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Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography: the PAGE study

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Abstract

Background: Fetal structural anomalies (FSA) detected by ultrasonography have a range of genetic aetiologies including chromosomal aneuploidy, copy number variations (CNVs) detectable by chromosomal microarrays (CMA) and pathogenic sequence variants in developmental genes. Investigations to detect aneuploidy and CNVs are routinely used for the investigation of FSA but information on the clinical utility of genome-wide next generation sequencing in the prenatal setting is limited.

Methods: Whole exome sequencing (WES) was performed, after exclusion of aneuploidy and large CNVs, on a prospective cohort of 392 fetuses with FSA and in 772 parental samples (380 case-parental trios and 12 case-parent dyads). Sequencing was interpreted based on a targeted developmental disorder virtual gene panel comprising 1536 genes. Genetic results relevant to the phenotype were validated and reported after the pregnancy was completed.

Findings: After bioinformatic filtering and prioritisation, 201 genetic variants representing 155 potential diagnoses were selected as "potential pathogenic variants" and reviewed by a multidisciplinary clinical review panel (CRP). A diagnostic genetic abnormality was identified in 34/392 cases (8·7%;95%CI:6·1-11·9%) and a further 12(3·1%) had a variant of uncertain significance (VUS) with potential clinical utility. Variant detection enabled syndromic and non-syndromic cases of fetal anomaly to be distinguished. Diagnostic variants were more common in fetuses with multisystem anomalies (more than one FSA) (16·9%(13/77)), cardiac anomalies (18·4%,(9/49)), skeletal anomalies (15·4%,(6/39)) and hydrops fetalis (10·5%,(2/19)) and less frequent in fetuses with isolated increased nuchal translucency (>4·0 mm) in the first trimester (1·1%, (1/88)).

Interpretation: WES facilitates genetic diagnosis in FSAs enabling more accurate prediction of fetal prognosis and risk of recurrence in future pregnancies. However the overall detection rate in a prospectively ascertained, unselected cohort is lower than that suggested by previous smaller-scale studies of highly selected phenotypes.

Research in context

Evidence before this study:

Genome wide sequencing strategies such as whole exome (WES) or whole genome sequencing greatly increase the diagnostic yield over postnatal chromosomal microarray analysis (CMA) in children with developmental disorders. Previous, relatively small studies have suggested that the addition of WES to CMA for the investigation of fetal structural anomalies diagnosed by prenatal ultrasound could enable a genetic diagnosis to be made in the majority of cases with a FSA. However most such studies have been retrospective and include a small number (<30 cases) of highly selected subgroups of FSA; they provide limited information regarding the likely diagnostic yield in clinical practice of the application of WES for FSAs.

Added value of this study

In a prospectively ascertained cohort of FSA detected using prenatal ultrasound (in which aneuploidy and large copy number variants had been excluded) the overall diagnostic yield was 8·7% after bioinformatic filtering of the WES and assessment by a multidisciplinary clinical review panel to reach consensus as to whether a WES finding was pathologic and causative. These data confirm that the addition of prenatal WES to CMA would increase the detection of genetic causes of FSA and provide important information on prognosis and future recurrence risks. The WES diagnostic yield varied with type of FSA suggesting that WES might be targeted to the phenotypic classes with highest clinical utility. The Prenatal Assessment of Genomes and Exomes study (PAGE) study also highlighted differences between the application of WES in the prenatal and postnatal period that will facilitate the translation of WES for FSA into clinical practice.

Introduction

Approximately 3% of pregnancies will have a sonographically detected fetal structural anomaly (FSA) which may range from a single minor defect to severe multisystem anomalies incompatible with life.(1) Genetic investigations play an important part in the evaluation and clinical triage of FSAs. For many years prenatal 'conventional' cytogenetic analysis was the first line investigation but more recently chromosomal microarray analysis (CMA) has been widely adopted to detect submicroscopic pathogenic copy number variations (CNVs).(2,3) Adding CMA testing to karyotyping increases the detection rate of chromosomal abnormalities by 3-5% .(2–4) FSAs may be associated with all types of genetic variation including aneuploidy, uniparental disomy (UPD), CNVs and intragenic mutations. There is increasing interest in the genome-wide sequencing strategies to investigate prenatally detected congenital abnormalities. Though prenatal whole genome sequencing (WGS) has been described,(5) whole exome sequencing (WES) or targeted gene panels has received more interest because of the lower cost, limited amounts of fetal DNA available, requirement for rapid turnaround and greater sequencing depth. (6–13). Previously we performed WES in 29 fetal-parental trios with an ultrasound detected FSA and identified a causative diagnosis in 10% of cases.(7) WES for the investigation of a range of FSA has been associated with a "diagnostic rate" of >50% but most previous studies have been small (≤30) and/or confined to highly selected subgroups (see supplementary Table 1).(14,15)

To define the potential utility of genome-wide sequencing strategies in prenatal diagnosis of FSA a large-scale sequencing project, the Prenatal Assessment of Genomes and Exomes study (PAGE; http://www.pageuk.org) was initiated. In this paper we report on the experience to date of the clinical review panel (CRP) in considering the results of WES from 392 probands (and parental samples) presenting with a wide range of FSAs and highlight the issues encountered to date that have implications for the translation of prenatal WES into clinical practice.

