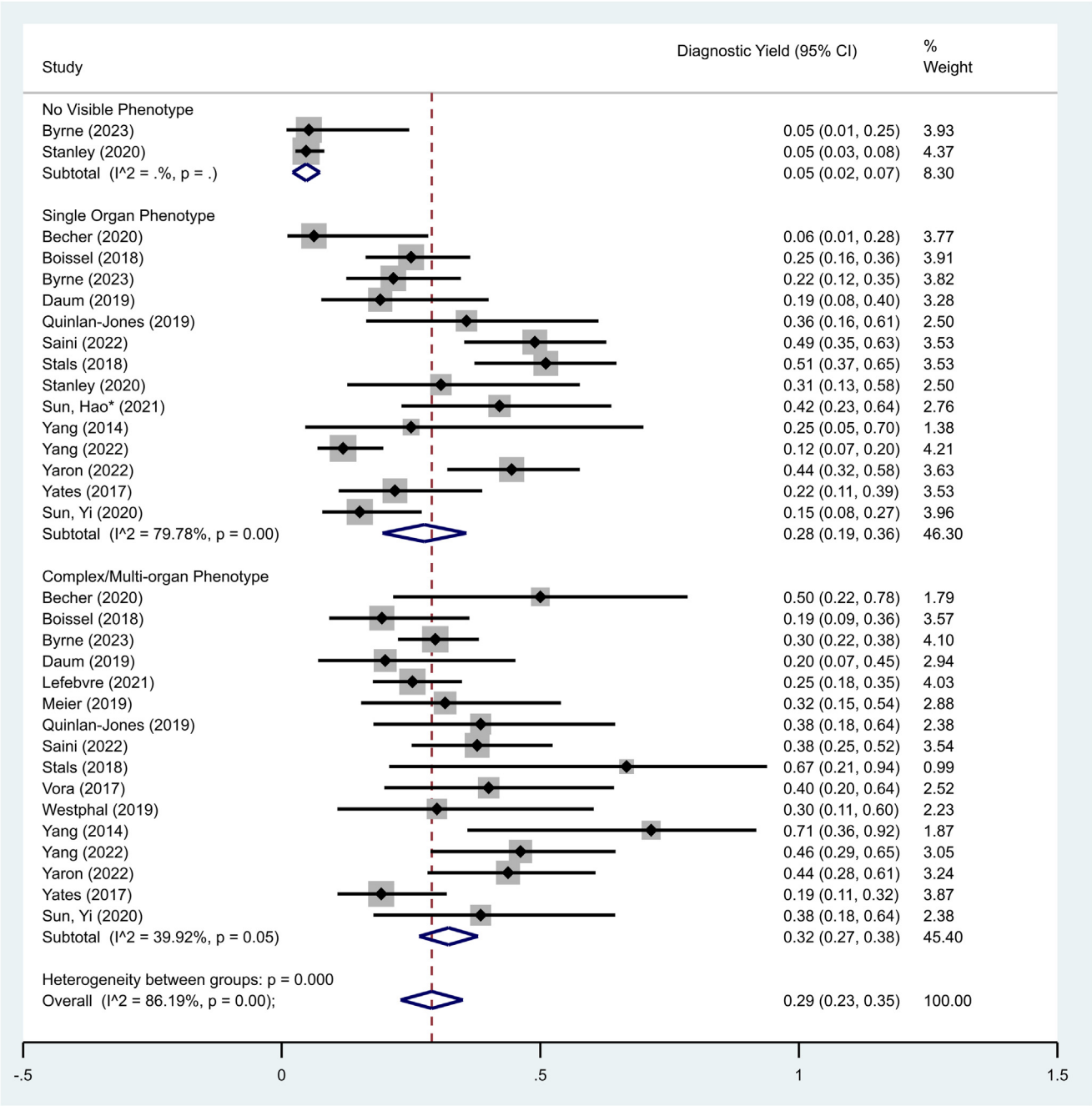

Figure 3 Forest plot showing diagnostic yield with studies grouped by the extent of phenotype manifestation (no phenotype apparent, single organ phenotype, complex/multi-organ phenotype). The meta-analysis was originally run including all available data. The analysis excluded cases from the following sub-groups/studies, because of inadequate sample size: no visible phenotype; Daum et al[7] and single-organ phenotype.[37] The meta-analysis was rerun manually excluding these data to improve readability of graphical representation. *All cases classified as cardiac-specific phenotype.

