Singleton exome sequencing of 90 fetuses with ultrasound anomalies revealing novel disease-causing variants and genotype-phenotype correlations

Mateja Smogavec1,2, Maria Gerykova Bujalkova1, Reinhard Lehner1, Jürgen Neesen1, Jana Behunova1, Gülen Yerlikaya-Schatten2, Theresa Reischer2, Reinhard Altmann3, Denisa Weis4, Hans-Christoph Duba3 and Franco Laccone1

© The Author(s) 2021

Exome sequencing has been increasingly implemented in prenatal genetic testing for fetuses with morphological abnormalities but normal rapid aneuploidy detection and microarray analysis. We present a retrospective study of 90 fetuses with different abnormal ultrasound findings, in which we employed the singleton exome sequencing (sES; 75 fetuses) or to a lesser extent (15 fetuses) a multigene panel analysis of 6713 genes as a primary tool for the detection of monogenic diseases. The detection rate of pathogenic or likely pathogenic variants in this study was 34.4%. The highest diagnostic rate of 56% was in fetuses with multiple anomalies, followed by cases with skeletal or renal abnormalities (diagnostic rate of 50%, respectively). We report 20 novel disease-causing variants in different known disease-associated genes and new genotype-phenotype associations for the genes KMT2D, MN1, CDK10, and EXOC3L2. Based on our data, we postulate that sES of fetal index cases with a concurrent sampling of parental probes for targeted testing of the origin of detected fetal variants could be a suitable tool to obtain reliable and rapid prenatal results, particularly in situations where a trio analysis is not possible.

European Journal of Human Genetics (2022) 30:428–438; https://doi.org/10.1038/s41431-021-01012-7

INTRODUCTION
Structural fetal abnormalities are detected by prenatal ultrasound in ~3–4% of pregnancies [1], and 8.4–18.2% of these cases are caused by an abnormal karyotype [2, 3]. Microarray analysis detects causal submicroscopic rearrangements in an additional 6.0–8.2% of cases [3–5]. The introduction of exome sequencing into prenatal diagnostics in recent years extended the diagnostic yield by 8.5–50% [6–9]. The most recent prenatal exome-sequencing studies suggest an increasing transition from proband exome analysis to trio exome analysis [9–11]. Along with the increased genetic detection rate, prenatal whole-exome sequencing (WES) can also reveal new and/or unexpected associations between fetal phenotypes and identified variants, considerably extending the spectrum of prenatal manifestations of disease-causing variants in specific genes. Despite the ongoing discussions regarding indications for exome sequencing in a prenatal setting [12–14], the challenges of interpreting variants of unknown significance or of de novo variants in potential candidate genes, and strategies for managing secondary findings, this method has already become an important diagnostic tool in pregnancies with fetal abnormalities.

METHODS
Patient selection
In this retrospective study, we included 90 fetuses with a normal Rapid Aneuploidy Detection (RAD) using Fluorescence in situ hybridization analysis and microarray analysis results, which was referred to as a genetic analysis from April 2015 to July 2020 as a clinical service. The cases included in this study have been selected of all consecutive fetuses with normal RAD and microarray testing undergoing prenatal genetic testing by referring gynecologists according to their best practice based on clinical assessment of fetal ultrasound phenotype suspicious of monogenic cause. Fetal samples were obtained by chorionic villus sampling (n = 29) and amniocentesis (n = 61). The gestational age at the time of testing ranged from 11 + 1 to 31 + 4 weeks, with the majority of fetuses being tested after the 20th week of pregnancy (n = 60 cases; 66.7%) and the rest of the fetuses (n = 30 cases; 33.3%) being tested before the 20th week of pregnancy with a peak in the 13th week of pregnancy. The fetuses showed various major and/or minor ultrasound abnormalities, including increased nuchal translucency (>3.5 mm), and lacked a previously detected causal aberration. When possible, parental blood samples were concurrently obtained for DNA extraction. If the parental samples for variant origin testing were immediately available, the collection-to-reporting turnaround time was ~3 weeks for all confirmatory methods used for detected variations as well as parental tests.
The 90 fetal samples sent to our laboratory for genetic analyses with the indication of fetal malformation received firstly a RAD analysis and secondly an array CGH. If both analyses showed a negative result, we proceeded to perform an sES (singleton exome sequencing) or in the first analyzed cases a multiple gene panels enriched for 6713 genes associated with Mendelian disorders. The reason for performing a singleton analysis was mostly due to approved costs of medical insurance for this analysis. Recently, we increasingly perform trio WES as the financial covering has improved.

**Exome-sequencing and bioinformatics analysis**

Fetal DNA extraction was performed according to standard protocols. For 15 fetal samples, multigene panel testing was enriched for 6713 genes associated with Mendelian disorders by means of the TruSight One Expanded Sequencing Panel Kit, and for 75 fetal samples, enrichment for whole-exome analysis was performed with the TruSeq Exome Kit, both from Illumina (San Diego, California, USA). DNA fragments were paired-end sequenced on an Illumina NextSeq500 system. We performed sES in 86 fetuses and trio analysis including parental samples in four fetuses.

The obtained sequencing reads were aligned to the NCBI human genome assembly (hg19) using the Burrows Wheeler Alignment Tool. Variant calling (HaplotypeCaller) was performed according to GATK best practice guidelines (available at https://gatk.broadinstitute.org/hc/en-us) for calling single-nucleotide variants, insertions, and deletions. The evaluation of the called variants was performed using VarSeq software from Golden Helix® (Bozeman, Montana, USA). The exome-wide average read coverage was 75, whereas that of the multigene panel was 110.

Variants were filtered based on minor allele frequency (MAF) using our in-house database including data from >1000 whole exomes and published disease-causing variants. Variants present as heterozygous in more than five cases or homozygous in more than three cases in the internal database were filtered out, followed by filtering based on the MAF listed in the Genome Aggregation Database (gnomAD) (MAF ≤ 0.5). The remaining variants were filtered for genes associated with HPO terms based on fetal malformations with predefined criteria, namely, read depth of >9, the current variant allele frequency of >0.35, and Phred scale base quality score >99. In a second step, the cutoff for the variant allele frequency was lowered to 0.19 to allow the detection of possible mosaicism. If this analysis did not reveal any possibly pathogenic or pathogenic variant, the data analysis was extended to the whole exome, where also non-OMIM disease-associated genes were analyzed.