Methods

Subject recruitment and sample processing: Two groups (the Fetal Medicine Unit, Birmingham Women's & Children's Foundation Trust/University of Birmingham and North East Thames Regional Genetics Laboratory, Great Ormond Street NHS Foundation Trust, London) coordinated patient recruitment through a network of 30 fetal medicine units across England and Scotland (Supplementary Figure 1). Following detection of a FSA at a routine ultrasound scan (USS) parents opting for invasive testing were offered the opportunity to participate in PAGE. All participants gave written informed consent and the study was approved by Research and Development offices at each of the participating institutions and by relevant Research Ethics Committees (NRES Committees:West Midlands-South Birmingham and London-Harrow). Parental blood samples were collected for DNA extraction and fetal DNA was obtained from chorionic villi, amniotic fluid or fetal blood remaining after routine investigations (cases were excluded when these revealed aneuploidy/CNV explaining the phenotype). DNA was extracted at the two coordinating centres and shipped to the Wellcome Sanger Institute for WES. Participants were informed that the results of PAGE genetic analyses would not be available during the current pregnancy and only results relevant to the USS-detected FSA would be reported back to parents. To ensure that a range of phenotypes were included, prior to study it was agreed that the number of recruits with a specific phenotype would be capped at 20% of the total.

Exome sequencing, variant detection and annotation: After WES, candidate pathogenic variants in a modified developmental disorder-associated gene list (see supplementary methods and supplementary Table 2)(16) were assessed and rare, protein altering variants where the inheritance pattern of the variant matches that of the gene were selected for clinical review. Sequencing data is available from the European Genome-phenome Archive (https://www.ebi.ac.uk/ega/).

Data interpretation and variant classification: Candidate pathogenic variants were reviewed and classified by a clinical review panel (CRP) comprising at least six participants (including at least a clinical geneticist, a fetal medicine specialist, two clinical scientists and a bioinformatician) from the study team and, usually, a clinical geneticist and clinical laboratory scientist from the recruitment centre. Initially CRP meetings were face-to-face but subsequently distant participants joined by Webex/teleconferencing. All participants reviewed anonymised variant annotation data and clinical findings through the Sapientia (Congenica, UK) software. The CRP reached a consensus decision regarding variant classification (pathogenic/likely pathogenic/variant of uncertain significance/likely benign/benign) and relationship to prenatal sonographic phenotype. Causative pathogenic/ likely pathogenic variants were confirmed by Sanger sequencing in a NHS accredited laboratory. Research reports were issued to the local clinical geneticist/fetal medicine

specialist. Other types of variants were not validated or reported except in the case of a VUS that the CRP considered to have potential clinical utility.

Statistical analysis: Variants were annotated with the probability of the relevant gene being loss of function intolerant (pLI).(17) pLI scores were compared using the Mann-Whitney U test. The diagnostic rate between different phenotypic classes was compared using Fisher's Exact test. All statistical analyses were conducted using R (version 3·1·3).

Results

Ultrasound (USS) anomalies

The 392 probands (164 female, 228 male) were categorised into 11 phenotypic classes according to the site of the USS-detected anomalies (see supplementary Table 3): abdominal(n=29), brain(n=38), cardiac(n=49), thoracic(n=12), facial(n=21), fetal hydrops(n=19), increased nuchal translucency(>4·0mm)(n=88), renal(n=12), skeletal(n=39), spinal(n=8) and complex/multisystem anomalies(≥2 FSAs detected) (n=77) (Supplementary Table 5).

Variant assessments

201 genetic variants, representing 155 potential diagnoses in 126 individuals were reviewed by the CRP (see Supplementary Table 4 and supplementary results). The mean number of potential diagnoses per proband considered by the CRP was 0·40 (range 0-4). For complete trios (n=380), this value was 0·37 (range 0-4), while for dyads (n=12) this value was 1·08 (range 0-4).

The CRP assessed variants in 126 different developmental disorder genes with a median of one case per gene, however, 14 genes were assessed in multiple cases (*KMT2D* in 4 cases, *FLNA* in 3, and *NRAS, CHD7, COL1A1, ZC4H2, ATP13A2, COL6A3, CHRNG, CDH23, HYDIN, BRCA1, RYR1 and COL18A1* in 2 each) (Figure 1A and 1B).

