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Molecular autopsy in maternal–fetal medicine

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Purpose: The application of genomic sequencing to investigate unexplained death during early human development, a form of lethality likely enriched for severe Mendelian disorders, has been limited.

Methods: In this study, we employed exome sequencing as a molecular autopsy tool in a cohort of 44 families with at least one death or lethal fetal malformation at any stage of in utero development. Where no DNA was available from the fetus, we performed molecular autopsy by proxy, i.e., through parental testing.

Results: Pathogenic or likely pathogenic variants were identified in 22 families (50%), and variants of unknown significance were identified in further 15 families (34%). These variants were in genes known to cause embryonic or perinatal lethality (*ALPL*, *GUSB*, *SLC17A5*, *MRPS16*, *THSD1*, *PIEZO1*, and *CTSA*), genes known to cause Mendelian phenotypes that do not typically include embryonic lethality (*INVS*, *FKTN*, *MYBPC3*, *COL11A2*, *KRIT1*, *ASCC1*, *NEB*, *LZTR1*, *TTC21B*, *AGT*, *KLHL41*, *GFPT1*, and *WDR81*) and genes with no established links to human disease that we propose as novel candidates supported by embryonic lethality of their orthologs or other lines of evidence (*MS4A7*, *SERPINA11*, *FCRL4*, *MYBPHL*, *PRPF19*, *VPS13D*, *KIAA1109*, *MOCS3*, *SVOPL*, *FEN1*, *HSPB11*, *KIF19*, and *EXOC3L2*).

Conclusion: Our results suggest that molecular autopsy in pregnancy losses is a practical and high-yield alternative to traditional autopsy, and an opportunity for bringing precision medicine to the clinical practice of perinatology.

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Key Words: embryonic lethality; genomic autopsy

INTRODUCTION

The diagnostic power of exome sequencing, with its coverage of the medically relevant genome, is well established.[1,2] One increasingly prominent advantage of exome sequencing is its potential to overcome clinical bias, best reflected in "reverse phenotyping" where the molecular diagnosis guides the clinical interpretation of the phenotype.[3] Reverse phenotyping need not be limited to instances where clinicians are unaware of the specific diagnosis, as it also encompasses true phenotypic expansion where the observed is distinct from the "typical" phenotype.[4,5]

Clinical phenotyping is an essential clinical skill that is often aided by supplemental diagnostic modalities. During embryonic and fetal stages (collectively referred to as embryonic development for simplicity), however, detailed phenotyping can be challenging. This can significantly hamper the provision of an accurate diagnosis in the setting of fetal malformations. More challenging are situations where no discernible abnormalities are detected despite evidence of fetal demise, or where the abnormalities are nonspecific. For example, nonimmune hydrops fetalis is a final common pathway of numerous fetal pathologies with a generally poor

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prognosis.[6] Early termination of pregnancies based on imaging studies that suggest lethal fetal malformations may occur at such early stages of pregnancy that the full phenotype of the underlying disorder may not appreciated. Additional challenges may arise when couples who lost one or more pregnancies in the past present for counseling with insufficient records and no access to stored fetal samples.

The contribution of genetics to fetal demise is incompletely understood. Until recently, chromosomal aberrations (karyotyping and more recently molecular karyotyping) were the only class of mutations that can be identified in a genomewide manner irrespective of the suspected clinical diagnosis.[7] More recently, however, it became also possible to conduct a genomewide search for likely causal point mutations with the advent of exome sequencing. Exome sequencing was first reported in the setting of recurrent fetal demise by Shamseldin et al. in 2012.[8] Several studies have since followed and showed the power of exome sequencing not only to expand the phenotype of known disease genes to include embryonic lethality, but to also reveal novel embryonic lethal genes in humans.[9–16] In this study, we describe our experience with exome sequencing in a large cohort of previously unpublished families who presented with various forms of fetal and perinatal lethality.