We routinely used six prediction tools for independent assessments of the potential pathogenicity of filtered missense variants (SIFT, Polyphen2 HVAR, MutationTaster, MutationAssessor, FATHMM, FATHMM MKL Coding), and variants with at least four predictions as damaging/pathogenic/likely pathogenic were selected. Loss-of-function variants were independently considered for further analysis. All variants were assessed for their interpretation in the ClinVar database.

Reported variants were classified based on the ACMG guidelines [15]. Among diagnostic variants, we reported likely pathogenic and pathogenic variants (Table 1), in addition, we also reported variants of uncertain significance. Among the secondary findings, we reported only pathogenic or likely pathogenic variants. All reported fetal variants were confirmed by Sanger sequencing. Where available, targeted testing of parental DNA to examine the origin of variants detected in fetal DNA was performed by Sanger sequencing. Pathogenic and likely pathogenic variants have been submitted to ClinVar with accession numbers from SCV000159005–SCV000159082.

In addition, a copy number variation (CNV) analysis was performed for all analyzed samples comparing the calculated coverage of each sequenced sample to the already existing coverage data, obtained from BAM-files, for all previously analyzed in-house samples. This analysis was also done by a supported module from VarSeq within the VarSeq software from Golden Helix®. The reported fetal CNVs were confirmed by qPCR (multiplexin deletion in EXOC3L2).

**RESULTS**

**Fetal phenotypes**

A large proportion of the fetuses showed either central nervous system (n = 28; 31.1%) or multiple structural anomalies (n = 25; 27.8%), followed by skeletal abnormalities (n = 16; 17.8%), increased nuchal translucency or fetal hydrops (n = 13; 14.4%) and renal abnormalities (n = 6; 6.7%). Two fetuses had an isolated complex heart defect. Fetuses with malformations in ≥2 organ systems were defined as having multiple abnormalities.

**Diagnostic outcomes**

We observed an overall diagnostic yield of 34.4% (31 of 90, Table 1). In 3.3% (3 of 90) of the fetuses, we detected variants of uncertain significance. The diagnostic rate varied considerably within different phenotypic subgroups, and the greatest yield was reached in fetuses with multiple anomalies (56.0%; 14 of 25), followed by skeletal abnormalities (50.0%; 8 of 16) and renal abnormalities (50.0%; 3 of 6). A lower diagnostic rate was found in those with central nervous system abnormalities (14.3%; 4 of 28) and increased nuchal translucency or fetal hydrops (7.7%; 1 of 13) (Fig. 1). In one fetus with an isolated complex heart defect, we detected a heterozygous de novo pathogenic variant in the KMT2D gene (1 of 2).

In 45.1% (14 of 31) of the cases, we detected biallelic disease-causing variants for diseases inherited in an autosomal recessive fashion, and in 45.1% (14 of 31) of the fetuses, we detected de novo disease-causing variants or autosomal dominant inherited disorders. In one fetus, the origin of the autosomal dominant disease-causing variant in the SF3B4 gene could not be defined because parental DNA was not available. Another two fetuses carried maternally inherited variants for X-linked disorders in the GP3 and ANOS1 genes. Two fetuses carried two diagnostic variants each (COL27A1 and PKD1 in one fetus and PTPN11 and PSEN in the second fetus).

Pathogenic and likely pathogenic variants as well as the fetal phenotypes are listed in Table 1.

**Further delineation of known phenotypes and unexpected findings**

Here, we report new possible phenotypic associations and further delineation of prenatal features in three known disease-causing genes, KMT2D, MN1, and CDK10, as well as in a candidate disease-causing gene, EXOC3L2.

A fetus diagnosed with multicystic and dysplastic enlarged kidneys and severe oligohydramnios had a novel variant c.5642_5644=1del in KMT2D of de novo origin, detected by sES (case 14 in Table 1).

A fetus with sonographic signs of shortened long bones in the 23rd week of pregnancy (length of all proximal long bones under the 3rd percentile) and a single umbilical artery confirmed by fetal MRI and no further abnormalities, had a novel nonsense variant in MN1 of de novo origin (c.3555C>A, p.Cys1185Ter) (case 1 in Table 1 and Fig. 2). A fetus of consanguineous parents showed cerebellar vermian hypoplasia, enlarged hypertrophic kidneys, oligohydramnios, and lung hypoplasia in the 24th week of pregnancy. One previous pregnancy of the same couple was terminated because of a similar severe phenotype and in another pregnancy, with anhydramnios a girl was born in the 25th week with Dandy-Walker malformation and encephalocoele, who died shortly afterward. Furthermore, the couple had one abortion and has two healthy children. For the current pregnancy, a trio whole-exome analysis was performed, and homozygous deletion of exons 3–5 in EXOC3L2 was detected by CNV analysis. The parents were both heterozygous carriers of this deletion (case 22 in Table 1 and Fig. 3).

In a fetus diagnosed with fetal hydrops, multicystic dysplastic kidneys, cardiomyopathy, lung hypoplasia, retrogastria, and hydrocephalus in the 18th week of pregnancy, we performed an sES that showed a known homozygous disease-causing variant of the canonical donor splice site (c.608+1 G > A) in the CDK10 gene. The parents were both heterozygous carriers of this disease-causing variant (case 26 in Table 1 and Fig. 4).
Table 1. Fetal phenotypes and identified pathogenic and likely pathogenic variants in our cohort.

