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A genomic autopsy identifies causes of perinatal death and provides options to prevent recurrence

Hamish Scott (hamish.scott@sa.gov.au)
SA Pathology https://orcid.org/0000-0002-5813-631X
Alicia Byrne
Centre for Cancer Biology, an alliance between UniSA and SA Pathology https://orcid.org/0000-0002-8141-1818
Peer Arts
Centre for Cancer Biology, an alliance between UniSA and SA Pathology https://orcid.org/0000-0002-6742-6239
Thuong Ha
Centre for Cancer Biology, an alliance between UniSA and SA Pathology
Karin Kassahn
SA Pathology
Lynn Pais
Broad Institute of MIT and Harvard
Anne O'Donnell-Luria
Broad Institute https://orcid.org/0000-0001-6418-9592
Milena Babic
Centre for Cancer Biology, an alliance between UniSA and SA Pathology
Mahalia Frank
Centre for Cancer Biology, an alliance between UniSA and SA Pathology
Jinghhua Feng
ACRF South Australian Cancer Genomics Facility, Centre for Cancer Biology, SA Pathology
Paul Wang
ACRF genomics facility, Centre for Cancer Biology
David Lawrence
ACRF genomics facility, Centre for Cancer Biology
Leila Eshraghi
Centre for Cancer Biology, an alliance between UniSA and SA Pathology
Luis Arriola
Centre for Cancer Biology, an alliance between UniSA and SA Pathology
John Toubia
Centre for Cancer Biology https://orcid.org/0000-0002-5135-6504
Hung Nguyen

SA Pathology
Genomic Autopsy Study Research Network
Centre for Cancer Biology, an alliance between UniSA and SA Pathology
George McGillivray
Victorian Clinical Genetics Services, Murdoch Children's Research Institute
Jason Pinner
Sydney Children’s Hospitals Network
Fiona McKenzie
Genetic Services of Western Australia
Rebecca Morrow
Centre for Cancer Biology, an alliance between UniSA and SA Pathology
Jillian Lipsett
Anatomical Pathology, SA Pathology, Women's and Children's Hospital
Nick Manton
Anatomical Pathology, SA Pathology, Women's and Children's Hospital
T Khong
Anatomical Pathology, SA Pathology, Women's and Children's Hospital https://orcid.org/0000-0002-2404-007X
Lynette Moore
Anatomical Pathology, SA Pathology, Women's and Children's Hospital
Jan Liebelt
Paediatric and Reproductive Genetics Unit, South Australian Clinical Genetics Service, Women’s and Children’s Hospital
Andreas Schreiber
SA Pathology https://orcid.org/0000-0002-9081-3405
Sarah King-Smith
Centre for Cancer Biology, an alliance between UniSA and SA Pathology
Tristan Hardy
SA Pathology
Matilda Jackson
Centre for Cancer Biology, an alliance between UniSA and SA Pathology
Christopher Barnett
South Australian Clinical Genetics Service

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Abstract

Perinatal death, of a fetus or newborn, is a devastating event for families. Following nationwide multicentre recruitment, we assessed 'genomic autopsy' as an adjunct to standard autopsy for 200 families who experienced perinatal death, and provided a definite or candidate genetic diagnosis in 105 families. From this understudied cohort, half of the (candidate) diagnoses were phenotype expansions or novel disease genes, revealing previously unknown *in-utero* presentations of existing developmental disorders, and genomic disorders that are likely incompatible with life. Among the definite diagnoses, 43% were recessively or dominantly inherited, posing a 25% or 50% recurrence risk for future pregnancies. Ten families used their diagnosis for preimplantation or prenatal diagnosis of 12 pregnancies, facilitating the delivery of ten healthy newborns and management of two affected pregnancies. We emphasize the clinical importance of genomic investigations of perinatal death, with short turn-around times, enabling accurate counselling and options for families to prevent recurrence.

Introduction

In developed countries, approximately 1% of pregnancies result in perinatal death, which is the collective term for the loss of a fetus (stillbirths >20 weeks or >400gm, including termination of pregnancy) or neonate (up to 28 days post birth) [1–4]. Despite advanced monitoring of pregnancies and increased access to healthcare, eight perinatal deaths are experienced per day in Australia, a figure that has not changed over the last two decades [2]. The devastating impact that perinatal death has on families and the wider community is often further compounded by the uncertainty of the cause of death, and the subsequent recurrence risk for future pregnancies [5–7]. Clinical testing to determine the cause of perinatal death currently involves the complex integration of family and obstetric history, radiographic imaging, macroscopic and histological examination of the body and placenta, along with laboratory investigations such as biochemistry, microbiology, and genetic testing [8,9]. While collectively these investigations are most likely to yield a clinical diagnosis, due to complex reasons, including the perceived invasiveness of perinatal autopsy, and religious or cultural beliefs, an autopsy is performed in less than 50% of perinatal death cases [1,4,10]. Congenital abnormalities are present in 1/3 of cases of perinatal death, either as the main determinant of fetal demise *in utero* (e.g. via hydrops fetalis) or more commonly, as the main precedent for a termination of pregnancy. Even with congenital abnormalities, for the majority of cases, an underlying aetiology is not determined by current standard-of-care practises. A further 10.5% of deaths remain completely unexplained despite extensive investigation [1,11].

A genetic aetiology is expected to underpin perinatal death due to congenital abnormality, along with many cases of unexplained death; often representing an extreme phenotype and one which is incompatible with life. Large chromosomal abnormalities, like autosomal trisomies and copy number variants (CNVs), account for 25-30% of cases with congenital abnormalities, and are routinely detected by microarray, performed as part of standard-of-care autopsy [11,12]. A further ~5% of cases are attributed to monogenic disorders, diagnosed in the clinical setting by single gene or gene panel testing. However,

these investigations are only performed if a specific phenotype is suspected [11]. As a result of the limited genetic investigation that is currently considered standard-of-care, the underlying aetiology of ~70% of congenital abnormality-related deaths remains unexplained, limiting the accuracy of counselling and restricting options to prevent recurrence.

A broader approach to identifying the molecular origins of congenital abnormalities and unexplained perinatal death, by exome sequencing (ES) or genome sequencing (GS), would allow the full range of large-scale genetic variation discernible by microarray, as well as single nucleotide variants (SNVs) and insertions/deletions (indels) to be identified [13].

Initially, our study focused on the analysis of a select cohort of perinatal death families, thus adding to other studies of this type and expanding our understanding of this clinical cohort [14–19]. However, as our study progressed, recruitment became contemporaneous, and families were recruited nation-wide. This also meant results could be reported using a diagnostically accredited pathway with turnaround times suitable for real-time clinical decision making, thus providing genomic information to assist in reproductive counselling of families.

