Supplementary Methods

DNA isolation

The DNA isolation from blood samples was performed by an automated DNA extractor according to its protocol (chemagic MSM I, PerkinElmer, Waltham, MA, USA). DNA isolation from amniotic cells and chorionic villi was performed using the Kit "EZ1&2 DNA Tissue" (Qiagen, Hilden, Germany), according to protocol, by the automated DNA extractor EZ1 Advanced XL (Qiagen).

Chromosomal Microarray (CMA)

The DNA was labeled with the SureTaq DNA Labeling Kit (Agilent, Santa Clara, CA, USA) and hybridized on a GenetiSure Cyto 4x180K CGH Microarray (Agilent) according to the manufacturer's instructions. The slides were scanned using an InnoScan 910 AL scanner (Innopsys, Carbonne, France) and processed by the analysis programs Mapix (Innopsys) and CytoGenomics, versions 5.1.2.1 and 5.3.0.14 (Agilent). The data was evaluated with the reference genome GRCh38.

Chromosomal Analysis and Fluorescence In Situ Hybridization

Metaphase preparation was carried out from heparin blood samples, as well as cultures from both chorionic villi and amniotic cells by standard methods.

Briefly, cells from heparin blood samples were cultured in LymphoGrow medium (CytoGen, Sinn, Germany), containing phytohemagglutinin as a mitogen, amniotic cells were cultured in Amniogrow plus medium (Cytogen, Sinn, Germany), CVS-cells were cultured in Chang medium D (Fujifilm, Minato, Japan).

After fixation, metaphases were dropped onto slides and then dried at 60 °C overnight. Metaphase chromosome spreads were evaluated by GTG banding using the karyotyping system Ikaros (MetaSystems, Altlussheim, Germany).

For FISH analyses, the probes RP11-213E22-green and RP11-577D9-orange (chromosome 7), as well as RP11-358H10-green and RP11-241M19-orange (chromosome 16) from Empire Genomics were used (Buffalo, NY, USA). All probes were used according to the manufacturer's instructions. Images were analyzed using the Isis Digital Imaging System (Metasystem Inc., Altussheim, Germany).

PCR and Sequencing

Where applicable, the breakpoints from the OGM analyses were confirmed and further specified, either by third-generation long-range sequencing using a MinION sequencer (Oxford Nanopore, Oxford, UK), or by Sanger sequencing on a Hitachi 3500xL Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The primers were designed according to the strategy described in Dremsek *et al.,* 2021. To position the primer as close as possible to the anticipated breakpoints, both OGM-data and CMA-data were incorporated in their design.

For analysis of P1, long-range PCRs were performed (amplicon of junction B/D*: fwd. primer: 5'-ggaggacaattttatcccccaggg-3' and rev. primer: 5'-gtgagccgtgagtttgccactat-3'; amplicon of junction D*/B*: fwd. primer: 5'-tcgttgacggtgaaatgctacgt-3' and rev. primer: 5'-gcagataacggagtgaggaaggc-3'). Following PCR amplification, the amplicon of junction B/D* (4 kbp in size) was prepared for Sanger sequencing using the primer 5'-acagctcactatagcagataggtgt-3', 5'-ttgcatcaggaacatgtggacct-3', 5'-ctggtcacaggcgcaaatcaaag-3', 5'-gtcagcaaaggagagaagcagct-3' and 5'-gcaggttggctctttcccaagta-3'. The amplicon of junction D*/B* (13 kbp in size) was prepared for Sanger sequencing using the primer 5'-agggaaaagagatgtgtaaaatactgt-3', 5'-agatgaggaagggcatctgac-3', 5'-tcaagttgtcattgtggtgaatt-3', 5'-cagatgccagcgctaagacgat-3', 5'-aggttattacacacccctcct-3', 5'-tgttcattatcactggccatcaga-3', 5'-aaggggaaacctcctgctactct-3', 5'-tgcacccactaacgtgtcatcta-3', 5'-gggttggttccaagtctttgcta-3', 5'-gctgaaactggatcccttcctta-3', 5'-tgtagggacatggatgaaattgg-3' and 5'-ccaaacaccgcatattctcactc-3'.

For analysis of P3, long-range PCR was performed (fwd. primer: 5'-ttaccacgaaagagcaaacggtga-3' and rev. primer: 5'-aacgttattccttccagtcacccac-3'). Following PCR amplification, the 9 kbp sized amplicon was prepared for sequencing on a MinION 106D flow cell according to the manufacturer's protocol (SQK-LSK109, Oxford Nanopore). For familial testing, PCRs were established, using the inversion-specific primer 5'-tgcctctgcttaataggaagttttgg-3' and 5'-cagccaataacgtgagtttaggagt-3' (resulting in a 1247 bp amplicon), as well as the wild-type primer 5'-cagccaataacgtgagtttaggagt-3' and 5'-ctgttgaaggacacaagctctggc-3' (resulting in a 778 bp amplicon) (see S.3).

MLPA Analysis

Multiplex ligation-dependent probe amplification (MLPA) was performed to verify gains detected in CMA and test the carrier-status of relatives. For MLPA, the DNA was hybridized with the probes and amplified according to the manufacturer's instructions. Fragment analysis of amplified DNA was performed on a Hitachi 3500xL Genetic Analyzer (Thermo Fisher), and data were processed with the SeqPilot (JSI, Ettenheim, Germany) analysis program. The MLPA probe sets used for the presented clinical cases were P034-B2, P035-B1 (P1) and P216-C1 (P3) (MRC-Holland, Amsterdam, NL).