| No. | Fetal phenotype | Gene | Variant | Reference sequence | Zygosity/inheritance | ACMG classification/criteria** | Disorder | Novel/known variant | ClinVar ID |
|-----|-----------------|------|---------|-------------------|----------------------|--------------------------------|----------|---------------------|-----------|
| 1   | NT > 3.5 mm, microgenia, mesomelia, syndactyly of fingers | SF3B4 | c.763_781del, p.(Pro255Cysfs*59) | NM_005850.4 | het, AD, unknown | 5 | PVS1/PM2-M/PP3-S | Acrofacial dysostosis 1, Nager type | Novel | SCV001519055 |
| 2   | Hydrocephalus (2nd pregnancy with the same malformations) | MPDZ | c.4171C>T, p.(Arg1391Ter) | NM_001330637.1 | comp, het, AR | 5 | PVS1/PM2-M/PP3-S | Hydrocephalus, congenital, 2, w/wo brain or eye anomalies | Known | VCV000667381 |
| 3   | Bilateral renal agenesis | ANOS1 | c.774del, p.(Trp258Cysfs*9) | NM_000216.3 | hemi, XL, mat inherited | 5 | Polycystic kidney disease 1 | Known | SCV001519057 |
| 4   | Left-sided diaphragmatic hernia, ascites, pleural effusion, susp. duplex kidneys | PTPN11 | c.218C>T, p.(Thr73Ile) | NM_002834.4 | het, AD, de novo | 5 | Noonan syndrome 1 | Known | VCV00013334 |
| 5   | Shortened limbs, multiple fractures | COL1A2 | c.1352G>A, p.(Gly451Asp) | NM_000089.3 | het, AD, de novo | 5 | osteogenesis imperfecta | Novel | SCV001519058 |
| 6   | NT > 7 mm, heart defect, multicystic dysplastic kidneys, shortened long bones, bilateral clubfoot, abnormal skull configuration | COL27A1 | c.4519C>T, p.(Arg1507Ter) | NM_032888.3 | hom, AR | 5 | Steel syndrome | Novel | SCV001519059 |
| 7   | Fetal hydrops | GUSB | c.1651C>T, p.(Gln551Ter) | NM_001009944.2 | hom, AD | 4 | Polycystic kidney disease 1 | Novel | SCV001519060 |
| 8   | Skeletal dysplasia, IUGR (length of long bones and fetal weight < 3rd percentile, small thorax) | CUL7 | c.5022_5025del, p.(Asp362_Phe370del) | NM_001168370.1 | hom, AR | 5 | 3-M syndrome 1 | Novel | SCV001519063 |
| 9   | Agenesis of corpus callosum, cerebellar hypoplasia, colpocephaly, transposition of great vessels, microgenia, fingers closed in fists, asymmetric shortened legs and malformed feet (2nd pregnancy with the same malformations) | COG6 | c.511C>T, p.(Arg171Ter) | NM_020751.2 | hom, AR | 5 | Congenital disorder of glycosylation, type III/Shaheen syndrome | Known | VCV000493007 |
| 10  | Skeletal dysplasia, shortened long bones, bilateral clubfoot | SLC26A2 | c.1020_1022del, p.(Val341del) | NM_000112.3 | comp, het, AR | 4 | Diastrophic dysplasia/Achondrogenesis 1B/Atelosteogenesis, type II | Known | VCV000004098 |
| 11  | Skeletal dysplasia, shortened long bones | SLC26A2 | c.749A>T, p.(Asp250Val) | NM_000112.3 | comp, het, AR | 4 | Diastrophic dysplasia/Achondrogenesis 1B/Atelosteogenesis, type II | Novel | SCV001519064 |