abnormality and therefore is not comparable to the general series. The other one, which also used trio sampling, had a relatively low diagnostic yield of 13% (95% CI: 3%-36%) but was a relatively early and small study.[26] Most of the nonphenotype-specific studies used ES with trio sampling; however, a substantial portion were limited to singleton samples. In the nonphenotype-specific series, the yield for ES using trio sampling was 31% (95% CI: 21%-40%), and the yield for ES on singleton samples was 32% (95% CI: 15%-48%), with large variability and heterogeneity. The studies that analyzed sufficient duo samples (2 parents as proxies when fetus DNA was not available) had a meta-analyzed overall yield of 52% (95% CI: 42%-63%).

Diagnostic accuracy or concordance

None of the studies assessed the diagnostic accuracy of molecular sequencing by formally estimating analytical or

Table 1 Concordance rates in identification of P/LP variants using ES interpretation with and without autopsy information and with or without phenotype restricted biofiltering

Study and Population	Diagnostic Yield (P/LP Variants) *n/N* (%)			Diagnostic Concordance (vs Autopsy + Unfiltered ES)
	ES[a] No Autopsy	Autopsy + Filtered ES[b]	Autopsy + Unfiltered ES	
Aarabi et al[18] Fetuses with structural birth defects and no findings on prenatal ES.	0/20 (0%)		1/20[c] (5%)	ES without autopsy: 95%
Aggarwal et al[21] Fetuses positive for variants. Retrospectively reanalyzed with filtered variant lists.	7/22 (32%)	12/22 (55%)	22/22 (100%)	ES without autopsy: 32% Autopsy + filtered ES: 55%
Bourgon et al[40] Subgroup of fetuses with identified variants (P/LP or VUS) from Lefebvre[31]	Solo sampling: 22/32 (69%) Trio sampling: 24/32 (75%)		Solo sampling: 24/32 (75%)	ES (solo) without autopsy: 92% ES (trio) without autopsy: 100%
Rinaldi et al[28] Cases with molecular diagnosis on ES + autopsy and unfiltered variant list.	6/19 (32%)	8/19 (42%)	19/19 (100%)	ES, no autopsy: 32% autopsy + filtered ES: 42%
Westphal et al[37] Fetuses with prenatal CHD diagnosis. ES analyzed once with prenatal information only and once with postnatal information.	3/18 (17%)		3/18 (17%)	ES, no autopsy: 100%

CHD, congenital heart defects; *ES*, exome sequencing; *P/LP*, pathogenic/likely pathogenic; *VUS*, variants of uncertain significance.
[a]ES bioinformatics analysis conducted using variant list filtered by prenatally observed phenotype and clinical information only.
[b]ES bioinformatics analysis conducted using variant list filtered by phenotype as observed on autopsy.
[c]An additional case with a P/LP variant was present in this cohort that was not identified using ES. The additional case was identified after retesting with a Sanger sequencing gene panel, requested by clinician because the distinct phenotypical features observed on autopsy were strongly suggestive of genetic pathology.

clinical validity. Most studies explicitly reported using Sanger sequencing (the gold standard for sequencing small sections of DNA) to verify all pathogenic variants as a quality control process; this approach minimizes the risk of false positives due to technological biochemical error.[22] Studies did not report concordance rates between Sanger verifications and NGS-identified variants; however, 1 case is reported in which repeat testing with Sanger sequencing (as part of a targeted gene panel) identified a pathogenic variant that ES did not—ie, a false negative on ES.[18]

A number of studies made observations comparing diagnostic results for a single cohort under alternative scenarios: (1) interpretation of ES in conjunction with autopsy information vs without[18,21,28,37,40] and (2) interpretation of ES with a strict bioinformatic filter (phenotype restricted approach) vs without (genotype first approach).[21,28] Table 1 presents concordance rates between alternative approaches.

