Dr. Jane Doe
Paul-Ehrlich-Str. 23
D-72076 Tübingen

Proband 1	XXX, XX
ID #	Female (*DD.MM.YYYY)
Sample receipt	xxx
Material	EDTA blood
Proband 2	XXX, XX
ID #	Male (*DD.MM.YYYY)
Sample receipt	xxx
Material	EDTA blood
Report date	xxx
Report-ID	R#

Family Planning Analysis Report

Order   Family planning analysis

Result: Report with Significant Findings

- No detection of a pathogenic repeat expansion in the gene *FMR1* in the female proband.

- No detection of carrier status of a pathogenic copy number variant in gene *SMN1* in both probands.

- **Detection of carrier status for a pathogenic variant in the female proband and a pathogenic intragenic deletion in gene *RTTN* in the male proband.**

Gene	Phenotype	Heredity	Allele 1	Allele 2	Normal	Intermediate	Premutation	Pathogenic for
FMR1	FXTAS (300623) FXS (300624) POF1 (311360)	XLD	27±1	29±1	5-44	45-54	55-200	>200 repeats

Gene	Variant	Zygosity female proband	Zygosity male proband	Heredity	MAF (%)	Classification
RTTN	**c.3705C>A; p.Tyr1235*** chr18:67776932 G>T (hg19)	**het.**	**-**	**AR**	**< 0.01**	**pathogenic**
RTTN	**deletion of exons 19-49** chr18:67671387-67809623del (hg19)	**-**	**het.**	**AR**	**-**	**pathogenic**

Information for the interpretation of this table can be found in section *Additional Information*.

Recommendation

Prenatal testing for the pathogenic variants in gene *RTTN* is possible upon genetic counselling.

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DIN EN ISO 15189:2014

Genetic Relevance

Both probands are heterozygous carriers of a pathogenic alteration in gene *RTTN*. Therefore, there is a disease risk of 25 % for future children of this couple.

Please note that the detected variants may be of relevance for at-risk family members.

Clinical Information and Variant Interpretation

RTTN, NM_173630.4

OMIM / Reference	Phenotype	Heredity
614833	Microcephaly, short stature, and polymicrogyria with seizures (MSSP)	AR

The gene *RTTN* encodes the protein rotatin, which is thought to play a role in cilia function (Faisst et al. 2002, PMID: 11900971). Changes in *RTTN* can cause an autosomal recessive inherited disorder manifested by polymicrogyria, lissencephaly, cerebellar hypoplasia, microcephaly, short stature, epileptic seizures and mental retardation including language development disorder, whereby the clinical spectrum can be very heterogeneous and variable (Kheradmand Kia et al. 2012, PMID: 22939636; Shamseldin et al. 2015, PMID: 26608784; Vandervore et al. 2019, PMID: 30879067).

RTTN, c.3705C>A; p.Tyr1235* (het.), ClinVar ID: 871897

ACMG/ACGS Criterion	Points	Description
PVS1	+8	The variant likely results in a loss (or truncation) of the protein, which is a known pathomechanism for *RTTN*-associated disease.
PM2	+2	This variant is listed in the gnomAD global population dataset with a very low frequency.
ACMG/ACGS Classification: pathogenic	+10	
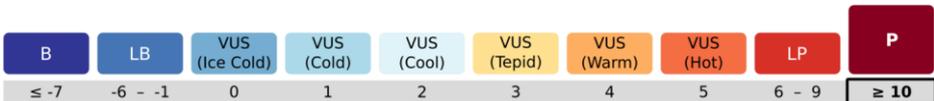
RTTN, deletion of exons 19-49 (het.)

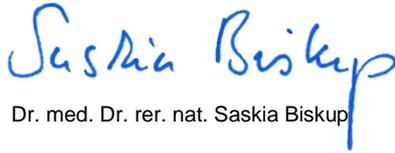| Evidence |
|---|
| This deletion results in the loss or truncation of a protein, which coincides with the known pathomechanism for the associated disease. |
| This aberration is not present in population databases or occurs at a very low allele frequency within the general population, which is consistent with reduced penetrance (gnomAD, DGV, DECIPHER). |

Classification: pathogenic

According to § 10 of the German Genetic Diagnostics Legislation, appropriate genetic counseling should be offered with all diagnostic genetic testing.

