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

Karen L. Stals¹, Matthew Wakeling², Júlia Baptista¹,², Richard Caswell¹, Andrew Parrish¹, Julia Rankin¹, Carolyn Tysoe¹, Garan Jones¹, Adam C. Gunning¹, Charu Deshpande⁶, Abhijit Dixi¹⁰, Jacqueline Eason¹⁰, Frances Elmslie¹¹, Andrew E. Fry¹², Alan Fryer¹³, Muriel Holder⁶, Tessa Homfray¹¹, Emma Kivuva³, Victoria McKay¹³, Ruth Newbury-Ecob¹⁴, Michael Parker¹⁵, Ravi Savarirayan¹⁰, Claire Seear¹⁰, Nora Shannon¹⁰, Deborah Shears⁸, Sarah Smithson¹⁴, Ellen Thomas⁶, Peter D. Tumpenny³, Vinod Varghese¹², Pradeep Vasudevan¹⁷, Emma Wakeling⁶, Emma L. Baple²,³,¹⁸, and Sian Ellard¹,²*

¹Molecular Genetics Department, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK
²Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK
³Clinical Genetics Department, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK
⁴Department of Clinical Genetics, Our Lady’s Children’s Hospital, Dublin, Ireland
⁵North West Thames Regional Genetics Service, London North West Healthcare NHS Trust, Harrow, UK
⁶Guy’s Regional Genetics Service, Guy’s and St Thomas’ NHS Foundation Trust, London, UK
⁷Oxford Regional Clinical Genetics Service, Northampton General Hospital, Northampton, UK
⁸Guy’s and St Thomas’ NHS Foundation Trust, London, UK
⁹Oxford Centre for Genomic Medicine, Oxford University Hospitals NHS Foundation Trust, Oxford, UK
¹⁰South West Thames Regional Genetics Service, St George’s University Hospitals NHS Foundation Trust, London, UK
¹¹Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK
¹²Department of Clinical Genetics, Liverpool Women’s NHS Foundation Trust, Liverpool, UK
¹³Sheffield Children’s Hospital, University Hospital, Sheffield, UK
¹⁴Victorian Clinical Genetics Services, Murdoch Children’s Research Institute, Melbourne, Vic, Australia
¹⁵Leicester Clinical Genetics, Women’s and Children’s Services, Leicester Royal Infirmary, Leicester, UK
¹⁶Women’s and Children’s Services, St George’s Hospital, Liverpool, UK
¹⁷Wessex Clinical Genetics Service, University Hospital Southampton NHS Foundation Trust, Southampton, UK
¹⁸Wessex Clinical Genetics Service, University Hospital Southampton NHS Foundation Trust, Southampton, UK

*Correspondence to: Sian Ellard, Institute of Biomedical and Clinical Science. E-mail: sian.ellard@nhs.net

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.

Funding sources: SE is a Wellcome Trust Senior Investigator.

Conflicts of interest: None declared
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.5,8 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 HGMD 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 (<0.01%) 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.10 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
Table 1  Summary of clinical information and results in the affected pregnancies where a genetic diagnosis was obtained

