SUPPLEMENTAL DATA

Supplemental note

Clinical Report of Family A: In Family A, the index individual (III-1) and her maternal uncle (II-3) showed several CFs of the VATER/VACTERL association: Individual III-1 presented with anorectal malformation (A; vestibular fistula), tetralogy of Fallot (C), esophageal atresia with tracheo-esophageal fistula (TE; Vogt type 3b), and unilateral renal agenesis (R). Her uncle (II-3) showed anorectal malformation (A), esophageal atresia with tracheo-esophageal fistula (TE; Vogt type 3b), unilateral renal agenesis (R), and aplasia of the right radial bone and pre-axial polydactyly of the left hand (L). Both individuals showed normal physical and neurocognitive development. At the time of assessment, the index individual was a student of good standing in high school and her uncle had a degree from university. Ultrasound examinations of I-1, I-2, II-2 and III-2 confirmed that these family members were unaffected regarding their internal organ status.

Supplemental figures and tables

Figure S1. Results of molecular karyotyping in individuals II-2 and II-1 (Family C)

(A) Chromosome Xp11.23p11.22 deletions of 1.07 Mb spanning chrX:g.49,375,617-50,447,320 involving eight genes and six microRNAs is depicted in Genome Studio.

(B) The respective genomic segment is aligned to RefSeq genes (according to hg19)

Figure S2. Exon structure of zebrafish shroom4 with Morpholino targets.

(A) Schematic depiction of shroom4 gene with transcripts shroom4-201 and shroom4-202 and targets of MO. The shroom4 MO is overlapping the exon (E) 2 splice donor site.
(B) RT-PCR amplified cDNA and Sanger sequencing showed excision of E2 for \textit{shroom4-201} and insertion of 41 base pairs (bp) from intron (I) 2 for \textit{shroom4-202}.

(C) Pictures of ethidium-bromide-stained gel after performing RT-PCR with zfl harvested at 2 dpf for MO and Ctrl MO. MO injected zfl show a weaker band at 397 bp and excision of E2 for \textit{shroom-201}. For \textit{shroom4-202} a weaker band can be seen at 255 bp and the insertion of 41 intronic bp is shown. \textit{eef1a1} is used as a housekeeping gene control indicating an equal expression of \textit{eef1a1} in both samples.

Table S1. Summary of genetic and clinical features in individuals with \textit{SHROOM4} variations

This supplementary table depicts the genetic and clinical features of previously described affected individuals. The table is divided in two subgroups: single nucleotide variants (SNV), comprising missense and nonsense variants, and structural variations, including deletions, duplications and complex rearrangements. Abbreviations are as follows: ID, intellectual disability; MRI, Magnetic resonance imaging