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Medical report written by: XXX

Proofread by: XXX

Validated by: XXX

With kind regards

Saskia Biskup

Dr. med. Dr. rer. nat. Saskia Biskup

Consultant for Human Genetics

Additional Information

Analyzed Regions	**Family planning panel analysis was performed for the two individuals described above using whole exome sequencing data.**

FMR1 (CGG-Repeat) analyzed for the female proband only, *SMN1* (Del/Dup)

AAAS, AARS1, AARS2, ABAT, ABCA12, ABCA3, ABCB11, ABCB4, ABCB7, ABCC6, ABCC8, ABCC9, ABCD1, ABCD4, ABHD12, ABHD5, ACACA, ACAD9, ACADM, ACADS, ACADSB, ACADVL, ACAN, ACAT1, ACD, ACE, ACO2, ACOX1, ACOX2, ACP5, ACSL4, ACTA1, ACTL6B, ACY1, ADA, ADA2, ADAM17, ADAM22, ADAMTS13, ADAMTS19, ADAMTS2, ADAMTSL2, ADAR, ADARB1, ADAT3, ADCY1, ADCY5, ADCY6, ADGRG1, ADGRG6, ADGRV1, ADK, ADPRS, ADSL, AFF2, AFG2A, AFG3L2, AGA, AGK, AGL, AGPAT2, AGPS, AGRN, AGT, AGTPBP1, AGTR1, AGXT, AHCY, AHI1, AIFM1, AIMP1, AIMP2, AIPL1, AIRE, AK2, AKR1D1, ALAD, ALDH18A1, ALDH1A3, ALDH3A2, ALDH4A1, ALDH5A1, ALDH6A1, ALDH7A1, ALDOA, ALDOB, ALG1, ALG11, ALG12, ALG13, ALG14, ALG2, ALG3, ALG6, ALG8, ALG9, ALMS1, ALOX12B, ALOXE3, ALPL, ALS2, ALX3, ALX4, AMACR, AMER1, AMN, AMPD1, AMPD2, AMT, ANK3, ANKLE2, ANKS6, ANO10, ANO5, ANOS1, ANTXR1, ANTXR2, AP1B1, AP1S1, AP1S2, AP3B1, AP3B2, AP3D1, AP4B1, AP4E1, AP4M1, AP4S1, APC2, APTX, AQP2, AR, ARFGEF2, ARG1, ARHGDIA, ARHGEF9, ARL13B, ARL3, ARL6, ARL6IP1, ARMC9, ARNT2, ARPC1B, ARSA, ARSB, ARSL, ARV1, ARX, ASAH1, ASCC1, ASL, ASNS, ASPA, ASPH, ASPM, ASS1, ATAD1, ATAD3A, ATCAY, ATIC, ATM, ATOH7, ATP13A2, ATP1A2, ATP2B3, ATP5F1D, ATP5MK, ATP6AP1, ATP6AP2, ATP6V0A2, ATP6V0A4, ATP6V1A, ATP6V1B1, ATP6V1E1, ATP7A, ATP7B, ATP8A2, ATP8B1, ATPAF2, ATR, ATRX, AUH, AVIL, B3GALNT2, B3GALT6, B3GAT3, B3GLCT, B4GALNT1, B4GALT1, B4GALT7, B4GAT1, B9D1, B9D2, BANF1, BBIP1, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, BCAP31, BCKDHA, BCKDHB, BCKDK, BCOR, BCS1L, BGN, BHLHA9, BIN1, BLM, BLNK, BLTP1, BMP1, BMP2, BMPER, BMPR1B, BOLA3, BPNT2, BRAT1, BRCA1, BRCA2, BRF1, BRWD3, BSCL2, BSND, BTD, BTK, BUB1B, C12orf57, C19orf12, C1QBP, C2CD3, C2orf69, CA2, CA5A, CA8, CABP2, CACNA1D, CAD, CAMK2A, CANT1, CAPN3, CARD11, CARMIL2, CARS2, CASK, CASQ2, CASR, CAV1, CAVIN1, CBS, CC2D1A, CC2D2A, CCBE1, CCDC103, CCDC115, CCDC22, CCDC39, CCDC40, CCDC47, CCDC65, CCDC8, CCDC88A, CCDC88C, CCN6, CCNO, CCNQ, CCT5, CD19, CD247, CD27, CD3D, CD3E, CD3G, CD40, CD40LG, CD55, CD70, CD79A, CD79B, CDC14A, CDC45, CDH11, CDH2, CDH23, CDH3, CDIN1, CDK10, CDK5RAP2, CDKL5, CDSN, CDT1, CENPF, CENPJ, CEP104, CEP120, CEP135, CEP152, CEP164, CEP290, CEP41, CEP55, CEP57, CEP63, CEP78, CEP83, CERS1, CERS3, CFAP298, CFAP300, CFAP410, CFAP418, CFL2, CFP, CFTR, CHAT, CHKB, CHM, CHMP1A, CHRDL1, CHRNA1, CHRNB1, CHRND, CHRNE, CHRNG, CHST14, CHST3, CHSY1, CHUK, CIB2, CIITA, CILK1, CISD2, CIT, CKAP2L, CLCN1, CLCN2, CLCN4, CLCN5, CLCN7, CLCNKB, CLDN1, CLDN10, CLDN14, CLDN16, CLDN19, CLIC5, CLMP, CLN3, CLN5, CLN6, CLN8, CLP1, CLPB, CLPP, CLRN1, CNKSR2, CNNM2, CNPY3, CNTNAP1, CNTNAP2, COA6, COA8, COASY, COCH, COG1, COG2, COG4, COG5, COG6, COG7, COL11A1, COL11A2, COL13A1, COL17A1, COL18A1, COL1A2, COL27A1, COL3A1, COL4A3, COL4A4, COL4A5, COL6A1, COL6A2, COL6A3, COL7A1, COL9A2, COLEC10, COLEC11, COLQ, COQ2, COQ4, COQ6, COQ7, COQ8A, COQ8B, COQ9, CORO1A, COX10, COX14, COX15, COX20, COX6A2, COX6B1, COX7B, COX8A, CPLANE1, CPLX1, CPS1, CPT1A, CPT2, CRADD, CRB1, CRB2, CRBN, CREB3L1,