<table>
<thead>
<tr>
<th>Case</th>
<th>Phenotype (HPO TERMS)</th>
<th>Affected Pregnancies</th>
<th>Consanguinity</th>
<th>Gene</th>
<th>HGVS Nomenclature</th>
<th>Variant Classification on Clinical Diagnostic Report</th>
<th>OMIM Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Hydrops fetalis, multiple joint contractures, pulmonary hypoplasia</td>
<td>≥2</td>
<td>N</td>
<td>RYR1</td>
<td>NM_000540.2 p. [Ser3074Phe]</td>
<td>Pathogenic</td>
<td>Fetal akinesia OMIM No. 180901</td>
</tr>
<tr>
<td>02</td>
<td>Arthrogryposis multiplex congenita, multiple pterygia</td>
<td>≥2</td>
<td>N</td>
<td>GLE1</td>
<td>NM_0103722.1 p. [Arg569His]</td>
<td>Likely pathogenic</td>
<td>Lethal congenital contracture syndrome 1/lethal arthrogryposis with anterior horn cell disease OMIM no 603371</td>
</tr>
<tr>
<td>03</td>
<td>Hydrops fetalis, agenesis of corpus callosum, hypertrophic cardiomyopathy, pulmonary hypoplasia, low-set ears, increased nuchal translucency, ventriculomegaly, wide anterior fontanel</td>
<td>≥2</td>
<td>N</td>
<td>MPPS22</td>
<td>NM_020191.2 p. [Arg170His]</td>
<td>Likely pathogenic</td>
<td>Combined oxidative phosphorylation deficiency 5 OMIM no 605810</td>
</tr>
<tr>
<td>04</td>
<td>Congenital microcephaly</td>
<td>1</td>
<td>N</td>
<td>CENPJ</td>
<td>NM_018451.4 p. [Glu9Ter]</td>
<td>Pathogenic</td>
<td>Autosomal recessive primary microcephaly 6 OMIM no 609729</td>
</tr>
<tr>
<td>07</td>
<td>Postaxial hand polydactyly, postaxial foot polydactyly, fibular aplasia, downslanted palpebral fissures, low-set ears, cleft palate, intestinal malrotation, abnormality of pancreas morphology, absentibia, preaxial foot polydactyly</td>
<td>≥2</td>
<td>N</td>
<td>IFTI22</td>
<td>NM_029985.3 p. [Ala688Asp]</td>
<td>Likely pathogenic</td>
<td>Craniofacial dysplasia 1 (CED1) OMIM no 218330</td>
</tr>
<tr>
<td>09</td>
<td>Absent hand (bilateral), absent toes, gastroschisis, intestinal malrotation, bilateral renal agenesis, ventriculomegaly, sex reversal</td>
<td>≥2</td>
<td>N</td>
<td>LRPI</td>
<td>NM_002334.3 p. [Asp606Asn]</td>
<td>Likely pathogenic</td>
<td>Cabant Lenz syndrome OMIM no 604270</td>
</tr>
<tr>
<td>11</td>
<td>Ambiguous genitalia, arthrogryposis multiplex congenita</td>
<td>≥2</td>
<td>N</td>
<td>ATRX</td>
<td>NM_000498.4 p. [Asp2177Asn]</td>
<td>Likely pathogenic</td>
<td>Mental retardation-hypotonic facies syndrome, X-linked OMIM no 309580</td>
</tr>
<tr>
<td>12</td>
<td>Hyperechogenic kidneys, polydactyly</td>
<td>≥2</td>
<td>N</td>
<td>BBS10</td>
<td>NM_024663.3 p. [Cys91fs]</td>
<td>Pathogenic</td>
<td>Bardet-Biedl syndrome 10 OMIM no 601418</td>
</tr>
<tr>
<td>13</td>
<td>Polyhydramnios, decreased fetal movement, arthrogryposis multiplex congenita, micrognathia, high palate, congenital hip dislocation, poor suck, focal seizures, increased serum lactate, hypoglycaemia</td>
<td>≥2</td>
<td>N</td>
<td>GLE1</td>
<td>NM_0103722.1 p. [Arg670Leu]</td>
<td>Likely pathogenic</td>
<td>Lethal congenital contracture syndrome 1/lethal arthrogryposis with anterior horn cell disease OMIM no 603371</td>
</tr>
<tr>
<td>14</td>
<td>Congenital microcephaly, agenesis of corpus callosum, cerebellar hypoplasia, 11 pairs of ribs</td>
<td>≥2</td>
<td>Y</td>
<td>SASS6</td>
<td>NM_194292.1 p. [Cys412Gln]</td>
<td>Likely pathogenic</td>
<td>Autosomal recessive primary microcephaly 14 OMIM no 609721</td>
</tr>
<tr>
<td>16</td>
<td>Arthrogryposis multiplex congenita, hydrops fetalis</td>
<td>≥2</td>
<td>N</td>
<td>RYR1</td>
<td>NM_000540.2 p. [Gly792Arg]</td>
<td>Likely pathogenic</td>
<td>Fetal akinesia OMIM no 180901</td>
</tr>
</tbody>
</table>

