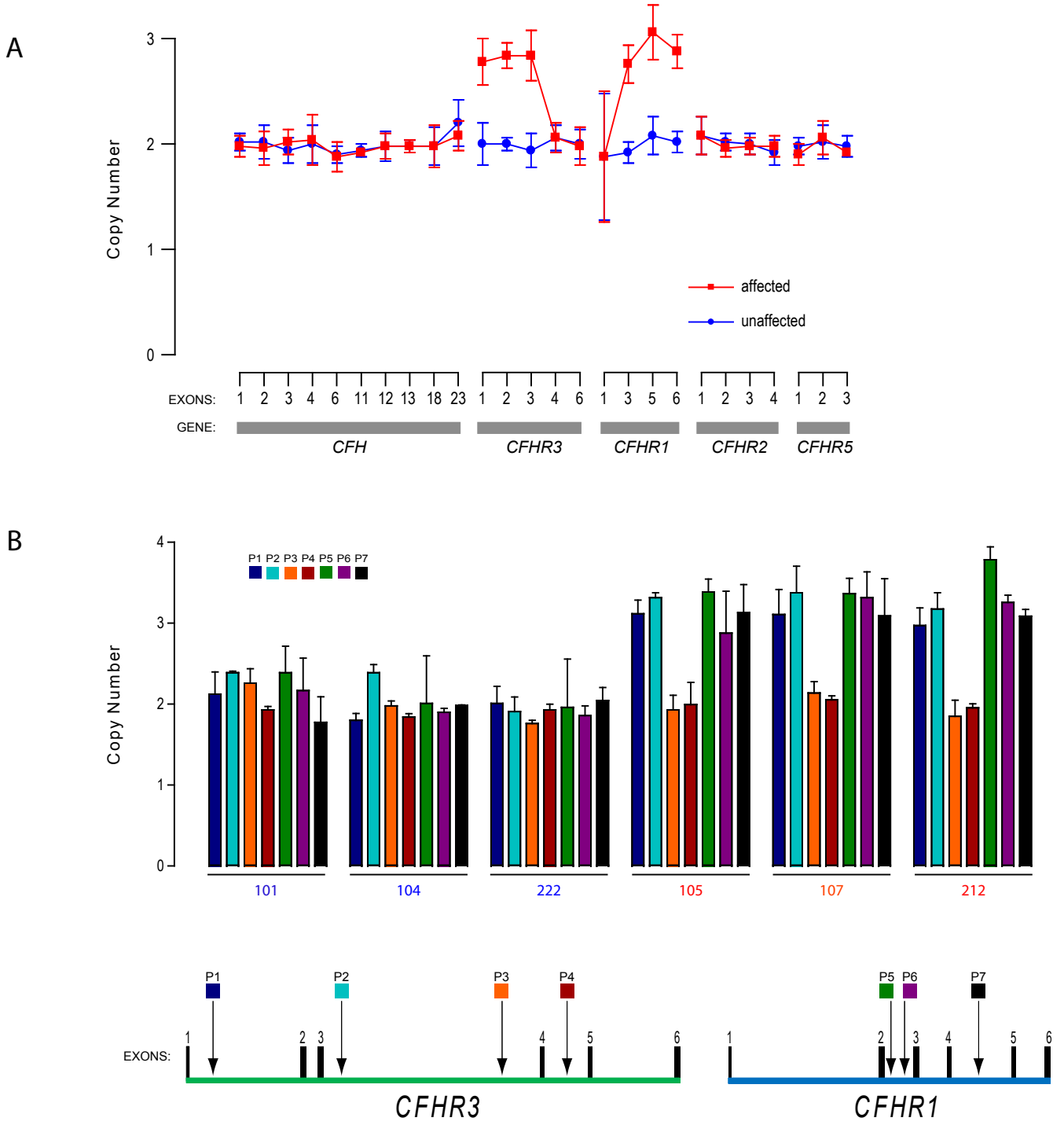


Malik et al., Supplemental figure 1

Supplemental figure 1. **Copy number assays using genomic DNA from affected and unaffected individuals.**

(A) Multiplex ligation-dependent probe assay (MLPA).

