ORIGINAL INVESTIGATION

Prenatal exome sequencing and chromosomal microarray analysis in fetal structural anomalies in a highly consanguineous population reveals a propensity of ciliopathy genes causing multisystem phenotypes

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Abstract
Fetal abnormalities are detected in 3% of all pregnancies and are responsible for approximately 20% of all perinatal deaths. Chromosomal microarray analysis (CMA) and exome sequencing (ES) are widely used in prenatal settings for molecular genetic diagnostics with variable diagnostic yields. In this study, we aimed to determine the diagnostic yield of trio-ES in detecting the cause of fetal abnormalities within a highly consanguineous population. In families with a history of congenital anomalies, a total of 119 fetuses with structural anomalies were recruited and DNA from invasive samples were used together with parental DNA samples for trio-ES and CMA. Data were analysed to determine possible underlying genetic disorders associated with observed fetal phenotypes. The cohort had a known consanguinity of 81%. Trio-ES led to diagnostic molecular genetic findings in 59 fetuses (with pathogenic/likely pathogenic variants) most with multisystem or renal abnormalities. CMA detected chromosomal abnormalities compatible with the fetal phenotype in another 7 cases. Monogenic ciliopathy disorders with an autosomal recessive inheritance were the predominant cause of multisystem fetal anomalies (24/59 cases, 40.7%) with loss of function variants representing the vast majority of molecular genetic abnormalities. Heterozygous de novo pathogenic variants were found in four fetuses. A total of 23 novel variants predicted to be associated with the phenotype were detected. Prenatal trio-ES and CMA detected likely causative molecular genetic defects in a total of 55% of families with fetal anomalies confirming the diagnostic utility of trio-ES and CMA as first-line genetic test in the prenatal diagnosis of multisystem fetal anomalies including ciliopathy syndromes.

Introduction
Fetal abnormalities are present in 3% of all pregnancies and are responsible for around 20% of all perinatal deaths (Vora and Hui 2018). Prenatal ultrasound scanning (USS) of the fetus may be used to detect a range of anomalies, from a single minor defect to a severe multisystem disorder (Lord et al. 2019). Chromosomal abnormalities, inherited and de novo genetic disorders and multifactorial conditions are well known to be major contributors to birth defects, although the aetiology of many congenital malformations remains unknown (Yates et al. 2017). Genetic diagnosis of fetal structural anomalies is an important step in the evaluation and clinical management of the fetus and managing the family’s expectations. Fetal anomalies can be associated with all types of chromosomal and genetic abnormalities that include aneuploidy, copy number variations (CNVs) and gene-specific pathogenic variants (Lord et al. 2019). For many years, prenatal diagnosis was performed by fetal USS followed by conventional karyotype and/or chromosomal
microarray analysis (CMA) using amniotic fluid, chorionic villus sampling or DNA extracted from cord blood. The addition of CMA in prenatal diagnosis increases the frequency of diagnosis of chromosomal abnormalities by 3–5% (Wapner et al. 2012). More recently, exome sequencing (ES) and genome sequencing (GS) have been applied to fetal molecular genetic diagnostics to improve diagnostic yield (Best et al. 2018; Talkowski et al. 2012). ES is becoming the first line molecular test for a huge range of genetic disorders and there is some evidence that it has improved the detection rate of prenatal diagnosis (Best et al. 2018). Prenatal genetic diagnosis using ES varies between 10 and 57% (Vora et al. 2017), and this huge range maybe attributed to variables in inclusion criteria, family history of prenatal anomalies, fetal USS abnormalities, trio (an affected proband with both parents) versus solo and other technical factors. Recently, a large study, which included ES in 610 fetuses with structural anomalies detected by fetal USS resulted in a diagnostic yield of 12.4%, including variants of unknown significance (Lord et al. 2019) while another similar-sized investigation resulted in a detection rate of 10% (Petrovski et al. 2019). The efficiency of trio-ES over solo-ES is widely accepted and the implementation of trio-ES in prenatal diagnosis leads to a higher detection rate (Dillon et al. 2018; Jelin and Vora 2018).

The Saudi Arabian population has a consanguinity rate of around 56% (Alkuraya 2013) with first-degree cousin marriages occurring in 33.6% compared to 22.4% of marriages with more distant relations (El-Mouzan et al. 2007). In prenatal hospital settings with the aid of fetal USS, the detection rate of genetic causes of underlying anomalies using ES is expected to be higher than previously reported, especially in a consanguineous population such as Saudi Arabia. Although many studies worldwide describe the diagnostic yield of ES in prenatal settings, no previous reports have studied prenatal cases from consanguineous couples using ES. This study demonstrates the diagnostic yield of trio-ES combined with CMA in fetuses with ultrasound structural anomalies in a highly consanguineous population and reveals a high rate of variants in ciliopathy syndrome genes accounting for multisystem fetal anomalies.

Materials and methods

Study cohort

A total of 119 fetuses from 119 unrelated families were recruited at the Maternal Fetal Medicine section of King Faisal Specialist Hospital and Research Centre in Riyadh, Saudi Arabia from November 2018 until January 2020. Inclusion criteria included pregnant women with a previous family history of congenital anomalies (defined as at least one previous fetus/child from the same family having congenital anomalies) and acceptance of invasive testing for identified structural anomalies or nuchal translucency in their fetus as detected by USS after 12 weeks of gestation. The parents, following informed written consent, participated in a family questionnaire to determine if there was known consanguinity, blood testing for DNA extraction and molecular genetic analysis (Figure S1). In the knowledge of a previous family history of congenital anomalies, all fetuses with structural anomalies detected by antenatal USS were recruited for clinical service diagnosis by trio-ES and CMA testing according to the American College of Obstetricians & Gynecologists (ACOG) guidelines (American College of Obstetricians & Gynecologists 2016). Fetal DNA was extracted from either chorionic villi, amniotic fluid, or cord blood. The study was conducted according to the Declaration of Helsinki and approved by the local institutional review board (IRB) (KFSHRC RAC# 2211035).

Prenatal USS examination, DNA extraction and maternal cell contamination (MCC)

Prenatal anatomy USS examination was performed for all families. Serial prenatal USS examinations started between 12 and 34 weeks gestation or for new referrals prenatal USS started at the first antenatal visit. Fetal anatomy was reported for features as previously described (Al-Hamed et al. 2016). Fetal death (FD) was defined as an intrauterine death after 10 gestational weeks, perinatal death (PD) within 7 days of birth and infant death (ID) within 1 year of birth.

DNA was extracted using the Gentra Systems PURE-GENE DNA Isolation kit (Qiagen, USA). Maternal cell contamination (MCC) was excluded in all fetal samples using the AmpFLSTR® Identifier® PCR Amplification Kit as described by the manufacturer (Applied Biosystems, Life Technologies).

Trio-ES and variant interpretation

Trio-ES was performed on fetal and parental genomic DNA using an Agilent Sureselect All Exons V5 (50 Mb) capture kit (Agilent Technologies; Santa Clara, CA, USA) as described previously (Monies et al. 2017). An in-house variant interpretation pipeline was used for variant interpretation as previously described (Monies et al. 2017). We used the ACMG guidelines-based websites Franklin by Gennox (https://franklin.genoox.com) and VarSome (Kopanos et al. 2019) (https://varsome.com) for variant interpretation. The variant classification of trio-ES results was based on ACMG guidelines and categorized into four groups:

(A): Pathogenic or Likely Pathogenic (P/LP) explaining the phenotype: all reported pathogenic variants and novel (not reported) loss of function variants in a gene where loss
of function is a known mechanism of disease according to ACMG guidelines, (B): Variants of unknown significance (VUS) that explain the phenotype: all extremely low frequency missense and intronic variants with uncertain significance in genes causing the phenotype, (C): Negative: where no potential variants were identified to describe the disease phenotype, and (D): Cases with chromosomal abnormalities solved by CMA.

HGMD® Professional 2020.2 and ClinVar databases (https://www.ncbi.nlm.nih.gov/clinvar/) were used as a reference for the previously reported variants. In addition to Online Mendelian Inheritance in Man (OMIM), the Genomics England PanelApp (https://panelapp.genomicsgland.co.uk/) was used to assess the genotype–phenotype correlation.

**Sanger sequencing**

Sanger sequencing validation was performed for all de novo, novel and low quality metric variants (depth of coverage, base quality and mapping quality). Oligonucleotide primers for PCR amplification of targeted variants were designed using Primer3 software (http://frodo.wi.mit.edu/) and synthesized in house. The amplified PCR products were sequenced using an ABI 3730xl capillary sequencer (Applied Biosystems, CA, USA) and sequences were analysed using Mutation Surveyor software V.3.24 (SoftGenetics LLC, State College, Pennsylvania, USA).