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CRIPT, CRLF1, CRPPA, CRTAP, CRYAA, CRYAB, CSF1R, CSF3R, CSPP1, CSTA, CSTB, CTC1, CTDP1, CTNNA2, CTNS, CTPS1, CTSA, CTSD, CTSK, CTU2, CUL4B, CUL7, CWC27, CWF19L1, CYB5R3, CYBA, CYBB, CYC1, CYP11A1, CYP11B1, CYP11B2, CYP17A1, CYP24A1, CYP27A1, CYP27B1, CYP2R1, CYP2U1, CYP4F22, CYP7B1, D2HGDH, DAG1, DARS1, DARS2, DBT, DCAF17, DCDC2, DCHS1, DCLRE1C, DCX, DDB2, DDC, DDHD1, DDHD2, DDR2, DDX11, DDX3X, DDX59, DEAF1, DEGS1, DENND5A, DGAT1, DGKE, DGUOK, DHCR24, DHCR7, DHDDS, DHH, DHODH, DHTKD1, DHX37, DIAPH1, DIS3L2, DKC1, DLAT, DLD, DLG3, DLL3, DLX5, DMD, DMP1, DMXL2, DNA2, DNAAF11, DNAAF3, DNAAF4, DNAAF5, DNAAF6, DNAH11, DNAH5, DNAH9, DNAJC12, DNAJC19, DNAJC21, DNAJC3, DNAJC6, DNM1L, DNM2, DNMT3B, DOCK2, DOCK6, DOCK7, DOCK8, DOK7, DOLK, DONSON, DPAGT1, DPH1, DPM1, DPM2, DPYD, DRC1, DSE, DSG1, DSP, DST, DSTYK, DUOX2, DUOXA2, DYM, DYNC2H1, DYNC2I1, DYNC2I2, DYNC2LI1, DYSF, EARS2, EBP, ECEL1, ECHS1, EDA, EDAR, EDARADD, EDN3, EDNRB, EFEMP2, EFL1, EFNB1, EGR2, EIF2AK3, EIF2AK4, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, EIF2S3, EIF4A3, ELAC2, ELMO2, ELMOD3, ELOVL4, ELP1, ELP2, EMC1, EMC10, EMD, EMG1, EML1, ENPP1, ENTPD1, EOGT, EPCAM, EPG5, EPM2A, EPRS1, EPS8, EPS8L2, ERAL1, ERBB3, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6, ERCC6L2, ERCC8, ERLIN1, ERLIN2, ESCO2, ESPN, ESRRB, ETFA, ETFB, ETFDH, ETHE1, EVC, EVC2, EXOC3L2, EXOSC3, EXOSC8, EXOSC9, EXPH5, EXT2, EXTL3, F10, F13A1, F2, F7, F8, F9, FA2H, FADD, FAH, FAM149B1, FAM20A, FAM20C, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FAR1, FARS2, FASTKD2, FAT4, FBLN5, FBP1, FBXL4, FBXO7, FCSK, FERMT3, FEZF1, FGA, FGB, FGD1, FGD4, FGF3, FGFR3, FGG, FH, FHL1, FIG4, FITM2, FKBP10, FKBP14, FKRP, FKTN, FLAD1, FLNA, FLNB, FLVCR1, FLVCR2, FOLR1, FOXE1, FOXE3, FOXL2, FOXN1, FOXP3, FOXRED1, FRAS1, FREM1, FREM2, FRMPD4, FRRS1L, FSHB, FTCD, FTL, FTO, FTSJ1, FUCA1, FUT8, FXN, G6PC1, G6PC3, GAA, GAD1, GALC, GALE, GALK1, GALNS, GALT, GAMT, GAN, GAS8, GATA1, GATM, GBA1, GBA2, GBE1, GCDH, GCH1, GCK, GCSH, GDAP1, GDF1, GDF5, GDF6, GDI1, GEMIN4, GFER, GFM1, GFM2, GFPT1, GHR, GIPC3, GJA1, GJB2, GJB3, GJB6, GJC2, GK, GLA, GLB1, GLDC, GLDN, GLE1, GLIS3, GLRX5, GLS, GLUL, GLYCTK, GM2A, GMPPA, GMPPB, GNB5, GNPAT, GNPTAB, GNPTG, GNRH1, GNRHR, GNS, GOLGA2, GORAB, GOSR2, GOT2, GPAA1, GPC3, GPC6, GPHN, GPSM2, GPT2, GPX4, GRHL2, GRHPR, GRIA3, GRID2, GRIK2, GRIN1, GRIP1, GRM1, GRM7, GRXCR1, GSS, GTF2H5, GTPBP3, GUCY1A1, GUCY2C, GUF1, GUSB, GYS1, GYS2, GZF1, HACD1, HACE1, HADH, HADHA, HADHB, HAMP, HARS1, HARS2, HAX1, HBB, HCCS, HCFC1, HDAC8, HEPACAM, HERC1, HERC2, HES7, HESX1, HEXA, HEXB, HFE, HGF, HGSNAT, HIBCH, HIKESHI, HINT1, HJV, HK1, HLCS, HMGCL, HMGCS2, HMX1, HNRNPH2, HOGA1, HOXA1, HOXC13, HPD, HPDL, HPGD, HPRT1, HPS1, HPSE2, HSD11B2, HSD17B10, HSD17B3, HSD17B4, HSD3B2, HSD3B7, HSPA9, HSPD1, HSPG2, HTRA2, HUWE1, HYAL1, HYCC1, HYDIN, HYLS1, IARS1, IARS2, IBA57, ICOS, IDS, IDUA, IER3IP1, IFIH1, IFNGR1, IFNGR2, IFT122, IFT140, IFT172, IFT27, IFT43, IFT52, IFT56, IFT74, IFT80, IFT81, IGBP1, IGF1, IGF1R, IGFBP7, IGHMBP2, IGSF1, IHH, IKBKB, IKBKG, IL10RA, IL11RA, IL12RB1, IL1RAPL1, IL1RN, IL21R, IL2RA, IL2RB, IL2RG, IL7R, ILDR1, INPP5E, INPP5K, INPPL1, INS, INSR, INTU, INVS, IPO8, IQCB1, IQSEC1, IQSEC2, IRAK4, IRF8, IRX5, ISCA1, ISCA2, ITCH, ITGA3, ITGA6, ITGA7, ITGA8, ITGB4, ITK, ITPA, ITPR1, IVD, JAGN1, JAK3, JAM2, JAM3, JUP, KARS1, KATNB1, KATNIP, KCNE1, KCNJ1, KCNJ10, KCNJ11, KCNMA1, KCNQ1, KCTD7, KDELR2, KDM5B, KDM5C, KDM6A, KIAA0586, KIAA0753, KIDINS220, KIF14, KIF1A, KIF1C, KIF7, KIFBP, KISS1R, KLHL15, KLHL40, KLHL41, KLHL7, KNL1, KPTN, KRT10, KRT14, KRT18, KRT5, KRT8, KY, L1CAM, L2HGDH, LAGE3, LAMA1, LAMA2, LAMA3, LAMB1, LAMB2, LAMB3, LAMC2, LAMC3, LAMP2, LARGE1, LARP7, LARS2, LAS1L, LAT, LBR, LDHA, LDLR, LFNG, LGI4, LHB, LHFPL5, LHX3, LIAS, LIFR, LIG4, LIMS2, LINS1, LIPA, LIPT1, LMBR1, LMBRD1, LMNA, LMOD3, LNPK, LONP1, LOXHD1, LPIN1, LPIN2, LPL, LRBA, LRP2, LRP4, LRP5, LRPPRC, LRRC56, LRTOMT, LTBP2, LTBP3, LTBP4, LYRM4, LYRM7, LYST, LZTFL1, LZTR1, MAB21L2, MAG, MAGI2, MAGT1, MALT1, MAMLD1, MAN1B1, MAN2B1, MANBA, MAOA, MAP3K20, MAPKBP1, MARS1, MARVELD2, MASP1, MAT1A, MATN3, MBOAT7, MBTPS2, MC2R, MCCC1, MCCC2, MCEE, MCM4, MCOLN1, MCPH1, MDH2, MECP2, MECR, MED12, MED17, MED23, MED25, MEFV, MEGF10, MEGF8, MEOX1, MESD, MESP2, MET, METTL23, METTL5, MFN2, MFRP, MFSD2A, MFSD8, MGAT2, MGME1, MGP, MICOS13, MICU1, MID1, MIPEP, MITF, MKKS, MKS1, MLC1, MLPH, MLYCD, MMAA, MMAB, MMACHC, MMADHC, MMP13, MMP2, MMP21, MMUT, MOCS1, MOCS2, MOGS, MPDU1, MPDZ, MPI, MPL, MPLKIP, MPV17, MPZ, MPZL2, MRE11, MRPL3, MRPL44, MRPS14, MRPS16, MRPS2, MRPS22, MRPS34, MSL3, MSMO1, MSN, MSRB3, MSTO1, MTFMT, MTHFD1, MTHFR, MTM1, MTMR2, MTO1, MTR, MTRFR, MTRR, MTTP, MUSK, MUTYH, MVK, MYBPC1, MYBPC3, MYD88, MYH11, MYH3, MYH7, MYL3, MYMK, MYO15A, MYO18B, MYO3A, MYO5A, MYO5B, MYO6, MYO7A, MYO9A, MYOD1, MYPN, MYSM1, NAA10, NADSYN1, NAGA, NAGLU, NAGS, NALCN, NANS, NARS1, NARS2, NAXD, NAXE, NBAS, NBN, NCAPD3, NCF1, NCF2, NCF4, NCKAP1L, NDE1, NDP, NDRG1, NDST1, NDUFA1, NDUFA10, NDUFA11, NDUFA12, NDUFA13, NDUFA2, NDUFA6, NDUFA9, NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5, NDUFAF6, NDUFAF8, NDUFB3, NDUFB8, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2, NEB, NECAP1, NECTIN1, NECTIN4, NEK1, NEK8, NEK9, NEMF, NEU1, NEUROG3, NEXMIF, NFASC, NFU1, NGF, NGLY1, NHEJ1, NHLRC1, NHP2, NHS, NIPAL4, NKAP, NKX3-2, NKX6-2, NMNAT1, NNT, NODAL, NONO, NOP10, NPC1, NPC2, NPHP1, NPHP3, NPHP4, NPHS1, NPHS2, NPR2, NR0B1, NR1H4, NRROS, NRXN1, NSDHL, NSMCE2, NSMCE3, NSUN2, NT5C2, NT5C3A, NTNG2,