While for some families a candidate diagnosis may still have value in guiding reproductive choice [20], robust validation of the causal relationship of novel variants and genes is crucial for widespread utility. Elucidating the molecular mechanisms that underlie perinatal lethality provides a unique opportunity to not only provide affected families with a genetic diagnosis, but to also identify and understand the genes and pathways critical for early human development.

In this study we investigated the utility of a genomic autopsy by prospectively offering ES or GS to families who had experienced perinatal death due to congenital abnormality (without a genetic diagnosis identified from standard-of-care testing) or where death was unexplained. The primary objective was to provide families with diagnoses, accurate recurrence risks, and options for prenatal diagnosis (PND) or preimplantation genetic diagnosis (PGD) in future pregnancies. Supporting this aim was the systematic use of MatchMaker Exchange (MME) to seek additional kindreds, in conjunction with adjunct investigations, such as RNA sequencing, and *in vitro* and *in vivo* studies, to implicate causality of novel variants and genes. Here, we report the results of our ongoing study on genomic autopsy and the subsequent clinical impact for the first 200 consecutively referred families (parent-proband trios or quads). We highlight the importance of reducing turnaround times for Mendeliome (known OMIM-Morbid genes) analysis for perinatal death so that the genetic diagnosis can inform future pregnancies. Furthermore, since perinatal death represents an understudied patient cohort, the high rate of variants in novel candidate genes and phenotype expansions require additional cases and experimental follow-up to establish a final diagnosis.

Results

Cohort characteristics

The cohort demographics including gender, gestational age (in Supplementary Figure 1), reason for referral, major organ system affected and detailed phenotypes with HPO terms are reported in Supplementary Tables 1 and 2.

Identification of pathogenic or Likely pathogenic variants from 'Mendeliome' analysis

Despite the clinical tests performed prior to inclusion in our study, the exome or genome trio/quad analysis resulted in a direct diagnosis for 42/200 families (21.0%). These diagnoses are American College of Medical Genetics (ACMG)-classified pathogenic (P) and likely pathogenic (LP) variants in established OMIM disease genes with clinical presentations that are completely or partially concordant with phenotypic spectrum known for the associated prenatal and/or postnatal disorders (Figure 1, Supplementary Table 2 and 3) [21,22]. For an additional 9/200 families, a genetic diagnosis was reached after further follow-up of a candidate variant was performed, ranging from identification of additional kindreds, RNA analysis, and in-vitro experiments, to the development of a mouse model (See Supplementary Results, Supplementary Figures 2-6, Supplementary Table 2 and 4, [23]). In 10 of the 51 solved cases (19.6%), the observed phenotype represented a phenotype expansion, because of either the phenotypic severity, the early in-utero presentation of the disorder, or due to previously undescribed clinical features present in the proband. Of the 51/200 families that received a genetic diagnosis, 58.8% (30/51) were caused by autosomal or X-linked *de novo* mutations (including one dual *de novo* diagnosis of Kabuki and Noonan syndromes in PED046), 29.4% (15/51) were recessive (5 homozygous and 11 compound heterozygous), 7.8% (4/51) were dominantly inherited with reduced penetrance and two were hemizygous for a maternally inherited X-linked recessive disease (Figure 1; green).

Identification of candidate variants from 'research' analysis

In addition to the direct diagnoses from Mendeliome analyses, we have identified strong candidate variants in 54 families who were prioritised for follow-up investigations. Of these families, 17 received a report on the variant(s) of unknown significance (VUS) impacting established (OMIM) disease genes in the probands with a clinical phenotype that overlaps with previously reported patients. For another 11 families, the candidate variants that were identified in known disease genes could not be classified since there was insufficient phenotypic overlap with previously described cases. The remaining 26 candidate variants were identified in potentially novel genes, which are considered genes of uncertain significance (GUS) and cannot yet be clinically classified or reported. The 54 candidate variants include 15 autosomal dominant *de novo* variants, 27 autosomal recessive variants (9 homozygous and 18 compound heterozygous), 7 X-Linked recessive variants, 3 autosomal dominant variants with reduced penetrance and two cases with suspicious digenic variants (Figure 1; yellow). For the remaining 47.5% (95/200) of families, no genetic diagnoses were identified, nor were any strong candidates prioritised for follow-up (Figure 1; white).

As part of our research analysis, we have prioritised candidate variants in 33 families for follow-up. These include variants in potentially novel disease genes (n=18), exhibiting novel phenotypes (n=5), leading to large phenotype expansions (n=5), or presenting with only partial phenotypic overlap (n=5). For the variants prioritised from research analysis, 66 variants in 50 genes were shared on gene matching platforms [24,25], yielding 11 matches with relevant genotype-phenotype overlap (Supplementary Table 5). One of these matched variants (a *de novo GNB2* variant in PED044) has recently been published and is now considered solved [23]. Collaborative patient cohort collection and experimental follow-up studies are currently ongoing for candidate variants in 10 genes.

Association between cohort demographics and selected (candidate) diagnoses

A multiple correspondence analysis (MCA) was performed to assess whether a subset of cohort demographics are associated with a candidate diagnosis (Supplementary Figure 7). Gestational weeks and major organ systems affected are the two sources that explain the largest proportion of variability in the study cohort (Supplementary Figure 7A-F). The number of gestational weeks and organ systems were reduced, by grouping similar categories together, which effectively allowed other cohort characteristics to be studied in association with a (candidate) genetic finding (Supplementary Figure 7 G-J). Based on the MCA factor maps, the characteristics of a proband that are more likely to yield a genetic diagnosis include: neonatal death, organ systems (cardiovascular, respiratory, global, hematopoietic and lymphatic), and multiple congenital abnormalities as reason for referrals (Supplementary Figure 7M). Conversely, the characteristics of a proband that are more likely to receive a negative exome finding include: stillbirth, either no major organs or urogenital and neurological systems affected, and single congenital abnormalities (Supplementary Figure 7M). Correspondingly, there is a minor subset of individuals within the study cohort that share similar demographics and a negative exome finding: males, recurrent family history and families studied under a quad analysis (Supplementary Figure 7N).

De novo follow-up by phasing and droplet digital PCR

Systematic follow-up of identified *de novo* variants was performed in order to more accurately define the recurrence risk. Phasing based on ES data or Oxford Nanopore Technologies (ONT) sequencing showed that 76% (22/3) of autosomal *de novo* variants occurred on the paternal allele, while only one out of four (25%) X-chromosomal *de novo* variants were traced to the paternal allele. Custom droplet digital PCR (ddPCR) showed (low-level) paternal post-zygotic mosaicism in blood for 4/43 (9.3%) of the GATK-called '*de novo*' mutations. For two of these (PED043 and PED084), the paternal sperm DNA showed increased variant allele frequencies of the disease-causing *de novo* mutations, thereby redefining the recurrence risks to 20.1% and 3%, respectively [26] (Supplementary Table 3 and Supplementary Figure 8).

