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Diagnosis of lethal or prenatal-onset autosomal recessive disorders by parental exome sequencing

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ABSTRACT

Objective Rare genetic disorders resulting in prenatal or neonatal death are genetically heterogeneous, but testing is often limited by the availability of fetal DNA, leaving couples without a potential prenatal test for future pregnancies. We describe our novel strategy of exome sequencing parental DNA samples to diagnose recessive monogenic disorders in an audit of the first 50 couples referred.

Method Exome sequencing was carried out in a consecutive series of 50 couples who had 1 or more pregnancies affected with a lethal or prenatal-onset disorder. In all cases, there was insufficient DNA for exome sequencing of the affected fetus. Heterozygous rare variants (MAF < 0.001) in the same gene in both parents were selected for analysis. Likely, disease-causing variants were tested in fetal DNA to confirm co-segregation.

Results Parental exome analysis identified heterozygous pathogenic (or likely pathogenic) variants in 24 different genes in 26/50 couples (52%). Where 2 or more fetuses were affected, a genetic diagnosis was obtained in 18/29 cases (62%). In most cases, the clinical features were typical of the disorder, but in others, they result from a hypomorphic variant or represent the most severe form of a variable phenotypic spectrum.

Conclusion We conclude that exome sequencing of parental samples is a powerful strategy with high clinical utility for the genetic diagnosis of lethal or prenatal-onset recessive disorders. © 2017 The Authors Prenatal Diagnosis published by John Wiley & Sons Ltd.
INTRODUCTION

Whole exome sequencing (WES) in the postnatal setting has a diagnostic yield of 25 to 37%.1–4 Trio exome sequencing is often considered to be the strategy of choice as this can identify both inherited and de novo variants but is also the most expensive and relies on availability of large quantities of good quality DNA samples for the affected child and both parents. Trio exome analysis is often not possible for couples with single or multiple pregnancies affected with rare lethal disorders due to limited DNA quantity and/or quality of fetal DNA (if only formalin-fixed paraffin-embedded tissue is available), leaving these couples without a diagnosis and limited reproductive choices.5 Several studies have demonstrated the utility of exome sequence analysis for fetuses where DNA quantity is not limited.6,7 In addition to DNA availability, other factors make diagnosis difficult in lethal fetal disorders, including the large number of potential genes, phenotypic variability, and the difficulty in accurately phenotyping a mid-gestation fetus. Single molecular tests may be guided by the limited phenotyping available, but testing single genes in a step-wise manner may exhaust the little fetal material that may be available. This approach often fails to make a diagnosis, and ultrasound diagnosis in the second trimester is the only potential option for many couples, with the consequence of prolonged uncertainty regarding the pregnancy outcome and the possibility of a late gestation termination.5,6 Where multiple pregnancies are affected, the disorders are very likely to be recessive, and a recurrence risk of at least 25% is given for each future pregnancy but with no prenatal test available for future pregnancies.

We previously described a strategy utilizing parental exome sequencing and the application of a set of filtering criteria as an alternative method to identify potentially pathogenic variants in shared genes in unrelated, unaffected parents.5 This strategy saves precious fetal DNA as only a small amount is used for co-segregation studies. Identification of a genetic diagnosis will enable prenatal diagnosis or preimplantation genetic diagnosis in subsequent pregnancies. This parental sequencing strategy has been applied in a recent study of prenatal-onset cases where the authors describe the approach as “molecular autopsy by proxy” and report a high diagnostic yield.7 Following our early work,5 we introduced a diagnostic service, and the current study is an audit of the first 50 couples referred for parental exome sequencing. The couples had at least 1 fetus affected with severe fetal malformations or underwent termination of a pregnancy for a severely disabling disorder. In contrast to our previous report, in this large case series, the analysis was not limited to unrelated couples or couples with 2 or more affected pregnancies. Variants were shortlisted for evaluation by using a bioinformatics pipeline to identify potentially recessive likely pathogenic variants in established disease-causing genes that were consistent with the clinical phenotype. Co-segregation studies were carried out by using the stored DNA samples from the affected fetus(es). Here, we highlight the effectiveness of this strategy and the benefits of integrating exome sequencing for lethal prenatal disorders into the diagnostic pathway for these couples.

