Supplementary Information

Heterozygous truncating variants in SUFU cause congenital ocular motor apraxia
Simone Schröder et al.

Material and Methods
Exome sequencing and variant screening

In family 1, trio-based exome sequencing (ES) of the affected subject III.6 and his parents (II.5 and II.6) was carried out using the NimbleGen SeqCap EZ Human Exome Library v2.0 enrichment kit (Roche) on an Illumina HiSeq4000 sequencer. In families 2 and 3, trio-based ES of the affected subjects and their parents was carried out using the Agilent SureSelect V6 (Agilent) on an Illumina HiSeq4000. ES data analysis and filtering of mapped target sequences was performed using the ‘Varbank’ exome analysis pipeline of the Cologne Center for Genomics (CCG, University of Cologne, Germany), and data were filtered for high-quality (coverage of more than 6 reads, a minimum quality score of 10), rare (minor allele frequency, MAF <1.0%) variants. In family 4, the coding region and flanking intronic regions were enriched using the SureSelect XT All Exon V7 in-solution technology (Agilent, Santa Clara, USA) and were sequenced using the Illumina NovaSeq system (Illumina, San Diego, USA). Illumina bcl2fastq2 was used to demultiplex sequencing reads. Adapter removal was performed with Skewer. The trimmed reads were mapped to the human reference genome (hg19) using the Burrows Wheeler Aligner and variants were called using inhouse software. Only SNVs and small indels in the coding regions and the flanking intronic regions (±8 bp) with a MAF <1.5% were evaluated. Minor allele frequencies were taken from public databases (gnomAD, dbSNP) and an in-house database. Known disease-causing variants (according to HGMD®) were evaluated in up to ±30 bp of flanking regions and up to 5% MAF. Evaluation was based on the ACMG guidelines for the interpretation of sequence variants. In the affected subject II.1 of family 5, all exons and adjacent exon-intron
boundaries of *SUFU* were analyzed by PCR and subsequent, bidirectional Sanger sequencing. In family 6, ES was performed for individual II.1 using the SureSelect V6 enrichment kit (Agilent), paired-end sequenced on an Illumina HiSeq4000 and analyzed using the pipeline of the Department of Human Genetics, Technical University of Munich, Germany, as described previously.\textsuperscript{13}

All detected *SUFU* variants were confirmed by PCR amplification and subsequent Sanger sequencing on an independent DNA sample and tested for co-segregation within the respective families using the BigDye terminator v3.1 chemistry (Thermo Fisher Scientific) on a 3500 Genetic Analyzer (Thermo Fisher Scientific).

**Cell culture and treatments**

Primary dermal fibroblasts established from affected subjects III.3 (family 1), II.1 (family 2), II.1 (family 3), II.1 (family 4) and five healthy control subjects were cultured in Dulbecco’s modified Eagle medium (DMEM, Gibco) supplemented with 10% fetal calf serum (FCS, Gibco) and antibiotics at 5% CO\textsubscript{2} and 37°C. Before treatment, cells were starved in DMEM supplemented with 0.1% FCS and antibiotics for 24 hrs. Treatment of cells was carried out with 100 nM Smoothened Agonist (SAG, Cayman Chemical), 1 µM vismodegib (Selleckchem) or a combination of both reagents for 16 hrs. SAG and vismodegib were solved in DMSO. DMSO-treated cells served as controls. Experiments were performed in biological triplicates.

**Cilia formation and immunofluorescence staining**

For analysis of cilia formation, fibroblasts were grown on coverslips to 90%-95% confluency. After serum starvation for 24 hrs, cells were fixed for 10 min with 4% paraformaldehyde at room temperature, permeabilized with 1x TBS containing 0.5% Triton X-100, and blocked with Tropix I-Block (Applied Biosystems) in 1x TBS containing 0.1% Triton X-100 (PBST) for
20 min. Thereafter, cells were stained with anti-acetylated tubulin (Sigma, T6793, 1:100) and anti-Smo (Abcam, ab38686, 1:500) antibodies overnight at 4°C, followed by incubation with Alexa488-conjugated anti-mouse (Jackson ImmunoResearch, 1:200) and Cy3-labeled anti-rabbit antibodies (Jackson ImmunoResearch, 1:200). Cells were mounted with ProLong Gold antifade reagent with DAPI (Thermo Fisher Scientific) and analyzed by confocal laser scanning microscopy (Olympus FLUOVIEW FV100, Olympus). Images were processed with Adobe Photoshop CS5.

**Real-time quantitative PCR**

Total RNA was extracted from dermal fibroblasts using TRIzol Reagent (Thermo Fisher Scientific). Synthesis of cDNA was performed using the Superscript II reverse transcriptase (Invitrogen) and random hexamer primers. Gene expression for *GLI1, GLI2, GLI3, HIP1,* and *PTCH1* was quantified by SYBR Green-based qRT-PCR assays on an ABI Prism HT 7900 Detection System instrument (Applied Biosystems) by using the primer pairs listed in Table S1. Data were analyzed by the standard curve method for relative quantification. Experiments were performed with two different passages per fibroblasts each analyzed in biological triplicates, which were measured in technical triplicates. Amplification of 18S rRNA and *HPRT* were used as endogenous controls for the normalization of target gene expression.

