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Supplemental Data

Next-Generation Sequencing of a 40 Mb Linkage Interval Reveals *TSPAN12* Mutations in Patients with Familial Exudative Vitreoretinopathy

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Figure S1. Coverage histogram for the complete 40.5-Mb FEVR locus (part 1)

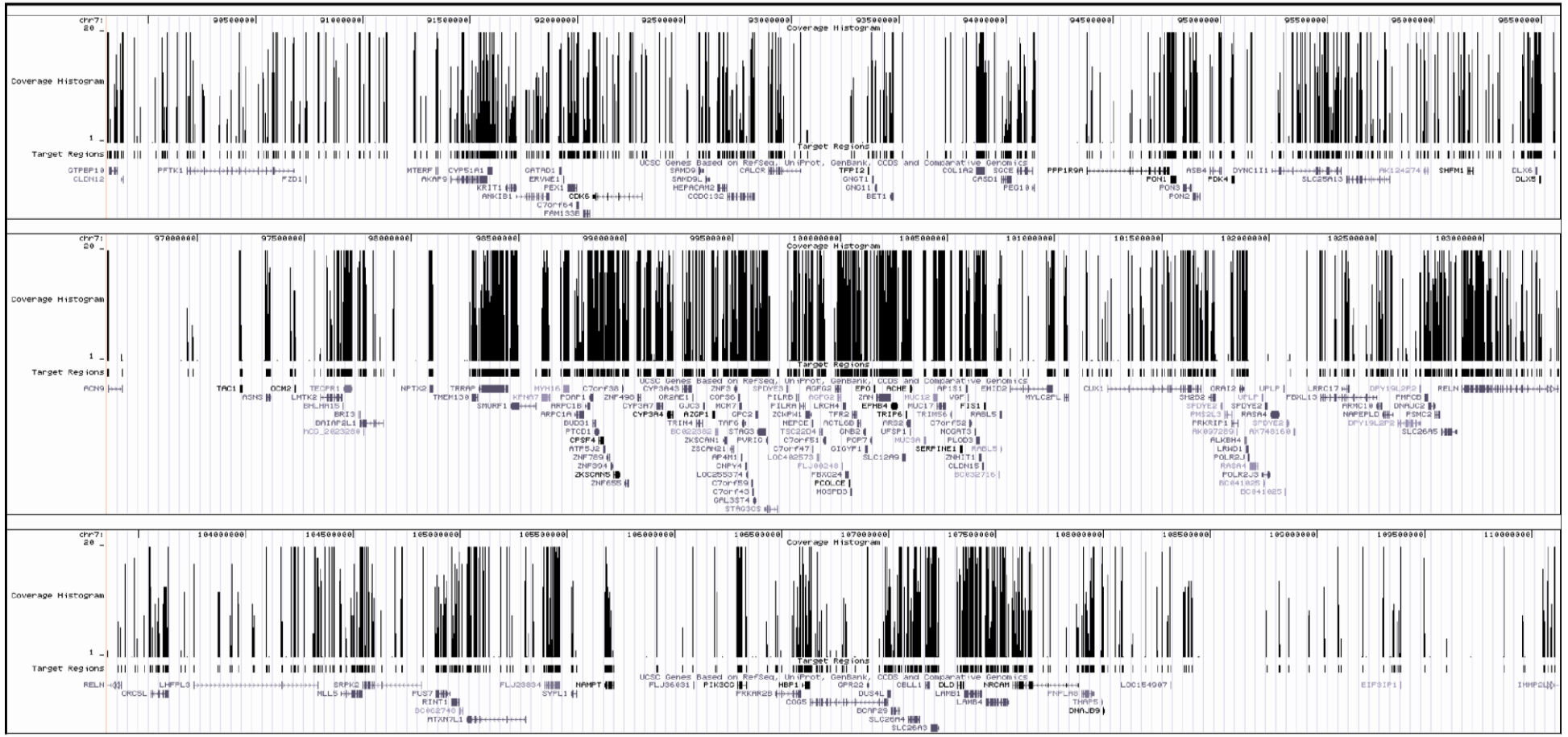
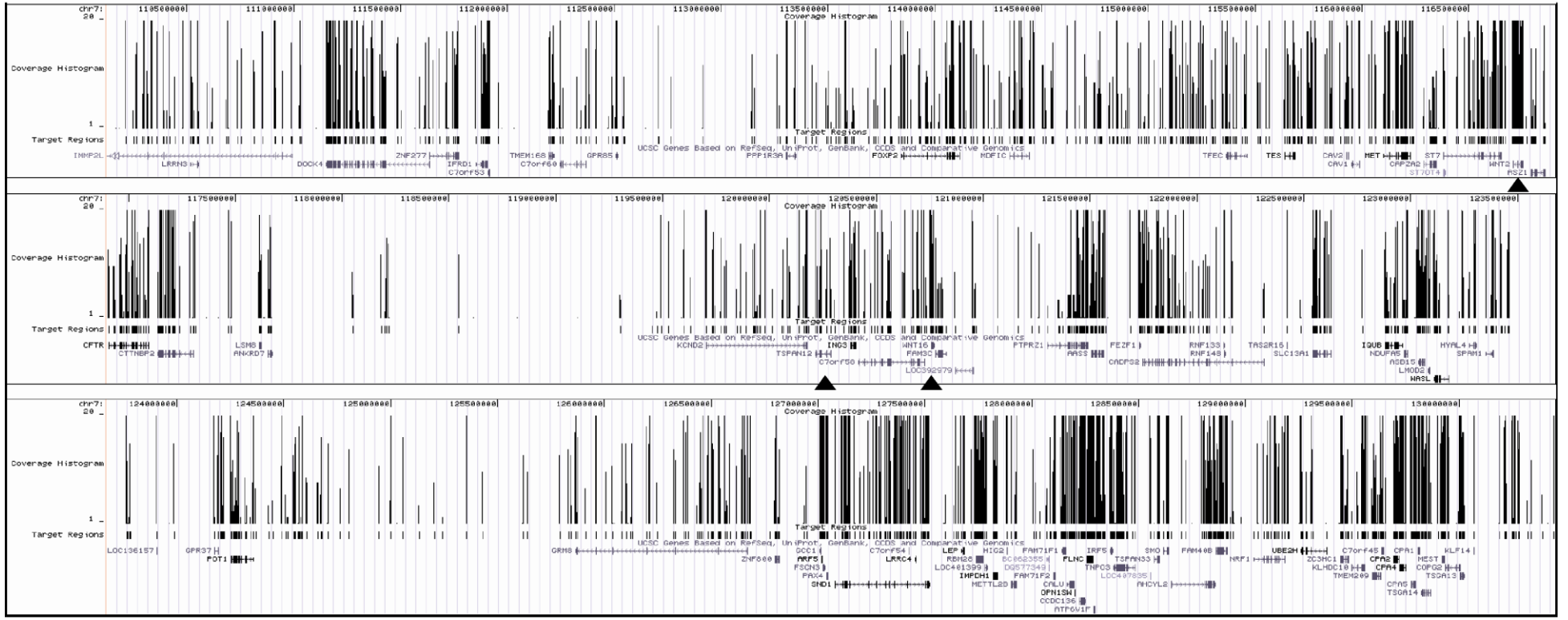
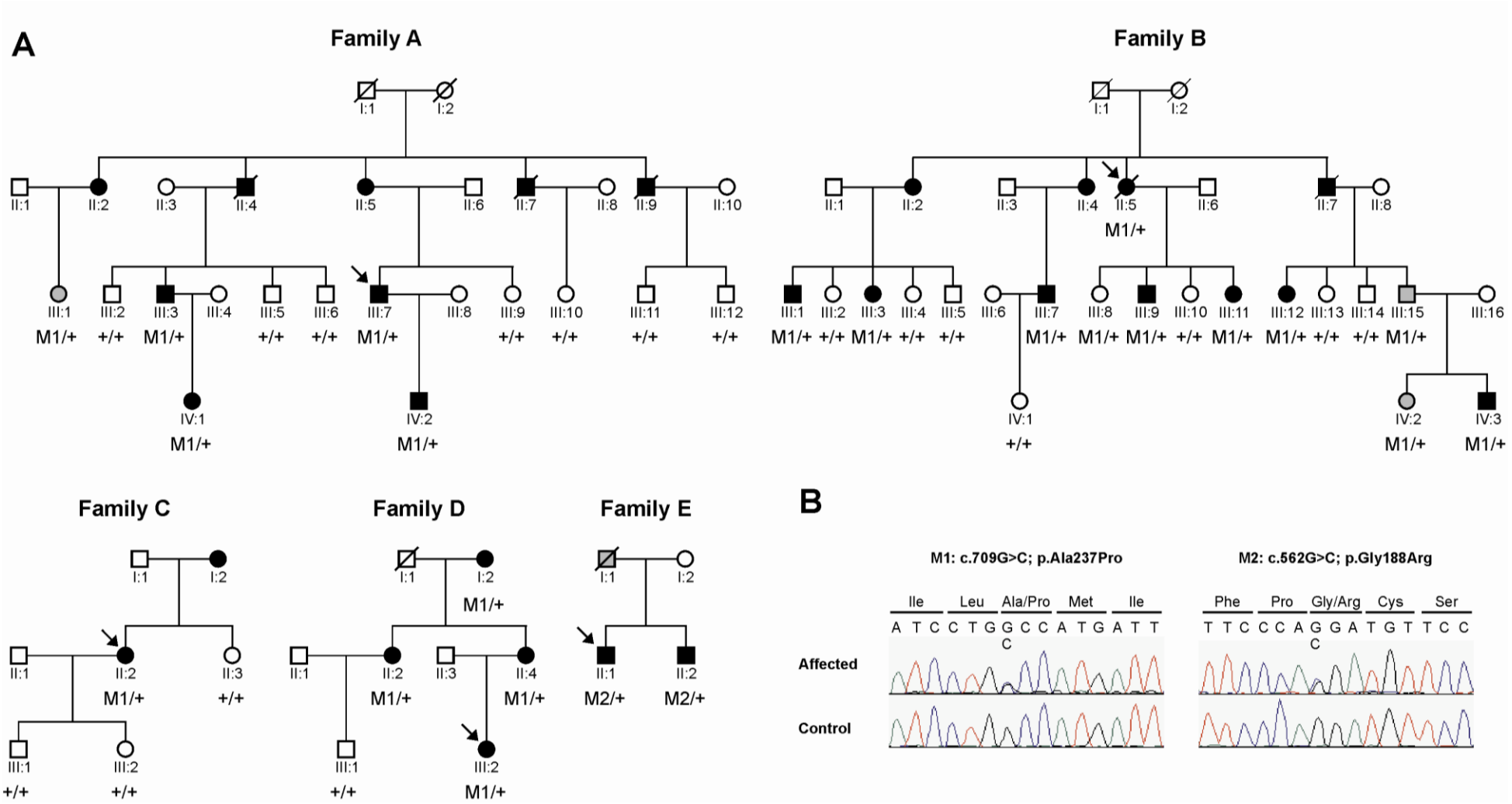