MATERIALS AND METHODS

Human subjects

We recruited pregnancies diagnosed with unexplained intrauterine fetal demise or terminated due to major unexplained fetal malformations regardless of family history. Fetuses diagnosed with lethal forms of nonimmune hydrops fetalis were also eligible for recruitment. Couples who had prior intrauterine fetal deaths or lethal nonimmune hydrops fetalis but with no available samples from those pregnancies were also included. Fetal samples were in the form of chorionic villus tissue, amniotic fluid, or umbilical blood as applicable. We only proceeded with downstream analysis when chromosomal aberrations were excluded using regular karyotype with or without noninvasive MaterniT GENOME test, which was outsourced to Sequenom Lab (Burlington, NC, USA) as a clinical test. Parental samples were always obtained as well as available normal siblings for segregation analysis. Informed consent was obtained from all participants in accordance with an institutional review board–approved protocol (KFSRHC RAC 2080006 and 2121053).

Exome sequencing and variant filtering

DNA samples were genotyped on the Axiom SNP chip (Affymetrix, Santa Clara, CA, USA) platform following the manufacturer's instructions followed by autozygome analysis using AutoSNPa (http://dna.leeds.ac.uk/autosnpa).[17] Runs of homozygosity that are >2 Mb in length were considered as surrogates of autozygosity where there is history of consanguinity as described before.[2,18] For exome analysis, samples were prepared according to the preparation guide of Agilent SureSelect Target Enrichment Kit (Santa Clara, CA, USA) and the resulting libraries were sequenced using the Illumina HiSeq2000 sequencer (Santa Clara, CA, USA). The Genome Analysis Toolkit (Broad Institute, MA, USA) was used for variant calling. Solo exome was performed whenever DNA samples from affected fetuses were available; otherwise duo exome on both parents was performed to look for shared heterozygous variants under an autosomal recessive model. Exome variants were prioritized using the following filters: homozygous and within autozygome (or heterozygous and within shared parental haplotype in the case of duo analysis), coding/splicing, rare (minor allele frequency <0.001 using 2,379 ethnically matched exomes and ExAC). Only candidate variants with confirmed segregation within the respective family by Sanger sequencing are reported in this study. All variants were classified using the American College of Medical Genetics and Genomics guidelines.[19]

RESULTS

A high diagnostic yield of molecular autopsy

We recruited and analyzed 44 families with unexplained intrauterine fetal death, lethal nonimmune hydrops fetalis, or severe fetal malformation syndromes necessitating termination of pregnancy. None of the recruited families received a specific diagnosis and all had normal chromosomal analysis. We did not perform molecular karyotyping using single-nucleotide polymorphism array (MaterniT GENOME is only a screening test and has a limited detection limit of >7 Mb). Although family history was not a requirement, we note that most of the recruited families (86%) presented with recurrence. This most likely reflects a referral pattern to our specialized referral perinatology center; i.e., families with recurrence are probably more likely to be referred for further evaluation.

Variants that potentially explain the lethal phenotype were identified in 84% of families. However, in only 50% of the families were we able to classify these variants as pathogenic or likely pathogenic, while in 34% the candidate variants had to be classified as variants of unknown significance according to the American College of Medical Genetics and Genomics guidelines (**Figure 1** and **Table 1**). These variants fall in three categories of genes (**Table 1**):

1. Genes known to present with perinatal demise (category I). Pathogenic/likely pathogenic variants were identified in *GUSB*, *SLC17A5*, and *CTSA* in five families. These three genes are known to cause metabolic diseases that often present as severe nonimmune hydrops fetalis. The same founder *THSD1* mutation that we had previously described was identified in one family.[10] *PIEZO1*, another gene only recently linked to nonimmune hydrops fetalis, was also mutated in one family.[16,20] Renal tubular dysgenesis is another lethal disorder that causes anuria and pulmonary hypoplasia, and was diagnosed retrospectively in 14DG1138 when exome sequencing revealed a biallelic mutation in *AGT*. We also report a family that had unexplained perinatal