METHODS

Subjects

The case series was composed of 50 couples who had had 1 or more fetal/neonatal losses or terminated pregnancies based on the presence of malformations detected by ultrasound scanning. Of these, 29 had 2 or more affected pregnancies. Known consanguinity was reported in 11 couples. Two couples had offspring that died at birth but were tested with this method due to limited DNA availability from the affected babies (cases 31 and 41). The couples were referred for exome sequencing from centers across the UK and Ireland. All patients provided informed consent for testing to identify a genetic cause of the disorder affecting their pregnancies. The clinical description provided for each case by the referring clinician was converted to human phenotype ontology (HPO) terms by using the ontobee browser (http://www.ontobee.org/) and HPO browser (http://human-phenotype-ontology.github.io/tools.html). The HPO terms for the case series are shown in Table S1.

Exome library preparation and sequencing

Genomic DNA samples were quantified according to the manufacturer’s instructions on the Qubit fluorimeter (Thermo Fisher Scientific, Massachusetts, USA) to determine that the minimum quantity of DNA required, 3000 ng, was available. The samples were fragmented by using the Bioruptor (Diagenode, Liège, Belgium) and indexed adaptors ligated before hybridization with the Agilent SureSelect All Exon capture kit (v4, v5, or v6) or Agilent SureSelect Focused exome kit (Santa Clara, CA, USA). Paired-end 100-bp reads were sequenced on a HiSeq 2500 (Illumina, San Diego, CA, USA) by using either the standard or the rapid run mode or paired-end 150-bp reads on the NextSeq500 (Illumina, San Diego, CA, USA) by using either a mid or high output flow cell. Approximately 12 whole exomes can be run per flow cell, to generate at least 60 million reads with >80X mean coverage and >98% of target bases at ≥20X. Samples were sequenced in multiple batches as and when samples were received for diagnostic testing. The Illumina HiSeq FASTQ sequencing reads were demultiplexed and aligned to the reference (GRCh37/Hg19) by using BWA-MEM (v0.7.12), converted to BAM format file and subjected to duplicate removal by using Picard (v1.129), GATK (v3.4-46) was used for indel realignment, variant calling, and quality filtering.

Variant annotation and filtering

Variants were annotated by using Alamut-Batch (v1.4.4), a variant call format file was inputted and all SNVs and indels were annotated by using a range of different variant and genomic databases, including HGM Professional.8 A bioinformatics pipeline was designed in-house to identify shared genes where both parents had a heterozygous potentially pathogenic variant and to identify X-linked recessive variants where appropriate (Figure 1). Variants with a MAF < 0.0001 (<0.1%) and 0.001 (<0.1%) in Exome aggregation consortium (ExAC http://exac.broadinstitute.org/) or the Exome variant server (EVS http://evs.gs.washington.edu/EVS/) were retained to produce a subset of very rare
variants and rare variants. Variants were restricted to nonsynonymous variants, those affecting the conserved splice sites or those within −50/+10 base pairs of flanking exons predicted by Alamut-Batch to affect splicing (5 tools were used: SpliceSiteFinder-like, MaxEntScan, NNSplice [Fruitfly], GeneSplicer, and Human Splicing Finder). Variants annotated as Pathogenic in HGMD Pro (all cases) or ClinVar (since 2016) were retained regardless of other filtering criteria. Copy number variants were identified by using read depth analysis with a modified version of R software package ExomeDepth (v1.1.8) and comparing the test sample against reference samples. Autosomal recessive variants were identified where parents either shared the same heterozygous variant or had different heterozygous variants in the same gene. Potential X-linked recessive variants in the mother were also shortlisted where only male pregnancies were affected. The bioinformatics pipeline is summarized in Figure 1.