**Chromosomal microarray analysis (CMA)**

CMA was performed in cases with a suspicion of chromosomal abnormalities including advanced maternal age (above 35 years), cases where there was no known consanguinity and for all cases with a negative trio-ES analysis except for five fetuses due to insufficient quantities of DNA (Figure S1). Genomic DNA was fragmented, amplified and hybridized to the array according to manufacturer’s guidelines (Affymetrix CytoScan® HD Array Kit). It contains 2.7 million markers across the genome covering 96% of the genes. Results were analysed with the Chromosome Analysis suite (ChAS, Affymetrix). Copy number variations (CNVs) with more than 50 kb (deletions) and 200 kb (duplications) are reported. CMA was performed on 67 cases out of 119 fetuses who met the above criteria. Classification of CNVs was based on the ACMG standards and guidelines for interpretation and reporting of constitutional CNVs (Riggs et al. 2020). CNVs were evaluated in terms of their pathogenicity and contribution to the fetal phenotype using the Database of Genomic Variants (DGV; http://dgv.tcag.ca/) and the Decipher database (https://www.deciphergenomics.org/). CMA is unable to detect small deletions/insertions, balanced chromosomal translocations and inversions and imbalances in the mitochondrial genome.

**Results**

A total of 119 fetuses from 119 Saudi Arabian families were investigated in this study by trio-ES analysis (Table 1). Sixty-seven fetuses were investigated by CMA in addition to trio-ES (Figure S1), including 13 cases of advanced maternal age, 32 negative trio-ES cases and 22 cases with no known consanguinity. Ninety-seven of the 119 enrolled families (81%) were known to be consanguineous. The average gestational age for prenatal testing was 26 weeks. Trio-ES with an average turnaround time of 31 days detected 59 cases (49%) with a confirmed diagnosis in Group A with pathogenic or likely pathogenic alleles (Table 2; Fig. 1) and 21 (18%) in Group B with variants of unknown significance that were in genes that likely explained the phenotype (Table 3). Trio-ES was negative in 32 (27%) cases. Seven cases had positive CMA results that were thought to be diagnostic of the fetal anomalies present (Table 4).

The phenotypic spectrum of fetuses varied in severity, most likely due to the underlying genes and variant type (Table S1). The majority of fetuses had severe multisystem phenotypes, hydrops fetalis, brain abnormalities or renal anomalies. Fetal death, perinatal death, infant death and

| Findings                                                                 | No. of cases | % AR | % AD |
|-------------------------------------------------------------------------|--------------|------|------|
| Total number of cases                                                  | 119          |      |      |
| Known consanguineous families                                          | 97 (81%)     |      |      |
| Cases with a diagnostic group A (P/LP) variant                         | 59 (49%)     | 54 (95.5%) | 5 (8.5%) |
| Cases with group B (VUS) variant (that may explain phenotype)          | 21 (18%)     | 18 (86%) | 3 (14%) |
| Cases with group C (Negative ES and CMA)                               | 32 (27%)     |      |      |
| Cases with group D (positive CMA findings that explains phenotype)     | 7 (6%)       |      |      |
| Cases with autosomal recessive inheritance                             | 72 (61%)     |      |      |
| Cases with autosomal dominant inheritance                               | 8 (7%)       |      |      |
| Cases with X-linked inheritance                                        | 0            |      |      |
| Family | Outcome | Maternal age | Consanguinity | Fetal structural defects | Exome sequencing results | Zygo- sity | Inheritance | SHGP (f) | gnomAD (f) | CMA results | Reference | ACMG classification |
|--------|---------|--------------|---------------|-------------------------|-------------------------|-----------|-------------|----------|------------|-------------|-----------|---------------------|
| FAM- 55 | Live- birth | 40 | Yes | Cardiac—hypo- plastic right heart | *IGFBP7* (NM_001553.3):c.830-1G > A | Hom | AR | 0.00139766 | 0 | Negative | rs866994863, (Abu- Safieh et al. 2011) | P |
| FAM- 33 | Live- birth | 29 | Yes | Central nerv- ous—hydro- cephalus and Dandy– Walker mal- formation | *CPLANE1* (NM_023073.3):c.7988_7989delGA (p.Gly2663Alafs*40) | Hom | AR | 0.00106609 | 0 | N/P | rs730882217, (Bach- mann-Gagescu et al. 2015) | P |
| FAM- 37 | ID | 21 | Yes | Central nerv- ous—hydro- cephalus | *POMT1* (NM_001136114.2):c.376C > T (p.Arg126*) | Hom | AR | 0.00011572 | 0.00000795 | N/P | rs398124247, RCV000081494 | P |
| FAM- 38 | Live- birth | 37 | Yes | Central nerv- ous—absent corpus cal- losum and hydrocepha- lus | *MPDZ* (NM_001261406.2):c.628C > T (p.Gln210*) | Hom | AR | 0.00070197 | 0 | N/P | rs372127610, (Shaheen et al. 2017) | P |
| FAM- 84 | ID | 40 | yes | Central nerv- ous—micro- cephaly | *CENPF* (NM_016343.4):c.1323+1G > A | Hom | AR | 0 | 0 | Negative | This study | P |
| FAM- 5 | Live- birth | 32 | Yes | Central nervous -microcephaly | *ASPM* (NM_001206846.1):c.1959_1962delCAAA (p.Asn653Lysfs*14) | Hom | AR | 0.00027962 | 0 | Negative | rs199422147, (Bond et al. 2003) | P |
| FAM- 46 | FD | 33 | Yes | Head/neck—cystic hygroma | *TTN* (NM_003319.4):c.28958G > A (p.Trp9653*) | Hom | AR/AD | 0.00014668 | 0 | Negative | rs1477101279, (Rob- erts et al. 2015) | P |
| FAM- 24 | FD | 30 | Yes | Fetal hydrops | *GUSB* (NM_000181.4):c.307C > T (p.Arg103Trp) | Hom | AR | 0.00061114 | 0.000016 | N/P | rs786205673, (Sham- seldin et al. 2015) | LP |
| FAM- 31 | TOP | 42 | Yes | Fetal hydrops | *CHRNA1* (NM_000079.4):c.685C > T (p.Arg229Cys) | Hom | AR/AD | 0.00017405 | 0.00000795 | N/P | rs74535427 | LP |
| FAM- 44 | TOP | 30 | Yes | Fetal hydrops | *NEB* (NM_001164507.1): c.1014_1015delinsT (p.Lys338Asnfs*9) | Hom | AR | 0 | 0 | Negative | This study | P |
| FAM- 48 | FD | 40 | No | Fetal hydrops | *PTPN11* (NM_002834.5):c.923A > G (p.Asn308Ser) | Het, de novo | AD | 0.00002928 | 0 | N/P | rs121918455, (Reuter et al. 2020) | P |
| FAM- 80 | ID | 36 | Yes | Fetal hydrops | *GUSB* (NM_000181.4):c.1429C > T (p.Arg477Trp) | Hom | AR | 0.001104766 | 0.00000199 | N/P | rs774393243, (Schwartz et al. 2003) | LP |
| FAM- 85 | FD | 23 | Yes | Fetal hydrops | *HBAI* and *HBA2* whole gene deletion | Hom | AR | N/A | Negative | RCV000050953, (Tamary and Dgany 1993) | P |
Table 2 (continued)