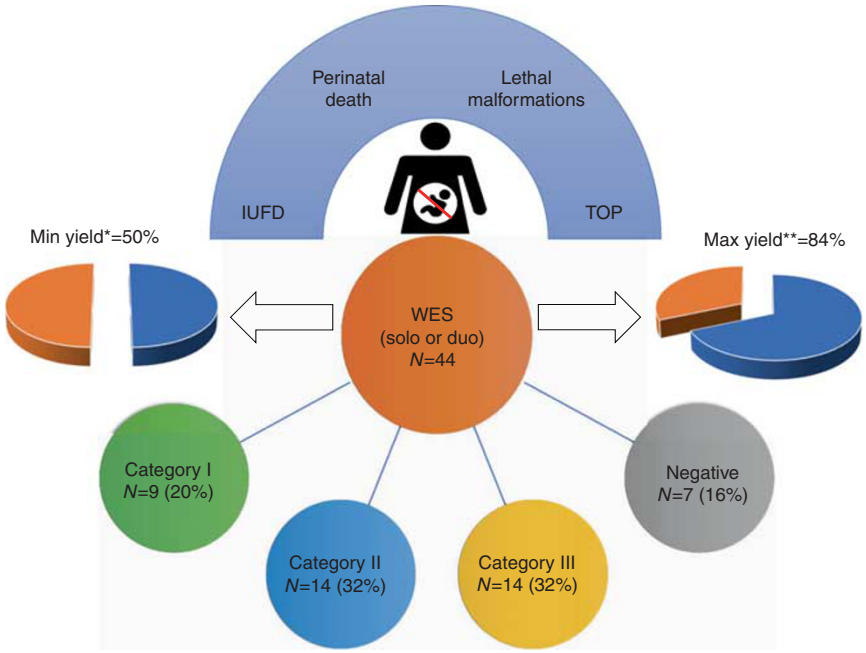

Figure 1 Workflow of the study. The three categories of variants are described in the text. IUFD, intrauterine fetal death; TOP, termination of pregnancy; WES, whole-exome sequencing; *yield estimate based on pathogenic/likely pathogenic variants only; **yield estimate based on all candidate variants.

death with severely abnormal mineralization in which we identified a novel *ALPL* variant that allowed the case to be relabeled as a lethal form of hypophosphatasia.

2. Genes for which perinatal demise represents phenotypic expansion (category II). We identified potentially causal variants in several genes known to cause severe pediatric muscle disease, namely *FKTN*, *KLHL41*, *NEB*, *GFPT1*, and *ASCC1*. We have previously shown that severe homozygous truncating *NEB* mutations can present as embryonic lethality.[10] *INVS* mutations typically cause isolated renal ciliopathy but we have observed a severe biallelic truncating mutation in one of our families that is consistent with the perinatally lethal phenotype observed in *Invs* knockout mouse.[21] Similarly, *TTC21B* mutations have been shown to cause two ciliopathy phenotypes in humans: nephronophthisis and asphyxiating thoracic dystrophy, in the setting of one mild allele in compound heterozygosity with a more severe allele.[22] In this study, we show that a biallelic likely loss of function mutation in this gene causes a very severe and lethal phenotype during pregnancy. In addition, we observed the first instance of recessive inheritance of three variants in strictly dominant genes that are linked to cardiac phenotypes: *LZTR1*, *MYBPC*, and *KRIT1*. *LZTR1* heterozygous mutations cause cardiomyopathy as part of a rare Noonan syndrome phenotype, whereas *MYBPC* is an established cause of dilated cardiomyopathy.[23,24] We suggest that the recessive inheritance of these variants may have resulted in severe cardiac involvement with resulting nonimmune hydrops fetalis. *KRIT1* (*CCM1*) dominant mutations have been linked to cerebral cavernous malformation.[25] Similar to the *LZTR1* and *MYBPC* variants described above, we suggest the possibility that the lethal phenotype we observed in 15DG2390 may be due to the recessive variant identified in *KRIT1* especially since the mouse knockout is embryonic lethal.[26] Lack of phenotype in the carrier phenotypes seems to suggest bona fide recessive inheritance as we described before.[27] However, we cannot rule out the possibility of inconspicuous clinical manifestations since parents declined imaging studies (echocardiography in the *LZTR1* and *MYBPC* families and brain magnetic resonance angiogram in the *KRIT1* family).