**Selection of candidate variants**

All genes on the variant shortlist were considered and initially reviewed via the Online Mendelian Inheritance in Man database (OMIM https://www.omim.org/) and PubMed (https://www.ncbi.nlm.nih.gov/pubmed/). Candidate variants in known disease-causing genes were identified for further investigation by comparison with the fetal phenotype. Previous reports of a variant were determined by using HGMD professional, ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and Locus-specific databases. In silico tools were accessed via Alamut Visual (versions 2.7.2-2.10) to predict pathogenicity of variants. Likely causative variants identified in this series were variants that were reported as likely pathogenic or pathogenic in the patient’s clinical diagnostic report. We have reviewed all the variant classifications by using the recently published American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology guidelines. In cases where no likely diagnosis was identified, we undertook further analysis of a curated gene panel from PanelApp (https://bioinfo.extge.co.uk/crowdsourcing/PanelApp/).

**Confirmation of results**

Possible disease-causing variants identified in parental DNA samples were confirmed by PCR/Sanger Sequencing for SNVs and indels or using the QX200 droplet digital PCR system (Bio-Rad, Hercules, California, USA) for CNVs. Fetal DNA samples were tested to establish co-segregation of the variant(s) with disease. DNA from at least 1 affected fetus (or neonate) was available for testing for each couple. PCR primer and droplet digital PCR primer/probe sequences are available on request.

**RNA extraction and reverse transcription**

For case 7, total RNA was extracted from PAXgene collection tubes (PreAnalytIX, Hombrechtikon, Switzerland) by using the PAXgene Blood miRNA Kit (Qiagen, Hilden, Germany). Complementary DNA (cDNA) was synthesized by using VILO SuperScript III RT-PCR system (Thermo Fisher Scientific, Massachusetts, USA) and fragment/sequence analysis performed by using primers designed against IFT122 exon 23 and 25. Products were visualized by electrophoresis on a 2% agarose gel.