Reproductive outcomes following diagnosis

Diagnoses or candidate diagnoses provided by a genomic autopsy were known to impact the reproductive planning of ten families (Table 1) of which eight had received a genetic diagnosis (six families with AR or XLR inheritance and two families with AD inheritance of variable penetrance/expressivity or parental mosaic variant); and the remaining two families used their candidate biallelic variants for PGD or PND (PED002 and PED040; Table 1). Additionally, families with identified true *de novo* inheritance reported relief at knowing it was unlikely to affect other children and confidence in trying for another pregnancy. The reproductive choices or plans for further children for most of the families is unknown. Of the ten families where reproductive planning was known to be impacted, five families used the information for PGD, facilitating the transfer of an unaffected embryo. From five separate pregnancies, for four couples, five healthy babies have already been delivered and a fifth couple did not become pregnant following transfer of a single unaffected embryo (Table 1). The remaining five families chose to use the information for PND, via chorionic villus sampling at 10-13 weeks' gestation, to enable relatively early identification of potential recurrence. From six separate pregnancies, four healthy babies have been delivered, although one child does carry the familial mutation for a disorder known to have variable penetrance. In the remaining two pregnancies the fetuses were found to carry the causative mutations, and in both cases the parents elected to continue the pregnancy with informed management (Table 1).

Discussion

The clinical efficacy of an ES or GS approach for the molecular diagnosis of postnatal developmental disorders is well documented, with diagnostic yields ranging from 10-70% dependent on the primary presentation [27,28]. More recent studies have also focused on fetuses with congenital abnormalities identified on ultrasound, and implied that quick turnaround times may aid couples when decisions on elective terminations of pregnancy are to be made [29–31].

However, genomic analyses have not yet been implemented in the clinical setting for cases of perinatal death. A small number of research projects in recent years have sought to elucidate the genetic causes of perinatal death by genomic analyses, returning diagnostic yields ranging from 14-57%[14–19,32], suggesting that many of these cases will have an identifiable monogenic basis. While differences in study design and inclusion criteria likely account for some of the variability in diagnostic rate, this discrepancy also reflects the challenges currently faced in the genomic analysis of disorders presenting prenatally. Due to the skewing of existing reference data sets to adult profiles of gene function and expression, interpreting and classifying genetic variants in a prenatal versus postnatal setting is often more challenging due to the current limitations of in utero phenotyping [21,33,34] and availability of appropriate databases and guidelines for fetal and neonatal classifications [21,35]. Traditional standards for variant classification [22] can also be difficult to apply as gestational age (and development) strongly influences whether the representative fetal organ(s) are sufficiently developed to display the characteristic phenotypic features associated with known genetic postnatal disorders [36] with limited databases and guidelines on the classification of fetal and neonatal phenotypes at specific gestational ages [21,35].

In this study, trio/quad ES or GS was prospectively applied for 200 consecutively referred families with perinatal death due to congenital abnormality or in which death was completely unexplained. All cases had undergone standard-of-care full autopsy investigations, and results from microarray and candidate gene testing were negative or non-contributory. Following Mendeliome analysis (n=42) and follow-up research (n=9), a (likely) pathogenic variant in a known gene was identified for 25.5% (51/200) of families and a candidate (VUS/GUS) identified in a further 27 % (54/200), demonstrating the clinical utility of this approach over conventional testing (Figure 1, Figure 2; Supplementary Table 2 and 3). Despite prior molecular investigation of suspected disorders based on phenotype, 60% (63/105) of (candidate) diagnoses were made in known OMIM disease genes, with retrospective phenotype review revealing significant overlap between the proband and the reported phenotype. A further 13.5% (14/104) of (candidate) diagnoses represented novel disease phenotype associations with known disease genes, and the remaining 26.9% (28/104) were made in potentially novel disease genes (Supplementary Table 2). anatomy

Within our cohort, the phenotypic distribution ranged from severe congenital abnormalities to unexplained fetal loss. Compared to the overall distribution of clinical subtypes of perinatal death[11], the overall cohort seems skewed towards fetuses with congenital abnormalities and the reported major organ systems (Figure 2, supp Figure 10) are enriched for essential organs detected on early fetal ultrasounds (i.e. brain, urogenital, skeletal and cardiovascular). After excluding cases that were diagnosed by the SNP arrays or gene panels that are part of standard-of-care in Australia, the genomic analyses also indicated a large heterogeneity in the genetic defects underlying perinatal death. Seven recurrently mutated genes (*FGFR2, KMT2D, ARSL, TUB1A1, NIPBL, USP9X,* and *ACTA1*) were the likely cause of perinatal death in fourteen families, with variants in the remaining 96 genes being observed only once in our cohort (Supplementary Table 2). While dual diagnoses are expected to contribute to (phenotype expansions of) developmental disorders in 5% of cases [37], reportable candidate variants in two genes were identified in only three families without phenotype expansions. In addition, candidates with possible digenic inheritance were detected in two of the families with a specific clinical presentation.

As observed in other studies on non-consanguineous cohorts of developmental disorders [28], the majority (58.8%; 30/51) of variants leading to definitive diagnoses occurred *de novo* in the proband. In contrast, the majority (62.9%; 34/54) of candidate variants are autosomal or X-linked recessively inherited variants. The different distribution in causative and candidate variants can (partially) be explained by the additional weight of the criteria for *de novo* variants (PS2) in the ACMG guidelines [22]. Despite studies showing that parental (post-zygotic) mosaicism is an important source of germline '*de novo*' variants in offspring, parents are currently counselled that a recurrence risk for *de novo* variants is ~1%. As expected based on earlier studies, the phasing experiments for *de novo* variants showed that ~75% of autosomal de novo variants occurred on the paternal allele. Systematic follow-up of *de novo* variants by ddPCR revealed that four parents were low-level mosaic in blood (and sperm) for the *de novo* variant, leaving 39 'true' *de novo* (candidate) variants, which have likely arisen after gametogenesis. Two paternal mosaic

variants indicated a higher recurrence risk compared to the 1% that is commonly counselled in the clinic; with 3% (PED084) and 20% (PED043) respectively [26].

As could be expected, families with informed or measured relatedness seem more likely to yield a (candidate) genetic diagnosis compared to families that are unrelated; 75% compared to 50.5% (not significant) (Supplementary Figure 9). Surprisingly, we identified more (likely) pathogenic variants in female probands (32%; 31/97) compared to male probands (19.4%; 20/103) (Supplementary Table 1 and 2). This may partially be explained by the higher proportion of more mature females recruited (34 females recruited in the last trimester of the pregnancy compared to 22 males). Fetuses of greater maturity are more likely to have a phenotype that is recognisable and more comparable to the phenotypic features in a young infant, for example, and are therefore easier to classify. There is also a higher number of *de novo* mutations in known disease genes in females (20) compared to male (10) probands in the cohort. Finally, of the 21 quads, in 14 families both siblings were male (66.7%) compared to five families with siblings of both genders (23.8%) and two (9.5%) with both females (Supplementary Figure 10). The number of (candidate) genetic findings from families analysed as quads is remarkably low, indicating that the likely autosomal or X-linked recessive genetic variants were not detected by ES or GS.