Of the 155 potential diagnoses (comprising 187 SNVs and indels, 12 CNVs and 2 UPDs) in 126 probands (32·1% of all cases analysed) considered, 34/392 probands were classed as harbouring likely pathogenic or pathogenic (LP/P) variant(s) relevant to the fetal anomaly (see Table 1), giving a diagnostic rate of 8·7% (95% CI 6·1 to 11·9%). Twenty (59%) of the cases with a diagnostic variant had a *de novo* mutation (11 truncating, 8 missense and 1 in-frame insertion), 13 cases had inherited the relevant mutation(s) (11 autosomal recessively inherited disorders and two dominantly inherited disorders) and the final diagnosis had a chromosome 15 UPD. Factors associated with an increased likelihood of a diagnostic variant were protein truncating variants in monoallelic genes (diagnostic in 11/13 (84·6%) cases compared to 26·7% (8/30) for *de novo* missense variants in monoallelic genes, and *de novo* variants in a monoallelic disease gene (diagnostic in 20/45 cases (44·4%)), compared to other potential diagnoses ((CNVs, inherited variants, UPDs) with 12·7% diagnostic rate (14/110)). Genes harbouring diagnostic SNVs and indels had a higher median pLI than non-diagnostic variants (diagnostic=0·91, non-diagnostic=0·74) although this difference did not reach statistical significance (P=0·1652).

Three of the 14 genes were considered by the CRP in multiple cases harboured diagnostic variants in two or more cases (*KMT2D* (n=3), *COL1A1* (n=2) and *CHRNG* (n=2)) (see Figure 1B). Cases with diagnostic *KMT2D* mutations (all *de novo* truncating) were present in multiple phenotypic classes (one multisystem, one cardiac and one hydrops).

To our knowledge, this PAGE cohort includes the first instances of the prenatal identification of mutations in *ANKRD11*, *ARCN1, CCDC103, COQ9, DNAH11, GATA4, MYCN, NR2F2, TAB2, TUBB* and *ZC4H2* (Table 2). Further details of selected cases are provided in Case Reports #1 and #3 (see supplementary material).

The diagnostic rate varied between the phenotypic groups (see figure 1C and supplementary Table 5). The highest diagnostic rates were in those with cardiac anomalies (18·4% (9/49), p=0·02 compared to all other phenotypes), multisystem fetal anomalies (16·9% (13/77), p = 0·01), skeletal anomalies (15·4% (6/39)) and hydrops fetalis (10·5%, (2/19)). Diagnostic rates in all other groups were <4% and the diagnostic rate in those with isolated increased nuchal translucency (>4·0mm) was significantly lower than in all the other phenotypes combined (1/88, p= 0·002).

Consequences of a positive diagnosis were a low recurrence risk in 21 cases (20 with *de novo* mutations in monoallelic disease genes and one UPD) and high recurrence risk in 13 cases with inherited variants (11 autosomal recessive and 2 dominant disorders). None of the diagnostic variants would currently, if detected in an ongoing pregnancy, have led to *in utero* fetal therapy. However, "in pregnancy" diagnosis could influence decisions about pregnancy outcome, e.g. in cardiac cases diagnostic variants were found in genes associated with postnatal extracardiac manifestations, including, learning disability (e.g. *KMT2D, ANKDR11*, *SOS1*, *CCDC103*). In pregnancy diagnosis might have enabled (in cases in which the parents choose to continue the pregnancy) better postnatal management (e.g. monitoring for neonatal hypoglycaemia in a Beckwith-Wiedemann syndrome fetus with exomphalos and a *CDKN1C* mutation) and it has been suggested that CoQ10 treatment might be helpful in COQ9-deficient children.(18)

Clinically relevant non-diagnostic findings

In addition to 34 WES-diagnosed cases, a further 12 cases had variants that were not considered diagnostic but merited further clinical and/or molecular investigations and were reported as "clinically-relevant-VUS" (see supplementary Table 6). These included a fetus with micrognathia, radial aplasia, ulnar and fibular hypoplasia, tibial and femoral shortening and an abnormal lumbar spine (probable hemivertebrae) on prenatal USS with compound heterozygous nonsense (c.2269C>T, p.Gln757Ter) and missense variants

(c.1580C>G, p.Thr527Arg) in *RECQL4.* Though the nonsense variant was considered pathogenic the missense substitution was classified as a VUS. Biallelic pathogenic variants in *RECQL4* are associated with radial aplasia/hypoplasia syndromes and further follow up was considered to be indicated. In another case (PP0722) a *de novo* missense *KMT2D* variant was detected in a fetus with a 6·7mm nuchal translucency in the 1st trimester (see Text Box 1). In a further case an apparently pathogenic *de novo* nonsense variant in *CHD7* was detected (PP1720) but the relevance to the prenatally detected brain ventriculomegaly was uncertain in the absence of any other features of CHARGE syndrome.