**ACMG classification/criteria: VUS = Variant of Uncertain Significance; PM1-M = Pathogenic; PM2-M = Probably Pathogenic; PM3-M = Likely Pathogenic; PM4-M = Likely Benign; PM5-M = Benign; PP1-S = Substantially Supportive of Pathogenicity; PP2-S = Supportive of Pathogenicity; PP3-S = Possibly Supportive of Pathogenicity; PP4-S = Weak Support for Pathogenicity; PP5-S = Not Supportive of Pathogenicity; PVS1 =Poor Validation; PVS2 = Poor Validation with Strong Evidence against Pathogenicity; PVS3 = Poor Validation with Strong Evidence for Pathogenicity; PS1 = Positive Support; PS2 = Positive Support with Strong Evidence against Pathogenicity; PS3 = Positive Support with Strong Evidence for Pathogenicity; PM1 = Pathogenic; PM2 = Probably Pathogenic; PM3 = Likely Pathogenic; PM4 = Likely Benign; PM5 = Benign; PP1 = Substantially Supportive of Pathogenicity; PP2 = Supportive of Pathogenicity; PP3 = Possibly Supportive of Pathogenicity; PP4 = Weak Support for Pathogenicity; PP5 = Not Supportive of Pathogenicity; PVS1 = Poor Validation; PVS2 = Poor Validation with Strong Evidence against Pathogenicity; PVS3 = Poor Validation with Strong Evidence for Pathogenicity; PS1 = Positive Support; PS2 = Positive Support with Strong Evidence against Pathogenicity; PS3 = Positive Support with Strong Evidence for Pathogenicity.**
| No. | Fetal phenotype                          | Gene    | Variant                           | Reference sequence | Zygosity/inheritance | ACMG classification/criteria** | Disorder                        | Novel/known variant | ClinVar ID     |
|-----|-----------------------------------------|---------|-----------------------------------|--------------------|----------------------|-----------------------------|--------------------------------|---------------------|-----------------|
| 12a | Polycystic kidneys, polyhydramnios     | HNF1B   | c.494G>A, p.(Arg165His)           | NM_000458.3        | het, AD, de novo     | 5                           | Renal cysts and diabetes syndrome | Known              | VCV00012647    |
| 13  | Agenesis of corpus callosum, hydronephrosis, bilateral clubfoot, abnormal hand position | KAT6B   | c.3903del, p.(Ser1303Valfs*31)    | NM_012330.3        | het, AD, de novo     | 4                           | Genitopatellar syndrome/ SBBYSS syndrome | Novel              | SCV001519065   |
| 14a | Multicystic, dysplastic enlarged kidneys, extreme oligohydramnios | KMT2D   | c.5642_5644+1del, p.(?)           | NM_003482.3        | het, AD, de novo     | 5                           | Kabuki syndrome 1                    | Novel              | SCV001519066   |
| 15  | Polycystic kidneys, dextrocardia       | BBS2    | c.263del, p.(Gly88Alafs*6)        | NM_031885.3        | hom, AR               | 5                           | Bardet-Biedl syndrome 2                | Known              | VCV000217434   |
| 16  | Thanatophoric dysplasia                | COL2A1  | c.2798G>A, p.(Gly933Glu)          | NM_001844.4        | het, AD, de novo     | 5                           | Platyspondylic lethal skeletal dysplasia, Torrance type/ Achondrogenesis 2 | Novel              | SCV001519067   |
| 17  | Brain malformation, bilateral clubfoot, pericardial effusion | VANGL1  | c.838del, p.(Leu280Ter)           | NM_138959.2        | het, AD, de novo     | 5                           | Caudal regression syndrome/Neural tube defects, susceptibility to | Novel              | SCV001519068   |
| 18a | Shortened long bones (< 3 percentile)  | MNI     | c.3555C>A, p.(Cys1185StTer)       | NM_002430.2        | het, AD, de novo     | 5                           | MNI-associated syndrome, CEBA syndrome | Novel              | SCV001519069   |
| 19# | NT > 3.5 mm, hydronephrosis, polycystic kidney, clubfoot, macrosomia, twin pregnancy, only one fetus with malformations | GPC3    | c.175+1G>T, p.(?)                | NM_004484.3        | hemi, XL, mat inherited | 5 | Simpson-Golabi-Behmel syndrome, type 1 | Novel              | SCV001519070   |
| 20  | Microcephaly, agenesis of the right kidney | NBN     | c.657_661del, p.(Lys219Asnfs*16) | NM_002485.4        | hom, AR               | 5                           | Nijmegen breakage syndrome            | Known              | VCV00006940    |
| 21  | Brain malformations, cardiomegaly (2nd pregnancy with the same malformations) | TREX1   | c.236_243dup, p.(Ser82Leufs*9)   | NM_033629.5        | hom, AR               | 5                           | Aicardi-Goutieres syndrome 1          | Known              | SCV001519071   |
| 22c | Suspected fetal ciliopathy, cerebellar vermis hypoplasia, hyperechogenic enlarged kidneys, oligohydramnios (3rd pregnancy with similar malformations) | EXOC3L2 | c.(90+1_91-1)_904+405-1del, p.(?) | NM_138568.3        | hom, AR               | 5                           | No OMIM disease association Publications: Meckel-Gruber-like syndrome | Novel              | SCV001519072   |
| 23  | Lissencephaly                          | PEX1    | c.1587+1G>A, p.(?)               | NM_000466.2        | comp, het, AR        | 5                           | Peroxisome biogenesis disorder 1 A/1B | Known              | VCV000371701   |
|     |                                         |         | c.2875C>T, p.(Arg959Ter)         |                    |                      | 5                           |                                      | Known              | VCV000371716    |
| 24  | Fetal hydrops, mild ventriculomegaly, microstomia, short fingers, syndactyly of all fingers on the one hand side, heart malformation, shorten long bones, bilateral curved femur bones | FRAS1   | c.4259G>A, p.(Trp1420Ter)        | NM_025074.6        | comp, het, AR          | 5                           | Fraser syndrome 1                        | Novel              | SCV001519073   |
|     |                                         | FRAS1   | c.6435C>T, p.(Arg2145Ter)        |                    |                      | 5                           |                                      | Novel              | SCV001519074   |
| No. | Fetal phenotype                                                                 | Gene   | Variant                            | Reference sequence | Zygosity/inheritance | ACMG classification/criteria** | Disorder                        | Novel/known variant | ClinVar ID          |
|-----|---------------------------------------------------------------------------------|--------|------------------------------------|--------------------|-----------------------|--------------------------------|---------------------------------|---------------------|-------------------|
| 25  | Suspected brain and brain vessel malformations, further abnormalities not specified | PTEN   | c.131G>A, p.(Gly44Asp)             | NM_000314.6        | het, AD, mat inherited | 5                              | Cowden syndrome 1/Macrocephaly and autism syndrome | Known              | VCV000427582       |
|     |                                                                                  | PTPN11 | c.922A>G, p.(Asn308Asp)            | NM_002834.4        | het, AD, de novo       | 5                              | Noonan syndrome 1              | Known              | VCV000013326       |
| 26  | Fetal hydrops, hydrocephalus, multicystic, dysplastic kidneys, lung hypoplasia, cardiomyopathy, retrognathia | CDK10  | c.608+1G>A, p.(?)                 | NM_052988.4        | hom, AR               | 5                              | Al Kaissi syndrome             | Novel              | VCV000440757       |
| 27  | Complex heart defect                                                            | KMT2D  | c.11320C>T, p.(Gln3774Ter)         | NM_003482.3        | het, AD, de novo       | 5                              | Kabuki syndrome 1              | Novel              | SCV001519075       |
| 28  | Suspected skeletal dysplasia, shortened long bones, malformed thorax with bowed ribs | COL1A1 | c.1201G>A, p.(Gly401Ser)           | NM_000088.3        | het, AD, de novo       | 5                              | Osteogenesis imperfecta        | Known              | VCV000425596       |
| 29  | Agenesis of corpus callosum                                                     | DCC    | c.3073C>T, p.(Arg1025Ter)          | NM_005215.3        | het, AD, mat inherited | 5                              | Mirror movements 1 and/or agenesis of the corpus callosum | Novel              | SCV001519076       |
| 30  | Skeletal dysplasia                                                              | COL1A1 | c.1930G>A, p.(Gly644Ser)           | NM_000088.3        | het, AD, de novo       | 5                              | Osteogenesis imperfecta, type II or III or IV | Known              | VCV000392573       |
| 31  | Multiple malformations with contraction of distal muscles of the hands, bilateral clubfoot, minimal skin edema | NEB    | c.24871-1G>C, p.(?)               | NM_001271208.1     | hom, AR               | 5                              | Nemaline myopathy 2            | Novel              | SCV001519077       |

*Tested via multigene panel encompassing 6713 genes.
**ACMG criteria are listed only for novel variants.

NT: nuchal translucency, IUGR: intrauterine growth retardation, AD: autosomal dominant inheritance, AR: autosomal recessive inheritance, XL: X-linked inheritance, Hom: homozygous, Het: heterozygous, Hemi: hemizygous, Comp het: compound het, proven trans phase of alleles. ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/; ACMG variant classification [15] (5 = pathogenic, 4 = likely pathogenic, 3 = variant of unknown significance, 2 = likely benign, 1 = benign); variants are described according to HGVS nomenclature.

Published separately in Clin. Pract. 2021, 11(1), 75–80; https://doi.org/10.3390/clinpract11010012.