With autopsy information, Aarabi et al[18] prospectively identified a pathogenic variant not identifiable in that case without autopsy. Similarly, Bourgon et al[40] (2022) (using positive and VUS cases identified from singleton testing in Lefebvre et al[31] 2021) conducted a blinded trial to estimate diagnostic rates in the absence of postmortem information; 92% of previously identified P/LP variants were reidentified with singleton DNA samples; however, all previously identified P/LP cases were reidentified when trio sampling was used.[40] Aggarwal et al[21] and Rinaldi et al[28] (although retrospective and unblinded) claimed substantially reduced yields (32%) without autopsy information.

With respect to the application of phenotype filtering and consistent with the concordance data presented, many of the study authors affirm their concerns regarding strict phenotype-based filtering because genetic causes of PND are often associated with incomplete or atypical genetic and phenotypic profiles.[5,22,41] A number of studies reported using a multistepped approach to analysis—filtering initially with narrow bio-informatic filters, then in cases without findings sequentially broader filters were applied with the goal of optimizing both accuracy and efficiency.[26]

Another variable associated with the interpretation process that may affect accuracy relates to the availability of follow-up research to investigate the pathogenic potential of individual VUS or candidate variants. Byrne et al[6] (2023) noted that yield increased by 5% because variants initially classified VUS or candidate genes were subsequently reclassified as LP/P following in vitro gene function testing (increasing yield in that series from 21% to 26%).[6]

Test acceptability and reliability

Not all publications reported data that enabled estimates of test completion rates. Of those that reported patient flow, attrition rates varied widely.

The rates of parental consent to fetal genomic testing in eligible cases reported in 3 studies were 27% (80/298),[34] 77% (652/843),[33] and 87% (90/103).[9] Although a particularly narrow population, Sun et al[34] (2020) accepted both singleton and trio samples and no explanation for the exceptionally low consent rate was identified. The pooled consent rate across the other two studies[9,33] is 79% (95% CI: 76%-83%).

Retrospective series reported more problems with DNA availability or quality, such as not being available, inadequate quantity, or degraded samples; in cases which were reported, only 60% (392/652)[33] and 81% (42/52)[21] of consenting cases had sufficient DNA to attempt sequencing. Prospectively recruited studies had fewer problems, with sufficient DNA for analysis collected on average in 92% (95% CI: 89%-96%) (RE model) of cases, across the studies that reported this.[9,20,24,31]

Multiple studies[5,25,27,32,42] included cases of "autopsy by proxy" using duo parental samples when the proband DNA was not available; this approach was used in the entire population in Stals et al[27] (2018).

Only 2 studies reported rates of successful sequencing; 1 with an 86% success rate (55/392 cases failed sequencing),[33] and 1 reported 91% success in which failure in 3 (of 33) cases was attributed to attempting sequencing with insufficient DNA.[24] Three studies reported quality control outcomes for sequencing data, with pass rates of 98% (95/97),[31] 88% (296/337; 41 failures were described as having insufficient base coverage and/or significant contamination),[33] and 76% (32/42).[21]

Extended clinical or societal utility

None of the studies with extractable data specific to the population of interest presented comparative quantitative information on extended aspects of utility.

Some studies highlighted case reports of downstream clinical consequences (eg, clinical management recommendations, actions, or outcomes); Byrne et al[6] (2023) reported on 5 families utilizing preimplantation genetic testing (PGT) and 5 utilizing prenatal diagnosis (PND). Guo et al[8] (2020) reported that 26 couples (of the population of 40) received PGD, and 3 healthy newborns had been delivered, and 4 couples were midpregnancy. Yaron et al[9] (2022) reported several couples were known to have utilized PGT subsequent to testing, and Becher et al[5] (2020) reported that 4 mothers were pregnant with a subsequent pregnancy at the time sequencing was conducted, which facilitated prenatal sequencing in 1 of those pregnancies. Daum et al[7] (2019) also reported a case of suspected germline mosaicism in which in vitro fertilization with PGT enabled selection of a nonaffected embryo. However, clinical follow-up information did not appear to be systematically collected in any of the studies; therefore, it was not possible to calculate rates of uptake of reproductive medicine technologies or subsequent pregnancy outcomes after molecular testing.