Adding the 12 cases with potentially clinically relevant variants to the 34 diagnostic cases gave a total of 46 (11·7%, 95%CI 8·7-15·3%) in which WES provided a clinically relevant result.

Ethical aspects of prenatal whole exome sequencing

A detailed study of the ethical issues surrounding WES in prenatal diagnosis is in progress and has been reported separately.(19) Issues noted in the cases reported here included (a).identification of potentially pathogenic variants that might carry a recurrence risk but were apparently unrelated to the detected FSA and so, in accordance, with the ethical approval not reported, (b).identification of a VUS in a relevant candidate gene. Postnatally this might be handled by more detailed phenotyping and/or periodic review but in the prenatal setting phenotypic information is generally more limited and delaying a diagnostic decision is usually not an option. This is particularly difficult if the FSA might have a benign prognosis (e.g. talipes equinovarus anomaly); (c). detection of a heterozygous pathogenic variant in a relevant developmental gene associated with autosomal recessive disease, (d) detection of a pathogenic variant predicting late-onset adult disease (e.g. increased breast cancer risk in a mother found to be a carrier of a Fanconi anaemia gene variant) that is not relevant to the fetal abnormality.

Pregnancy outcome data

Pregnancy outcome was available in 316/392 cases (80·6%). In 88 the parents opted for termination, 7 ended in miscarriage, there were 13 stillbirths, 9 neonatal deaths, and 199 were live born babies. Of the 34 cases with a WES diagnosis, post mortem or postnatal follow up was available in 31 cases (91·2%) and was consistent with the molecular diagnosis. To our knowledge a postnatal genetic diagnosis hasn't been made in any of the cases reviewed by the CRP and designated as having a variant without clinical relevance. However a diagnosis of Noonan syndrome was made in a child with a maternally inherited pathogenic *RIT1* variant (NM_006912·5(RIT1):c.284G>C (p.Gly95Ala)). This variant was not considered by the CRP as it did not pass bioinformatic filtering because the mother was designated as normal phenotype. The diagnostic yield for P/LP variants was significantly higher in cases with fetal demise (19/117) (miscarriage

(0/7), termination of pregnancy (15/88), stillbirth (2/13), neonatal death (2/9) than in those cases resulting in a live birth (12/199): 16·2% versus 6·0% (P=0·005).

Discussion

In this large prospectively collected series of 392 probands with FSA detected by prenatal USS in a fetal medicine centre we identified a relevant diagnostic genetic variant in a developmental disorder gene in 8·7% (34/392). In addition, in a further 12 cases a genetic variant considered to be of potential clinical utility was identified and reported and so overall 11·7% (46/392) of cases had a diagnostic or potentially clinically relevant variant. Previously published WES studies of FSA cohorts have reported diagnostic rates >50% [14] but most studies comprise small numbers of highly selected cases and the designation of variants as diagnostic was less stringent. The largest previous study reported in full performed WES on 84 deceased fetuses with diagnostic results in 20%. [11] Our lower diagnostic yield reflects differences in ascertainment strategies as we prospectively recruited all suitable cases and then undertook WES without genetic review (after aneuploidy and large CNVs were excluded) whereas Yates et al [11] studied deceased fetuses after termination of pregnancy or spontaneous fetal death. Around half of our cohort were live born (and had a lower diagnostic yield than cases with fetal demise) and the diagnostic yield in our cases associated with fetal demise was close to that reported by Yates et al.[11] The results of genome-wide sequencing in unselected idiopathic FSA are especially relevant when considering the potential for translating WES into clinical practice. We note that in a meeting abstract Wapner et al reported a causal pathogenic variant in 7·5% of sequential cases of FSA (a further 5·5% had a karyotype or CMA anomaly). [20]

WES diagnostic yields in FSA are significantly less than that reported (up to 43%) in children with developmental disorders despite a similar sequencing and interpretation strategy.[16,21] This reflects differences in ascertainment as the postnatal cohort was selected after assessment by clinical genetics specialists (and therefore enriched for likely monogenic disorders), whereas the PAGE cohort includes manifestations such as isolated large nuchal translucency, isolated talipes and neural tube defects all of which are known to have a low association with a monogenic aetiology. Additionally, greater imprecision in prenatal versus postnatal phenotyping may also contribute (e.g. postnatally expert dysmorphology developmental assessment is more readily available and can facilitate variant interpretation and increase diagnostic yield).