NTRK1, NUBPL, NUDT2, NUP107, NUP133, NUP188, NUP62, NUP88, NUP93, NYX, OBSL1, OCLN, OCRL, ODAD1, ODAD2, OFD1, OGDH, OPA1, OPA3, OPHN1, ORAI1, ORC1, ORC4, ORC6, OSGEP, OSTM1, OTC, OTOA, OTOF, OTOG, OTOGL, OTUD5, OTUD6B, OTULIN, OXCT1, OXR1, P3H1, PAH, PAK3, PAM16, PANK2, PAPSS2, PARN, PARS2, PAX3, PC, PCBD1, PCCA, PCCB, PCDH12, PCDH15, PCDH19, PCK1, PCNT, PCSK1, PCYT1A, PCYT2, PDE10A, PDE6D, PDE6G, PDHA1, PDHB, PDHX, PDP1, PDSS1, PDSS2, PDZD7, PEPD, PERCC1, PET100, PEX1, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, PEX2, PEX26, PEX3, PEX5, PEX6, PEX7, PFKM, PGAP1, PGAP2, PGAP3, PGK1, PGM1, PGM3, PHEX, PHF6, PHF8, PHGDH, PHKG2, PHYH, PI4KA, PIBF1, PIEZO1, PIEZO2, PIGA, PIGB, PIGG, PIGK, PIGL, PIGN, PIGO, PIGP, PIGQ, PIGS, PIGT, PIGV, PIGY, PIK3CD, PIK3R1, PIP5K1C, PISD, PITX3, PJVK, PKD1L1, PKHD1, PKLR, PLA2G6, PLAA, PLCB1, PLCB4, PLCE1, PLEC, PLEKHG2, PLEKHG5, PLG, PLK4, PLOD1, PLOD2, PLOD3, PLP1, PLPBP, PLS3, PLVAP, PMM2, PMP22, PMPCA, PMPCB, PNKP, PNP, PNPLA1, PNPLA6, PNPLA8, 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WDPCP, WDR19, WDR35, WDR4, WDR45, WDR45B, WDR62, WDR73, WDR81, WFS1, WHRN, WNK1, WNT1, WNT10A, WNT10B, WNT2B, WNT3, WNT4, WNT7A, WRAP53, WRN, WWOX, XIAP, XPA, XPC, XRCC2, XRCC4, XYLT1, XYLT2, YARS2, YIF1B, ZAP70, ZBTB24, ZC3H14, ZC4H2, ZDHHC9, ZFYVE26, ZIC3, ZMPSTE24, ZNF335, ZNF711, ZNHIT3 (Family planning V.1)

