Next-generation sequencing of a 40 Mb linkage interval reveals TSPAN12 mutations in patients with familial exudative vitreoretinopathy.

Familial exudative vitreoretinopathy (FEVR) is a genetically heterogeneous retinal disorder characterized by abnormal vascularisation of the peripheral retina, often accompanied by retinal detachment. To date, mutations in three genes (FZD4, LRP5, and NDP) have been shown to be causative for FEVR. In two large Dutch pedigrees segregating autosomal-dominant FEVR, genome-wide SNP analysis identified an FEVR locus of approximately 40 Mb on chromosome 7. Microsatellite marker analysis suggested similar at-risk haplotypes in patients of both families. To identify the causative gene, we applied next-generation sequencing in the proband of one of the families, by analyzing all exons and intron-exon boundaries of 338 genes, in addition to microRNAs, noncoding RNAs, and other highly conserved genomic regions in the 40 Mb linkage interval. After detailed bioinformatic analysis of the sequence data, prioritization of all detected sequence variants led to three candidates to be considered as the causative genetic defect in this family. One of these variants was an alanine-to-proline substitution in the transmembrane 4 superfamily member 12 protein, encoded by TSPAN12. This protein has very
recently been implicated in regulating the development of retinal vasculature, together with the proteins encoded by FZD4, LRP5, and NDP. Sequence analysis of TSPAN12 revealed two mutations segregating in five of 11 FEVR families, indicating that mutations in TSPAN12 are a relatively frequent cause of FEVR. Furthermore, we demonstrate the power of targeted next-generation sequencing technology to identify disease genes in linkage intervals.