Although not explicitly investigated, the "value of knowing" associated with diagnostic testing was indirectly described in 3 articles. One study performed a mixed-methods assessment and follow-up with 15 mothers to identify expectations and have an understanding of the impact of ES on future decisions; all expressed understanding and felt that having ES was a good decision.[36] Another study also reported that parents considered that there was positive psychological value associated with knowledge,[5] and 1 further study reported families with a low risk of recurrence experiencing relief.[6]

Some authors expressed the opinion that molecular sequencing investigation into fetal malformation and/or PND was providing valuable insight not only for the cases at hand but also for advancing medical science more broadly. Repositories of phenotypes and genetic findings are highly valuable for understanding human development and causes of pathology when solving future cases, particularly in fetuses and neonates in which phenotypes are less well characterized and understood.[23,34,35,41]

One of the nonextractable studies reported that genetic findings from ES had beneficial social justice outcomes because they enabled resolution of medical disputes for 2 families of deceased newborns.[43]

Secondary and incidental findings

Some studies specifically excluded secondary and incidental findings from reports, whereas others provided a choice at enrolment and reported medically relevant secondary findings to parents who had consented. A study with mixed PND and PN enrolment reported that 91% of participants chose to receive secondary findings and the rate of secondary findings was 3% (4/115).[44] Medically relevant genetic findings included familial hypercholesterolemia, familial hypertrophic cardiomyopathy, *PTEN* hamartoma tumor syndrome, and *BRCA1* hereditary breast and ovarian cancer syndrome. Secondary findings in which a parent was subsequently referred to further medical follow-up occurred in 3 out of 30 (10%) cases in the study by Westphal et al[37] (2019); however, this was specifically in a cohort in which fetuses had cardiac abnormalities. Another study reported 1 parent diagnosed with familial hypercholesterolemia felt this information was beneficial to his long-term health.[36] The remaining data included only isolated examples with unknown clinical consequence.[5,45]

Harms

A number of study authors raised potential concerns associated with incidental genetic findings (including nonpaternity) and uncertain findings (eg, particularly when faced with subsequent pregnancies); however, none recorded harms in a quantitative manner. Becher et al[5] (2020) provided examples of negative experiences, including 1 in which the waiting time for sequential testing was described

as a burden and another complex case with high uncertainty and "information overload" for both the health professionals and parents such that the parents refused genetic testing in a subsequent pregnancy.

Health economic findings

Some studies reported the costs of sequencing; however, none comprehensively measured costs associated with complete management pathways or assessed the cost-effectiveness of genomic testing.

Vora et al[36] (2017) identified that women in the highest socioeconomic group had statistically higher presequencing genomic knowledge compared to their lower income counterparts, and another study noted that the "out-of-pocket" costs applied in their series may be biased toward families with high motivation to reach molecular diagnosis and available financial resources.[30] Similarly, in studies not quantitively analyzed, one noted that the personal costs associated with testing may affect accessibility,[46] and another reported initial referrals for 2 postmortem fetuses were withdrawn for financial (personal or insurance related) reasons.[47]

Discussion

There is little doubt that ES/GS can provide valuable explanations for the cause of MCA and unsolved PND in a substantial portion—perhaps a third of cases. The likelihood of resolution does vary substantially based on individual case circumstances, with lower success in cases with fewer genetic clues, but high success in cases with typical phenotypes and familial risk factors. Having a diagnosis can be of both immediate and ongoing value to parents. In cases of de novo genetic disease, parents can be reassured of a low likelihood of recurrence, and in cases which a genetic cause is inherited, the knowledge enables decisive action to prevent recurrence. Highly meaningful outcomes—healthy babies in couples who had repeated pregnancy losses—have been reported because of the ability to target use of reproductive medicine techniques when disease causing inherited variants have been identified.