General Remarks	Additional variants may be present within regions which were not analyzed (e.g. introns, promoter and enhancer regions and long repeats). A lower specificity enrichment and/or inaccurate variant calling cannot be ruled out for homologous regions with multiple genomic copies. The occurrence of low frequency somatic mosaicism cannot be reliably assessed using a pipeline optimized for germline variant detection, and may therefore remain undetected. Moreover, detection of large deletions and duplications is not guaranteed by next-generation high-throughput sequencing. Based upon our sequencing data, as well as MLPA analysis (*SMN1*) we did not detect any large deletions or duplications within the analyzed genes. The classification of variants may change in the future due to new evidence or improvements in scientific understanding.

Information for the interpretation of the tables

Heredity: AD – autosomal dominant, AR – autosomal recessive, XL – X-linked, mito – mitochondrial

MAF: The **minor allele frequency** describes the least frequent allele at a specific locus in a given population. For mitochondrial variants, the population frequency (MAF column) is based on the homoplasmic frequency within a reference population (gnomAD).

Classification: Variant classification is based on ACMG, ACGS-2020v4.01, and ClinGen SVI WG guidelines (Richards et al., 2015, PMID: 25741868; Ellard et al., 2020, Association for Clinical Genomic Science; https://clinicalgenome.org/working-groups/sequence-variant-interpretation/). The weighting of criteria and their modification follows the current ACGS guidelines in the strength levels *very strong* (+ 8), *strong* (+/- 4), *moderate* (+/- 2), and *supporting* (+/- 1). According to the respective category (pathogenic or benign) and criterion strength, positive or negative points are assigned as mentioned above (Tavtigian et al., 2020, PMID: 32720330). Variants of uncertain significance (VUS) are subcategorized into *hot, warm, tepid, cool, cold, and ice cold* VUS according to their likelihood of reaching a pathogenic classification in the future. Posterior probability decreases from 90% to 10% in this order (Ellard et al., 2020, Association for Clinical Genomic Science). If a variant reaches the classification pathogenic, after checking of all benign criteria, not necessarily all other applicable criteria are listed.

The chromosomal positions of variants listed in the report refer to the human reference genome hg19.

Methods

Repeat analysis: A repeat spanning polymerase chain reaction (PCR) was performed to determine the number of CGG repeats in the 5'-UTR region of the gene *FMR1*. Additionally, a repeat-primed PCR method specifically designed to detect larger repeat expansions was performed. This was followed by separation and sizing of the PCR fragments by capillary electrophoresis. The test was performed according to the standard protocol of the AmplideX®-FMR1-mPCR method. Since pathogenic *FMR1* repeat expansions are inherited maternally, only the sample of the female proband was examined.

Copy Number Analysis: Deletion and duplication analysis of the gene *SMN1* was performed using MLPA (MRC Holland P021-B1) as relative quantification in comparison to a reference sample DNA (a probemix does not necessarily contain probes for all exons of a certain gene).

If pathogenic alterations are present within a gene which are not the result of copy number changes (e.g. SNVs), these cannot be detected via MLPA unless covered by variant-specific probes, and therefore cannot be ruled out.

MLPA analysis cannot determine the allele configuration of copy number variants. In rare cases, the presence of an unexpected copy number distribution, e.g. a gene duplication on one allele and a deletion on the other allele, may lead to false negative results.

Sequencing: Protein-coding regions, as well as flanking intronic regions and additional disease-relevant non-coding regions, were enriched using in-solution hybridization technology, and were sequenced using the Illumina NovaSeq 6000/NovaSeq X Plus system.