Following Mendeliome analysis alone 74.5% (149/200) of families did not receive a definitive diagnosis, of which 47.5% (95/200) remained without a selected candidate for follow-up. The lowest diagnostic yield was observed for cases of perinatal death without congenital abnormalities, of which 91.7% (11/12) remained without a diagnosis, and 66%; (8/12) are unresolved without a candidate (Figure 2, Supplementary Table 2, Supp Fig 1). Notably, there were no LP/P variants identified in previously reported “sudden death” genes in this cohort (e.g. long QT syndrome), despite previous reports suggesting that variants in these genes contribute to unexplained stillbirths [38,39]. Overall, the placental genome remains an under-explored area in the understanding of unexplained perinatal death and is an ongoing focus of our research [40]. While the MCA pinpointed demographic characteristics that influence the diagnostic yields, larger datasets are required to reach statistical significance for these analyses.

Considering the unique perinatal death cohort described in this study, and the high proportion (24%; 23/96) of diagnoses and candidate variants in (potentially) novel disease genes, follow-up research will remain an important adjunct to clinical genomic analyses. This research is required to characterise all fetal lethal developmental disorders resulting from genetic variants in genes intolerant to variation (i.e. the intolerome) [32]. These candidate variants in potentially novel disease genes were prioritised by gene constraint scores and phenotypic overlap (e.g. early lethality) observed in mouse knock-out models and/or similar presentations observed by gene matching approaches (Supplementary Table 6).

The phenotype expansions observed in our cohort include severe prenatal presentations of postnatal disorders, additional clinical manifestations within the same organ system, and other affected organ systems, of which the latter may also be explained by other genetic factors that were missed in our analysis. Interestingly, phenotype expansions were mostly observed in patients with autosomal recessive variants (n=8) compared to autosomal dominant (n=3) and X-linked recessive (n=2) variants (Supplementary Table 2). Generally, the phenotypic presentations in our cohort were more severe compared to what is reported in the literature (Supplementary Table 2; PED012, PED042, PED043)[26,41], which may be explained by the resulting variant impact, i.e complete loss-of-function versus reported missense variants which may be partial LoF or hypomorphic variants.

Our results strongly support a clinical role for genomic testing in elucidating the cause of perinatal death, particularly when congenital abnormalities are present. In 21% of cases, genomic autopsy provided a clear diagnosis where standard autopsy could not. Despite this, standard autopsy remains a valued assessment of perinatal death due to its ability to identify non-genetic cause(s) of death (e.g., congenital abnormalities of diabetic embryopathy or evidence of CMV infection), and the advantage of utilising anatomical and histological information obtained at autopsy for the interpretation of candidate variants. Therefore a genomic autopsy is best implemented alongside current standard-of-care measures to help improve diagnostic rates for perinatal death.

In 43 families, the identified (candidate) diagnoses were *de novo* variants without significant (>1% allele balance) parental mosaicism, and the minimal associated recurrence risk comforted couples for their future pregnancies. In addition to these 43 families, an inherited (candidate) diagnosis was identified in 62 families. Of these, autosomal recessively inherited (candidate) diagnoses were identified in 43 families and X-linked recessive variants in an additional 9; giving a 25% recurrence risk. Indeed, 22.5% (45/200) of families in this study had experienced recurrent perinatal death due to congenital abnormality or unexplained cause (Supplementary Table 1 and 2). Following genomic analysis, a (candidate) diagnosis was identified for 49% (22/45) of these recurrent families, providing options to reduce the risk of further recurrences in future pregnancies.

During the course of our study, at least 24 couples conceived a subsequent pregnancy prior to receiving a result from genomic analysis, providing impetus to reduce turnaround times to improve clinical utility. Driven by this knowledge, and our aim to maximise the impact of our study for clinical care and decision making, we have adjusted our workflows and protocols to provide clinically accredited reports within three months (Figure 3).

Following genomic analysis, 10 families are known to have used the information for reproductive planning, with 5 electing for PGD and 5 electing for PND, facilitating 10 healthy pregnancies and 2 early detections of recurrence (PND) (Table 1). This outcome also highlights the importance of equitable access to high quality assisted reproductive services. Interestingly, one family in this study chose to use

PND (PED040, 2 pregnancies) based solely on a candidate variant (VUS)[42], resulting in the birth of 2 unaffected children (Table 1). A particularly impressive example of the utility of a molecular diagnosis though, is family PED005 who after 4 consecutive pregnancies affected with Meckel Syndrome were able to use PGD to facilitate the birth of two unaffected children from two separate cycles (Table 1).

We conclude that clinical implementation of a genomic autopsy as part of the current standard-of-care in the investigation of perinatal death would help to reduce the incidence of stillbirth and newborn death by providing options to prevent recurrence. However, an integrated clinical-diagnostic-research setting is beneficial since perinatal death represents an understudied cohort and genetic causes of early lethality remain to be discovered.

Declarations

WEB RESOURCES

AnnotSV: https://lbgi.fr/AnnotSV

BCFtools: http://samtools.github.io/bcftools

ClinVar: https://www.ncbi.nlm.nih.gov/clinvar

GATK: https://gatk.broadinstitute.org

gnomAD: https://gnomad.broadinstitute.org

The Human Phenotype Ontology: https://hpo.jax.org/app

IGV: https://software.broadinstitute.org/software/igv

NCBI RefSeq: https://www.ncbi.nlm.nih.gov/refseq

Manta: https://github.com/Illumina/manta

OMIM: https://omim.org

Seqr: https://seqr.broadinstitute.org

SnpEff: http://snpeff.sourceforge.net

VariantGrid: https://variantgrid.com

VEP: https://ensembl.org/info/docs/tools/vep

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COMPETING INTERESTS

The authors declare no competing interests.