3. Genes with no established role in human disease (category III). In 14 families, interesting variants were identified in genes not known to cause human disease, but have been shown to cause embryonic lethality in animal models. *KIAA1109* was found to be independently mutated in two families with a remarkably similar phenotype of hydrocephalus and arthrogryposis. We had previously suggested KIAA1109 as a novel disease gene in humans based on a single family that similarly presented with recurrence of hydrocephalus and arthrogryposis.[4] The two families presented here as well as additional families and the zebrafish model confirm the candidacy of *KIAA1109* as a bona fide disease gene in humans with phenotypes ranging from embryonic lethality to less severe and viable forms of intellectual disability and arthrogryposis (revision in preparation). Similarly, we have recently suggested *EXOC3L2* as a

Table 1 Summary of the study cohorts and their molecular findings

ID	Phenotype	Analysis	Recurrence	Consanguinity	Variant	Zygosity	Mutation effect	Class	ACMG score	ACMG classification	Variant reference	Justification of candidacy (category III)
14DG1255	NIHF	Solo	No	Yes	GUSB:NM_000181:exon9:c.1429C > T:p.ARG477Trp	Homozygous	Missense (Polyphen = probably_damaging(1),SIFT = deleterious(0), CADD = 35)	I	PS1,PM2,PP3	Likely pathogenic	CM960803	
15DG1257	NIHF	Duo	Yes	Yes	MOCS3:NM_014484:exon1:c.1027C > T:p.Arg343*	Homozygous	Protein truncation	III	VUS	VUS	This study	KO is lethal in yeast (PMID: 14551258)
16DG0787	NIHF	Solo	Yes	Yes	MYBPC3:NM_000256.3:c.2449C > T:p.Arg817Trp	Homozygous	Missense mutation (Polyphen = probably_damaging(1), SIFT = deleterious(0), CADD = 34)	II	PS1,PM2, PP1,PP3	Likely pathogenic	CM1516385	
15DG1390	NIHF and CHD	Solo	Yes	Yes	MYBPHL:NM_001010985:exon2:c.212T > G:p.V71G,	Homozygous	Missense (Polyphen = possibly_damaging(0.7),SIFT = deleterious(0), CADD = 26)	III	VUS	VUS	This study	KO mouse has defective cardiac function (http://circres.ahajournals.org/content/119/Suppl_1/A391)
15DG1889	NIHF	Solo	Yes	Yes	PRPF19:NM_014502:exon10:c.794A > G:p.Lys265Arg	Homozygous	Missense (Polyphen = probably_damaging(0.934),SIFT = deleterious (0.01),CADD = 27.6)	III	VUS	VUS	This study	KO mouse is embryonic lethal (PMID: 27626380)
15DG1117	IUFD	Duo	Yes	Yes	VPS13D:NM_018156:exon17:c.2020C > G:p.Arg674Gly	Homozygous	Missense (Polyphen = probably_damaging (0.936),SIFT = deleterious (0.01),CADD = 31)	III	VUS	VUS	This study	KO mouse is embryonic lethal (PMID: 27626380)