ACMG criterion applied:
PV51: Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease.
PV2-S: De novo (both maternity and paternity confirmed) in a patient with the disease and no family history, used at a strong level.
PV1-M: Located in a mutational hot spot and/or critical and well-established functional domain without benign variation, used at a moderate level.
PV2-M: Absent from controls in gnomAD database, used at a moderate level.
PV3-M: For recessive disorders, detected in trans with a pathogenic variant, used at a moderate level.
PV2-S: Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease, used at supporting level.
PV3-S: Multiple lines of computational evidence support a deleterious effect on the gene or gene product, used at the supporting level.

There are no corresponding identifiers to patient numbers.
Table 2. Reported secondary findings in our cohort.

| Case no. | Fetal phenotype | Gene and reference sequence | Identified variant | Disorder | Disorder inheritance, variant zygosity and origin | ACMG classification/criteria** | Novel/known variant | ClinVar ID |
|----------|----------------|-----------------------------|-------------------|---------|-------------------------------------------------|-------------------------------|-------------------|------------|
| 6*       | NT > 7 mm, heart defect, multicystic dysplastic kidneys, shortened long bones, bilateral clubfoot, abnormal skull configuration | SBDS NM_016038.2 | c.258-2T>C, p.(?) | Shwachman-Diamond syndrome | AR, hom, mother also homozygous, father heterozygous (parents are consanguineous) | 5 Known | VCV000003196.14 |
| 8*       | Skeletal dysplasia, IUGR (length of long bones and fetal weight < 3rd percentile, small thorax) | KMT2D NM_003482.3 | c.5468-1G>A, p.(?) | Kabuki syndrome 1 | AD, het, maternally inherited | 5 Novel | SCV001519078 |
| 20       | Microcephaly, agenesis of right kidney | BRCA2 NM_000059.3 | c.7350_7354del, p.(Asn2450Lysfs*2) | Breast-ovarian cancer, familial, 2 | AD, het, paternally inherited | 5 Known | VCV000254601.2 |
|          | DUOX2 NM_014080.4 | c.605_621del, p. (Gln202Argfs*93) | | Thyroid dyshormonogenesis 6 | AR, hom | 5 Known | SCV001519079 |

In all listed fetuses a diagnostic variant of underlying malformation has been identified.
*Tested with a panel encompassing 6713 genes.
NT nuchal translucency, IUGR intrauterine growth retardation, AD autosomal-dominant inheritance, AR autosomal recessive inheritance, Hom homozygous, Het heterozygous, ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/.
**ACMG criteria are listed only for novel variants, ACMG variant classification [15] (5 = pathogenic, 4 = likely pathogenic, 3 = variant of unknown significance, 2 = likely benign, 1 = benign); variants are described according to HGVS nomenclature.
There are no corresponding identifiers to patient numbers.
ACMG criterion applied:
PVS1: Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease.
PM2-M: Absent from controls in gnomAD database, used at a moderate level.
Cases with two diagnostic variants
Two fetuses in our cohort carried two diagnostic variants each. In case 6, a fetus with nuchal transparency >7 mm, a heart defect, multicystic dysplastic kidneys, shortened long bones, bilateral clubfoot, and an abnormal skull configuration, we detected a homozygous nonsense COL27A1 variant as well as a homozygous missense variant in PKD1. The parents were both heterozygous carriers of both variants. The COL27A1 variant is suggested to be responsible for the skeletal phenotypic features, whereas the homozygous variant in PKD1 is considered to be a hypomorphic dominant allele causing features of autosomal recessive polycystic kidney disease [16]. In case 25, we identified a known PTPN11 disease-causing variant of de novo origin as well as a known disease-causing variant in the PTEN gene inherited from a previously undiagnosed mother. The 34 years old mother did not show any manifestations of Cowden syndrome and her family history regarding PTEN-associated tumors was unremarkable.

Cases with potentially diagnostic variants of uncertain significance
In addition to 31 fetuses with confirmed genetic diagnosis, three fetuses had inconclusive results. A fetus with lissencephaly carried a heterozygous novel missense variant in TUBA1A (NM_006009.3:c.680T>A, p.(Leu227Gln); SCV001519080). In a fetus with an isolated complex heart defect, we identified heterogeneous missense variants in two genes (ASXL2: NM_018263.4:c.2847T>G, p.(Asn949Lys); SCV0001519080). In the other fetus, with a similar phenotype, we could identify a previously unreported de novo disease-causing variant in the SBDS gene carried by the father as well as known maternal variant, which was not shown to be any manifestations of Shwachman-Diamond syndrome and her family history regarding SBDS-associated tumors was unremarkable.

Secondary findings
Every data set was proofed for secondary findings. We detected and reported four secondary findings in three fetuses (detection rate of 3.3%; including two variants in one fetus). These variants are listed in Table 2. In all three of these fetuses, the main cause of the anomalies was detected. A paternally inherited loss-of-function variant in BRCA2 in case 20 was the only variant in a medically actionable gene identified [17, 18]. A secondary finding in this fetus was a homozygous frameshift variant in DUOX2 leading to congenital hypothyroidism. Case 6 carried a known splicing variant in the SBDS gene in a homozygous state, in addition to the two diagnostic variants in COL27A1 and PKD1 discussed above. The testing of the consanguineous parents revealed a heterozygous carrier status in the father and, surprisingly, homozygosity of the SBDS variant in the mother, thus confirming the diagnosis of Shwachman-Diamond syndrome (SDS) in the fetus and the mother. The phenotypic spectrum of SDS has been shown to be rather broad, and asymptomatic individuals have also been described [19]. The contribution of the SBDS variant to some of the fetal phenotypic features (especially shortened long bones) cannot be fully excluded. However, given the familial setting, we consider it as a secondary finding. In case 8, which had a diagnostic variant in CUL7, we also identified a maternally inherited novel splicing variant in the KMT2D gene as a secondary finding. The phenotyping of the mother for potential symptoms of Kabuki syndrome was unfortunately not possible.