The strict focus on postmortem diagnosis distinguishes this review from other previously published systematic reviews of molecular DNA sequencing to identify anomalies in the prenatal setting.[15,48-51] Including cases in which PND occurred without apparent congenital anomalies reduced the overall yield in this review. Although the overall diagnostic yield of 33% (95% CI: 27%-40%) was essentially very similar to the 31% (95% CI: 26%-36%) yield found in a recent comprehensive review of prenatal sequencing,[48] all cases in that review had a structural anomaly and many cases were ongoing pregnancies. Leung et al[51] (2018) reported a higher postnatal/postmortem yield of 36% (95% CI: 31%-50%) (which includes live neonates) but interestingly found a significantly lower "prenatal" yield of only 20% (95% CI: 11%-29%; $P < .05$).

In some studies,[6,26,28] the population was drawn from a larger population that had accessed targeted gene panels before study enrolment; however, the nature and extent of prior gene testing was not well described other than to confirm that only undiagnosed cases were included. Therefore, the rate of SNVs identified by ES or GS in "targeted-gene-test-negative" populations would plausibly be lower than the prevalence of SNVs that would be identified if ES or GS were performed before (in place of) targeted gene analysis.

The higher yields associated with consanguinity and family history are consistent with observations in miscarriage and prenatal settings.[30,52] Unsurprisingly, phenotype is also highly relevant. Consistent with prenatal data,[48] the highest yields of P/LP variants were in skeletal anomalies and lymphatic/nonimmune hydrops fetalis , muscular/neuromuscular, and complex multiorgan presentations. The differing likelihoods of a genomic finding for the different physical presentations of anomalies is potentially more clinically relevant (for example, when counseling) compared to an "overall" yield across heterogeneous cohorts.[53] Notably, a substantially lower but consistent rate of genomic diagnosis occurred in PND cases with no apparent abnormality. This may be an important consideration when counseling parents; the likelihood of "no finding" is particularly high, but in the rare cases that a genetic cause is found, the value of providing an explanation, in the absence of other "clues," may be particularly high for personal and social reasons.

Theoretically, differences in the sampling and the extent of sequencing should also result in small differences in diagnostic yield: GS is able to identify pathogenic variants in the noncoding (intron) areas of DNA not evident on ES and therefore should generate a higher yield.[5,54] However, this was not apparent in this review, and a meaningful comparison between sequencing methods could not be made. Similarly, analysis of a trio of DNA samples (proband and parents) is considered more likely to identify a result compared to singleton/proband-only testing because potentially relevant de novo and compound heterozygous variants are more obvious.[55] This was apparent within Bourgon et al[40] but not reflected in the meta-analyzed yields across studies in which a 1% difference and wide, entirely overlapping confidence intervals were observed because of the substantial heterogeneity across studies. In the studies that reported on both singleton and trio testing,[7,22] allocation was not random. Both studies allocated cases to singleton testing if an autosomal recessive condition were considered likely on the basis of family history, consanguinity, or typical phenotype. The highest yield was observed in the duo (parent proxy) sampling when this method would be expected to have a lower yield because it cannot identify any de novo pathogenic variants that may have caused the fetal outcome. Overall, this suggests that confounding factors, likely those directly associated with the case selection (clinical risk factors, extent of laboratory use for research vs commercial practice, etc) have much more significant

impact on yield estimates compared to the effect of ES vs GS or trio vs singleton sampling.

Although higher yields for postmortem series have been associated with the increased severity of cases (severe/complex phenotypes being more lethal),[15] the yield concordance when sequencing is interpreted with and without postmortem information suggests that the higher yield rates are also due to increased diagnostic sensitivity. There is no defined "reference standard" for ES or GS sequencing to identify SNVs, but interpretation of the studies that compared approaches with and without autopsy findings consistently identified more relevant variants when autopsy findings were available.[18,21,28,40] Postmortem examination can identify morphologies/pathologies not apparent on prenatal scans or superficial examination, for example, Aggarwal et al[21] (2020) notes the role of histopathology in 8 of 22 diagnosed cases. Comprehensive physical autopsy findings provide more scope for efficient recognition of the genotype-phenotype associations necessary for making a positive finding.[23,45] Although physical autopsy is not essential to conduct the genomic autopsy, the combination provides a superior investigative approach.