| Family | Outcome | Maternal age | Consanguinity | Fetal structural defects | Exome sequencing results | Zygodity | Inheritance | SHGP (f) | gnomAD (f) | CMA results | Reference | ACMG classification |
|--------|---------|--------------|---------------|-------------------------|-------------------------|----------|-------------|----------|------------|-------------|----------|-------------------|
| FAM-88 | Live-birth | 24 | Yes | Fetal hydrops | *FZD6* (NM_003506.4):c.869A > G (p.Tyr290Cys) | Hom | AR | 0.0005772 | 0.0 | N/P | rs786205672, (Shamseldin et al. 2015) | | LP |
| FAM-94 | Live-birth | 31 | Yes | Fetal hydrops | *THSD1* (NM_018676.4):c.617G > A (p.Cys206Tyr) | Hom | AR | 0.00103919 | 0 | N/P | rs786205669, (Shamseldin et al. 2015) | | P |
| FAM-114 | ID | 27 | Yes | Fetal hydrops | *NEB* (NM_004543.5):c.11077-1G > A | Hom | AR | 0.00028717 | 0 | N/P | rs886041851, (Monies et al. 2017) | | P |
| FAM-1 PD | PD | 23 | Yes | Multisystem phenotype—midline cleft lip, cardiac anomalies, short long bones, unilateral kidney agenesis | *INTU* (NM_015693.4):c.1499A > C (p.Glu500Ala) | Hom | AR | 0.00014845 | 0 | Negative | rs1360128571, (Toriyama et al. 2016) | | LP |
| FAM-3 PD | PD | 35 | Yes | Multisystem—abnormal hands, talipes and hydronephrosis | *DCHS1* (NM_003737.4):c.2225T > A (p.Leu742*) | Hom | AR/AD | 0.00014845 | 0 | Negative | This study (VCV001177398) | | P |
| FAM-10 ID | ID | 33 | Yes | Multisystem—atrial septal defect, ventricular septal defect, cardiomegaly, micrognathia | *TMEM94* (NM_014738.6):c.606dupG (p.Ile203Aspfs*4) | Hom | AR | 0 | 0 | Negative | (Al-Hamed et al. 2020) | | P |
| FAM-11 TOP | TOP | 39 | Yes | Multisystem—cerebellar vermis agenesis, Dandy walker malformation and skull defect | *AHI1* (NM_017651.4):c.2361G > A (p.Trp787*) | Hom | AR | 0.00005649 | 0 | Negative | This study (VCV001029630) | | P |
| FAM-13 PD | PD | 20 | Yes | Multisystem—encephalocele, bilateral polycystic kidneys | *CC2D2A* (NM_001080522.2):c.3084delG (p.Lys1029Argfs*3) | Hom | AR | 0.00211623 | 0.00000829 | N/P | rs386833749, (Shaheen et al. 2016c) | | P |
Table 2 (continued)

| Family | Outcome | Maternal age | Consanguinity | Fetal structural defects | Exome sequencing results | Zygo- | Inheritance | SHGP (f) | gnomAD (f) | CMA results | Reference | ACMG classification |
|--------|---------|--------------|---------------|-------------------------|--------------------------|-------|-------------|----------|-------------|-------------|----------|----------------------|
| FAM-21 | FD      | 40           | Yes           | Multisystem—bilateral cleft hands, bilateral cleft foot, no stomach and poor shaped chest, generalized oedema, fetal akinesia | AGRN (NM_198576.4):c.4922dupA (p.Asn1641Lysfs*230) | Hom   | AR          | 0        | 0.0        | Negative    | This study  | LP                   |
| FAM-27 | PD      | 31           | Yes           | Multisystem—ventriculomegaly, occipital encephalocele, hypoplastic cerebellum and absent vermis, bilateral polycystic kidney | CC2D2A (NM_001080522.2):c.3084delG (p.Lys1029Argfs*3) | Hom   | AR          | 0.00211623 | 0.00000829 | N/P         | rs386833749, (Shaheen et al. 2016c) | P |
| FAM-29 | PD      | 33           | Yes           | Multisystem—partial agenesis of corpus callosum, contractures, bilateral rocker bottom feet, micrognathia | ERCC5 (NM_000123.4):c.2427delT (p.Asp809Glufs*24) | Hom   | AR          | 0.00122476 | 0.00000398 | N/P         | rs777455688, RCV000985070 | P |
| FAM-53 | PD      | 24           | Yes           | Multisystem—anhydramnios, abnormal kidney, hypoplastic lung and Dandy–Walker syndrome | NEK8 (NM_178170.3):c.515dupC (p.Glu173*) | Hom   | AR          | 0        | 0          | N/P         | This study  | P |

SHGP = Sporadic Human Genetics Project; gnomAD = Genome Aggregation Database; CMA = Copy Number Variation Analysis.
| Family Outcome | Maternal Age | Con-sanguinity | Fetal structural defects | Exome sequencing results | Zygo-... |
|---------------|-------------|----------------|--------------------------|-------------------------|----------|
| FAM-56 TOP    | 23          | Yes            | Multisystem—dolichocep... | WNT7A (NM_004625.4):c.874C>T (p.Arg292Cys) | Hom AR   |
|               |             |                |                          |                         | 0.0002429 0 Negative rs104893835, (Mutlu et al. 2016) |          |
| FAM-59 ?      | 25          | Yes            | Multisystem—enlarged ech... | CC2D2A (NM_001080522.2):c.3084delG (p.Lys1029Argfs*3) | Hom AR   |
|               |             |                |                          |                         | 0.00211623 0.00000829 N/P rs386833749, (Shaheen et al. 2016c) |          |
| FAM-62 ?      | 30          | Yes            | Multisystem—multiple congenital anomalies, bilateral enlarged echogenic kidneys, anhydramnios, polydactyly, clubfoot | TCTN2 (NM_024809.5):c.1286dupA (p.Asn429Lysfs*2) | Hom AR   |
|               |             |                |                          |                         | 0 0 N/P rs1555293215 P |          |
| FAM-64 FD     | 29          | Yes            | Multisystem—multiple fetal anomalies, encephalocele, bilateral polycystic kidneys | CC2D2A (NM_001080522.2):c.4531 T>C (p.Trp1511Arg) | Hom AR   |
|               |             |                |                          |                         | 0.00047378 0 N/P (Al-Hamed et al. 2016) |          |
Table 2 (continued)