KFMC-306	Anhydramnios, cystic hygroma, bilateral renal agenesis, enlarged and cystic liver	Solo	Yes	Yes	TTC21B:NM_024753.4:c.1176_1185+1del	Homozygous	Splicing	II	PVS1,PM2	likely pathogenic	This study	
13DG0010	Hydrocephalus and holoprosencephaly (terminated pregnancy)	Solo	Yes	Yes	WDR81:NM_001163809:c.845G > A:p.Gly282Glu	Homozygous	Missense (Polyphen = probably_damaging(1),SIFT = deleterious(0), CADD = 26)	II	PM2,PP2, PP3,PP4,PP5	likely pathogenic	HGMD CM150814	
12DG1901	Holoprosencephaly (terminated pregnancy)	Solo	Yes	Yes	No obvious candidate						This study	
14DG1138	Anhydramnios, bilateral echogenic kidneys, stillbirth	Solo	No	Yes	AGT:NM_000029:c.104G > A:p.Arg35Gln	Homozygous	Missense (PolyPhen = probably_damaging(0.999);SIFT = deleterious(0)	II	PM2,PP3,PP4	VUS	This study	
15DG0174	IUFD, dilated 3rd and lateral ventricles, absent choroid plexus dilated posterior fossa, hypoplastic cerebellum, signs of hydrops fetalis	Solo	Yes	Yes	FKTN:NM_006731:c.78C > G:p.Tyr26*	Homozygous	Protein truncation	II	PVS1,PM2	Likely pathogenic	This study	
030hc	NIHF		Yes	Yes	FEN1:NM_004111.5:c.704G > A:p.Cys235Tyr	Homozygous	Missense (Polyphen = probably_damaging(0.97),deleterious(0), CADD = 27)	III	VUS	VUS	This study	KO mouse is embryonic lethal (PMID: 27626380)
603hc	IUFD, unilateral renal agenesis, unilateral polycystic kidney, lung hypoplasia	Solo	Yes	Yes	HSPB11:NM_016126.2:c.183dup:p.(Leu62Alafs*14)	Homozygous	Protein truncation	III	VUS	VUS	This study	KO mouse is embryonic lethal (PMID: 27626380)
15DG1840	IUFD, hypoplastic cerebellum, absent choroid plexus, micrognathia, hydrops fetalis, fixed flexed deformities in upper and lower limbs	Solo	Yes	Yes	No obvious candidate							
15DG0610	NIHF	Solo	Yes	Yes	CTSA:NM_000308:c.575T > C:p.Leu192Pro	Homozygous	Missense (Polyphen = probably_damaging(0.979),SIFT = deleterious(0), CADD = 28.2)	I	PM1,PM2, PP1,PP3	Likely pathogenic	This study	
17-0409	Recurrent lethal hydrocephalus	Trio	Yes	No	KIF19: NM_153209:c.788G > A: p.Arg263His.KIF19: NM_153209.3: c.1906T > G:p.Tyr636ASP	Comp het	Missense (Polyphen = possibly_damaging(0.80) deleterious(0), CADD = 29/ Polyphen = possibly_damaging(0.60) deleterious(0.02), CADD = 26.7)	III	VUS	VUS	This study	KO mouse is embryonic lethal (PMID: 27626380)
15DG0877	NIHF	Solo	Yes	Yes	SLC17A5:NM_012434:c.738_741del:p.246_247del	Homozygous	Protein truncation (partial)	I	PVS1,PM2	Likely pathogenic	This study	
15DG1315	Micromelia, craniofacial dysmorphia	Solo	Yes	Yes	COL11A2:NM_080679:c.1550dupC:p.Pro517fSer	Homozygous	Protein truncation	II	PVS1,PM2	Likely pathogenic	This study	