**Statistical analysis**

qRT-PCR data analysis and determination of statistical differences were performed using the software GraphPad Prism 6 by nonparametric Mann–Whitney testing. Values were considered significant when p < 0.05.
Figure S1
Expression of Hedgehog signaling signature genes in COMA-patient derived dermal fibroblasts

A
Figure S1 B
**Legend to Figure S1**

Quantitative real-time PCR-based expression analyses of the Hedgehog signaling signature genes GLI1, GLI2, GLI3, HIP and PTCH1 normalized to 18S rRNA (A) or HPRT (B) expression levels, respectively, of controls (N=5, grey) and COMA-patient derived fibroblasts (COMA) (N=4, red). Shown results represent data of 2 different cellular passages per fibroblast culture each analyzed in biological triplicates (grey circles) that were measured in technical triplicates. Treatment of cells was carried out with 100 nM Smoothened Agonist (SAG, Cayman Chemical), 1µM Vismodegib (Selleckchem), a combination of both reagents or DMSO only for 16 hrs. Total mean value +/- SEM of all analyzed samples are indicated in black. Significant differences were tested by nonparametric Mann-Whitney tests. *p< 0.05; **p< 0.01; ***p< 0.001.
| target | primer | sequence | amplicon |
|--------|--------|----------|----------|
| GLI1   | forward | 5'-AGC TAC ATC AAC TCC GGC CA-3' | 116 bp |
|        | reverse | 5'-GCT GCG GCG TTC AAG AGA-3' |          |
| GLI2   | forward | 5'-AAG CCC TTC AAG GCG CAG TA-3' | 170 bp |
|        | reverse | 5'-TCG TGC TCA CAC ACA TAT GGC TT-3' |          |
| GLI3   | forward | 5'-GCC AGC GCA GCC CCT AT-3' | 128 bp |
|        | reverse | 5'-CGG CCT GGC TGA CAG CCT-3' |          |
| HIP    | forward | 5'-ATG GTG GGT TGT GCT TTC CA-3' | 130 bp |
|        | reverse | 5'-CAG AAG CAG TTG TGT TTG TGC T-3' |          |
| PTCH1  | forward | 5'-GAG GTT GGT CAT GGT TAC ATG GA-3' | 196 bp |
|        | reverse | 5'-TGC TGT TCT TGA CTG TGC CAC C-3' |          |
| HPRT   | forward | 5'-TGG CGT CGT GAT TAG TGA TG-3' | 134 bp |
|        | reverse | 5'-CGA GCA AGA CGT TCA GTC CT-3' |          |
| 18S    | forward | 5'-CGCAAATTACCCACTCCCCG 3' | 81 bp |
|        | reverse | 5'-TTC CAA TTA CAG GGC CTC GAA-3' |          |
# Congenital Ocular Motor Apraxia (COMA) questionnaire

Reporting physician: _____________________________________________

## Patient data

**Date of birth:**

- Patient #:……..
- Month/year _____ / __________

**Sex:**

- □ female
- □ male

## Birth

**Gestational age:**

- □ term
- □ preterm (…. weeks GA)

**Perinatal complications:**

- □ no
- □ yes
  - If any, which? _______________________________

## Family history

**Other family members affected:**

- □ no
- □ yes
  - If any, who? _______________________________

**Consanguinity of parents:**

- □ no
- □ yes

## Developmental data

**Age at unaided walking:**

- _____ years _____ months

**Speech delay:**

- □ no
- □ yes

## Ocular findings

**Ocular motor apraxia:**

- Onset at age _____ months

**Course:**

- □ attenuating
- □ normal
- □ increasing

**Jerking head movements:**

- □ no
- □ yes, at age ....

**Nystagmus:**

- □ no
- □ yes, at age....

**Involvement of vertical eye movements:**

- □ no
- □ yes, at age....

## Neurological findings

**Ataxia:**

- □ no
- □ yes, □ trunc, □ limbs

**Muscular hypotonia:**

- □ no
- □ yes

**Cognitive development:**

- □ normal
□ impaired

In case psychological test results available:
□ intellectual disability (IQ<70)
□ learning disability (IQ<85)
□ normal (IQ>85)

Epilepsy:
□ no
□ yes

Organ involvement

Hepatic involvement:
□ no
□ yes

Elevated liver enzymes
□ no
□ yes
ALT: ___________ U/l
AST: ___________ U/l

Renal involvement:
□ no
□ yes

Elevated serum creatinine:
□ no
□ yes
creatinine: ______ mg/dl or ______ μmol/l

Polyuria/Polydipsia:
□ no
□ yes
□ unknown

Other clinical findings

Irregular breathing pattern in neonatal age (i.e. apnoe, tachypnoe):
□ no
□ yes
□ unknown

Retinal anomaly (i.e. chorioretinal coloboma?)
□ no
□ yes
□ unknown

Dysmorphic facial features?
□ no
□ yes
If any, which? _______________________________
_____________________________________________

Skeletal features (i.e. polydactyly)?
□ no
□ yes
If any, which? _______________________________
_____________________________________________

Other clinical symptoms/abnormalities?
□ no
□ yes
If any, which? _______________________________
_____________________________________________

Previous genetic testing:
□ none
□ these tests were performed:
_____________________________________________
_____________________________________________
_____________________________________________
This questionnaire used in this study was published previously as additional file with reference 12:

Wente S., et al. Nosological delineation of congenital ocular motor apraxia type Cogan: an observational study. Orphanet J Rare Dis. 2016;11(1),104. doi:10.1186/s13023-016-0486-z