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AUTHOR CONTRIBUTIONS

ABB, PA and TH drafted the manuscript. ABB, PA, TH, MB, MRJ, SLK-S, CPB and HSS coordinated the study, with ABB, MB and HN managing samples. TH, JF, PW, DL, LE and LA processed genomic data, with ABB, PA, TH, LP, AO'D-L, MSBF and RM performing data analyses. KSK, TSEH and HSS implemented a clinically accredited pathway for analysis and reporting. TH, LE, LA and JT processed transcriptomic data, with TH performing data analysis. AWS supervised processing of genomic and transcriptomic data. TH performed the multiple correspondence analysis. PA performed phasing and ddPCR assays. RM, JL, NM, TYK and LM performed routine autopsy investigations. GM, JP, FM TSEH, JEL, and CPB provided clinical care. ABB, PA, TH, KSK, MRJ, AO'D-L, RM, TSEH, SLK-S, CPB and HSS contributed to interpretation

and discussion of results. HSS and CPB conceived and supervised the study and should both be considered senior authors. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

Sequence data has been deposited at the European Genome-phenome Archive (currently preparing upload) which is hosted by the European Bioinformatics Institute. All implicated variants have been submitted to ClinVar (currently preparing upload). All unique materials and datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Online Methods

Ethics declaration and consent to participate

This study was performed as part of the NHMRC and GHFM-MRFF funded Genomic Autopsy Study, and was approved by the Human Ethics Committee of the Women's and Children's Health Network, South Australia, Australia (HREC/15/WCHN/35) and the Melbourne Health Human Research Ethics Committee as part of the Australian Genomics Health Alliance protocol: HREC/16/MH/251. Informed consent for genomic analysis and participation in study protocols was obtained from parents, and all research was conducted in accordance with the Declaration of Helsinki.

Study design

The definition of perinatal death varies globally [43]. For this study, we included cases of stillbirth and neonatal death occurring between 20 weeks' gestation and 28 days post-partum, as well as euploid miscarriages occurring between 13 and 20 weeks' to reflect all gestations at which a 'standard-of-care' autopsy can be performed [9]. Consistent with Australian definitions, terminations of pregnancy (TOPs) for congenital abnormality were included alongside spontaneous deaths due to congenital anomaly or where death was unexplained. Routine autopsy, including collection of obstetric and family history, and anatomical pathology of the fetus and placenta, amongst other investigations, was performed for all cases allowing detailed phenotypic information to be obtained. Microarray was performed for all cases, with single gene or panel testing performed where indicated by a specific phenotype. For inclusion in the study, previous standard-of-care testing must not have yielded a genetic diagnosis that could explain the disease phenotype. The first 200 consecutively referred families between 2016 and 2021 (179 trios and 21 quads; Supplementary table 1-3) are included in this study.

Sample preparation and genomic sequencing

Details on nucleic acid isolation, sequencing methodologies and data processing can be found in the supplementary methods. Details on sample types per pedigree are summarised in Supplementary Table 7.

Genomic variant analysis

The analysis and interpretation approach utilised in this study is summarised in Supplementary Figure 1 and full details are provided in the supplementary methods. In brief, initial variant filtering selected for rare, protein altering variants (small variants: gnomAD and in-house frequencies ≤1%, max 5 homozygotes for recessive and ≤0.01%, max 5 heterozygotes for dominant; CNVs: no reciprocal overlap with known benign CNVs ≥70%), consistent with any plausible inheritance model. Variants were prioritised for further interpretation based on *in silico* pathogenicity predictions, sequence conservation scores, protein function and expression, and known disease associations (human and animal).

RNA analysis for confirmation of splice effects

RT-PCR or Poly(A) RNA-seq was performed to aid the interpretation of variant effect for candidate causative variants in five families predicted to affect splicing or to result in whole exon deletions/duplications. Further details are provided in the Supplementary Methods and Supplementary Table 4.

Multiple correspondence analysis (MCA)

MCA was computed using the FactoMineR package [44] based on 200 supplementary individuals and 8 active variables from Supp Table 2-3. The results from the MCAs were then visualised using the factoextra R package [45]. Interpretations of the factor map, finding variables associated with a genetic diagnosis, were made based on two complementary MCAs covering 8 active variables and 27 categories.

Identification of additional kindreds

In 33 unrelated families, we submitted a total of 39 different variants to Matchmaker exchange (MME) through the software Matchbox or GeneMatcher portal [25,46]. These variants of uncertain significance in existing disease genes were submitted for cases seeking phenotype or inheritance expansion, different to the reported evidence, and/or for genes with less than two reported cases at the time of curation. In addition, genes of uncertain significance with no human disease gene associations, excluding those with susceptibility risks or limited clinical validity (via ClinGen and PanelApp Australia), were submitted based on one or more of the following evidence: gene-wide or regional constraints, spatial expression in relevant disease tissues or organs, and phenotypes from animal models.

Follow up of *de novo* mutations by phasing and droplet digital PCR

A total of 46 causative and candidate *de novo* variants in 44 families were followed up by phasing and/or droplet digital PCR (ddPCR), full details are provided in the supplementary methods. In brief, phasing was performed for 33 de novo variants using existing ES data (n=14), long-range PCR and Oxford Nanopore sequencing (n=16), or ddPCR (n=2) to determine the allelic origin of the variant. After exclusion of three variants for which no assay could be designed (PED046 - SOS2, PED162 - EWSR1 and PED187 - TUBA1A) ddPCR was performed on 43 de novo variants as a follow-up test to assess possible parental mosaicism and to aid in defining recurrence risk.

Variant classification, reporting and diagnostic outcome

All variants of interest were classified according to ACMG guidelines [21,22], and research reports were issued to the referring clinician at the completion of both first (Mendeliome) and, where performed, second (research) pass analysis. Only variants classified as a variant of uncertain significance (VUS), likely pathogenic (LP) or pathogenic (P) (ACMG Class 3-5) and relevant to the proband's phenotype were reported, with detailed gene- and variant- level curation information included to support interpretation of clinical utility. Further details are provided in the supplementary methods.

Tables

Pedigree ID	*Genomic autopsy outcome*	*Inheritance [gene]*	*Reproductive history prior to genomic autopsy*	*Assisted reproductive choice*	*Outcome*
001	*Solved*	*Autosomal recessive – hom [FGFR2]*	*1 affected pregnancy*	*PND (x1)*	*1 affected pregnancy (liveborn)*
002	*Candidate*	*Autosomal recessive – hom digenic [DNAJB11/TMEM212]*	*2 affected pregnancies*	*PGD (x1)*	*1 unaffected child*
005	*Solved*	*Autosomal recessive – comp. het [MKS1]*	*4 affected pregnancies*	*PGD (x2)*	*2 unaffected children*
013	*Solved*	*Autosomal recessive – comp. het [PIBF1]*	*1 affected pregnancy*	*PGD (x1)*	*Single unaffected embryo failed to implant*
017	*Solved*	*Autosomal recessive – comp. het [TPI1]*	*1 affected pregnancy*	*PGD (x1)*	*1 unaffected child*
040	*Candidate*	*Autosomal recessive – comp. het [LAMC3]*	*1 affected pregnancy*	*PND (x2)*	*2 unaffected children*
043	*Solved*	*Autosomal dominant – paternal mosaic [PBX1]*	*1 affected pregnancy*	*PND (x1)*	*1 affected pregnancy (stillborn)*
051	*Solved*	*Autosomal dominant – variable penetrance [ZFPM2]*	*2 affected pregnancies. Maternal grandmother & great-uncle also affected*	*PND (x1)*	*1 unaffected child (but has familial variant)*
056	*Candidate*	*X-linked recessive – maternal [ARSL]*	*2 affected pregnancies*	*PGD (x1)*	*1 unaffected child (girl)*
098	*Solved*	*Autosomal recessive – comp. het [POLG]*	*2 affected pregnancies*	*PND (x1)*	*1 unaffected child*