**RESULTS**

Heterozygous pathogenic or likely pathogenic variants in both partners were identified in 26/50 couples (52%), and where 2 or more fetuses were affected, a genetic diagnosis was obtained in 18/29 (62%) cases (Table 1 and Figures 2A
| Case | Phenotype (HPO TERMS)                                                                 | Affected Pregnancies | Consanguinity | Gene       | HGVS Nomenclature                                         | Variant Classification on Clinical Diagnostic Report | OMIM Diagnosis                          |
|------|-----------------------------------------------------------------------------------|----------------------|---------------|------------|----------------------------------------------------------|-----------------------------------------------------|-----------------------------------------|
| 01   | Hydrops fetalis, multiple joint contractures, pulmonary hypoplasia                  | ≥2                   | N             | RYR1       | NM_000540.2 p.[Ser3074Phe] [?]; [9221C>T]; [141302A>G] | Pathogenic                                         | Fetal akinesia OMIM No. 180901          |
| 02   | Arthrogryposis multiplex congenita, multiple pterygia                               | ≥2                   | N             | GLE1       | NM_01037221.1 p.[Arg569His] [?]; [1706G>A]; [878+1G>T] | p.(Arg569His)11 Likely pathogenic p.(Val617Met)11 Likely pathogenic | Lethal congenital contracture syndrome 1/lethal arthrogryposis with anterior horn cell disease OMIM no 603371 |
| 03   | Hydrops fetalis, agenesis of corpus callosum, hypertrophic cardiomyopathy, pulmonary hypoplasia, low-set ears, increased nuchal translucency, ventriculomegaly, wide anterior fontanel | ≥2                   | N             | MPRSS22    | NM_020191.2 p.[Arg170His] [?]; [509G>A]; [878+1G>T] | p.(Arg170His)11 Likely pathogenic c.(878+1G>T) Pathogenic | Combined oxidative phosphorylation deficiency 5 OMIM no 605810 |
| 04   | Congenital microcephaly                                                             | 1                    | N             | CENPJ      | NM_018451.4 p.[Glu9Ter] [?]; [25G>T]; [2911C>T] | Pathogenic                                         | Autosomal recessive primary microcephaly 6 OMIM no 609279 |
| 07   | Postaxial hand polydactyly, postaxial foot polydactyly, fibular aplasia, downslanted palpebral fissures, low-set ears, cleft palate, intestinal malrotation, abnormality of pancreas morphology, absent tibia, preaxial foot polydactyly | ≥2                   | N             | IFT122     | NM_053985.3 p.[Ala688_Asp694delinsGlyVal] [?]; [2063_2082delinsGCCGTO]; [3099A>G] | Likely Pathogenic                      | Cranioectodermal dysplasia 1 (CED1) OMIM no 218330 |
| 09   | Absent hand (bilateral), absent toes, gastrochisis, intestinal malrotation, bilateral renal agenesis, ventriculomegaly, sex reversal | ≥2                   | N             | LRPR       | NM_002334.3 p.[Asp606Asp][Gly29Glu] [?]; [1816G>A]; [1886G>A] | Likely Pathogenic                      | Cenani-Lenz syndrome OMIM no 604270     |
| 11   | Ambiguous genitalia, arthrogryposis multiplex congenita                              | ≥2                   | N             | ATRX       | NM_000489.4 p.[Asp2177Ala] [?]; [6530A>C]; [1886G>A] | Likely Pathogenic                      | Mental retardation-hypotonic facies syndrome, X-linked OMIM no 309580 |
| 12   | Hypercholesterogenic kidneys, polydactyly                                           | ≥2                   | N             | BBS10      | NM_024685.3 p.[Cyto911][c.2377dup][dup] | Pathogenic13,14                             | Bardet-Bied syndrome 10 OMIM no 601418 |
| 13   | Polyhydramnios, decreased fetal movement, arthrogryposis multiplex congenita, micrognathia, high palate, congenital hip dislocation, poor suck, focal seizures, increased serum lactate, hypoglycemia | ≥2                   | N             | GLE1       | NM_01037221.1 p.[Ser194Ala] [?]; [581G>A]; [2009G>T] | Likely Pathogenic                      | Lethal congenital contracture syndrome 1/lethal arthrogryposis with anterior horn cell disease OMIM no 603371 |
| 14   | Congenital microcephaly, agenesis of corpus callosum, cerebellar hypoplasia, 11 pairs of ribs | ≥2                   | Y             | SASS6      | NM_194292.1 p.[Glu12452Glu][c.1239A>G][14344G>A] | Likely Pathogenic                      | Autosomal recessive primary microcephaly 14 OMIM no 609321 |