(Continues)
<table>
<thead>
<tr>
<th>Case</th>
<th>Phenotype (HPO TERMS)</th>
<th>Affected</th>
<th>Consanguinity</th>
<th>Gene HGVS Nomenclature</th>
<th>Variant Classification on Clinical Diagnostic Report</th>
<th>OMIM Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Hand polydactyly, foot polydactyly, multiple renal cysts, enlarged kidneys, hyperechogenic kidneys</td>
<td>1</td>
<td>Y</td>
<td>BBSP</td>
<td>Likely Pathogenic</td>
<td>Bardet-Biedl syndrome 9 OMIM no 615986</td>
</tr>
<tr>
<td>18</td>
<td>Short long bones, neonatal respiratory distress, short ribs, anterior rib cupping, thoracic hypoplasia, abnormality of the clavicle</td>
<td>1</td>
<td>N</td>
<td>DYNC2H1</td>
<td>Likely Pathogenic</td>
<td>Short rib polydactyly type II OMIM no 603297</td>
</tr>
<tr>
<td>19</td>
<td>Bilateral renal agenesis, oligohydramnios, pulmonary hypoplasia, hypotrophic cardiomyopathy, and aterias, aplasia, aplasia/Hypoplasia of the fallopian tube</td>
<td>≥2</td>
<td>N</td>
<td>FRAS1</td>
<td>c.55302A&gt;G; Pathogenic p. (Gly2004Ser) Pathogenic</td>
<td>Fraser syndrome OMIM no 607830</td>
</tr>
<tr>
<td>20</td>
<td>Bilateral renal agenesis, oligohydramnios</td>
<td>≥2</td>
<td>Y</td>
<td>ITGA8</td>
<td>Pathogenic</td>
<td>Renal hypoplasia/aplasia 1 OMIM no 604063</td>
</tr>
<tr>
<td>21</td>
<td>Intrauterine growth retardation, hypertelorism, low-set ears, megalencephaly, ventriculomegaly</td>
<td>≥2</td>
<td>N</td>
<td>B3GLCT</td>
<td>c.660+1G&gt;A Pathogenic</td>
<td>Peters plus syndrome OMIM no 261540</td>
</tr>
<tr>
<td>27</td>
<td>Occipital meningoencephalocele, cystic renal dysplasia, polydactyly, 2-3 toe syndactyly, low-set ears, female external genitalia in individual with 46,XY karyotype, pulmonary hypoplasia</td>
<td>1</td>
<td>N</td>
<td>MKS1</td>
<td>c.1408-34_1408-6del[del] Pathogenic</td>
<td>Meckel syndrome 1 OMIM no 249000</td>
</tr>
<tr>
<td>28</td>
<td>Ventriculomegaly</td>
<td>≥2</td>
<td>N</td>
<td>POMGNT1</td>
<td>Likely Pathogenic</td>
<td>Muscular dystrophy-dystroglycanopathy type A3 OMIM No. 253280</td>
</tr>
<tr>
<td>29</td>
<td>Arthrogryposis multiplex congenita</td>
<td>1</td>
<td>Y</td>
<td>ERCC5</td>
<td>Pathogenic</td>
<td>Cerebrooculofacioskeletal syndrome 3 OMIM no 616570</td>
</tr>
<tr>
<td>31</td>
<td>Cerebellar hypoplasia</td>
<td>1</td>
<td>N</td>
<td>DIO2C2</td>
<td>Pathogenic</td>
<td>Ponto-oclocerebral hypoplasia, type B OMIM no 614678</td>
</tr>
<tr>
<td>34</td>
<td>Bilateral renal dysplasia, multiple renal cysts</td>
<td>≥2</td>
<td>N</td>
<td>E1FA</td>
<td>Pathogenic</td>
<td>Glutaric acidemia IIA OMIM no 231069</td>
</tr>
<tr>
<td>37</td>
<td>Tetraamelia, hypoposphatasia, absent septum pelvicium, abnormal cortical gyration</td>
<td>≥2</td>
<td>N</td>
<td>TRP11</td>
<td>Pathogenic</td>
<td>Achondrogenesis, type IA OMIM no 2000600</td>
</tr>
<tr>
<td>39</td>
<td>Arthrogryposis multiplex congenita, fetal alinesia sequence</td>
<td>≥2</td>
<td>Y</td>
<td>NEK9</td>
<td>Pathogenic</td>
<td>Lethal contracture syndrome type 10 OMIM no 617022</td>
</tr>
<tr>
<td>41</td>
<td></td>
<td>≥2</td>
<td>N</td>
<td>ATAD3A</td>
<td>Pathogenic</td>
<td>Harel-Yoon syndrome OMIM no 617183</td>
</tr>
</tbody>
</table>
Table 1 (Continued)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>Spinal dysraphism, encephaly, hydrocephaly, microcephaly, calvarial hypoplasia, optic atrophy, renal hypoplasia, megalencephaly, microphthalmia, genital hypoplasia, Arnold-Chiari malformation, anhydramnios</td>
<td>NM_000331.5 [c.1098G&gt;A]</td>
<td>TMEM67</td>
<td>[c.1098G&gt;A]</td>
<td>p.Glu363Lys</td>
<td>Mutatio likely pathogenic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>Cerebellar hypoplasia, ventriculomegaly, spastic paraplegia, myoclonus, and facial weakness</td>
<td>NM_018136.4 [c.1260_1266[del]</td>
<td>ASPM</td>
<td>[c.1260_1266[del]</td>
<td>p.(Gln421fs)23</td>
<td>Pathogenic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cases 1 and 2 have been described previously.† For ACMG classification Table 51. The reference is cited for variants previously reported in the literature.