Table 1: *The genomic outcomes of our study have informed management for 12 future pregnancies in 10 families, of which 5 elected preimplantation genetic diagnosis (PGD) and 5 had prenatal diagnosis (PND).*

References

1. AIHW. *Stillbirths and neonatal deaths in Australia*. https://www.aihw.gov.au/reports/mothers-babies/stillbirths-and-neonatal-deaths-in-australia (2020).
2. Flenady, V. *et al.* Stillbirths: recall to action in high-income countries. *Lancet* **387**, 691–702 (2016).
3. Hug, L., Alexander, M., You, D., Alkema, L. & UN Inter-agency Group for Child Mortality Estimation. National, regional, and global levels and trends in neonatal mortality between 1990 and 2017, with scenario-based projections to 2030: a systematic analysis. *Lancet Glob Health* **7**, e710–e720 (2019).
4. Donna L. Hoyert, E. C. W. G. *Cause of Fetal Death: Data From the Fetal Death Report, 2014*. vol. 65 https://www.cdc.gov/nchs/data/nvsr/nvsr65/nvsr65_07.pdf (2016).
5. Homer, C. S. E., Malata, A. & Ten Hoope-Bender, P. Supporting women, families, and care providers after stillbirths. *Lancet* **387**, 516–517 (2016).
6. Heazell, A. E. P. *et al.* Stillbirths: economic and psychosocial consequences. *Lancet* **387**, 604–616 (2016).
7. Select Committee on Stillbirth Research and Education. Senate inquiry on stillbirth research. (2018).
8. Nijkamp, J. W. *et al.* Perinatal death investigations: What is current practice? *Semin. Fetal Neonatal Med.* **22**, 167–175 (2017).
9. Flenady, V. *et al.* Perinatal Society of Australia and New Zealand clinical practice guideline for care around stillbirth and neonatal death. *Perinatal Society of Australia and New Zealand* **3**, 56–72 (2018).
10. Elizabeth S Draper, Ian D Gallimore, Lucy K Smith, Alan C Fenton, Jennifer J Kurinczuk, Peter W Smith, Thomas Boby, Bradley N Manktelow. *Perinatal Mortality Surveillance Report - UK Perinatal Deaths for Births from January to December* 2018. https://www.npeu.ox.ac.uk/assets/downloads/mbrrace-uk/reports/perinatal-surveillance-report-2018/MBRRACE-UK_Perinatal_Surveillance_Report_2018_-_final_v3.pdf (12/2020).
11. SA Health Prevention and Population Health Branch. *Maternal and Perinatal Mortality in South Australia 2017*. (2019).
12. Reddy, U. M. *et al.* Karyotype versus microarray testing for genetic abnormalities after stillbirth. *N. Engl. J. Med.* **367**, 2185–2193 (2012).
13. Stavropoulos, D. J. *et al.* Whole-genome sequencing expands diagnostic utility and improves clinical management in paediatric medicine. *npj Genomic Medicine* **1**, 15012 (2016).
14. Alamillo, C. L. *et al.* Exome sequencing positively identified relevant alterations in more than half of cases with an indication of prenatal ultrasound anomalies. *Prenat. Diagn.* **35**, 1073–1078 (2015).
15. Yates, C. L. *et al.* Whole-exome sequencing on deceased fetuses with ultrasound anomalies: Expanding our knowledge of genetic disease during fetal development. *Genet. Med.* **19**, 1171–1178 (2017).
16. Shamseldin, H. E. *et al.* Molecular autopsy in maternal-fetal medicine. *Genet. Med.* **20**, 420–427 (2018).

17. Stals, K. L. *et al.* Diagnosis of lethal or prenatal-onset autosomal recessive disorders by parental exome sequencing. *Prenat. Diagn.* **38**, 33–43 (2018).
18. Armes, J. E. *et al.* Application of Whole Genome Sequencing Technology in the Investigation of Genetic Causes of Fetal, Perinatal, and Early Infant Death. *Pediatr. Dev. Pathol.* **21**, 54–67 (2018).
19. Quinlan-Jones, E. *et al.* Molecular autopsy by trio exome sequencing (ES) and postmortem examination in fetuses and neonates with prenatally identified structural anomalies. *Genet. Med.* **0**, 1–9 (2018).
20. Harris, S., Gilmore, K., Hardisty, E., Lyerly, A. D. & Vora, N. L. Ethical and counseling challenges in prenatal exome sequencing. *Prenat. Diagn.* **38**, 897–903 (2018).
21. Monaghan, K. G. *et al.* The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). *Genet. Med.* (2020) doi:10.1038/s41436-019-0731-7.
22. Richards, S. *et al.* Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **17**, 405–424 (2015).
23. Tan, N. B. *et al.* Recurrent de novo missense variants in GNB2 can cause syndromic intellectual disability. *J. Med. Genet.* (2021) doi:10.1136/jmedgenet-2020-107462.
24. Philippakis, A. A. *et al.* The Matchmaker Exchange: A Platform for Rare Disease Gene Discovery. *Hum. Mutat.* **36**, 915–921 (2015).
25. Sobreira, N., Schiettecatte, F., Valle, D. & Hamosh, A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. *Hum. Mutat.* **36**, 928–930 (2015).
26. Arts, P. *et al.* Paternal mosaicism for a novel PBX1 mutation associated with recurrent perinatal death: Phenotypic expansion of the PBX1-related syndrome. *Am. J. Med. Genet. A* **182**, 1273–1277 (2020).
27. Rehm, H. L. Evolving health care through personal genomics. *Nat. Rev. Genet.* **18**, 259–267 (2017).
28. Retterer, K. *et al.* Clinical application of whole-exome sequencing across clinical indications. *Genet. Med.* **18**, 696–704 (2016).
29. Lord, J. *et al.* Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. *Lancet* **393**, 747–757 (2019).
30. Petrovski, S. *et al.* Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. *Lancet* **393**, 758–767 (2019).
31. Deden, C. *et al.* Rapid whole exome sequencing in pregnancies to identify the underlying genetic cause in fetuses with congenital anomalies detected by ultrasound imaging. *Prenat. Diagn.* **40**, 972–983 (2020).
32. Stanley, K. E. *et al.* Causal Genetic Variants in Stillbirth. *N. Engl. J. Med.* **383**, 1107–1116 (2020).
33. Vora, N. L. & Hui, L. Next-generation sequencing and prenatal ’omics: advanced diagnostics and new insights into human development. *Genet. Med.* **20**, 791–799 (2018).