We found higher diagnostic yields with cardiac anomalies, complex/multisystem anomalies, skeletal anomalies, and, to a lesser extent, hydrops fetalis but detection rates were <4% with other types of anomalies. *KMT2D* likely pathogenic variants were the most frequent diagnostic finding and were associated with a variety of phenotypes including multisystem anomalies (PP1843), isolated complex cardiac defect (PP1864) and fetal hydrops and cystic hygroma (PP1573). *KMT2D* mutations cause Kabuki syndrome which is characterised by developmental delay, epilepsy, cardiac, genitourinary and

musculoskeletal anomalies and distinctive facial features.[22–25] Although the presentation of Kabuki syndrome with fetal hydrops has been reported previously, the distinctive facial dysmorphology is less apparent in infancy than older children and diagnosis may be difficult in the early postnatal period.[25,26]

To date prenatal WES studies have implicated a large number of developmental genes but 19 genes (including *KMT2D*) have been reported in multiple studies (Supplementary Table 1). To our knowledge, we report the first prenatally diagnosed cases of mutations in genes associated with isolated (*NR2F2, TAB2*) and syndromic congenital heart disease (primary ciliary dyskinesia/*CCDC103;* KBG syndrome/*ANKRD11*)) (see Table 2), so in cases of prenatally diagnosed cardiac defect, WES can provide important information on the non-cardiac prognosis. Other genes for which prenatally diagnosed cases had not apparently been reported included *ARCN1, COQ9, MYCN, TUBB* and *ZC4H2* (see Text Box #1 and #3 and Table 2).

To maximise diagnostic yield we analysed variants in the 1421 developmental disorder genes included in the DDG2P panel (www.ebi.ac.uk/gene2phenotype) (downloaded 25/4/17), plus 116 genes identified as being associated with a prenatal presentation from the literature (Supplementary Table 2). This resulted in about a third of trios having at least one potential diagnostic finding. When implementing WES into clinical practice, there is a strong argument for curating the DDG2P list to remove genes not associated with FSA (e.g. causes of non-syndromic learning disability) and using smaller phenotype-specific virtual gene panels in order to reduce the number of VUS that are irrelevant to the FSA. Careful thought is also required as to which FSA cases should be investigated by WES/WGS. For non-specific FSAs which can be associated with a normal outcome (e.g. talipes equinovarous, resolving ventriculomegaly or an isolated small nuchal translucency) not only might the diagnostic rate of WES be small but finding a VUS could be problematic in the absence of such a non-specific phenotype.

The PAGE study protocol does not, currently involve real time "in pregnancy" variant interpretation and reporting but decisions on variant classification/validation/reporting were based on information that would have been available for an ongoing pregnancy and provided insights into the challenges for translating prenatal WES into clinical practice. For rapid and efficient variant prioritisation fetal-parental trio analysis is clearly preferable to fetus-only WES as trios enables rapid identification of *de novo* variants in monoallelic developmental disorder genes and defines whether heterozygous pathogenic variants in biallelic genes are *in cis* or *in trans.* Optimal variant interpretation requires a multidisciplinary approach and detailed clinical information, including the prenatal USS and family history should be available to the CRP (the importance of family history was illustrated by the finding of a familial *MYCN* variant (Text Box 3) and a case of familial Noonan syndrome that was not referred to the CRP). Performing WES in a large-

scale/central sequencing facility (as in PAGE) provides consistency of methodology and bioinformatic pipelines but makes face-to-face CRP meetings more difficult. We found that a virtual CRP enabled all relevant specialists (including the clinical staff who will communicate the results to the parents) to discuss the cases and reach a consensus. Currently, PAGE results are only communicated to women and their partners after the pregnancy is complete but our experience has highlighted some of the ethical issues that would be encountered in clinical practice (see above). Many potential ethical issues (e.g. incidental findings, non-paternity etc.) are not unique to prenatal WES/WGS and can be managed according to standard policies but it is essential that parents receive clear information regarding which findings might (and might not) be reported.[19] The identification and addressing of practical ethical issues within the PAGE project illustrates the value of embedded ethics research and also highlights the importance of ethics support and training for health professionals for the successful introduction of the PAGE study protocols into clinical practice. It also highlights the need for guidelines for clinical implementation as have been published recently by the International Society of Prenatal Diagnosis. [27]

It is critical that the clinical and molecular data from prenatal WES is added to a confidential database and shared widely in an anonymous manner (e.g. in DECIPHER for PAGE variants) so that variant interpretations can be improved and the prenatal genotype-phenotype associations defined. Such databases should be international to facilitate rapid accumulation of data.