Phenotype-specific bioinformatic filters increase the efficiency of analysis by rapidly screening out many variants of no significance from the shortlist, and it has been suggested that this also reduces the likelihood of false positives,[56] but the studies in this review suggest that phenotype-based filtering may result in missed diagnoses (reduce sensitivity), particularly in the fetal setting in which phenotypes may not be fully developed. Although instances of variant reclassification are reported,[36,57] no studies were identified that assessed the clinical validity of diagnoses based on designations of pathogenicity in this setting. Clinical validation of diagnoses of cause of death, based on rare genetic variation, is a difficult research proposition. As a result, despite growing clinical experience and acceptance, uncertainty around the true accuracy of ES/GS interpretation in this clinical context remains unestimated.

Neither public acceptance nor practical feasibility of DNA sequencing appeared to be significant barriers to the use of this technology, with the apparent consent rate of around 85% being higher than PND autopsy consent rates.[4] DNA availability was infrequently problematic in prospectively designed series (4%-9% of cases had inadequate quantity/quality DNA vs up to 48% in historical series), and in cases which fetal DNA was not available, the option to analyze parent DNA as a "genomic autopsy by proxy" was utilized in multiple studies, including the entire cohort in Stals et al[27] (2018).

The clinical impacts beyond a diagnostic finding were generally not well reported. It was apparent that positive findings of a genetic variant enabled reasonably confident estimates to be made on the risk of recurrence for subsequent pregnancies and also facilitated PGT or early prenatal diagnosis in some cases,[6] but follow-up data on the overall extent to which this occurred, or subsequent pregnancy outcomes, were very limited. Change-in-management studies assessing the impact of testing on family planning and PGT/PND uptake would be helpful in this regard. Long-term research or modeling may be required to estimate the full downstream costs and benefits associated with such outcomes. Similarly, although a "value of knowing" may be implicit in a cause-of-death investigation, (with the exception of Vora et al[36]) little attempt to systematically verify or characterize such value for parents was apparent. Testing resulted in what may be described as a "burden of knowing" for 1 case.[5] Although a review conducted in the prenatal setting[48] identified 3 studies in which the clinical impact of negative findings was described, no studies in the PND investigation described the impact (benefit or harm) associated with negative findings. More research on the broader spectrum of implications for parents is necessary to comprehensively describe the potential risks and benefits should genomic sequencing become a routine investigation.

The fact that we were unable to differentiate between postmortem and prenatal test interpretation in many studies and excluded them from the meta-analyses is a potential limitation of this review that substantially reduced the total volume of data and the precision of the estimates. Having said that, however, the presented series of postmortem cases is, to our knowledge, the largest presented and meta-analyses. The differing study designs and eligibility criteria of the case series included in the meta-analyses meant that there were persistently high levels of heterogeneity between studies, and although we attempted to explain this, it could not be explored fully because of the limitations in the available data.

Conclusion

The value of ES or GS when investigating TOPFA and PND has been demonstrated repeatedly in terms of diagnostic yield, although long-term follow-up information on the impact in families is less well quantified. Generating analytical or clinical validity data in this setting is difficult and was not attempted in any of the identified studies; thus, the potential for inaccurate diagnostic findings, although apparent, remains unquantified. Similarly, little consideration has been given to potential harms that may be associated with correct or incorrect findings. The lack of comprehensive information in areas beyond diagnostic yield presents a challenge to health technology assessment and decision makers considering the funding of these technologies. Further analysis and attempts to identify the benefits, concerns, and costs at societal level are warranted to determine the appropriate place of genomic autopsy in clinical practice.

Data Availability

All data utilized in this systematic review were from published studies and supplemental files made available with

published studies. The corresponding author can be contacted regarding clarification of data sources, if required.

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Conflict of Interest

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Additional Information

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