DISCUSSION
Diagnostic yield, prioritization strategy, and new genotype–phenotype associations
In our retrospective study of a broad spectrum of fetal structural abnormalities in 90 prenatal cases referred to our institute and investigated primarily with sES, we observed an overall diagnostic yield of 34.4%. Several studies have shown the diagnostic utility of prenatal WES in fetuses with different ultrasound abnormalities [20]. The detection yield varies widely according to the selection criteria, number of genes investigated within WES analysis, variant prioritization, singleton versus trio analysis, number of investigated cases, and parental consanguinity. The first studies with a
smaller number of fetal cases (<15) initially showed a diagnostic yield of above 40% [21, 22]; recent studies with 103 and 105 prenatal cases reported lower diagnostic rates, of 20.6 and 19% [11, 23], and another two studies with 234 and 610 prenatal cases recorded even lower diagnostic yields of 8.5% and 10%, respectively [9, 10]. One feature common to all the published data on prenatal WES is a higher detection yield in fetuses with multiple structural anomalies and skeletal anomalies than in fetuses with other types of structural or isolated anomalies [11, 24]. This trend is also observed in our data (diagnostic rate with multiple malformations of 56.0% and with skeletal anomalies of 52.9%). However, the two prospective studies with the largest number of investigated fetuses and a fetal-parent trio exome strategy have a significantly lower diagnostic yield, as in our retrospectively assessed cohort [9, 10]. This may be in part due to the conservative approach of variant classification used by Petrovski and coworkers, which may lead to the underreporting of variants. Lord et al. selected only a subset of genes associated with developmental disorders, and the study criteria aimed to include a particular spectrum of phenotypes so that the number of fetuses with any specific phenotype was capped at ~20% of the ongoing total. Avoiding the pre-filtering of the analyzed gene set according to OMIM phenotypic associations may be of particular importance as shown in our case 22. This case had a biallelic loss-of-function variant of the EXOC3L2 gene, which is not included in the virtual gene panel adopted by Lord et al. and has not yet been

Fig. 3 Pedigree of the consanguineous couple carrying a heterozygous deletion of exons 3, 4, and 5 in EXOC3L2 and ultrasound findings of the fetus with EXOC3L2 deletion in homozygous state. Both parents (I:1 and I:2) carry a heterozygous deletion of exons 3, 4, and 5 in EXOC3L2, which has been detected in the fetus from the last pregnancy (II:6) in a homozygous state (A). A male fetus in the first pregnancy (II:1) showed renal dysgenesis. The pregnancy has been interrupted in the 22nd gestational week. A healthy boy has been born from the 2nd pregnancy (II:2). In the 3rd pregnancy, an anhydramnios has developed and a girl has been born spontaneously in the 25th gestational week with a Dandy-Walker malformation and an encephalocoele, who died shortly after the birth (II: 3). From the 4th pregnancy, a healthy girl has been born in the 39th gestational week (II:4). The 5th pregnancy ended in an abortion in the 15th gestational week (II:5). The fetus of the last pregnancy (II:6, index case) with a homozygous deletion in EXOC3L2 showed in the 30 + 5 gestational week lung hypoplasia, hypoplastic vermis cerebelli (B: Ve – Vermis, Po – Pons, Mo – Medulla oblongata; cc 15,8 mm (<5. perc), ap 11,4 mm (25. perc.), brainstem-vermis angle: 38,2°) and hyperechogenic, enlarged kidneys (C).

Fig. 4 Ultrasound of the fetus with homozygous CDK10 variant. At 16th gestational week, the fetus manifested several abnormalities in different organ systems: widened lateral (>10 mm) and 3rd brain ventricles (A); cardiomegaly and overrotation of the heart to the left (B); enlarged and hyperechogenic kidneys (C).
variants with prenatally diagnosed multicystic or polycystic
fenestrations for dominant versus recessively inherited diseases. An
onsus for what is the detection rate for disease-causing variants, 30
ective and straightforward approach in rapid detection of de novo variants as well as defining the mono-
various renal malformations in fetal kidneys as well as extreme oligohydramnios and biallelic variants in
from receiving the sample to the
development of ciliopathy (http://www.informatics.jax.org/allele/MGI:5548646) [28]. Paradoxically, the use of trio exome analysis can also lead to a lower detection rate as sES analysis. Trio exome analysis is indeed the most effective and straightforward approach in rapid detection of de novo variants as well as defining the mono- or biallelic position of two variants. However, depending on the filtering strategy used, inherited pathogenic variants could be missed, as in our case 25 with a maternally inherited variant of the PTEN gene or in our case 29 with a maternally inherited variant in the DCC gene. Familial, previous unknown variants are an important factor to consider in genetic counseling for further family planning.

Our high detection rate of 34.4% for pathogenic and/or likely pathogenic variants is comparable to the detection rate reported by Normand et al. [8] in fetuses with at least one structural anomaly; although the high detection rate cannot be fully explained, there are several aspects that may contribute to this outcome. The prioritization and assessment strategy is likely one element of pivotal importance. We used a hypothesis-driven strategy for prioritization of the variants that included different filters for dominant versus recessively inherited diseases. An additional reason for the high detection rate may be a selection of fetuses with major structural malformations at the highly specialized feto-maternal unit, although this selection was based on general clinician expertise and not on any predefined criteria. Our detection rate is, however, in line with the diagnostic rate of 36.7% in an exome study carried out during the first 100 days of life in neonates with severe disease [29]. The similarity of our prenatal yield and the reported postnatal detection rate is encouraging and may be considered to confirm the validity of our bioinformatics approach and final assessment strategy. Each institution and laboratory develops its own pipeline, which depends on the number of investigated genes, bioinformatics assessment, and final assessment strategy. This could also explain the large variability in diagnostic yields and turnaround times from receiving the sample to the final report.