In addition to informing the current pregnancy management, WES/WGS can provide insight into recurrence risks and enable future prenatal genetic testing or preimplantation diagnosis (40% of PAGE cases with a diagnostic finding were associated with a high recurrence risk). Though many WES diagnoses were caused by low recurrence risk *de novo* mutations, such cases can be associated with a small increased recurrence risk from gonadal mosaicism and non-invasive prenatal diagnosis using analysis of circulating cell free fetal DNA in maternal plasma can be offered at an early stage of future pregnancies. [28]

In conclusion, we report the largest study to date of WES for unselected FSA. Though the diagnostic yield is lower than that suggested by smaller, mostly retrospective, studies on selected groups, adding WES to CMA substantially increases the number of USS-detected FSA cases in which a genetic diagnosis can be made and improves the prognostic information that can be provided. This can have important implications for prognosis and recurrence risks. It seems inevitable that WES/WGS will be applied increasingly for investigating FSAs but large scale studies with careful curation of clinical and genomic data will greatly facilitate the challenges of incorporating WES/WGS into prenatal diagnostics.

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Author Contributions:

The study was conceived and designed by MEH, LSC, MDK, ERM and DJMcM. Whole exome data was analysed by JL, RYE and molecular genetic studies were performed by EP and RK. Variant interpretation and assessment was undertaken by core members of the clinical review panel (JL, DJMcM, RYE, GR, SH, EQJ, DW, LJ, RS, MKD, LSC, MEH and ERM.) and local centre representatives (SB, GJC, IRB, KEC, LC, RE, AH, STH, TH, RAL, RNE, KP, OQ, SR, ER, DT, MJT, PCV, AW, DGW, PW, HW). ERM and DJMcM wrote the first draft, JL produced the figures and undertook statistical analysis and all authors critically reviewed iterations of the manuscript and approved the final draft for submission.

Tables

Table 1: Details of Diagnostic Variants classified by the PAGE Clinical Review Panel (see text for details) (hom=homozygous)

PP_ID	Phenotype	Gene	VEP	Inheritance	Zygosity
PP0602	Large NT>4.0	chr15 UPD	chr15 UPD	UPD	NA
PP0258	Abdominal	MYCN	missense	inherited from affected parent	Heterozygous
PP1753	Multisystem	CDKN1C	frameshift_variant	inherited	Heterozygous
PP0555	Multisystem	EVC2	frameshift_variant (hom)	inherited	Homozygous
PP0318	Skeletal	CHRNG	frameshift_variant (hom)	inherited	Homozygous
PP0342	Multisystem	CHRNG	frameshift_variant x 2	inherited	Heterozygous
PP0390	Cardiac	CCDC103	missense_variant (hom)	inherited	Homozygous
PP1627	Multisystem	PIEZO1	missense_variant x 3	inherited	Heterozygous
PP1780	Multisystem	TCTN2	splice_acceptor	inherited	Homozygous
PP0659	Multisystem	RAPSN	splice_donor_variant	inherited	Homozygous
PP0513	Cardiac	DNAH11	stop gained	inherited	Homozygous
PP1795	Multisystem	COQ9	stop_gained (hom)	inherited	Homozygous
PP2000	Multisystem	RYR1	stop_gained + frameshift_variant	inherited	Heterozygous
PP0087	Skeletal	DYNC2H1	stop_gained x 2	inherited	Heterozygous
PP1711	Facial	SF3B4	frameshift	de novo	Heterozygous
PP1750	Cardiac	ANKRD11	frameshift_variant	de novo	Heterozygous
PP0333	Cardiac	GATA4	frameshift_variant	de novo	Heterozygous
PP2033	Cardiac	CHD7	frameshift_variant	de novo	Heterozygous
PP1726	Cardiac	TAB2	frameshift_variant	de novo	Heterozygous
PP1573	Hydrops	KMT2D	frameshift_variant	de novo	Heterozygous
PP0204	Skeletal	ZC4H2	frameshift_variant	de novo	Heterozygous
PP2009	Skeletal	ARCN1	frameshift_variant	de novo	Heterozygous
PP1462	Multisystem	BRAF	missense	de novo	Heterozygous
PP1579	Brain	TUBB	missense_variant	de novo	Heterozygous
PP0184	Cardiac	NR2F2	missense_variant	de novo	Heterozygous
PP0174	Multisystem	NRAS	missense_variant	de novo	Heterozygous
PP1408	Multisystem	SOX9	missense_variant	de novo	Heterozygous
PP2015	Multisystem	FLNB	missense_variant	de novo	Heterozygous
PP0792	Skeletal	COL1A1	missense_variant	de novo	Heterozygous
PP1934	Skeletal	COL1A1	missense_variant	de novo	Heterozygous
PP1892	Cardiac	SOS1	protein_altering_variant	de novo	Heterozygous
PP1864	Cardiac	KMT2D	splice_donor_variant	de novo	Heterozygous
PP2039	Hydrops	NIPBL	stop_gained	de novo	Heterozygous
PP1843	Multisystem	KMT2D	stop_gained	de novo	Heterozygous