Table 1 continued

Affected

<table>
<thead>
<tr>
<th>Variant Classification</th>
<th>On Clinical Diagnostic Report</th>
<th>OMIM Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>p.(Asp606Asn)</td>
<td>OMIM no 607361</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>p.(Gly629Glu)</td>
<td>OMIM no 608716</td>
</tr>
</tbody>
</table>

Copy number variants were identified as likely disease-causing in 2 families; an ETTA exon 11 deletion (case 34) and an ATAD3A exon 3 to 4 deletion (case 41). Four couples had causative variants in recently discovered genes where there is only 1 publication or <3 cases previously reported: ATAD3A, ITGA8, NEK9, and SASS6. Well-described founder mutations in GLEI, BBS9, B3GLCT, MKS1, and EXOSC3 were identified in 5 families. The MKS1 variant was identified through a gene panel analysis as the MAF is greater than 0.001, and the nomenclature recorded in HGMD Pro for this founder mutation is discordant with the Alamut annotation.

Rare LRP4 missense variants, p.(Asp606Asn) and p.(Gly629Glu), were identified in case 9. Pathogenic variants in this gene are a known cause of Cenani-Lenz syndrome, which is characterized by syndactyly/oligodactyly and kidney abnormalities. Sanger sequencing confirmed that the affected fetus was compound heterozygous for the missense variants. In silico tools supported pathogenicity of both missense variants, which lie within conserved LRP4 receptor class B repeat 3 and 4, respectively, of LRP4. The PROSITE database entry for the LRP4 receptor class B repeat (entry PS51120) contains 683 repeats from 62 different proteins; alignment of LRP4 repeats 3 and 4 with the consensus sequence shows that the p.(Asp606Asn) and p.(Gly629Glu)
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), 24 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 B3GALT1). 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.27 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.24 A recent report of truncating variants causing a severe lethal prenatal form28,29 is consistent with
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 syndrome24 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.(Gly629Glu) may have a more severe effect, consistent with 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 1,30 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).31 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.32 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.10 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.33 Homozygous NEK9 variants were identified in family 39 who were known to be consanguineous and had 2 affected pregnancies with arthrogryposis and fetal akinsia sequence. In 2016, this gene was linked to lethal contracture syndrome type 10 in 2 Irish traveler families.34 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.35 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 of inheriting the variants causative of the recessive condition that had already presented. We note that if there had been sufficient DNA available for a trio analysis, this additional finding might not have been detected. The couple found the information on the incidental finding useful and informative.

There are multiple reasons why likely disease-causing variants were not identified in 24 of the 50 families reported here. It is reasonable to assume an autosomal recessive pattern of inheritance in a family with multiple affected offspring or in a consanguineous pairing, but a different mode of inheritance cannot be excluded. Recent case reports describe families with multiple affected children with megacistrosis microcolon intestinal hypoperistalsis syndrome, due to de novo disease-causing variants in the ACTG2 gene, suggestive of gonadal mosaicism.36 Similarly, diagnostic exome sequencing in 13 families with known consanguinity indicated that over 38% of positive results were not autosomal recessive.37 One of the 11 couples tested who were known to be consanguinuous each had a different missense variant in the BB39 gene that was compound heterozygous in their affected fetus. Other explanations for not identifying a diagnosis include that the disorder is not monogenic, the pathogenic variant may be noncoding, the pathogenic variant may be a structural variant, or...
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.2

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.

ACKNOWLEDGEMENTS

The authors thank Anne-Marie Bussell, Michael Day, Lewis Pang, and Jessica Settle of The Royal Devon and Exeter NHS Foundation Trust and Matthew Johnson of the University of Exeter Medical School for their assistance.

REFERENCES


SUPPORTING INFORMATION
Additional Supporting Information may be found online in the supporting information tab for this article.