Table 1 Continued

ID	Phenotype	Analysis	Recurrence	Consanguinity	Variant	Zygosity	Mutation effect	Class	ACMG score	ACMG classification	Variant reference	Justification of candidacy (category III)
14DG1950	IUFD	Solo	Yes	No	MS4A7:NM_206939:c.305C > A:p.Ser102*	Homozygous	Protein truncation	III	VUS	VUS	This study	Gene depleted for deleterious homozygous variants
15DG0599	NIHF	Solo	Yes	Yes	SERPINA11:NM_001080451:c.643+1G > A	Homozygous	Splicing	III	VUS	VUS	This study	Gene depleted for deleterious homozygous variants
16DG1276	NIHF	Solo	Yes	Yes	LZTR1:NM_006767:c.2317G > A:p.Val773Met	Homozygous	Missense (Polyphen = probably_ damaging(0.999),SIFT = deleterious(0), CADD = 33)	II	PM2,PP2,PP3	VUS	This study	
15DG0933	NIHF	Solo	Yes	Yes	SLC17A5:NM_012434:c.1111+1G > A	Homozygous	Splicing	I	PVS1,PM2	Likely pathogenic	This study	
13DG0259	NIHF	Solo	Yes	Yes	FCRL4:NM_031282:c.847+1G > A	Homozygous	Splicing	III	VUS	VUS	This study	Gene depleted for deleterious homozygous variants
15DG0934	NIHF	Duo	Yes	Yes	No obvious candidate							
SC1319	IUFD, fetal akinesia	Solo	Yes	Yes	ASCC1:NM_001198800:c.710+1G > A	Homozygous	Splicing	II	PVS1,PM2	Likely pathogenic	This study	
16DG0940	Post fossa malformation (terminated pregnancy)	Solo	Yes	Yes	EXOC3L2:NM_138568:c.122T > A:p.Leu41Gln	Homozygous	Missense (Polyphen = eprobably_ damaging(1) SIFT = deleterious(0), CADD = 24)	III	PM2,PP1, PP2,PP3,PP4	Likely pathogenic	This study	Previously reported candidate in lethal ciliopathy (PMID: 27894351)
15DG1901	NIHF	Solo	Yes	Yes	THSD1:NM_018676:c.617G > A:p.Cys206Tyr	Homozygous	Missense (Polyphen = probably_ damaging(0.999),SIFT = deleterious(0). CADD = 27)	I	PS1,PM2	Likely pathogenic	HGMD CM155511	
16DG1603	NIHF	Solo	Yes	Yes	NEB:NM_001164507:c.20974delA:p.Val6993Serfs*8	Homozygous	Missense (Polyphen = benign(0.365), SIFT = deleterious(0.01),CADD = 23)	II	PVS1,PM2	Likely pathogenic	This study	
15DG0986	NIHF, very short long bones, partial agenesis of the corpus callosum	Duo	No	Yes	MRPS16:NM_016065::c.331C > T:p.Arg111*,	Homozygous	Protein truncation	I	PS1,PS3,PM2	Pathogenic	HGMD CM043302	
15DG2390	NIHF	Solo	Yes	Yes	KRIT1:NM_194455:c.992A > G:p.Tyr331Cys	Homozygous	Missense (Polyphen = possibly_ damaging(0.907SIFT = deleterious (0.02),CADD = 25.2)	II	PM1,PM2, PP1,PP3	Likely pathogenic	This study	
15DG1933	Hydrocephalus, hypoplastic cerebellum, skin edema and bilateral talipes, two previous IUFDs at 6 months of pregnancy with similar presentation of severe hydrocephalus, spina bifida, and polyhydramnios	Solo	Yes	Yes	KIAA1109:NM_015312:c.12067G > T:p.Glu4023*,	Homozygous	Protein truncation	III	VUS	VUS	This study	Previously reported candidate in lethal hydrocephalus (PMID: 25558065)
16DG0291	NIHF	Solo	No	Yes	ASCC1:NM_001198800:c.871+1G > A	Homozygous	Splicing	II	PVS1,PM2	Likely pathogenic	This study	
16DG0493	NIHF	Solo	No	Yes	PIEZO1:NM_001142864:c.1264C > T:p.Gln422*	Homozygous	Protein truncation	I	PVS1,PM2	Likely pathogenic	This study	
16DG0673	IUFD, ventriculomegaly, ACC, hypoplastic vermis, thickened cardiac wall, and fixed flexion deformities of the extremities	Solo	Yes	No	No obvious candidate							
16DG1142	NIHF	Solo	Yes	Yes	SVOPL:NM_174959:c.205delT:p.Phe69Leufs*28	Homozygous	Protein truncation	III	VUS	VUS	This study	SV2 KO mouse is embryonic lethal
15DG0595	Hydrocephalus and limb malformations (terminated pregnancy)	Solo	Yes	Yes	KIAA1109:NM_015312:c.11250-1G > A	Homozygous	Splicing	III	VUS	VUS	This study	Previously reported candidate in lethal hydrocephalus (PMID: 25558065)
15DG2472	IUFD	Duo	Yes	Yes	No obvious candidate							
14DG1030	Polyhydramnios, talipes, cerebellar hypoplasia (terminated pregnancy)	Solo	Yes	Yes	KLHL41:NM_006063::c.176G > C:p.Arg59Pro	Homozygous	Missense (Polyphen = probably_ damaging(0.999),SIFT = deleterious(0), CADD = 30)	II	PM1,PM2, PP2,PP3	Likely pathogenic	This study	
16DG1084	NIHF	Solo	Yes	Yes	GUSB:NM_000181:c.1429C > T:p.Arg477Trp	Homozygous	Missense (Polyphen = probably_ damaging(1),SIFT = deleterious(0), CADD = 35 /CM960803).	I	PS1,PM2,PP3	Likely pathogenic	HGMD CM960803	

