

## **Supplemental material:**

Methods: The ultrasound equipment used for ultrasound examination were a GE Voluson E10.

For genetic investigations:

### *Array-CGH:*

Oligonucleotide array-CGH was performed using the Agilent Human Genome CGH microarray 4x180K (Agilent Technologies, Santa Clara, CA, USA). The experiment was performed according to version 7.5 (June 2016) of the protocol provided by Agilent (Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis).

### *MLPA:*

The Salsa® MLPA lot P187 “Holoprosencephaly” was used according to the quantification protocol from MRC-Holland.

### *Exome sequencing:*

#### *Sequencing technologies*

All samples were prepared with the Agilent V7 Exome preparation kit and sequenced on an Illumina NextSeq 550 sequencer using 2x75 bp sequencing kits. Briefly, gDNA is first fragmented by enzymatic protocol and DNA ends are modified with adaptor-tag for target enrichment. After amplification and purification, adaptor-ligated libraries are hybridized to SureSelect Focused Exome and captured prior to indexing. Samples are then pooled for multiplexed sequencing.

#### *Bioinformatics pipeline*

Raw sequencing data were mapped to the GRCh37/hg19 reference genome with the bwa-mem aligner (Li et al 2009). The identification of variants have been performed using Freebayes and the GATK (Unified Genotyper and Haplotype Caller) (Van der Auwera 2013). When possible, the calling has been conducted in a family structure.

Variants were annotated using ANNOVAR (Wang *et al*, 2010, build of February 2016) based on RefSeq genes, known variation from dbSNP144, and Clinvar and frequencies from 1000 Genomes Project , esp6500 project, Exac (Lek *et al* 2016) and kaviar (Glusman *et al*. 2011) database.

The functional consequence of missense coding variants has been assessed using dbNSFP v.3.0 (Liu *et al*, 2015) that includes several deleteriousness prediction algorithms (SIFT, PolyPhen2 HDIV, PolyPhen2 HVAR, LRT, MutationTaster, MutationAssessor, FATHMM, MetaSVM, MetaLR, VEST, CADD, GERP++, DANN, and PROVEAN). In order to facilitate the interpretation of these results, a summarizing value reflecting the percentage of predictions that classify this variant as deleterious has been calculated. For splice site prediction annotations we used SpliceSiteFinder-like, MaxEntScan, NNSPLICE, and GeneSplicer.

The variants were further prioritized based on the variant type, the deleteriousness predictions, OMIM and ClinVar informations, and the potential candidate genes list.