| 16   | Arthrogryposis multiplex congenita, hydrops fetalis                                 | ≥2                   | N             | RYR1       | NM_000540.2 p.[Glu4782Arg] [?]; [14344G>A] | Likely Pathogenic                      | Fetal akinesia OMIM no 180901           |
| Case | Phenotype (HPO TERMS)                                                                 | Affected Pregnancies | Consanguinity | Gene HGVS Nomenclature | Variant Classification on Clinical Diagnostic Report | OMIM Diagnosis |
|------|--------------------------------------------------------------------------------------|---------------------|---------------|------------------------|-----------------------------------------------------|-----------------|
| 17   | Hand polydactyly, foot polydactyly, multiple renal cysts, enlarged kidneys, hyperechogenic kidneys | 1                   | Y             | BBSP                   | Likely Pathogenic                                   | Bardet-Biedl syndrome 9 OMIM no 615986 |
| 18   | Short long bones, neonatal respiratory distress, short ribs, anterior rib cupping, thoracic hypoplasia, abnormality of the clavicle | 1                   | N             | DYNC2H1                | Likely Pathogenic                                   | Short rib polydactyly type II OMIM no 603297 |
| 19   | Bilateral renal agenesis, oligohydramnios, pulmonary hypoplasia, hypertrophic cardiomyopathy, and atresia, aplasia, aplasia of the uterus, aplasia/Hypoplasia of the fallopian tube | ≥2                  | N             | FRAS1                  | c.5530_2A>C Pathogenic p. (Gly2004Ser) Pathogenic | Fraser syndrome OMIM no 607830 |
| 20   | Bilateral renal agenesis, oligohydramnios                                            | ≥2                  | Y             | ITGA8                  | Pathogenic                                           | Renal hypoplasia/aplasia 1 OMIM no 604063 |
| 21   | Intrauterine growth retardation, hypertelorism, low-set ears, macrosomaphaly, ventriculomegaly | ≥2                  | N             | B3GLCT                 | c.660+1G>A Pathogenic                               | Peters plus syndrome OMIM no 261540 |
| 27   | Occipital meningoencephalocele, cystic renal dysplasia, polydactyly, 2-3 toe syndactyly, low-set ears, female external genitalia in individual with 46,XY karyotype, pulmonary hypoplasia | 1                   | N             | MKS1                   | c.1408-34_1408-6del Pathogenic                      | Meckel syndrome 1 OMIM no 249000 |
| 28   | Ventriculomegaly                                                                     | ≥2                  | N             | POMGNT1                | Likely Pathogenic                                   | Muscular dystrophy-dystroglycanopathy type A3 OMIM No. 253280 |
| 29   | Arthrogryposis multiplex congenita                                                    | 1                   | Y             | ERCC5                  | Pathogenic                                           | Cerbobrodulocostoskeletal syndrome 3 OMIM no 166570 |
| 31   | Cerebellar hypoplasia                                                                 | 1                   | N             | DIOSC2                 | p.(Gly31Ala) Pathogenic                             | Pontocerebellar hypoplasia, type 1B OMIM no 614678 |
| 34   | Bilateral renal dysplasia, multiple renal cysts                                      | ≥2                  | N             | ETFA                   | p.(Arg223Ter) Pathogenic Exon 11 deletion Likely Pathogenic | Glutaric acidemia II A OMIM no 231658 |
| 37   | Tetraamelia, kyphoscoliosis, absent septum pellucidum, abnormal cortical gyration     | ≥2                  | N             | TRP11                  | Pathogenic                                           | Achondrogenesis, type IA OMIM no 200060 |
| 39   | Arthrogryposis multiplex congenita, fetal akinesia sequence                           | ≥2                  | Y             | NEK9                   | Pathogenic                                           | Lethal contracture syndrome type 1 OMIM no 617022 |
| 41   |                                                                                  | ≥2                  | N             | ATAD3A                 | Pathogenic                                           | Hazel-Yoon syndrome OMIM no 617183 |