34. Cao, J. *et al.* A human cell atlas of fetal gene expression. *Science* **370**, (2020).
35. Rivera-Muñoz, E. A. *et al.* ClinGen Variant Curation Expert Panel experiences and standardized processes for disease and gene-level specification of the ACMG/AMP guidelines for sequence variant interpretation. *Hum. Mutat.* **39**, 1614–1622 (2018).
36. Aarabi, M. *et al.* Importance of complete phenotyping in prenatal whole exome sequencing. *Hum. Genet.* **137**, 175–181 (2018).
37. Posey, J. E. *et al.* Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation. *N. Engl. J. Med.* **376**, 21–31 (2016).
38. Crotti, L. *et al.* Long QT Syndrome–Associated Mutations in Intrauterine Fetal Death. *JAMA* **309**, 1473–1482 (2013).
39. Cuneo, B. F. *et al.* Mothers with long QT syndrome are at increased risk for fetal death: findings from a multicenter international study. *Am. J. Obstet. Gynecol.* **222**, 263.e1–263.e11 (2020).
40. Coorens, T. H. H. *et al.* Inherent mosaicism and extensive mutation of human placentas. *Nature* **592**, 80–85 (2021).
41. Byrne, A. B. *et al.* Pseudodiastrophic dysplasia expands the known phenotypic spectrum of defects in proteoglycan biosynthesis. *J. Med. Genet.* (2020) doi:10.1136/jmedgenet-2019-106700.
42. De Angelis, C. *et al.* Compound heterozygous variants in LAMC3 in association with posterior periventricular nodular heterotopia. *BMC Med. Genomics* **14**, 64 (2021).
43. Barfield, W. D. Standard Terminology for Fetal, Infant, and Perinatal Deaths. *Pediatrics* **137**, e20160551–e20160551 (2016).
44. Husson, F., Le, S. & Pagès, J. Exploratory Multivariate Analysis by Example Using R. (2017) doi:10.1201/b21874.
45. Kassambara, A., Mundt, F. & Others. Factoextra: extract and visualize the results of multivariate data analyses. *R package version* **1**, 337–354 (2017).
46. Arachchi, H. *et al.* matchbox: An open-source tool for patient matching via the Matchmaker Exchange. *Hum. Mutat.* **39**, 1827–1834 (2018).

Figures

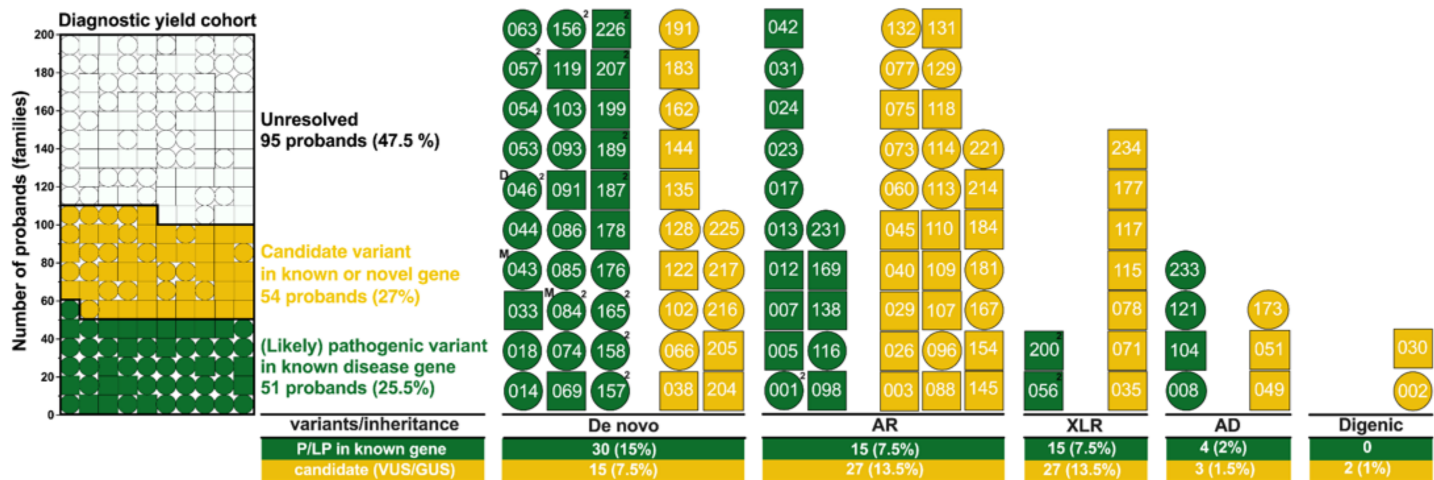

Figure 1: *The diagnostic yield of a genomic approach in our perinatal death cohort of 200 probands and the inheritance model observed. Corresponding pedigree number is contained within each proband symbol. AD: Autosomal Dominant, AR: Autosomal recessive, GUS: Gene of uncertain significance, LP: Likely pathogenic, P: Pathogenic, XLR: X-linked recessive, VUS: Variant of uncertain significance, ^{D}: Dual diagnosis, ^{M}: Parental mosaic indicated a recurrence risk over 1%, ^{2} Families with recurrently mutated genes, ○ female proband, □ male proband.*

Figure 1

See figure for legend.