| Family | Outcome | Maternal age | Consanguinity | Fetal structural defects | Exome sequencing results | Zygo- | Inherit- | SHG (f) | gnomAD (f) | CMA results | Reference | ACMG classification |
|--------|---------|--------------|---------------|--------------------------|--------------------------|-------|---------|---------|------------|-------------|-----------|------------------|
| FAM-65 | PD      | 21           | Yes           | Multisystem—Dandy-Walker malformation and bilateral renal cysts | TMEM67 (NM_153704.6):c.2290C > T (p.Arg764*) | Hom   | AR      | 0       | 0.00000398 | N/P         | rs751517725, (Bachmann-Gugescu et al. 2015) | P |
| FAM-66 | PD      | 34           | Yes           | Multisystem—encephalocele, polycystic kidneys | TMEM237 (NM_001044385.3):c.325C > T (p.Arg109*) | Hom   | AR      | 0       | 0.00000962 | N/P         | rs565778005, (Monies et al. 2017) | P |
| FAM-70 | PD      | 26           | Yes           | Multisystem—microcephaly, abnormal face, hypotelorism, intrauterine growth restriction | CTU2 (NM_001012759.3):c.873G > A (p.Thr291Thr) | Hom   | AR      | 0.00126187 | 0.00000929 | Negative | rs769481947, (Shaheen et al. 2019) | P |
| FAM-81 | FD      | 24           | Yes           | Multisystem—Cloverleaf skull, polycystic kidneys, polydactyly | TMEM231 (NM_001077416.2):c.823G > A (p.Val275Ile) | Hom   | AR      | 0.00059787 | 0.00000401 | N/P         | rs397514753, (Shaheen et al. 2013) | LP |
| FAM-92 | ID      | 36           | Yes           | Multisystem—Intrauterine growth retardation, unilateral renal agenesis, agenesis of corpus callosum and micrognathia | CTU2 (NM_001012759.3):c.873G > A (p.Thr291Thr) | Hom   | AR      | 0.00126187 | 0.00000929 | Negative | rs769481947, (Shaheen et al. 2019) | P |
| Family Outcome | Maternal age | Consanguinity | Fetal structural defects | Exome sequencing results | Zygo- sity | Inheritance | SHGP (f) | gnomAD (f) | CMA results | Reference | ACMG classification |
|---------------|--------------|---------------|--------------------------|--------------------------|----------|-------------|----------|-----------|-------------|-----------|---------------------|
| FAM-102 PD    | 30           | Yes           | Multisystem— micrognathia, ventricular septal defect, left sided hydrothorax, polyhydramnios, hydrops fetalis | TMEM94 (NM_014738.6):c.2729-2A > G | Hom AR | 0.00022268  | 0        |            | Negative (Al-Hamed et al. 2020) |            | P                   |
| FAM-109 ID    | 30           | Yes           | Multisystem— encephalocele, micrognathia, cystic hygroma, thoracic hypoplasia, lumbo-sacral scoliosis, short femur, short humerus, and talipes | HSPG2 (NM_005529.7):c.3518delG (p.Gly1173Valfs*101) | Hom AR | 0           | 0        |            | N/P        | This study (VCV001177407) |            | P                   |
| FAM-112 ID    | 23           | Yes           | Multisystem—ventriculomegaly, congenital heart disease, multicystic kidney dysplasia | POMT1 (NM_001077366.2):c.118 +1G > T | Hom AR | 0.00098368  | 0.00000795 | N/P       | rs746823238, (Geis et al. 2019) |            | P                   |
| FAM-116 PD    | 24           | Yes           | Multisystem— cerebellar hypoplasia, distended bladder, echogenic kidney, oligohydramnios | CTU2 (NM_001012759.3):c.873G > A (p.Thr291Thr) | Hom AR | 0.00126187  | 0.00000929 | N/P       | rs769481947, (Shaheen et al. 2019) |            | P                   |
| Family ID | Outcome | Maternal age | Consanguinity | Fetal structural defects | Exome sequencing results | Zygodity | Inheritance | SHGP (f) | gnomAD (f) | CMA results | Reference | ACMG classification |
|-----------|---------|--------------|---------------|------------------------|-------------------------|----------|-------------|----------|------------|-------------|-----------|---------------------|
| FAM-117   | PD      | 30           | Yes           | Multisystem—hydramnios, absent kidney, absent bladder and absent stomach | FANCD2 (NM_001018115.3):c.520C>T (p.Arg174*) | Hom      | AR          | 0.0000689 | 0.00000398 | N/P         | rs1449126896 | P                   |
| FAM-111   | PD      | 33           | Yes           | Neuromuscular—fetal akinesia syndrome | TTN (NM_001267550.2):c.40238dupA (p.Tyr13414Valfs*3) | Hom      | AR/AD       | 0         | 0          | N/P         | This study (VCV001177408) | P                   |
| FAM-2     | PD      | 30           | Yes           | Renal—hydramnios megacystis, hydrourerter, echogenic kidney and enlarged polycystic kidney, bladder outflow obstacle | FOXF1 (NM_001451.3):c.225C>G (p.Tyr75*) | Het, de novo | AD         | 0.00003368 | 0          | Negative (Stankiewicz et al. 2009) | P                   |
| FAM-16    | FD      | 32           | Yes           | Renal—bilateral renal agenesis | CD151 (NM_001039490.1):c.493C>T (p.Arg165*) | Hom      | AR          | 0.0050505 | 0.00012    | N/P         | This study (VCV001028736) | P                   |
| FAM-40    | Livebirth | 32         | Yes           | Renal—bilateral hydronephrosis | TBX18 (NM_001080508.3):c.692_693insT (p.Glu233Glyfs*19) | Het      | AD          | 0         | 0          | Negative (VCV001177415) | P                   |
| FAM-45    | PD      | 22           | Yes           | Renal—bilateral echogenic kidneys, ascites | DIS3L2 (NM_152383.5):c.1810C>T (p.Gln604*) | Hom      | AR          | 0.0012051 | 0          | 10q11 microduplication (VCV000970918) | P                   |
| FAM-60    | PD      | 27           | Yes           | Renal—polycystic kidney disease | NPHP3 (NM_153240.5):c.2694-2_2694-1delAG | Hom      | AR          | 0.00191342 | 0.000275 | N/P         | rs751527253, (Shaheen et al. 2016c) | P                   |
| FAM-61    | ID      | 29           | Yes           | Renal—cystic kidney disease | NPHP3 (NM_153240.5):c.457C>T (p.Gln153*) | Hom      | AR          | 0         | 0.00000401 | N/P         | rs751828098, (Sun et al. 2016) | P                   |
| Family | Out- | Mater- | Con- | Fetal structural defects | Exome sequencing results | Zygo- | Inher- | SHGP (f) | gnomAD (f) | CMA results | Reference | ACMG classification |
|--------|------|--------|------|--------------------------|-------------------------|-------|--------|----------|------------|-------------|-----------|---------------------|
| FAM-63 ID 26 Yes | Renal—anhydramnios, bilateral echogenic kidneys and absent stomach and bladder | *NPHP3* (NM_153240.5):c.2694-2_2694-1delAG | Hom | AR | 0.00191342 | 0.000275 | N/P | rs751527253, (Shaheen et al. 2016c) | P |
| FAM-96 PD 27 Yes | Renal—hyper-echogenic multicystic kidney dysplasia, anhydramnios | *PKHD1* (NM_138694.4):c.4644delC (p.Tyr1549Thrfs*43) | Hom | AR | 0 | 0 | Negative | This study (VCV001177406) | P |
| FAM-100 PD 25 Yes | Renal—poly-cystic kidneys and oligohydramnios | *NPHP3* (NM_153240.5):c.2694-2_2694-1delAG | Hom | AR | 0.00191342 | 0.000275 | N/P | rs751527253, (Shaheen et al. 2016c) | P |
| FAM-101 PD 36 Yes | Renal—poly-cystic kidneys | *PKHD1* (NM_138694.4):c.5237-92G > A / *PKHD1* (NM_138694.4):c.4870C > T (p.Arg1624Trp) | Comp-Het | AR | 0.00006540 / 0.00317221 | 0.0 / 0.000151 | Negative | rs560227398 / rs200391019, (Vivante et al. 2017) | VUS |
| FAM-104 ID 35 Yes | Renal—bilateral enlarged echogenic kidneys, pulmonary hypoplasia | *NPHP3* (NM_153240.5):c.2694-2_2694-1delAG | Hom | AR | 0.00191342 | 0.000275 | Negative | rs751527253, (Shaheen et al. 2016c) | P |
| FAM-7 TOP 26 No | Skeletal—Poly-hydramnios, proboscis nose, severe ventriculomegaly, bilateral short humerus & femur, short both tibia & fibula | *TBC1D32* (NM_152730.6):c.3724C > T (p.Arg1242*) | Hom | AR | 0.00018557 | 0.0000121 | Negative | rs748798523, (Alsa- han and Alkuraya 2020) | P |
| Family | Outcome | Maternal age | Consanguinity | Fetal structural defects | Exome sequencing results | Zygosity | Inheritance | SHGP (f) | gnomAD (f) | CMA results | Reference | ACMG classification |
|--------|----------|--------------|---------------|--------------------------|--------------------------|----------|-------------|----------|-----------|-------------|-----------|---------------------|
| FAM-14 | Livebirth | 27 | Yes | Skeletal—kyphosis, cleft foot/toes, wrist abnormalities, both elbows fixed, flexed joint, and abnormal nose | MYH3 (NM_002470.4):c.2501 T > C (p.Phe834Ser) | Het, de novo | AD/AR | 0.00003012 | 0 | N/P | This study (VCV001177399) | P |
| FAM-47 | TOP | 31 | Yes | Skeletal—fetus with short long bones, and skeletal dysplasia | IL6ST (NM_001190981.2):c.841C > T (p.Arg281*) | Hom | AR | 0.00029691 | 0 | N/P | rs1580817729, (Chen et al. 2020) | P |
| FAM-69 | Livebirth | 24 | Yes | Skeletal—absent left radius, absence of left thumb, syndactyly left hand | BLM (NM_000057.4):c.175_176delGT (p.Val59Ilefs*4) | Hom | AR | 0.00008438 | 0 | Negative | This study (VCV001177404) | P |
| FAM-75 | PD | 27 | Yes | Skeletal—skeletal abnormalities, club foot | CANT1 (NM_138793.4):c.902_906dupGCGCC (p.Ser303Alafs*21) | Hom | AR | 0 | 0.00000846 | Negative | rs58776895, (Bertoli-Avella et al. 2021) | P |
| FAM-77 | ID | 31 | Yes | Skeletal—polydactyly, head/femur length ratio | NKX3-2 (NM_0011189.4):c.225delC (p.Gly76Valfs*48) | Hom | AR | 0 | 0 | Negative | This study | LP |
| Family | Outcome | Maternal age | Consanguinity | Fetal structural defects | Exome sequencing results | Zygodity | Inheritance | SHGP (f) | gnomAD (f) | CMA results | Reference | ACMG classification |
|--------|----------|--------------|---------------|------------------------|-------------------------|----------|-------------|----------|------------|-------------|-----------|---------------------|
| FAM-90 | ?        | 31           | No            | Skeletal—Skeletal dysplasia, lumbar kyphosis, narrow chest, short humerus, short tibia, short femur, femoral bowing, hypoplasia of the ulna, fibular, radius | FGFR3 (NM_001163213.1):c.746C > G (p.Ser249Cys) | Het, de novo | AD/AR | 0 | 0 | Negative | rs121913483, RCV000017742 | P |
| FAM-99 | ID       | 36           | Yes           | Skeletal—Skeletal dysplasia | DYNC2H1 (NM_001080463.2):c.970C > T (p.Leu324Phe) & ARSB (NM_000046.5):c.455G > A (p.Arg152Gln) | Hom, Hom | AR, AR | 0 / 0.0002214 | 0.0 / 0.0000437 | N/P | DYNC2H1: This study (VCV001031040) / ARSB: rs776814144 (RCV000985065.1) | LP |

FD fetal death, PD perinatal death, ID infant death, TOP termination of pregnancy. ? unknown, Hom homozygous, Het heterozygous, N/P not performed, f frequency (SHGP, Saudi Human Genome Project and gnomAD, Version: 2.1.1)
termination of pregnancy represent the vast majority of pregnancy outcomes in the study (Fig. 2).