Figure S1. Coverage histogram for the complete 40.5-Mb FEVR locus (part 2)



Coverage histogram for the whole region targeted for next-generation sequencing, displayed in 6 panels. This figure shows a UCSC genome browser view with genomic position, custom tracks representing the sequence coverage that was obtained, the target region (e.g. regions covered on the enrichment array), and the UCSC gene track. The array design included all known exons, untranslated regions, microRNAs and highly conserved regions complemented with the complete genomic sequence of the candidate genes *WNT2* and *WNT16*. The positions of the genes *WNT2*, *WNT16* and *TSPAN12* are indicated with a black arrowhead. In total, 3,048 exons from 338 RefSeq genes and 313 UCSC genes as well as 14 microRNAs and 23 non coding RNAs were targeted.

Figure S2. Pedigree structure and mutation segregation in all families with *TSPAN12* mutations



A) Complete pedigrees for families A to E, and the segregation of the mutations identified in these families. M1/+ indicates heterozygous carriers of the p.Ala273Pro mutation, M2/+ heterozygous carriers of the p.Gly188Arg mutation, whereas +/+ indicates individuals with two wild-type alleles. All affected individuals in the families carry the respective causative mutations. In family A, the clinical status of individual III:1 was uncertain, as well as for individual III:15 and IV:2 in family B (indicated in grey). These three individuals all carried the mutation, as did the healthy individual III:8 in family B, suggesting non-penetrance. Probands in each family are indicated with an arrow.

B) Sequence chromatograms for the two mutations in *TSPAN12*. The upper two chromatograms show the heterozygous mutations, whereas the lower two indicate the sequences detected in controls. The corresponding amino acids are indicated above the sequence traces. M1 and M2 correspond to the mutations as described in panel A.

Table S1. General statistics of the sequencing run

Coverage statistics		Variant statistics	
Number of bases sequenced	51,440,891	Number of variants detected	1,915
% bases mapped	94.4%	Known SNPs (dbSNP130)	1,749
% bases mapped to or near (500bp) targets	90.5%	Unknown variants in genes	106
Average coverage of the targets	13.2x	Unknown variants in exons	21
% targets covered at least 1x	94.8%	Unknown missense variants	17
% targets covered at least 5x	79.8%	Unknown variants in splice sites	2
% targets covered at least 10x	56.5%	Unknown substitutions	139
% targets covered at least 20x	20.9%	Unknown indels	27

Left side: statistics on the target sequence coverage for the complete run. Right side: statistics on the detected variants in the region on chromosome 7 by the Roche 454 software.

Table S2. Primer sequences of *TSPAN12*

Exon	Forward (5'>3)	Reverse (5'>3')	Amplicon Length (bp)
1A	ggagggcaacaaagcaataa	cagctgcacaaactctcagc	397
1B	ctgggtgagagggacaagaa	gtcattcaaaccccgcttc	645
2	gatgaggcgatgtctcgaat	gtggctgcagaaaaccagag	332
3	ttcaagatgcagcaaatggt	ccaaaagatcaaggaagagca	307
4	tgctatgtcttgggtgcatt	tcactgctccctaatcttgtga	291
5	caagtctgtctttgaggagccta	gcggagtgaaaatgaactaaca	230
5'	cgaagagagtcatttggcaat	ccagagacagggagaaaatatga	393
6	tgcagttgcttcaaagagca	caggcttaatttttcagcacag	631
7	gccatggtgttcctttactaca	ggccttttacatttagacagagaag	430
8	tctcatcagctttccctgaga	tgtccaggtggtgacttatgac	646

Primers used for amplification and sequence analysis of human *TSPAN12*.