Table 2: PAGE cases with diagnostic variants in genes without previous prenatal phenotype descriptions
AVSD= atrioventricular septal defect; ASD= atrial septal defect

Gene	Postnatal phenotype	Reference [number of postnatal cases in cited references]	Prenatal USS PAGE findings (PAGE ID)
ANKRD11	*KBG syndrome, Coffin-Siris-like syndrome*: intellectual disability, macrodontia, facial dysmorphisms, skeletal anomalies, short stature, hearing loss, recurrent middle palatal abnormalities	[89][(29–31)]	Atrioventricular canal defect (PP1750)
ARCN1	Severe micrognathia, microcephaly, short stature with rhizomelic shortening, joint laxity, and mild developmental delay and, in some cases (each one case): cardiac defect, cleft palate	[n=4][(32)]	Absent/Hypoplastic radius, ulnar hyoplasia, fubular hypoplasia, short tibia, femur and humerus (PP2009)
CCDC103	*Primary ciliary dyskinesia* (upper and lower airway infections, sinusitis, bronchiectasis, dextrocardia/ *situs inversus,* AVSD, immotile sperm).	[14][(33–36)]	Complex univentricular heart, double outlet right ventricle (DORV), transposition great arteries (TGA), pulmonary stensosis, likely right atrial isomerism (PP0390)
COQ9	Neonatal encephalopathy with lactic acidosis, seizures, global developmental delay, hypertrophic cardiomyopathy, renal tubular dysfunction	[n=2] [(18,37)]	dilated loops of bowel, cardiomegaly, pericardial effusion, fetal growth restriction, anhydramnios (PP1795)
MYCN	*Feingold syndrome* (oesophogeal and duodenal atresias, microcephaly, learning disability, digital anomalies: brachymesophalang/ syndactyly); cardiac defects, renal anomalies,	[77][(38)]	**See Text Box** 3 (PP1579)
NR2F2	AVSD, ASD, hypoplastic left heart syndrome, coarctation of the aorta, tetralogy of Fallot; congenital diaphragmatic hernia	[11] [(39,40)]	Abnormal 4 chamber view of heart (PP0184)
TAB2	*Frontometaphyseal dysplasia;* hypertelorism, wide nasal bridge, micrognathia, hearing loss, congenital heart defects (variable), scoliosis, upper limb contractures	[15][(41–44)]	Increased NT (8.0 mm) PP1726

TUBB	Microcephaly, structural brain anomalies (dysmorphic basal ganglia, abnormalities of the corpus callosum, and brainstem hypoplasia), learning disability, circumferential skin creases, cleft palate, short stature	[6][45,46]	**See Text Box 1** (PP1579)
ZC4H2	Arthrogryposis multiplex congenita, kyphosis/scoliosis, severe learning disability	[5][47]	Fixed extended knees, rocker bottom feet, flat forehead (PP204)

Figure legends

Figure 1. A. Number of potential diagnoses per case reviewed by clinical review panel, B. Number of potential diagnoses reviewed by the clinical review panel by gene for all genes harbouring a diagnostic variant and for all genes considered for more than one proband (regardless of diagnostic status), C. Proportion of cases for each phenotypic class for all cases, for cases with a diagnostic variant(s) and for cases considered by the clinical review panel with non-diagnostic variants.

Figure 2: Pregnancy outcomes (live birth/miscarriage/termination of pregnancy/stillbirth or neonatal death/lost to follow up (LTFU) or unknown) for different phenotypic classes of fetal anomaly. Total number of cases for each group were abdominal (n=29), brain(n=38), cardiac (n= 49), thoracic (n=12), facial (n=21), fetal hydrops (n=19), increased nuchal translucency (>4.0mm) (n=88), renal (n=12), skeletal (n=39), spinal (n=8) and complex/multisystem anomalies (n=77)