Table 1 Continued

ID	Phenotype	Analysis	Recurrence	Consanguinity	Variant	Zygosity	Mutation effect	Class	ACMG score	ACMG classification	Variant reference	Justification of candidacy (category III)
12DG1042	NIHF	Solo	Yes	Yes	INVS:NM_014425:c.753T > G:p.Tyr251*	Homozygous	Protein truncation	II	PVS1,PM2	Likely pathogenic	This study	
15DG2545	IUFD	Duo	Yes	Yes	No obvious candidate							
15DG1281	NIHF	Solo	Yes	No	No obvious candidate							
15DG1287	Lethal skeletal dysplasia with severe hypomineralization	Solo	No	Yes	ALPL:NM_001177520:c.1195G > A:p.Glu399Lys	Homozygous	Missense (PolyPhen = probably_damaging(1), SIFT = deleterious(0), CADD = 35)	I	PM1,PM2, PP3,PP4	Likely pathogenic	This study	
16DG1654	Recurrent IUFD and two perinatal deaths with severe hypotonia and arthrogryposis	Duo	yes	Yes	GFPT1:NM_001244710:c.686-1G > A	Homozygous	Splicing	I	PVS1,PM2	Likely pathogenic	This study	

ACC, agenesis of corpus callosum; ACMG, American College of Medical Genetics and Genomics; CADD, combined annotation-dependent depletion; CHD, congenital heart disease; IUFD, intrauterine fetal death; KO, knock-out; NIHF, nonimmune hydrops fetalis; PMID, PubMed ID; SIFT, sorting intolerant from tolerant; VUS, variant of unknown significance.

candidate for a lethal phenotype that resembles Meckel–Gruber syndrome (severe posterior fossa malformation with kidney enlargement) based on one family.[28] In this cohort, we report another family with a very similar presentation and a different biallelic *EXOC3L2* mutation, which appears to confirm the disease link.

We have also encountered variants in genes with no established or suggested disease links in humans, several of which encode proteins with essential functions. For example, *FEN1* encodes a flap endonuclease that removes 5′ overhangs in DNA repair and processes the 5′ ends of Okazaki fragments in the lagging strand during DNA synthesis, and is highly conserved down to archaebacteria.[29] Its deficiency causes embryonic lethality in mouse.[30] Similarly, *KIF19* encodes a microtubule-depolymerizing kinesin that negatively regulates ciliary length and its deficiency causes a lethal form of hydrocephalus in mouse associated with elongated cilia.[31] A full list of category III genes and justification for their candidacy are provided in **Table 1**. All variants that survived our filtering strategy in all study families are included in **Supplementary Table S1** online.

