Identification of deep-intronic variants in *CCM1*, *CCM2* and *CCM3*

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Cerebral cavernous malformations (CCM; OMIM 116860, 603284, 603285) are vascular lesions of the central nervous system that occur in familial (autosomal dominant) and isolated forms. CCMs are characterized by dysfunctional cell-cell junctions of the vascular endothelium. Clinical symptoms include recurrent headaches, seizures and hemorrhagic stroke. Loss-of-function mutations have been identified in three genes: *CCM1*, *CCM2* and *CCM3*.

Despite stringent inclusion criteria, standard genetic screening of the coding regions and the adjacent exon-intron boundaries fails to identify causative mutations in 13 % of familial and 43 % of isolated cases. Therefore, twenty mutation-negative index cases were selected for NGS-based resequencing of the entire genomic regions of *CCM1-3* to detect deep-intronic variants.

A long-range PCR approach for target enrichment and library preparation using Nextera XT kit (Illumina®) was applied. By paired-end-sequencing on a MiSeq benchtop sequencer (2x250 cycles; Illumina®), 98% of the target region were covered by a minimum of 50x. A total of 862 variants were detected of which 51 were unique. Splice site predictions and global scores like CADD (Combined Annotation Dependent Depletion) indicated functional relevance for 14 rare or previously not identified variants. Transcript analyses are ongoing to verify the suspected effect on splicing.

This approach proves to be an efficient and cost effective method to detect probably disease causing mutations not only in exonic but also in deep-intronic and regulatory regions of the known *CCM* genes and might be an alternative to Sanger sequencing in CCM diagnostics.