Table 1 (Continued)
Table 1 (Continued)

| Gene | Affected | Phenotype (HPO TERMS) | HGVS Nomenclature | Consanguinity | Consequence | OMIM Diagnosis |
|------|----------|-----------------------|-------------------|---------------|-------------|----------------|
| ATM | 47 | Hypertrophic cardiomyopathy, cerebellar hypoplasia, vertebroplasia, arachnoid cyst, renal anomalies, genital anomalies, cataracts | [NM_018136.4 c.[1038G>T]; p.(Gln421fs)] | Y | Homozygous Pathogenic | OMIM 608716 |
| BBS7 | 15 | Microcephaly, facial anomalies, cataracts, deafness, intellectual disability | [NM_211130.4 c.[3452G>A]; p.(Glu115Lys)] | Y | Homozygous Pathogenic | OMIM 213800 |
| BBS9 | 1 | Cerebellar hypoplasia, ventriculomegaly, microcephaly, microphthalmia, hypertelorism, facial anomalies, syndactyly/oligodactyly | [NM_138260.4 c.[1701G>A]; p.(Glu567Lys)] | Y | Homozygous Pathogenic | OMIM 601097 |
| CENPJ | 2 | Cerebellar hypoplasia, metopic ridge, speech delay, growth delay | NM_006297.3 c.[1112G>T]; p.(Glu371Val) | N | Compound heterozygous | OMIM 606648 |
| CENPJ | 2 | Cerebellar hypoplasia, metopic ridge, speech delay, growth delay | NM_006297.3 c.[1112G>T]; p.(Glu371Val) | N | Compound heterozygous | OMIM 606648 |
| CEP290 | 2 | Autosomal dominant retinitis pigmentosa, hearing impairment, intellectual disability, ataxia | NM_021994.3 c.[1604C>T]; p.(Ser535Phe) | N | Compound heterozygous | OMIM 609628 |
| CEP290 | 2 | Autosomal dominant retinitis pigmentosa, hearing impairment, intellectual disability, ataxia | NM_021994.3 c.[1604C>T]; p.(Ser535Phe) | N | Compound heterozygous | OMIM 609628 |
| CEP290 | 2 | Autosomal dominant retinitis pigmentosa, hearing impairment, intellectual disability, ataxia | NM_021994.3 c.[1604C>T]; p.(Ser535Phe) | N | Compound heterozygous | OMIM 609628 |
| CEP290 | 2 | Autosomal dominant retinitis pigmentosa, hearing impairment, intellectual disability, ataxia | NM_021994.3 c.[1604C>T]; p.(Ser535Phe) | N | Compound heterozygous | OMIM 609628 |

For ACMG classification, see Table S1. The reference is included for variants previously reported in the literature.
variants lie at positions 40 and 20 of the repeat motif, respectively (Figure 4). Inspection of the PROSITE sequence logo indicated that aspartic acid and glycine are the most commonly observed amino acids at these positions (at frequencies of ~68% and ~79%, respectively), whereas the variant residues were absent or present at very low frequencies (≤1.5%). By comparison, a previously reported pathogenic LRP4 missense variant, p.(Asp529Asn), occurs at position 7 of the motif within the second LDLR receptor class B repeat, and although aspartic acid is most commonly seen at this position (~81% frequency in the sequence logo), the variant residue asparagine is observed in 41/683 sequences in the PROSITE entry (6%). This suggests that the p.(Asp529Asn) is more likely to be tolerated than the variants identified in this couple and thus have a milder effect on structure and function of the repeat resulting in a less severe phenotype.

Messenger RNA analysis was undertaken for a couple with novel IFT122 variants (case 7). The paternal variant was a large insertion-deletion that results in the net loss of 5 amino acids in exon 17 of the IFT122 gene and is predicted to result in significant changes to the arrangement of secondary structure motifs. The maternal variant was a base substitution, c.3039+4A>G, in intron 24 (Figure 5A), and the splice prediction tools suggest a reduction in the strength of the 5′ acceptor site of between 9.1% and 30%. Sanger sequencing of PCR products generated by using primers designed to amplify from exon 23 to exon 25 of cDNA from the carrier mother is consistent with exon 24 skipping (Figure 5B).

An additional, or unsolicited, finding was identified in 1 consanguineous couple who are both heterozygous carriers of a pathogenic frameshift variant, c.3503_3504del, p.(Leu1168fs) in the GNPTAB (NM_024312.4) gene. This is a previously reported founder mutation, and recessive variants are a known cause of Mucolipidosis II alpha/beta.25,26

**DISCUSSION**

Using the parental autosomal recessive strategy, a potential genetic diagnosis was established in 26/50 couples, giving a
diagnostic yield of 52%. This increases to 18/29 (62%) in those couples who have had 2 or more pregnancies affected with lethal or prenatal-onset disorders. Potentially pathogenic variants were identified in 24 different genes, consistent with the known genetic heterogeneity of these rare disorders. By using the parental DNA for exome sequencing, we can conserve the precious DNA from the affected pregnancies; using a small quantity for directed variant confirmation.