The majority of cases were solved by pathogenic variants in known ciliopathy genes with autosomal recessive inheritance pattern. Pathogenic (homozygous) variants of *NPHP3* were found in 5 fetuses, with 4 of them (FAM-60, FAM-63, FAM-100, FAM-104) sharing the same homozygous allele c.2694-2_2694-1delAG suggesting that these families are interrelated. The same allele was previously noted in Saudi families with ciliopathy syndromes (Shamseldin et al. 2020). Similarly, in *CC2D2A* pathogenic (homozygous) variants were found in four fetuses with severe multisystem phenotypes including encephalocele, four of whom had the identical allele c.2694-2_2694-1delAG suggesting that these families are interrelated. The same allele was previously noted in Saudi families with ciliopathy syndromes (Shamseldin et al. 2020). Similarly, in *CC2D2A* pathogenic (homozygous) variants were found in four fetuses with severe multisystem phenotypes including encephalocele, four of whom had the identical allele c.3084delG again suggesting these families may all be founder related. Consistent with this, the identical allele was reported in four Saudi families with Meckel syndrome/ Joubert syndrome phenotypes (Shamseldin et al. 2020). Pathogenic biallelic variants were also detected in more than one case for the known ciliopathy genes *PKHD1* and *TMEM94*. A shared and known pathogenic allele in *CTU2* (c.873G > A; p.Thr291Thr) (Shaheen et al. 2019) was identified in three cases with multiple anomalies including brain and renal phenotypes and corresponds to a likely founder allele in the Saudi Arabian population.

A total of 16 novel pathogenic variants and 7 novel VUS were detected in 22 different genes (Figure S2). Novel variants included missense, frameshift, nonsense and splicing defects and correlated closely with the phenotypes observed. Most cases with fetal or perinatal death can be explained by the detected pathogenic loss of function variants.

**Group A: pathogenic or likely pathogenic variants explaining the phenotype**

59 fetuses were identified with pathogenic or likely pathogenic variants explaining the phenotype (Table 2). 55 biallelic variants (54 homozygous and 1 compound heterozygous) pathogenic variants were detected in this group. 45 of them were predicted loss of function variants and 16 were novel. In FAM-45 trio-ES detected a loss of function variant...
| Family   | Outcome | Maternal age | Consanguinity | Fetal structural defects | Exome sequencing results                                                                 | Zygosity | Inheritance | SHGP (f) | gnomAD (f) | CMA results | References                        | Varsome Prediction | Franklin Prediction |
|----------|---------|--------------|---------------|--------------------------|------------------------------------------------------------------------------------------|----------|-------------|----------|------------|------------|-----------------------------------|-------------------|---------------------|
| FAM-15   | Livebirth | 31           | No            | Fetal hydrops            | PIEZO1 (NM_001142864.4):c.1792G > A (p.Val598Met)                                      | Het      | AR/AD       | 0.00007422 | 0          | N/P        | Gnanasambandam et al. (2018) | VUS               | VUS                 |
| FAM-17   | FD      | 31           | Yes           | Fetal hydrops            | EPHB4 (NM_004444.5):c.337G > A (p.Val113Ile)                                           | Hom      | AD          | 0.00048248 | 0.000305   | N/P        | rs55866373                       | B                 | LB                  |
| FAM-57   | ID      | 28           | Yes           | Fetal hydrops            | ACD (NM_001082486.1):c.1088-5_1088-3delCTC                                          | Hom      | AR/AD       | 0.00022268 | 0.000004   | N/P        | rs74719169 VUS VUS VUS VUS VUS VUS |
| FAM-118  | ID      | 25           | Yes           | Fetal hydrops            | PIEZO1 (NM_001142864.4):c.6211T > C (p.Cys2071Arg)                                   | Hom      | AR/AD       | 0          | 0          | N/P        | This study (VCV001177414)        | VUS               | VUS                 |
| FAM-6    | PD      | 29           | Yes           | Multisystem              | MYBPC3 (NM_002566.3):c.1321G > A (p.Glu441lys)                                       | Hom      | AR/AD       | 0.00132996 | 0.000138   | N/P        | rs193922377 LP VUS VUS VUS VUS |
| FAM-18   | PD      | 21           | No            | Multisystem              | LDLD (NM_001088.5):c.163G > A (p.Val51Ile)                                           | Hom      | AR          | 0.00041359 | 0          | N/P        | This study (VCV001177410)        | VUS               | VUS                 |
| FAM-28   | PD      | 22           | Yes           | Multisystem              | CEP290 (NM_025114.4):c.4151G > A (p.Arg1384His)                                      | Hom      | AR/AD       | 0.00010912 | 0.000030   | N/P        | rs143152287 VUS VUS VUS VUS VUS |
| FAM-68   | PD      | 30           | Yes           | Multisystem              | FRAS1 (NM_025074.7):c.10775A > G (p.His3592Arg)                                      | Hom      | AR          | 0.00048123 | 0.00000813 | Negative   | rs1011371729 LB VUS VUS VUS VUS |
| FAM-73   | ID      | 32           | Yes           | Multisystem              | RNASEH2C (NM_032193.4):c.190C > T (p.Arg64Trp)                                       | Hom      | AR          | 0          | 0.00000799 | Negative   | rs766270515 VUS VUS VUS VUS VUS |
| FAM-74   | TOP     | 29           | Yes           | Multisystem              | CENPF (NM_016343.4):c.2545C > G (p.Leu849Val)                                        | Hom      | AR          | 0.00029691 | 0          | Negative   | rs1000186164 LB VUS VUS VUS VUS |
| FAM-89   | PD      | 28           | Yes           | Multisystem              | MA51P1 (NM_139125.4):c.1358G > A (p.Gly453Asp)                                      | Hom      | AR          | 0          | 0.00000796 | Isochromosome 12p (Pallister-Killian syndrome) | rs777260336 VUS VUS VUS VUS VUS |
| FAM-113  | Livebirth | 31           | Yes           | Multisystem              | TTC8 (NM_00128782.1):c.107G > A (p.Cys36Tyr)                                         | Hom      | AR          | 0.0001823  | 0          | N/P        | rs1595959305 LP VUS VUS VUS VUS VUS |
| FAM-115  | FD      | 38           | Yes           | Multisystem              | TUBA8 (NM_019443.3):c.958C > T (p.Arg320Thr) and LZTR1 (NM_006767.4):c.1055A > C (p.Tyr352Ser) | Hom      | AR/AD       | 0.0001276  | 0.00011334 | 0.0000516  | Negative TUBA8: rs140202346 LZTR1: rs368689599 VUS VUS VUS VUS VUS |
| FAM-9    | PD      | 33           | Yes           | Renal                    | CILK1 (NM_014920.5):c.105G > A (p.Met35Ile) and PKHD1 (NM_133896.4):c.2180A > G (p.Asn727Ser) | Hom      | AR          | 0.00013773 | 0.00020603 | 0.0          | Negative CILK1: This study (VCV001177409) /PKHD1: rs772504090 VUS VUS VUS VUS VUS |
| FAM-51   | ID      | 36           | No            | Renal                    | SALLI (NM_002968.3):c.1759C > A (p.Pro587Thr)                                       | Het      | AD          | 0.00006027 | 0          | N/P        | This study (VCV001177412)        | VUS               | VUS                 |
DIS3L2:c.1810C > T; p.Gln604*) on chromosome 2q37.1 and CMA detected a 10q11 microduplication. The variant in DIS3L2 is associated with Perlman syndrome, which is consistent with prenatal USS findings in this fetus, showing fetal ascites and enlarged kidneys. The 10q microduplication is a well-defined and rare genetic occurrence that may lead to severe central hypotonia, mild ataxia, moderate developmental delay and mild dysmorphic features that would be unlikely to be detected antenatally (Manolakos et al. 2014). 4 heterozygous de novo variants were detected in four fetuses. The de novo heterozygous variant (MYH3:c.2501T > C; p.Phe834Ser) was novel (Figure S2), whilst the other three heterozygous variants in PTPN11, FGFR3, and FOXF1 have been reported previously (Abu-El-Haija et al. 2018; Rump et al. 2006; Stankiewicz et al. 2009; Tartaglia et al. 2004; Toydemir et al. 2006) and were reported to act in an autosomal dominant pattern. The heterozygous variants in FOXF1 and MYH3 were detected in consanguineous families while variants of PTPN11 and FGFR3 were presented in non-consanguineous families (Table 2, Table S2).

