Whole Exome Sequencing (WES)

To rapidly identify the disease causing variant, four exomes of Family 1 were sequenced (Fig.1A, left) after whole-exome enrichment (IntegraGen, Evry, France). Exons of DNA samples were captured and investigated as shown before with in-solution enrichment methodology (SureSelect Human All Exon Kits version 3, Agilent, Massy, France) and next-generation sequencing (NGS) (Illumina HISEQ, Illumina, San Diego, CA, USA). Image analysis and base calling were performed with Real Time Analysis software (Illumina). Genetic variation annotations were performed by an in-house pipeline (IntegraGen) (1, 2).

Targeted Next-Generation Sequencing (NGS)

The affected index cases from Family 2 and 3 (Fig.1A, middle and right) were sequenced by targeted NGS (MedGenome Labs Pvt. Ltd., Bengaluru, India) for the fourteen CSNB, Oguchi disease and fundus albipunctatus [MIM 258100, MIM 613411] candidate genes (*GRM6*, NM_000843; *NYX*, AJ278865; *TRPM1*, NM_002420.4; *GPR179*, NM_001004334.2; *LRIT3*, NM_198506.4; *CACNA1F*; *CABP4*; *CACNA2D4*; *RHO*, NM_000539.3; *PDE6B*, NM_000283.3; *GNAT1*, NM_144499.2; *SLC24A1*, NM_004727.2; *SAG*, NM_000541.4 [MIM 181031] and *GRK1*, NM_002929.2 [MIM 180381]). Targeted regions were captured using Agilent SureSelect custom designed probes and sequenced on Illumina HiSeq 2000 (Illumina, San Diego, CA, USA). The targeted NGS data analysis was performed by standard bioinformatics pipeline. The raw reads were filtered and aligned with hg19 reference sequence from UCSC Genome Browser (http://genome.ucsc.edu/) database. The variants were called and annotated using Genome Analysis TKLite-2.3-9 toolkit.

Investigation of annotated sequencing data

Results of WES and targeted NGS were provided per sample on ERIS server (http://eris.integragen.com/) or tabulated text files. Stringent filtering was applied to identify

the disease causing variants among 19,117 variants for WES and among 200 variants for targeted NGS. For Family 1, we included only variants present at a maximum frequency between 0 and 0.5 % in Exome Variant Server (EVS) (3), 1000 Genome Project (4) and HapMap (5) databases (1, 2, 6). The results were further filtered so that only homozygous, hemizygous or compound heterozygous variants in coding regions or splice sites (the first five nucleotides and the last five nucleotides of each intron) remained in the index patient and affected brother, which were heterozygous in the parents. For Family 2 and 3, homozygous, compound heterozygous or hemizygous variants only present at a maximum frequency between 0 and 0.5% in the coding regions and splice junctions of the fourteen CSNB, Oguchi disease and fundus albipunctatus candidate genes were filtered in EVS, 1000 Genome Project and Human Gene Mutation (7) databases. Allele frequency of identified variants was further checked in dbSNP, EVS and Exome Aggregation Consortium (ExAC, Cambridge, MA, USA: http://exac.broadinstitute.org/) databases. Identified amino acid substitutions were evaluated in respect to the conservation (UCSC Genome Browser, GRCh37/hg19), pathogenicity predictions (Sorting Intolerant from Tolerant (SIFT) (8): http://sift.jcvi.org/, PolyPhen-2 (9): http://genetics.bwh.harvard.edu/pph2/, and MutationTaster2 (10): http://www.mutationtaster.org/) and *in-silico* splice-site predictions (Alamut Visual, Interactive Biosoftware, Rouen, France). The most likely disease causing variants were confirmed by Sanger sequencing and co-segregation analyses (1, 2). In Family 2 and 3, the suspected disease causing variants were further screened in 100 healthy ethnically matched control individuals (200 chromosomes).

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