In most cases, the reported phenotypes in these families were consistent with that reported in the literature. Some cases identified a diagnosis that would not usually be readily identified in the prenatal period. A diagnosis of Peters-plus syndrome was made in family 21 after the identification of the founder mutation, c.660+1G>A, in the B3GLCT gene (formerly known as B3GALTL). A diagnosis of Peters-plus syndrome is often made postnatally; prenatal diagnosis has been reported but is generally more difficult due to variable and nonspecific findings. Severe hydrocephalus was the only indication of this diagnosis. Similarly, the fetuses with the POMGNT1 homozygous missense variant had presented with ventriculomegaly, an early feature of muscle-eye-brain disease (case 28).

Other cases confirmed an extension of a known phenotype or evidence of a genotype-phenotype correlation. LRP4 missense variants, p.(Asp606Asn) and p.(Gly629Glu), were identified in case 9. Pathogenic missense or splicing LRP4 variants are a known cause of Cenani-Lenz syndrome, which is characterized by a less severe phenotype than was seen in our patient, of syndactyly/oligodactyly and kidney abnormalities. A recent report of truncating variants causing a severe lethal prenatal form is consistent with

Figure 4. The upper panel shows an alignment of the YWTD repeats 2 to 4 of human LRP4 from PROSITE entry PS51120 (gaps removed), highlighting the variants identified in case 9, p.(Gly629Glu) (yellow) and p.(Asp606Asn) (blue) and the previously reported p.(Asp529Asn) (green). Representation of the sequence logo below indicates that the wild-type residues are the most commonly seen, whereas the variant residues are either absent or present at a low frequency (≤1.5%).

Figure 5. (A) Location of the IFT122 variant, NC_000003.11(NM_052985.3)c.3039+4A>G, in intron 24. (b) Sequence electropherogram of the IFT122 RT-PCR product demonstrating the effect of this splicing variant in the maternal RNA. The primers used were designed over the suspected breakpoint (exon23-exon25 boundary) so only the variant allele was amplified.
Cenani-Lenz syndrome being the result of hypomorphic variants. This suggested that some missense variants could have a severe impact on protein function and result in a severe lethal prenatal phenotype. The p.Asp606 and p.Gly629 residues are located within the highly conserved LR4 YWTD motif. Variant residues at these positions are either absent or rarely observed in this sequence and are unlikely to be tolerated within the repeat region. A missense variant, p.(Asp529Asn), reported in a patient with Cenani-Lenz syndrome is observed at a frequency of ~6%, and although this variant is known to be pathogenic, it is clearly compatible with life. We predict that both p.(Asp606Asn) and p.(Gly629Ceu) may have a more severe effect, consistent with the observed lethality in the case of the fetuses with compound heterozygosity for these 2 variants.

Similarly, recessive pathogenic IFT122 variants are known to cause Cranioectodermal dysplasia 1, which is a nonlethal condition where patients present with craniofacial, skeletal, and ectodermal abnormalities. One couple has been reported with recurrent pregnancy losses and compound heterozygosity for 2 IFT122 mutations (a missense and frameshift). More recently, IFT122 biallelic variants, a missense and a frameshift, were identified in a male infant born prematurely at 31/40 weeks of gestation with a typical phenotype of short-rib polydactyly type IV. We found compound heterozygous IFT122 variants in 1 family (case 7) with a skeletal dysplasia. The paternally inherited variant was a large insertion-deletion in exon 17 of IFT122. In-frame insertions and deletions in nonrepetitive regions of greater than 1 amino acid are considered more likely to disrupt protein function than a missense variant alone; the larger the deletion or insertion, the more likely it is to be pathogenic. These large in-frame insertions/deletions are rare in population databases reflecting the fact that they generally have deleterious effects on protein structure and folding, particularly when they overlap or occur within regions of secondary structure. In silico tools predicted aberrant splicing due to a maternal variant, c.3039+4A>G in intron 24, and messenger RNA studies supported that prediction with skipping of exon 24 observed in a maternal blood sample. Thus, evidence suggests that these 2 variants are likely to be pathogenic and provide a plausible explanation of the phenotype seen in their affected offspring. This case also illustrates the extended spectrum of phenotypes associated with known disorders identified due to exome sequencing.