Along with the abnormal USS findings, the molecular genetic findings in this study supports the pathogenicity of the following genes in early-onset phenotypes which include fetal structural abnormalities: AGRN (Geremek et al. 2020), MPDZ (Saugier-Veber et al. 2017), IL6ST (Schwerd et al. 2017), TBC1D32 (Alsahan and Alkuraya 2020), CANT1 (Laccone et al. 2011), FZD6 (Shamseldin et al. 2015) and TMEM94 (Al-Hamed et al. 2020). These genes are not commonly known to cause fetal abnormalities and our findings confirm the role of these genes in fetal structural abnormalities.

Our data also support previous findings of an association of biallelic NEK8 mutations with a multisystem ciliopathy phenotype (Al-Hamed et al. 2016; Frank et al. 2013). The fetus FAM-53, with a homozygous nonsense mutation in NEK8 had multisystem features consistent with a ciliopathy syndrome which included hypoplastic lung, Dandy–Walker malformation and enlarged cystic-dysplastic kidneys.

There were several instances where the phenotype we report extends the clinical disease spectrum associated with a known gene alterations. The homozygous pathogenic variant c.830-1G > A in IGFBP7 has previously been associated with autosomal recessive retinal arterial macroaneurysm with supravalvular pulmonic stenosis (Abu-Safieh et al. 2011). In this study, we observed a hypoplastic right heart in the fetus (FAM-55) with a homozygous pathogenic IGFBP7 variant. In FAM-16 where fetal USS indicated bilateral renal agenesis and pericardial effusion, we detected a homozygous loss of function variant of CD151 (NM_001039490.2: c.493C > T; p.Arg165*). Biallelic alterations in CD151 have been associated with nephropathy with pretibial epidermolysis bullosa and deafness (Vahidnezhad et al. 2018), CD151 (NM_006953.4):c.53-1G > A; Hom AR/AD 0.00116036 0 N/P This study (VCV001177413) LP VUS VUS (DIS3L2:c.1810C > T; p.Gln604*) on chromosome 2q37.1 and CMA detected a 10q11 microduplication. The variant in DIS3L2 is associated with Perlman syndrome, which is consistent with prenatal USS findings in this fetus, showing fetal ascites and enlarged kidneys. The 10q microduplication is a well-defined and rare genetic occurrence that may lead to severe central hypotonia, mild ataxia, moderate developmental delay and mild dysmorphic features that would be unlikely to be detected antenatally (Manolakos et al. 2014). 4 heterozygous de novo variants were detected in four fetuses. The de novo heterozygous variant (MYH3:c.2501T > C; p.Phe834Ser) was novel (Figure S2), whilst the other three heterozygous variants in PTPN11, FGFR3, and FOXF1 have been reported previously (Abu-El-Haija et al. 2018; Rump et al. 2006; Stankiewicz et al. 2009; Tartaglia et al. 2004; Toydemir et al. 2006) and were reported to act in an autosomal dominant pattern. The heterozygous variants in FOXF1 and MYH3 were detected in consanguineous families while variants of PTPN11 and FGFR3 were presented in non-consanguineous families (Table 2, Table S2).

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encodes a cell surface glycoprotein that is involved in cellular processes, including cell adhesion and it may regulate integrin trafficking and/or function. Importantly, an 11-year-old sibling of FAM-16 was also homozygous for the **CD151** variant and has a severe nephropathy leading to end-stage kidney disease requiring renal transplantation, implicating this gene in early-onset renal phenotypes that may include congenital anomalies. The fetus FAM-2 with a heterozygous de novo variant in **FOXF1** presented with a severe congenital anomalies of the kidney and urinary tract phenotype (Table 1) rather than the more typical alveolar capillary dysplasia with misalignment of pulmonary veins. Genitourinary findings have, however, been previously associated with heterozygous nonsense and frameshift mutations in **FOXF1** as well as microdeletions in 16q24.1q24.2 affecting the FOX gene cluster (**FOXF1, FOXC2, FOXL1**) (Stankiewicz et al. 2009). Mutations in **BLM** are typically associated with anomalies, 12 fetuses had skeletal anomalies, 2 fetuses had head / neck anomalies, 1 fetus had abdominal anomalies, 1 fetus had cardiac anomalies and 1 fetus had neuromuscular anomalies.

Table 4  Fetal anomaly cases with positive chromosomal microarray analysis (CMA) findings

| Family | Outcome | Maternal age | Consanguinity | CMA results                                      | Exome sequencing results | Fetal structural defects |
|--------|---------|--------------|---------------|-------------------------------------------------|--------------------------|-------------------------|
| FAM-4  | PD      | 43           | No            | Trisomy 18 (pathogenic)                         | Negative                 | Multisystem             |
| FAM-43 | Livebirth | 20          | No            | Distal Trisomy 14q syndrome (pathogenic)        | Negative                 | Multisystem             |
| FAM-45 | PD      | 22           | Yes           | 10q11 microduplication (pathogenic, but unlikely to contribute to antenatal phenotype) | **DIS3L2:**NM_152383.5:c.1810C>T; p.Gln604* (Homozygous) | Renal                   |
| FAM-67 | PD      | 32           | No            | Trisomy 18 (pathogenic)                         | Negative                 | Multisystem             |
| FAM-78 | ID      | 33           | Yes           | Mosaic Trisomy 15 (pathogenic)                  | Negative                 | Multisystem             |
| FAM-79 | ID      | 27           | Yes           | Male fetus with duplication of short arm of X chromosome, Xp11.4 (VUS) | Negative                 | Central nervous         |
| FAM-89 | PD      | 28           | Yes           | Isochromosome 12p (Pallister-Killian syndrome) (pathogenic) | **MASP1:** NM_139125.4:c.1358G>A; p.Gly453Asp (Homozygous, VUS) | Multisystem             |
| FAM-93 | FD      | 25           | Yes           | Turner syndrome (45,X) (pathogenic)             | Negative                 | Multisystem             |
| FAM-105| FD      | 26           | Yes           | Turner syndrome (45,X) (pathogenic)             | Negative                 | Fetal hydrops           |

*FD* fetal death, *PD* perinatal death, *ID* infant death, *VUS* variant of uncertain significance

Fig. 2  Pregnancy outcomes associated with different fetal structural anomalies. 48 fetuses had multisystem anomalies, 21 fetuses had hydrops, 21 fetuses had renal anomalies, 12 fetuses had brain malformations, 12 fetuses had skeletal anomalies, 2 fetuses had head / neck anomalies, 1 fetus had abdominal anomalies, 1 fetus had cardiac anomalies and 1 fetus had neuromuscular anomalies.
Bloom syndrome, a rare genodermatosis disorder characterised by genomic instability and cancer predisposition (Hafsi et al. 2021). In this cohort, one family (FAM-69) with a homozygous frameshift variant (c.175_176delGT; p.Val59Ilefs*4) in BLM was detected (Table 1). A prenatal growth deficiency is a prominent feature of BLM mutations but the skeletal features noted in in FAM-69 (absent left radius, absent left thumb and syndactyly of hand) are atypical. Biallelic mutations in POMT1 result in a dystroglycanopathy (Walker–Warburg syndrome) resulting in congenital muscular dystrophy with brain and eye involvement. Genital hypoplasia has also been reported (Beltrán-Valero de Bernabé et al. 2002). Consistent with this, FAM-112, with a homozygous pathogenic variant in POMT1 (c.118 + 1G > T) had features of brain, heart and kidney involvement.

**Group B: VUS explaining the phenotype**

Twenty-one fetal anomaly cases were identified with VUS likely explaining the phenotype (Table 3). The phenotypes included four fetuses with hydrops fetalis, nine with multisystem anomalies, four with renal and four with skeletal anomalies. A total of 23 VUS were identified (some patients having more than 1 VUS) of which 7 variants were novel. Biallelic VUS variants were detected in 20 cases, while monoallelic variants were detected in PIEZO1, HOXD13 and SALL1 in non-consanguineous families (FAM-15, FAM-39 and FAM-51) with non-immune hydrops fetalis, skeletal dysplasia and multicystic kidneys, respectively. Pathogenic alleles in HOXD13 and SALL1 genes which encode developmentally important transcription factors, cause similar phenotypes to the patients we describe (Ibrahim et al. 2013; Kohlhase et al. 1998).

