

Supplementary Materials and Methods

Sanger Sequencing

Genomic DNA samples from blood were analyzed for variants in *BLM* and *RMI1* by using specific primers for the regions of interest including the exon-intron boundaries. Following amplification, Sanger sequencing reactions were carried out with the Big Dye Terminator v3.1 (Applied Biosystems, USA) according to the instructions of the manufacturer, followed by capillary electrophoresis on an ABI 3500XL sequencer (Applied Biosystems, USA). Detailed primer sequences and PCR conditions are available upon request. When a variant was detected, the parents were also investigated for the corresponding region in the same way.

Microcephaly panel sequencing

An NGS-based in-house microcephaly panel (Agilent Technologies, Santa Clara, CA) covering 93 microcephaly-related genes including the intron-exon boundaries was used for the diagnosis of one patient (www.humangenetik-umg.de). List of the genes included in this panel is given in Supplementary Table 1. DNA was extracted from blood and enriched with the SureSelect QXT method, PCR-amplified, and sequenced on the Illumina NextSeq system. The sequencing data was evaluated in comparison to the reference sequence (from www.ensembl.org) with the Sequence Pilot software (jsi medical systems GmbH; Version 5.0.0 Build 506). Detected unknown variants were evaluated by computer programs such as SIFT¹ (<https://sift.bii.a-star.edu.sg>), MutationTaster² (www.mutationtaster.org), PolyPhen2³ (<http://genetics.bwh.harvard.edu/pph2/>), M-CAP (<http://bejerano.stanford.edu/mcap/>), Human Splicing Finder (<http://www.umd.be/HSF3/HSF.shtml>), BDGP Splice Site Prediction (https://fruitfly.org/seq_tools/splice.html), or with the Alamut program (Interactive Biosoftware). The identified BS phenotype-related variants were confirmed via Sanger sequencing.

Whole Exome Sequencing

DNA samples were enriched using Agilent SureSelect Human All ExonV6 r2 kit and sequenced on an Illumina HiSeq4000 sequencer. The variants were analyzed using the “Varbank” pipeline from Cologne Center for Genomics (CCG) by applying the following filter criteria⁴: coverage of >6 reads, quality score of >10, allele frequency $\geq 25\%$, and a minor allele frequency <0.1% in the 1000Genomes database and the Exome Variant Server (EVS; NHLBI Exome Sequencing Project).

References

1. Sim N-L, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Res.* 2012;40(W1):W452-W457. doi:10.1093/nar/gks539
2. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods.* 2014;11(4):361-362. doi:10.1038/nmeth.2890
3. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods.* 2010;7(4):248-249. doi:10.1038/nmeth0410-248
4. Neuhofer CM, Funke R, Wilken B, et al. A Novel Mutation in PIGA Associated with Multiple Congenital Anomalies-Hypotonia-Seizure Syndrome 2 (MCAHS2) in a Boy with a Combination of Severe Epilepsy and Gingival Hyperplasia. *Mol Syndromol.* 2020;11(1):30-37. doi:10.1159/000505797

Supplementary Table 1. List of genes of the BS/microcephaly multigene panel.

Gene	Location	Gene	Location	Gene	Location
<i>ANKLE2</i>	12q24.33	<i>ERCC2</i>	19q13.32	<i>PAFAH1B1 (LIS1)</i>	17p13.3
<i>ARX</i>	Xp21.3	<i>ERCC4</i>	16p13.12	<i>PALB2</i>	16p12.2
<i>ASPM</i>	1q31.3	<i>ERCC5</i>	13q33.1	<i>PCNA</i>	20p12.3
<i>ATR</i>	3q23	<i>ERCC6</i>	10q11.23	<i>PCNT</i>	21q22.3
<i>ATRIP</i>	3p21.31	<i>ERCC6</i>	10q11.23	<i>PHC1</i>	12p13.31
<i>B9D1</i>	17p11.2	<i>ERCC8</i>	5q12.1	<i>PHF6</i>	Xq26.2
<i>B9D2</i>	19q13.2	<i>FAAP95</i>	Xp22.2	<i>PHF9</i>	2p16.1
<i>BLM</i>	15q26.1	<i>FANCA</i>	16q24.3	<i>PIEZO2</i>	18p11.22-11.21
<i>BRAT1</i>	7p22.3	<i>FANCC</i>	9q22.32	<i>PPM1D</i>	17q23.2
<i>BRCA2</i>	13q13.1	<i>FANCD2</i>	3p25.3	<i>RAD50</i>	5q31.1
<i>BRIP1</i>	17q23.2	<i>FANCE</i>	6p21.31	<i>RAD51C</i>	17q22
<i>CASC5</i>	15q15.1	<i>FANCF</i>	11p14.3	<i>RBBP8</i>	18q11.2
<i>CASK</i>	Xp11.4	<i>FANCI</i>	15q26.1	<i>RELN</i>	7q22.1
<i>CC2D2A</i>	4p15.32	<i>GMNN</i>	6p22.3	<i>RMI1</i>	9q21.32
<i>CDC6</i>	17q21.2	<i>HNRNPU</i>	1q44	<i>RNU4atac</i>	2q14.2
<i>CDK5</i>	7q36.1	<i>KATNB1</i>	16q21	<i>RPGRIP1L</i>	16q12.2
<i>CDK5RAP2</i>	9q33.2	<i>KIF11</i>	10q23.33	<i>RTTN</i>	18q22.2
<i>CDK6</i>	7q21.2	<i>KIF14</i>	1q32.1	<i>SASS6</i>	1p21.2
<i>CDT1</i>	16q24.3	<i>KMT2B</i>	19q13.12	<i>SLX4</i>	16p13.3
<i>CENPE</i>	4q24	<i>LAMB1</i>	7q31.1	<i>STIL</i>	1p33
<i>CENPJ</i>	13q12.12-q12.13	<i>LIG4</i>	13q33.3	<i>TCTN2</i>	12q24.31
<i>CEP135</i>	4q12	<i>MCPH1</i>	8p23.1	<i>TMEM216</i>	11q12.2
<i>CEP152</i>	15q21.1	<i>MFSD2A</i>	1p34.2	<i>TMEM231</i>	16q23.1
<i>CEP290</i>	12q21.32	<i>MKS1</i>	17q22	<i>TMEM67</i>	8q22.1
<i>CEP63</i>	3q22.2	<i>NBN</i>	8q21.3	<i>TRAIP</i>	3p21.31
<i>CKAP2L</i>	2q14.1	<i>NHEJ1</i>	2q14.1	<i>TUBA1A</i>	12q13.12
<i>DCX</i>	Xq23	<i>NIN</i>	14q22.1	<i>UBE2T</i>	1q32.1
<i>DDX11</i>	12p11.21	<i>NPHP3</i>	3q22.1	<i>WDR62</i>	19q13.12
<i>DHCR7</i>	11q13.4	<i>ORC1</i>	1p32.3	<i>XRCC4</i>	5q14.2
<i>DNA2</i>	10q21.3	<i>ORC4</i>	2q23.1	<i>XRCC9</i>	9p13.3
<i>ERCC1</i>	19q13.32	<i>ORC6</i>	16q11.2	<i>ZNF335</i>	20q13.12