A major challenge of prenatal WES is the limited knowledge and developmental phase-specific detection of the fetal phenotypes associated with known disease-causing genes and with non-OMIM disease-associated genes. We detected a heterozygous de novo variant in the KMT2D gene in fetal case 14 with multicystic and dysplastic enlarged kidneys and extreme oligohydramnios. Among patients with KMT2D disease-causing variants, 30–40% develop urinary tract abnormalities, with hydrenephrosis being the most frequent [30, 31]. Renal dysplasia is deemed to be part of the spectrum of Kabuki syndrome (OMIM #147920) in ~15–21% of patients [32, 33]. To our knowledge, the association of KMT2D variants with prenatally diagnosed multicystic or polycystic enlarged kidneys is a very rare finding [34]. However, their possible role in cystic kidney formation can be hypothesized because KMT2D protein is known to be expressed in the metanephros of mice (http://www.informatics.jax.org/) as well as in the human kidney (https://www.proteinatlas.org/ENSG00000167548-KMT2D/tissue). A second particular challenge was case 18, with a de novo MN1 truncation variant at its C-terminus. The MN1 gene was only recently associated with CEBALID syndrome ( Craniofacial defects, dysmorphic ears, structural brain abnormalities, expressive language delay, and impaired intellectual development; OMIM #618774) [35, 36]. The published cases with CEBALID syndrome carried de novo C-terminal MN1 truncation variants that were suggested to act in a dominant-negative or gain-of-function manner. To date, no case with shortening of the proximal long bones associated with MN1 variant has been described. Because of the de novo origin, the loss-of-function variant, and the possibility that nonsense-mediated mRNA decay is triggered in our fetal case, we propose it to be probably associated with the fetal skeletal phenotype in our case. This could be a novel or a fetus-specific presentation of the variant in the MN1 gene, but we cannot exclude an independent, undetected genetic cause of the underlying shortening of long bones. Both above-mentioned examples highlight the challenge of fetal genotype-phenotype correlations, which are still in an early stage. Owing to the developing nature and time-limited clinical assessment of prenatal malformations, there are inherent difficulties in phenotype-guided genetic analysis and prompt detection of a meaningful genotype via WES.

Variants of unknown significance, secondary findings, and missed variants

Additional issues in prenatal genetic diagnostics are the presence of VUS as well as the detection of secondary pathogenic findings. The possibility of detecting secondary findings in actionable genes should be discussed with the parents during genetic counseling prior to the analysis, although the stress and anxiety of the situation may lead to a misunderstanding of the consequences of their decision. In three fetal cases, we detected and reported four secondary findings (detection rate of 3.3%; including two variants in one fetus). Even more challenging are cases with the presence of VUS without a clear genetic etiology of the malformation and cases in which supplementary investigations are required, prolonging the period of uncertainty.

A further concern in prenatal exome analysis is the unknown proportion of missed, unidentified, or assessed-as-non-pathogenic variants. As a recent example, an unreported homozygous nonsense variant in the ARMC9 gene was identified in a family with a second affected pregnancy with the same phenotype before adequate information in the medical literature was available to make a genetic diagnosis [10]. In such cases, the knowledge of an association between a specific gene and a phenotype at the time of analysis may be a relevant issue; it is also possible that the bioinformatics pipeline will filter out a causative variant in a known disease-causing gene. This potential risk could hinder the more widespread addition or first-line use of exome analysis to the spectrum of routine prenatal diagnostics, as failing to identify the causative variant can have major legal consequences. In addition, a well-defined strategy assessing this aspect is necessary so that the involved clinicians can counsel the family appropriately.

Study limitations

This study was based on coded data available from our internal clinical database. As such, we recognize several limitations. The first limitation of this study is its retrospective character with the inclusion of a subset of fetuses that were referred for prenatal sES after prior negative RAD and array CGH analysis based only on the clinician’s decision according to the assessment of the ultrasound phenotype. As a result, our cohort may represent a selected population of fetuses with malformations in which there was a higher probability of an underlying monogenic cause. This type of selection bias would tend to inflate the diagnostic yield. The second limitation of this study is our inability to access additional clinical information on all pregnancies in our cohort. As a result, we cannot indicate what percentage of pregnancies was terminated, how many children were born, and how was the phenotypic evolution after birth. We are also unable to access
information or do a segregation analysis in the families that might have allowed us to confirm or refute a particular diagnosis. This further information may have allowed us to reclassify variants of unknown significance. This limitation could be minimized in a prospective study in which full access to all medical records would be possible.

In conclusion, our retrospective study confirms the validity of exome analysis as a prenatal diagnostic tool that offers families additional opportunities to end the diagnostic odyssey during pregnancy. A very important requirement before even starting the WES analysis is that the precise description of fetal ultrasound and/or even fetal MR results should be available to the genetic laboratory. With the improvement of bioinformatics tools for the detection of CNVs, exome or genome analyses could become the routine first-tier analyses after negative RAD results in prenatal diagnostic workflows.