In FAM-28, a homozygous missense change in CEP290 (c.4151G > A; p.Arg1384His) was noted in a fetus with a multisystem ciliopathy-like phenotype. There is a reported pathogenic variant (c.4150C > T; p.Arg1384Cys) which affects the identical amino acid position (Monies et al. 2017), providing moderate evidence of pathogenicity of the variant p.Arg1384His according to ACMG guidelines (PM5). In addition, we identified a presumed loss of function variant of ACD in FAM-57 (ACD: c.1088-5_1088-3delCTC, homozygous) who demonstrated non-immune hydrops fetalis (NIHF), polyhydramnios, and hypertrophied cardiac muscle. Defects in ACD are typically associated with dyskeratosis congenital (Guo et al. 2014). Although no previous association between NIHF and defects in ACD gene have been reported, we postulate that the severe form of bone marrow failure could explain this phenotype.

We identified a novel homozygous variant in UPK3A gene (UPK3A:NM_006953:exon2:c.53-1G > A) in a fetus that displayed anhydramnios, bilateral echogenic kidneys and cardiomegaly. Compound heterozygous variants in UPK3A have been associated with congenital nephrotic syndrome (Liu et al. 2019) as well as congenital anomalies of the kidney and urogenital tract (Jenkins et al. 2005).

**Group C: negative trio-ES cases**

There were 32 cases (27%) where trio-ES was unable to detect molecular cause of fetal abnormalities with a wide range of structural phenotypes. CMA was performed on 27 of these 32 cases and a likely molecular genetic cause for the underlying phenotype was found in 7 cases (detailed below). For the remaining five cases, CMA testing was not performed due to insufficient DNA. Unsolved cases were a broad mixture of phenotypes including NIHF, cystic kidney disease, multisystem disorders and central nervous system disorders (Fig. 2, Table S1).

**Group D: positive CMA cases**

A total of nine fetal cases had a genetic abnormality identified by CMA, of which eight were likely to be pathogenic and seven were likely to be associated with the presenting antenatal phenotype (Table 4). Seven cases were initially negative for trio-ES and genetic defects were identified using CMA. The case (FAM-89) is from a consanguineous family with polyhydramnios and other multisystem anomalies. Trio-ES detected a homozygous missense VUS in MAS3P1 on chromosome 3 while CMA identified an additional abnormality in chromosome 12 (Isochromosome 12p, detailed below).

FAM-45 is a consanguineous family in whom we identified a homozygous loss of function variant in DIS3L2 (c.1810C > T; p.Gln604*) on chromosome 2 in addition to a CMA abnormality (10q11 microduplication) which is associated with postnatal rather than prenatal phenotypes. The chromosomal abnormalities detected were all are of known pathogenicity. Chromosomal abnormalities included Trisomy 18, Trisomy 14q syndrome, Trisomy 15 and Turner syndrome (Table S1). One case (FAM-79) was exceptional where the CMA finding (a duplication of a region on the short arm of chromosome X (Xp11.4)) is of uncertain significance in a male fetus with an encephalocoele.

**Families with more than one variant**

In a consanguineous population, pathogenic variants in more than one gene can be expected in a family (AIAbdi et al. 2021). These findings are very important for prevention and establishing appropriate prenatal management for future pregnancies. Five fetuses (4.7%) were identified to have more than one potentially disease causing genetic diagnosis.
FAM-45 had both a positive ES result (DIS3L2 nonsense variant, associated with Perlman syndrome that corresponds to the fetal USS findings of echogenic kidneys and moderate ascites) and pathogenic CMA findings (10q11 microduplication) which is a known mechanism in patients with neurological abnormalities (Stankiewicz et al. 2012). FAM-89 had a positive CMA result (Isochrromosome 12p, consistent with the genetic diagnosis of Pallister-Killian syndrome) combined with a VUS in MASP1 which is associated with 3MC syndrome. Both defects could have pathogenic implications for the fetal phenotype (Lawson et al. 2020; Salzano et al. 2018).

FMA-99 had two pathogenic homozygous missense alleles in DYN2CH1 and ARSB; and both would be predicted to contribute to the phenotype of skeletal dysplasia. FAM-9 and FAM-115 both had two homozygous VUS alleles. FAM-9 showed bilateral enlarged kidneys with anhydramnios. Two homozygous VUS, c.105G > A; p.Met35Ile in CILK1 (ICK gene) and c.2180A > G; p.Asn727Ser in PKHD1 were detected. Both missense variants shared the same block of homozygosity in the short (p) arm of chromosome 6 and both could explain the echogenic kidney phenotype. The c.2180A > G; p.Asn727Ser variant in PKHD1 has been reported previously, but according to HGMD and ClinVar with uncertain significance (Wojcik et al. 2019). However, the CILK1 variant is novel and according to ACMG guidelines, should be classified as a VUS.

In FAM-115 fetal USS indicated multisystem abnormalities and both VUS variants TUBA8: c.958C > T; p.Arg320Trp and LZTR1: c.1055A > C; p.Tyr352Ser could participate in the complex fetal phenotype.

**Postnatal fetal phenotypes**

At least 22 cases (18.5%) of our cohort were liveborn and survived the perinatal period, indicating the high disease severity of the cohort. 11 had class A variants, 2 with class B variants and 1 with class-D chromosomal abnormality. Clinical phenotypes in genetically solved cases surviving the perinatal period were re-assessed postnatally (Table 5). Postnatal phenotypes confirmed fetal USS findings and showed good concordance, with patients exhibiting expected phenotypes consistent with their molecular diagnosis.

**Discussion**

In this prenatal cohort of 119 fetuses affected with wide range of fetal structural anomalies, we detected a diagnostic (pathogenic/likely pathogenic) molecular genetic variant in 49% of fetuses. In addition, we identified a potentially clinically relevant variant which was classified by ACMG criteria as a VUS in 21 (18%) fetuses. Previous large studies identified diagnostic genetic variants in 12.5% (Lord et al. 2019) and 10% (Petrovski et al. 2019) of fetuses with fetal structural anomalies. The differences in diagnostic yields reflect the differences in study inclusion criteria where we recruited families with a history of fetal anomalies. Therefore, high diagnostic yield of our study can be attributed to the previously identified family history of fetal anomalies, consanguineous marriages often involving first cousins, and tribal structure of the Saudi Arabian population leading to several likely founder effect or shared pathogenic alleles. What is very striking is the large number of recessive ciliopathy genes identified in our cohort. There is very little overlap with the previously reported UK cohort (Lord et al. 2019), where the leading diagnostic genetic causes of structural fetal anomalies included KMT2S, CHD7 and PTPN11 all inherited as autosomal dominant conditions. The only overlapping genetic causes between this study and the data presented here included FGFR3 (identification of heterozygous variants) and PIEZO1. We saw shared pathogenic alleles within ciliopathy genes NPHP3 and CC2D2A. NPHP3 is now the commonest genetic cause of infantile nephronophthisis (Chaki et al. 2011; Srivastava et al. 2017; Tory et al. 2009) and may also lead to severe antenatal phenotypes including Meckel syndrome (Bergmann et al. 2008). Pathogenic variants in CC2D2A are also associated with a spectrum of ciliopathy phenotypes which include Meckel syndrome (Tallila et al. 2008). All the fetuses with CC2D2A mutations in our cohort had encephaloceles and cystic kidney disease consistent with Meckel syndrome.

The shared allele in CTU2, c.873G > A (pThr291Thr) is apparently synonymous allele, but has been previously shown to cause loss of the wild-type transcript and leads to a splicing defect leading to a frameshift and premature protein truncation (Shaheen et al. 2016b) and has been shown to be a founder mutation in Saudi Arabian patients (Shaheen et al. 2016a). Recognition of disease causing synonymous variants is important as these may be easily an inadvertently discarded by filtering steps in genetic pipelines (Olinger et al. 2021).