DISCUSSION

Lack of consensus definition of fetal deaths makes it challenging to compare the estimates obtained by different epidemiological surveys. For example, while the World Health Organization uses a broad definition of fetal death that spans the entire pregnancy, many registries employ a more restrictive gestational-limited (e.g., 20 weeks) or size-limited (e.g., 500 g) definition.[32] A large epidemiological survey in the United States concluded that fetal mortality rate is 1 per 160 pregnancies, and that the cause is unexplained in up to 75%.[32] It has been suggested that the percentage of unexplained cases can be reduced by thorough and systematic evaluation including autopsy of the fetus and placenta.[33] Unfortunately, autopsy is only possible in practice in a very small percentage of cases (11.7%) due to several factors.[32]

In this study, we show that molecular autopsy in the form of exome sequencing has several advantages when compared to the traditional approach. First, not only does molecular autopsy have a high diagnostic rate, but it also provides a precise mutational cause rather than a broad etiological classification. This level of precision is essential for accurate genetic counseling and for the pursuit of preventative options in future pregnancies such as preimplantation and prenatal diagnosis. Second, we note the practical advantage of molecular autopsy over classical autopsy, which was declined by all study participants. While it is possible that families opposed to classical autopsy were more likely to seek recruitment for our molecular autopsy study, it is worth highlighting that autopsy is very rarely authorized by parents in general in our society. It should be noted, however, that classical autopsy and molecular autopsy can be complementary since the former can help refine the phenotype and guide the interpretation of the latter.

Another major practical advantage is the potential of molecular autopsy to reveal the likely cause even when no samples are available from the affected pregnancy. In our cohort, duo-exome analysis in seven couples revealed potential causes of previous fetal demise in four (57%, but only two or 29% if we only consider pathogenic/likely pathogenic variants), including one with no recurrence who were found to harbor the same truncating *MRPS16* variant that was reported once in a Palestinian family with an identical phenotype.[34] Despite the potential of molecular autopsy by proxy through duo-exome sequencing, lack of direct confirmation of the candidate variant in the deceased fetus is an obvious limitation.

In addition to the practical utility of molecular autopsy, its potential in revealing novel developmentally essential genes in humans is noteworthy. In a previous study, we have shown that by selectively targeting recurrent pregnancy loss we were able to identify seven novel candidates in 19 families.[10] In this study, we report the identification of 13 novel candidates in 44 additional families. The nearly consistent proportion of families that harbor candidate variants in genes with no established role in human diseases seems consistent with the notion that the number of embryonic lethal genes in humans is large and includes many that have yet to be characterized.[35] This is further supported by Dickinson et al.,[36] who found that 410 of the first 1,751 unique gene knockouts in mouse are embryonic lethal (23%) and several candidates overlap with our study.

We note that lack of molecular karyotyping in the study cohort may have led to missed pathogenic copy number variants. That all the identified mutations in this study are autosomal recessive may reflect an ascertainment bias since 86% of the recruited families had history of recurrence. However, the unbiased experience of our molecular diagnostic lab that processes fetal samples from high-risk pregnancies irrespective of family history suggests that de novo dominant mutations are only seen in 13%.[37] Thus, it is possible that autosomal recessive lethal mutations are indeed more common in our highly consanguineous population compared to outbred populations where de novo mutations were the most common cause of fetal malformations.[38] This would be consistent with our experience with the genetics of another genetically heterogeneous disorder associated with marked reduction in reproductive fitness, i.e., intellectual disability, where $>80\%$ of the causal mutations in our population are recessive compared to outbred populations in which nearly all mutations are de novo dominant.[39] Therefore, it is imperative that complementary efforts involving molecular autopsy should be pursued both in inbred and outbred populations to fully catalog genes that are important in early human development.

In conclusion, we show in this study that molecular autopsy is a practical and high-yield approach to investigate the cause of fetal demise, sometimes even when no fetal samples are available. Our study expands the phenotypic spectrum of several known disease genes and provides evidence that they can express phenotypically as fetal deaths. We also highlight a number of genes as potential candidates for early human development pending future confirmation. We show that the promise of precision medicine ushered in by genome sequencing is inclusive of families that experience pregnancy loss.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

The authors declare no conflict of interest.

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