DATA AVAILABILITY
The data sets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES
1. Persson M, Cnattingius S, Villamor E, Söderling J, Pasternak B, Stephansson O, et al. Risk of major congenital malformations in relation to maternal overweight and obesity severity: cohort study of 1.2 million singleton. BMJ. 2017;357:j2563.
2. Kessler RG, Sanseverino MT, Leistner-Segal S, Magalhães JAA, Giugliani R. Prenatal diagnosis of fetal chromosomal abnormalities: report of an 18-year experience in a Brazilian public hospital. Genet Mol Biol. 2009;31:289–383.
3. Fu F, Li R, Li Y, Nie ZO, Lei T, Wang D, et al. Whole exome sequencing as a diagnostic adjunct to clinical testing in fetuses with structural abnormalities. Ultrasound Obstet Gynecol. 2018;51:493–502.
4. Wapner RJ, Martin CL, Levy B, Baillie BC, Eng CM, Zachary JM, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med. 2012;367:217–26.
5. Fiorentino F, Napoletano S, Caiazzo F, Sessa M, Bono S, Spiziziino L, et al. Chromosomal microarray analysis as a first-line test in pregnancies with a prior low risk for the detection of submicroscopic chromosomal abnormalities. Eur J Hum Genet. 2013;21:725–30.
6. Vora NL, Powell B, Brandt A, Strande N, Hardisty E, Lyerly AD, Vora NL, et al. Prenatal exome sequencing in anomalous fetuses: new opportunities and challenges. Genet Med. 2017;19:1207–16.
7. Yates CL, Monaghan KG, Copenheaver D, Retker K, Scuffins J, Kucera CR, et al. Whole-exome sequencing on deceased fetuses with ultrasound anomalies: expanding our knowledge of genetic disease during fetal development. Genet Med. 2017;19:1171–8.
8. Normand EA, Braxton A, Nassef S, Ward PA, Vetrini F, He W, et al. Clinical exome sequencing for fetuses with ultrasound abnormalities and a suspected Mendelian disorder. Genome Med. 2018;10:74.
9. Lord J, McMullan DJ, Eberhardt RY, Rinck G, Hamilton SJ, Quinlan-Jones E, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. Lancet. 2019;393:747–57.
10. Petrovski S, Aggarwal V, Giordano JL, Stosis M, Wouk K, Bier L, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet. 2019;393:759–66.
11. Vora NL, Gilmore K, Brandt A, Gustafson C, Strande N, Ramkisson L, et al. An approach to integrating exome sequencing for fetal structural anomalies into clinical practice. Genet Med. 2020;22:954–61.
12. Harris S, Gilmore K, Hardisty E, Lyerly AD, Vora NL. Ethical and counseling challenges in prenatal exome sequencing. Prenat Diagn. 2018;38:897–903.
13. International Society for Prenatal Diagnosis, Society for Maternal and Fetal Medicine, Perinatal Quality Foundation. Joint Position Statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (POF) on the use of genome-wide sequencing for prenatal diagnosis. Prenat Diagn. 2018;38:8–9.
14. Mone F, Quinlan-Jones E, Kibby MD. Clinical utility of exome sequencing in the prenatal diagnosis of congenital anomalies: a review. Eur J Obstet Gynecol Reprod Biol. 2018;231:19–24.
15. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24.
16. Vujic M, Heyer CM, Aris E, Hopp K, Markoff A, Omdal C, et al. Incompletely penetrant PKD1 alleles mimic the renal manifestations of ARPKD. J Am Soc Nephrol. 2010;21:1097–102.
17. Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing. 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med. 2017;19:249–55.
18. Pujol P, Vande Perre P, Faivre L, Sanlaville D, Corsini C, Baetschi B, et al. Guidelines for reporting secondary findings of genome sequencing in cancer genes: the SFMPG recommendations. Eur J Hum Genet. 2018;26:1732–42.
19. Myers KC, Bolyard AA, Otto B, Wong TE, Jones AT, Harris RE, et al. Variable clinical presentation of Shwachman-Diamond syndrome: update from the North American Shwachman-Diamond Syndrome Registry. J Pediatr. 2014;164:866–70.
20. Abou Tayoun A, Mason-Suares H. Considerations for whole exome sequencing unique to prenatal care. Hum Genet. 2020;139:1149–59.
21. Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, et al. Molecular findings among patients referred for clinical whole-exome sequencing. JAMA. 2014;312:1870–9.
22. Alamillo CL, Powi Z, Fanwell K, Shahmirzad L, Weltmer EC, Turicy J, et al. Exome sequencing positively identified relevant alterations in more than half of cases with an indication of prenatal ultrasound abnormalities. Prenat Diagn. 2015;35:1073–8.
23. Chen M, Chen J, Wang C, Chen F, Xie Y, Li Y, et al. Clinical application of medical exome sequencing for prenatal diagnosis of fetal structural abnormalities. Eur J Obstet Gynecol Reprod Biol. 2020;251:119–24.
24. Lefebvre M, Bruel AL, Tisserant E, Bourgon N, Dufourd Y, Collardeau-Frachon S, et al. Genotype-first in a cohort of 95 fetuses with multiple congenital abnormalities: when exome sequencing reveals unexpected fetal phenotype-genotype correlations. J Med Genet. 2020. https://doi.org/10.1136/jmedgenet-2020-108687.
25. Shaheen R, Szymbanska K, Basu B, Patel N, Ewida N, Faqeeh E, et al. Characterizing the morbid genome of ciliopathies. Genome Biol. 2016;17:242.
26. Shamseldin HE, Kurdi W, Almusaffa F, Alnemer M, Alkaff A, Babay Z, et al. Molecular autopsy in maternal-fetal medicine. Genet Med. 2018;20:420–7.
27. Shalata A, Lauhasurayotin S, Li H, Hebert D, Dhanraj S, et al. Biallelic mutations in EXOC3L2 cause a novel syndrome that affects the brain, kidney and blood. J Med Genet. 2019;56:340–6.
28. Wilson R, Geyer SH, Reissig L, Rose J, Szumawska D, Hardman E, et al. Highly variable penetrance of abnormal phenotypes in embryonic lethal knockout mice. Wellcome Open Res. 2017;1:1.
29. Meng L, Parmmi M, Saronwala A, Magoulas P, Ghazi AR, Vetrini F, et al. Use of exome sequencing for infants in intensive care units: ascertainment of severe single-gene disorders and effect on medical management. JAMA Pediatr. 2017;171:e173438.
30. Kawame H, Hannibal MC, Hudjins L, Pagon RA. Phenotypic spectrum and management issues in Kabuki syndrome. J Pediatr. 1999;134:840–5.
31. Li Y, Bogershausen N, Alanyan Y, Simsek Kiper PO, Plume N, Keupp K, et al. A mutation screen in patients with Kabuki syndrome. Hum Genet. 2011;130:715–24.
32. Courcet JB, Faivre L, Michot C, Burguet A, Perez-Martín S, Alik E, et al. Clinical and molecular spectrum of renal malformations in Kabuki syndrome. J Pediatr. 2013;163:742–6.
33. Armstrong L, Abd El Moneim A, Alick K, Aughton DJ, Baumann C, Braddock SR, et al. Further delineation of Kabuki syndrome in 48 well-defined new individuals. Am J Med Genet A. 2005;132a:265–72.
34. So PL, Luk HM, Cheung KW, Hui W, Chung MY, Mak ASL, et al. Prenatal phenotype of Kabuki syndrome: a case series and literature review. Prenat Diagnosis. 2021;41:1089–100.
35. Miyake N, Takahashi H, Nakamura K, Iidori B, Hikari Y, Koshimizu E, et al. Gain-of-function MN1 truncation variants cause a recognizable syndrome with craniofacial and brain abnormalities. Am J Hum Genet. 2020;106:13–25.
36. Mak CCC, Doherty D, Lin AE, Vegas N, Cho MT, Viot G, et al. MN1 C-terminal truncation syndrome is a novel neurodevelopmental and craniofacial disorder with partial rhombencephalosynapsis. Brain. 2020;143:55–68.

ACKNOWLEDGEMENTS
We are very thankful to all families participating in this study. No funds, grants, or other support were received. The authors have no financial or proprietary interests in any material discussed in this article.

COMPETING INTERESTS
The authors declare no competing interests.
ETHICAL APPROVAL
This study was approved by the Ethics Committee of the Medical University of Vienna (1370/2019). Written informed consent was obtained for all fetuses included in the study.

ADDITIONAL INFORMATION
Correspondence and requests for materials should be addressed to Mateja Smogavec.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.