Fetal phenotypic evaluations are limited and largely dependent on imaging systems (Fetal USS or fetal MRI). Several factors impact fetal phenotyping such as imaging instrument, maternal body habitus, fetal position, and gestational age (Oates and Taylor 2016). 3D ultrasound is very valuable in the first trimester of pregnancy, but other instruments such as fetal MRI and echocardiography are useful in special cases. We were limited in evaluating postnatal phenotype of all born fetuses due to many factors that include the fact that our centre is a tertiary referral centre and that the majority of pregnancies resulted in termination, fetal death or perinatal death. The high rate for fetal and infant death observed suggests that participants in the study had
| FAM#  | Age (months) | Gender | Mutation                          | Zygosity | CMA  | Fetus phenotype                                                                 | Postnatal fetal phenotype                                                                 |
|-------|--------------|--------|-----------------------------------|----------|------|--------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| FAM-5 | 20           | Female | *ASPM* (NM_001206846.1):c.1959_1962delCAA (p.Asn653Lysfs*14) | Hom/AR   | Negative | Microcephaly                                                                | Microcephaly                                                                            |
| FAM-14| 21           | Male   | *MYH3* (NM_002470.4):c.2501T > C (p.Phe834Ser) | Het, de novo | N/P | Kyphosis, cleft foot/toes, wrist abnormalities, both elbows fixed, flexed joint, abnormal nose | Bilateral club foot, hip flexion, deformity of both knees and deformity of upper limbs |
| FAM-33| 26           | Female | *CPLANE1* (NM_023073.3):c.7988_7989delGA (p.Gly2663Alafs*40) | Hom/AR   | N/P  | Hydrocephalus, Dandy–Walker malformation                        | Developmental delay, hydrocephalus                                                    |
| FAM-38| 23           | Male   | *MPDZ* (NM_001261406.2):c.628C > T (p.Gln210*) | Hom/AR   | N/P  | Absent corpus callosum                                           | Congenital hydrocephalus                                                                  |
| FAM-40| 22           | Male   | *TBX18* (NM_001080508.3):c.692_693insT (p.Glu233Glyfs*19) | Het/AD   | Negative | Bilateral hydronephrosis                                         | Bilateral hydronephrosis                                                                  |
| FAM-55| 21           | Male   | *IGFBP7* (NM_001553.3):c.830-1G > A | Hom/AR   | Negative | Hypoplastic right heart                                      | Right ventricular hypoplasia                                                           |
| FAM-69| 23           | Female | *BLM* (NM_000057.4):c.175_176delGT (p.Val59Ilefs*4) | Hom/AR   | Negative | Absent left radius, absence of left thumb, syndactyly left hand | Oesophageal atresia with transoesophageal fistula repaired, imperforate anus, absent thumb, absent radius |
| FAM-88| 18           | Male   | *FZD6* (NM_003506.4):c.869A > G (p.Tyr290Cys) & | Hom/AR   | N/P  | Non-immune hydrops fetalis                                     | Asymptomatic                                                                             |
| FAM-94| 14           | Male   | *THSD1* (NM_018676.4):c.617G > A (p.Cys206Tyr) | Hom/AR   | N/P  | Non-immune hydrops fetalis                                     | Asymptomatic                                                                             |
| FAM-15| 24           | Female | *PIEZO1* (NM_001142864.4):c.1792G > A (p.Val598Met) | Het/AR, AD | N/P | Non-immune hydrops fetalis                                     | Respiratory difficulties                                                                 |
| FAM-113| 21          | Male   | *TTCP* (NM_001287872.1):c.107G > A (p.Cys36Tyr) | Hom/AR   | N/P  | Polydactyly, Echogenic kidney, Oligohydramnios                      | Bardet-Biedl syndrome with multiple congenital anomalies: congenital heart disease, Bilateral polycystic kidney with chronic kidney disease stage III, ambiguous genitalia with under virilized male |
unusually severe fetal anomalies that may have been more likely to have a genetic cause.

Providing prenatal exome on a clinical basis is a challenging scenario; such services require analysis by a multidisciplinary team of perinatal practitioners and laboratory specialists. Another challenge in prenatal exome analysis is that only some genetic disorders give rise to a prenatal phenotype, and the fact that many genes critical to human development have yet to be identified (Vora and Hui 2018). In addition, the difficulty of detailed phenotyping, limited timeframe and genetic counselling of variants of unknown significance (VUS) may add extra challenges to prenatal exome diagnosis (Yates et al. 2017). Therefore, to overcome such challenges, careful selection criteria should be considered for cases of prenatal ES (Lord et al. 2019). These criteria should include a detailed phenotype of the fetus, considering ES as a phenotype-driven test, family medical history, trio-ES over solo-ES, rapid turnaround time, pre and post-test counselling and clinical utility of a prenatal diagnosis.

In prenatal settings where ES is available, we urge the use of trio instead of solo to improve variant interpretation. De novo and compound heterozygous variants in addition to potential VUS require familial segregation and confirmation, which can be ascertained by trio-ES. Although the average turnaround time of ES testing in this study was 31 days, we believe that up to 15 days turnaround time would be both desirable and achievable. The turnaround time of prenatal ES has to be rapid to provide proper counselling, management of the current pregnancy, and options for future reproduction. The information provided by genetic testing to people has received increased attention. In prenatal settings, a combination of USS and molecular genetics findings has facilitated management of pregnancy. Clinical utility of genetic findings in this cohort has translated to families in the following ways: first, by providing preventive measures and post-test counselling and clinical utility of a prenatal diagnosis.

The current study supports previous findings that autosomal recessive pathogenic variants play a more common role in the Saudi Arabian consanguineous population as compared to outbred populations where de novo variants are the most common cause of fetal malformations (Li et al. 2017). The association between genetic inbreeding in the Saudi population and disease mortality and morbidity has previously been noted (Al Husain and Al Bunyan 1997) and its effect on reproductive health including postnatal mortality and rates of congenital malformations has also been noted (Tadmouri et al. 2009). In this cohort, de novo mutations account for around 4% of cases with fetal abnormalities. Interestingly, no X-linked genes were encountered in this cohort.

Most ciliopathy genes detected in group A (AHI1, CC2D2A, NEK8, TCTN2, TMEM67, TMEM237, and TMEM231) showed multisystem manifestation leading to fetal or perinatal death. In our cohort, most fetuses with loss of function variants in cilia genes showed echogenic enlarged kidneys, encephalocele, skeletal dysplasia and polydactyly phenotypes as reported before, and with anhydramnios or oligohydramnios being common observations in such fetuses (Al-Hamed et al. 2016). The prenatal recognition of a ciliopathy syndrome affecting a fetus is often amenable due to subtle imaging findings in kidneys, brain and extremities (Braun and Hildebrandt 2017).

Although CMA was performed on 67 cases, positive results explaining the prenatal phenotype were found in 8 cases only (12% of tested cases), which support the prioritisation of performing trio-ES as the initial genetic analysis in consanguineous populations. In terms of diagnostics, CMA and trio-ES should be seen as complementary modalities and negative findings form one approach should prompt investigation with the other (Findley and Northrup 2021). An advantage of CMA is its prompt turn around time (typically 7 days) and its ability to detect genome wide microdeletions and microduplications. Diagnostic yields in cohorts of patients with prenatally diagnosed congenital heart disease are around 7% (Jansen et al. 2015). Other studies report a CMA detection of clinically significant CNVs in 10–15% of cases in fetuses with multiple major anomalies (Donnelly et al. 2014; Lee et al. 2012), in keeping with our own CMA findings. We recognise that the number of disease causing CNVs in this cohort may be potentially higher than we report, as many cases were not investigated using CMA. Trio-ES accompanied by CNV analysis is widely used in many genetic laboratories for prenatal diagnosis. A NGS-based CNV detection can be performed in parallel with exome sequence analysis and using this approach it is possible to detect a wide range of CNV sizes (Wang et al. 2018). The drawback of this approach is the limitation of short-read NGS for CNV calling in addition to other factors that can impact the ability to detect CNVs such as sample quality, uniformity and high sequencing coverage.

Despite ES and its combination with CMA where possible, a significant proportion of cases (32 out of 119, 27%) remained genetically unsolved. These cases could be further investigated by conventional karyotyping or whole genome sequencing, which is becoming more widely available in mainstream genetic diagnostics (Turro et al. 2020).

In conclusion, the revolution of NGS has increased our ability to improve feto-maternal medicine diagnostics. Trio-ES detected molecular defects in around half of our cohort, families with fetal structural anatomic anomalies
and a family medical history of congenital anomalies. There was a propensity of multisystem phenotypes with genetic diagnoses in ciliopathy associated diseases. A trio-ES approach to antenatal structural anomalies provides an accurate diagnosis and can be used to predict recurrence rates and to make possible planning for future pregnancies.

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Author contributions MHA-H, JAS and FI conceived the study and participated in its design, coordination, drafted and revised the manuscript. WK, KK, MT, MAN, NS and MM participated in the clinical diagnosis of the cases. MHA-H, RR, MA, ZR, ND, KR, JAS and FH participated in exome analysis. AO, WA, AS, RA, LQ, HB, AA, NM and AK carried out all technical aspects of molecular diagnosis. GM, WM, SD, SA, BS, HB, and MA participated in the collection of data of enrolled cases. JAS revised the manuscript. All authors read and approved the final manuscript.

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Data availability All data generated during this study are included in this published article and its supplementary information files.

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

Ethical approval The study was approved by the Research Advisory Council at King Faisal Specialist Hospital and Research Centre (KFSH&RC), Riyadh, Saudi Arabia (RAC# 2211035).

Consent to participate Informed consent was obtained from all individual participants included in the study.

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Code availability Not applicable.

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