NGS based CNV-Calling: Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth (only applicable for nuclear encoded genes). Briefly, we used reference samples to create a model of the expected coverage that represents wet-lab biases as well as inter-sample variation. *CNV calling* was performed by computing the sample's normalized coverage profile and its deviation from the expected coverage. Genomic regions are called as variant if they deviate significantly from the expected coverage. Copy number variants are named according to current ISCN guidelines. NGS based CNV-Calling will not detect copy number neutral structural variants such as balanced translocations, inversions, uniparental heterodisomy or low-level mosaicism. Aberrations within the pseudoautosomal region (PAR) cannot be detected with high accuracy. The integration site of duplications cannot be determined by NGS based CNV-Calling.

Please note that next generation sequencing based detection of copy number variations has lower sensitivity/specificity than a direct quantification method, e.g. MLPA. Copy-neutral structural aberrations cannot be detected using this method (e.g. balanced translocations and balanced inversions). The absence of reported CNVs therefore does not ultimately guarantee the absence of CNVs.

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Family planning report | XXX, XX (*DD.MM.YYYY) and XXX, XX (*DD.MM.YYYY) | Page 6 of 7

Computational Analysis: 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. Reads mapping to more than one location with identical mapping score were discarded. Read duplicates that likely result from PCR amplification were removed. The remaining high-quality sequences were used to determine sequence variants (single nucleotide changes and small insertions/deletions). The variants were annotated based on several internal as well as external databases.

Diagnostic data analysis: Variants were classified and reported based on ACMG/ACGS-2020v4.01 guidelines (Richards et al., 2015, PMID: 25741868, Ellard et al., 2020, Association for Clinical Genomic Science).

Only variants (SNVs/Small Indels) in the coding region and the flanking intronic regions (±8 bp) with a minor allele frequency (MAF) < 1.5% are evaluated. Known disease-causing variants (according to HGMD) are evaluated in up to ±30 bp of flanking regions and up to 5% MAF. Minor allele frequencies are taken from public databases (e.g. gnomAD) and an in-house database. Candidate CNV calls are evaluated manually. Potentially pathogenic findings are validated with a second method, like MLPA, on a case-by-case basis.

Family planning analysis: Variants (SNVs/CNVs) found in the probands were compared and filtered for the following cases 1) both probands are heterozygous carriers of pathogenic/likely pathogenic variants in the same gene located on an autosome; 2) the female proband is carrier of a pathogenic/likely pathogenic variant in a gene located on the X-chromosome; 3) one proband is carrier of a pathogenic/likely pathogenic variant in a gene known to underly maternal/paternal genetic imprinting. 4) one proband is carrier of a pathogenic/likely pathogenic variant in mosaic state, which is a possible causative factor for a severe early childhood autosomal dominant inherited disorder. Analysis is limited to severe childhood-onset phenotypes. Variants of uncertain significance may be considered in cases where a second pathogenic/likely pathogenic variant is detected in the other proband. Family planning analysis is only applicable for couples where both probands are not affected by genetic disease at the time of analysis. This medical report does not rule out a baseline risk for the occurrence of genetic diseases in children of this couple.

97.77%, and 97.9% of the targeted regions were covered by a minimum of 30 high-quality sequencing reads per base for the female and male proband, respectively.

The evaluation of variants is dependent on available scientific information at the time of analysis. The medical report contains all variants not classified as uncertain, benign or likely benign according to current literature. *In silico* predictions were performed using the programs MetaLR (Dong et al., 2015, PMID: 25552646), PrimateAI (Sundaram et al., 2018, PMID: 30038395), and SpliceAI (Jaganathan et al., 2019, PMID: 30661751). This prediction can be complemented with additional *in silico* predictions in individual cases.

Variants are named according to the HGVS recommendations without any information regarding the cis or trans configuration.

The sample fulfilled our quality criteria upon arrival and during/after each processing step in the laboratory.

The procedure described above was developed and validated in-house (Laboratory developed test; LDT).

Communication, dissemination and usage of this report for scientific purposes is only permitted in accordance with the German Genetic Diagnostics Legislation.

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