Figure 1

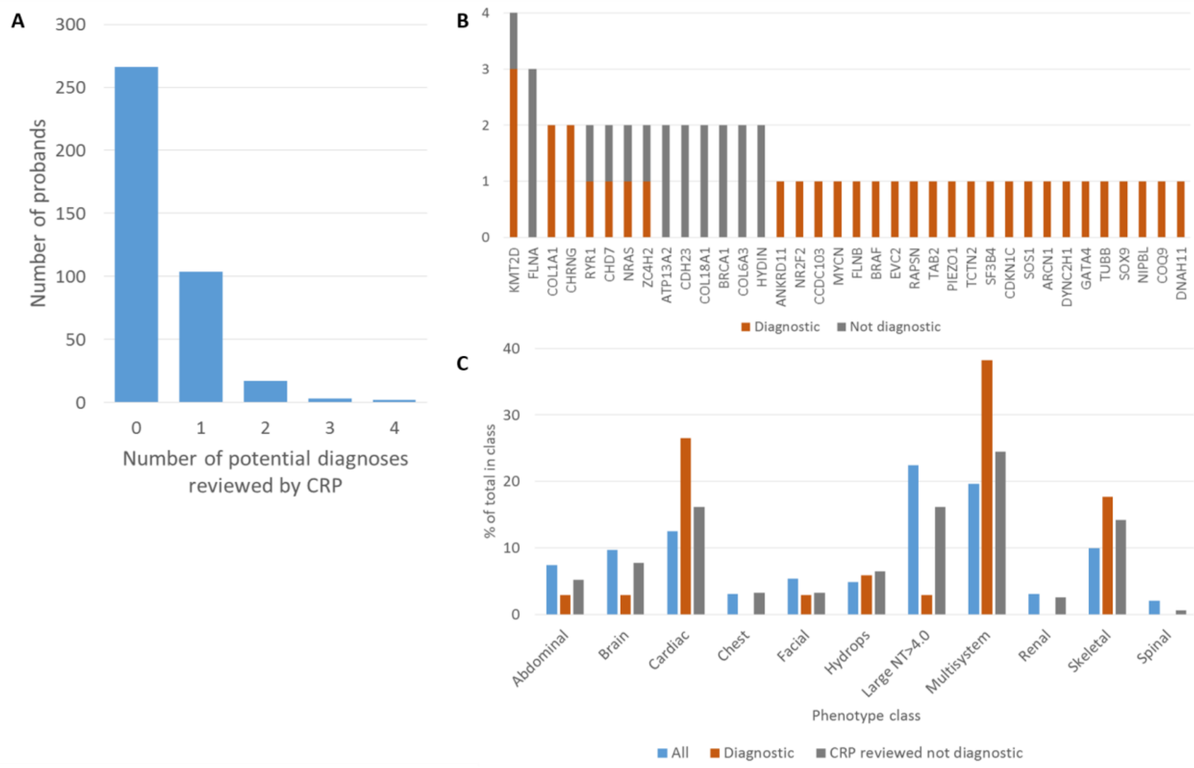
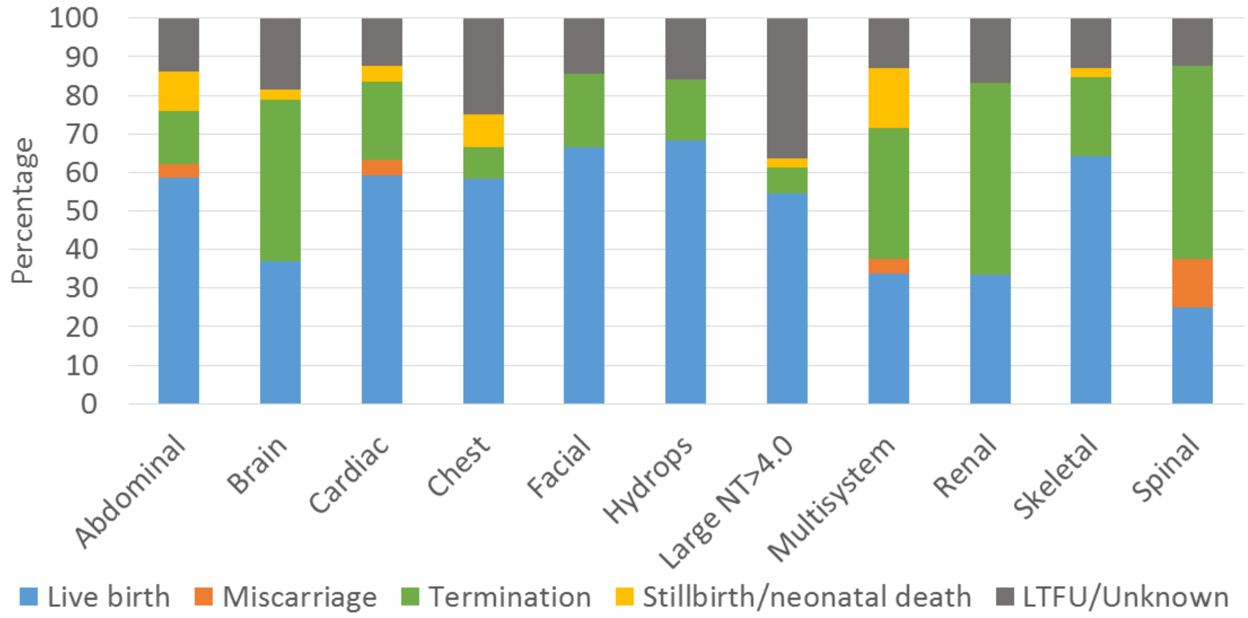


Figure 2:



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