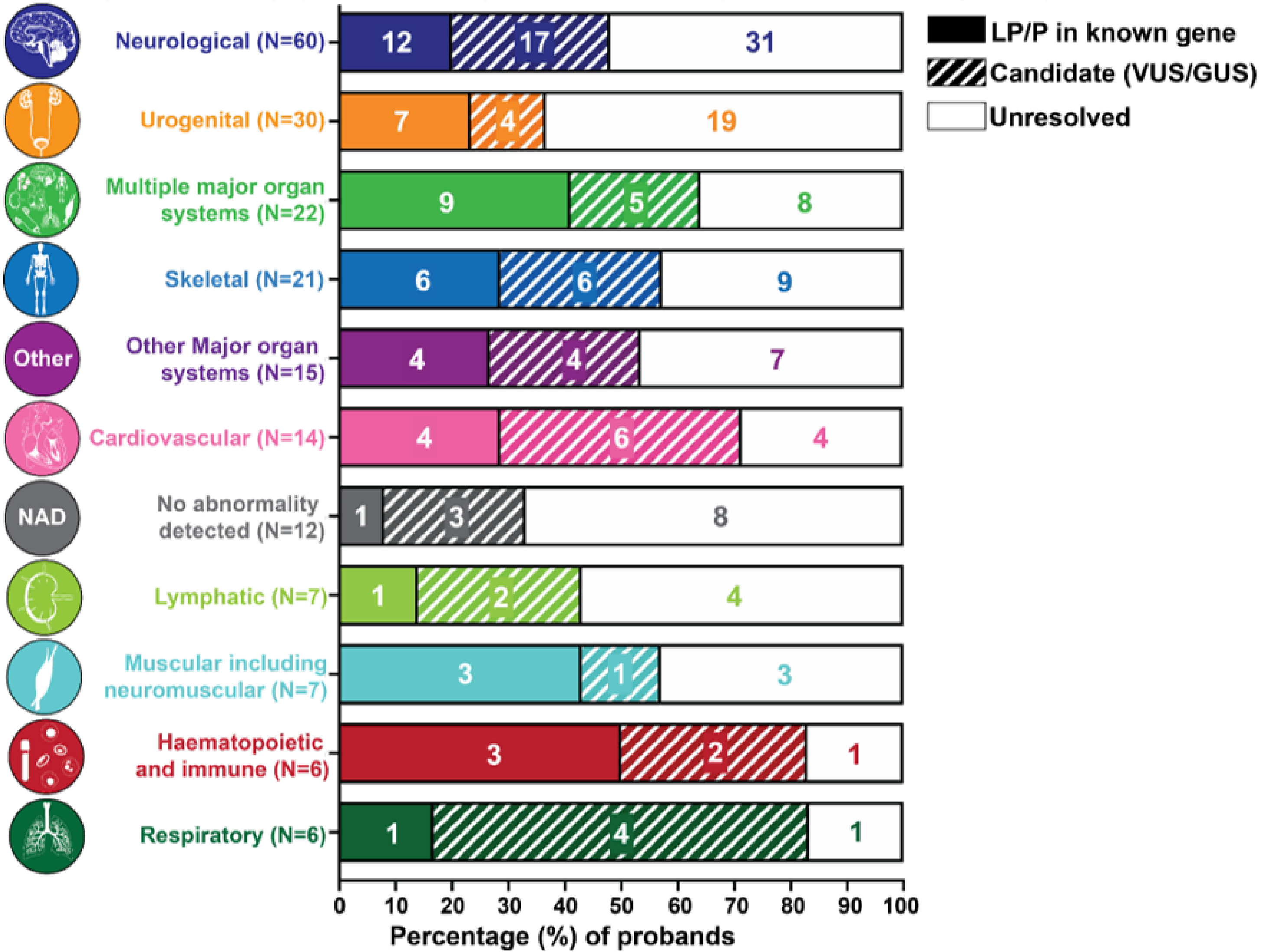

Figure 2

The distribution of probands and the percentage (candidate) diagnoses per major affected organ system based on ACMG classification. LP: Likely pathogenic, P: Pathogenic, VUS: Variant of uncertain significance, GUS: Gene of uncertain significance (i.e. novel gene).

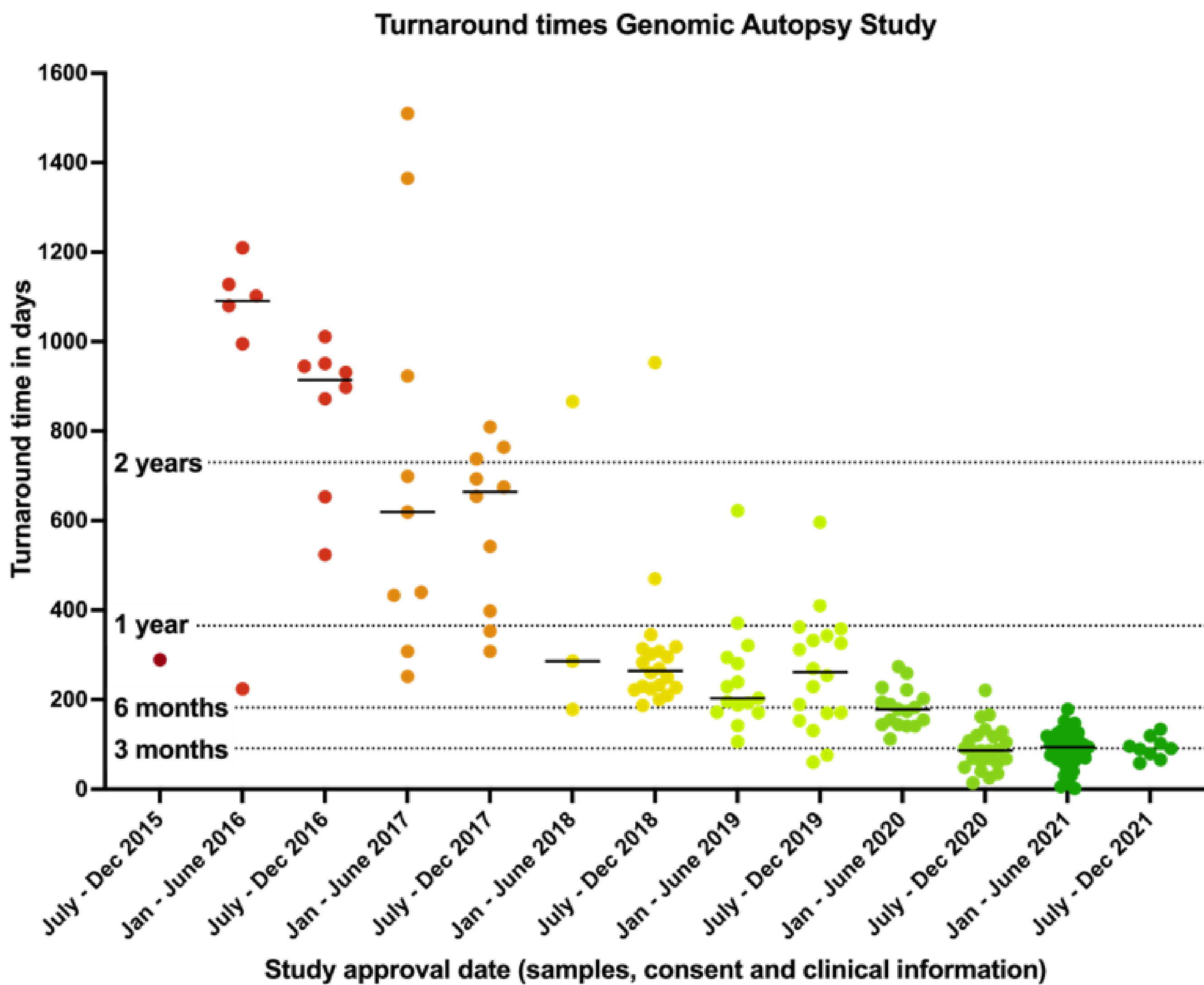

Figure 3

The reduction in turnaround times as our research study progressed towards a diagnostically accredited test for the genomic investigation of perinatal death

Supplementary Files

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