In 3 families, a “clinical exome” test was performed where a subset of the exome was sequenced (~6110 genes) and a genetic diagnosis was established in 2 of the 3 families. We recommend that WES is undertaken for the undiagnosed couple because in our cohort, 3 of the 26 diagnoses made by using the whole exome capture would have missed using this “clinical exome” approach targeting 6,110 genes associated with human diseases (genes known at the time of the capture design in 2013).

A novel SASS6 variant, p.(Glu412Gly), was identified in both partners of a couple who had 2 pregnancies with primary microcephaly (case 14). This gene has recently been identified by homozygosity mapping using WES in a large consanguineous Pakistani family with multiple family members affected with microcephaly. Homozygous NEK9 variants were identified in family 39 who were known to be consanguineous and had 2 affected pregnancies with arthrogryposis and fetal akinesia sequence. In 2016, this gene was linked to lethal contracture syndrome type 10 in 2 Irish traveler families. Couple 41 suffered 2 pregnancy losses at birth; both babies had no respiratory effort and congenital hypertrophic cardiomyopathy. Both affected offspring were compound heterozygous for an ATAD3A novel missense variant and a deletion of exons 3 and 4. Monoallelic and biallelic variants have recently been reported to cause Harel-Yoon syndrome, which is characterized by global developmental delay, hypotonia, optic atrophy, axonal neuropathy, and hypertrophic cardiomyopathy. Compared with the whole exome test, the clinical exome is cheaper, yields a shorter list of variants for analysis, and may give higher coverage across known disease genes but, as demonstrated in these 3 cases, has the disadvantage of not including the most recently discovered, or not yet identified, disease genes.

Using this strategy, only shared genes where both parents harbor a rare variant are included in the analysis; this limits the risk of detection of a variant in a later-onset dominant disorders, such as cancer predisposition genes. However, this approach does not exclude the possibility of revealing carrier status for other autosomal recessive disorders and this risk is increased in consanguineous couples. In one of the consanguineous couples in this case series, we identified an additional, unrelated finding where both parents were heterozygous for a founder mutation in the GNPTAB gene. As this is a clearly pathogenic variant, we discussed it with the referring clinician who agreed to disclose the finding due to the 1 in 4 risk for this couple having a child affected with Mucolipidosis II alpha/beta in addition to the 1 in 4 risk for this couple having a child affected with Mucolipidosis II alpha/beta in addition to the 1 in 4 risk for this couple having a child affected with Mucolipidosis II alpha/beta in addition to the 1 in 4 risk for this couple having a child affected with Mucolipidosis II alpha/beta in addition to the 1 in 4 risk for this couple having a child affected with Mucolipidosis II alpha/beta in addition to the 1 in 4 risk for this couple having a child affected with Mucolipidosis II alpha/beta in addition to the 1 in 4 risk for this couple having a child affected with Mucolipidosis II alpha/beta in addition to the 1 in 4 risk for this couple having a child affected with Mucolipidosis II alpha/beta.
the disease gene may not yet be associated with a disease phenotype, the causative gene may be poorly covered by the capture, or pathogenic variants could have been filtered out by the bioinformatic strategy/software. Interpretation of variants is based on information that is currently available, and this is likely to change as knowledge increases. One of the advantages of WES is that the data can be reanalyzed in the future as new disease genes are annotated.  

CONCLUSION

In summary, we demonstrate that parental exome sequencing is a powerful tool to diagnose lethal or prenatal-onset recessive fetal disorders. For those couples where insufficient fetal DNA is available for exome sequencing, this strategy provides the opportunity of a genetic diagnosis to provide preimplantation or prenatal diagnosis for future pregnancies.

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