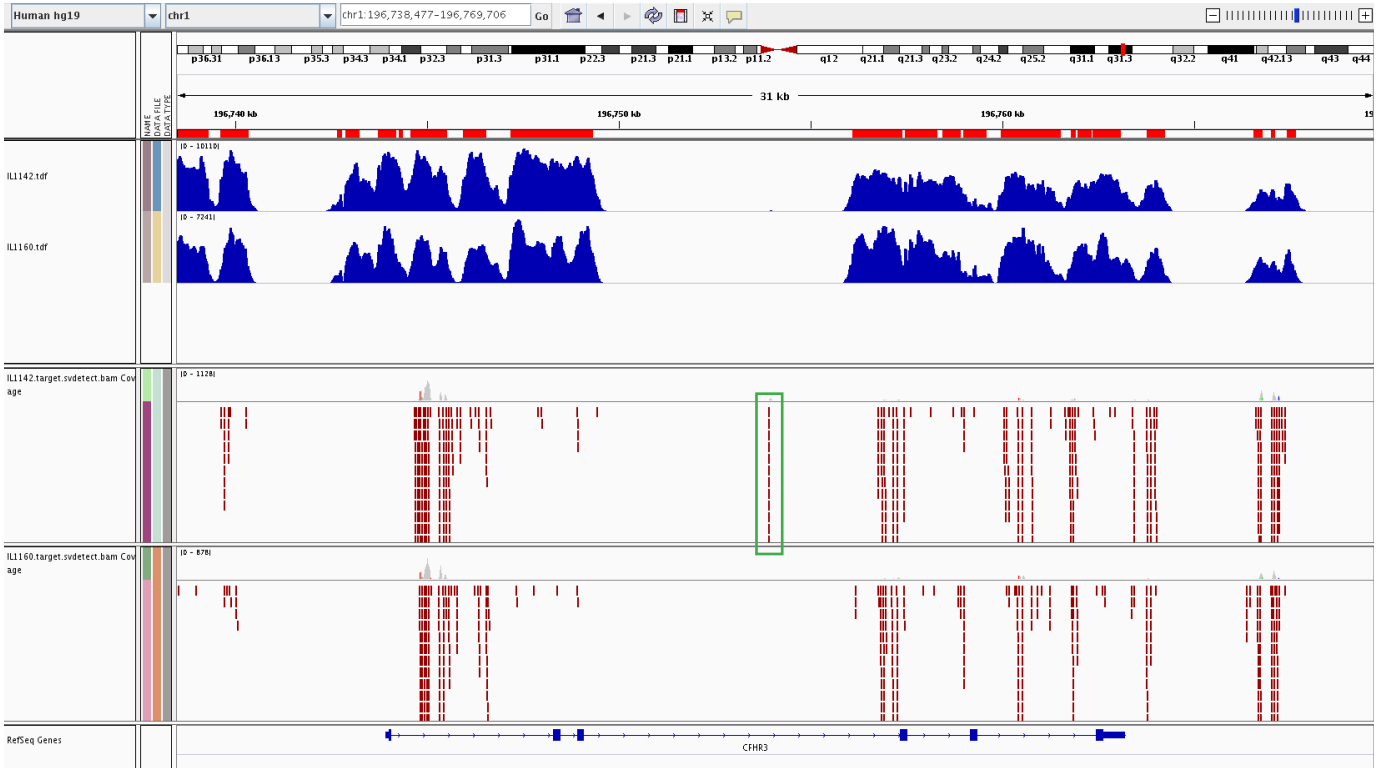
Probes used targeted complement factor H (*CFH*) exons 1, 2, 3, 4, 6, 11, 12, 13, 18, 23; complement factor H-related 3 (*CFHR3*) exons 1, 2, 3, 4, 6; complement factor H-related 1 (*CFHR1*) exons 1, 3, 5, 6; complement factor H-related 2 (*CFHR2*) exons 1, 2, 3, 4; and complement factor H-related 5 (*CFHR5*) exons 1, 2 and 3 (MLPA P236-A1, MRC Holland, www.mlpa.com). Genomic DNA was processed according to manufacturer's instructions. Assay readings were validated using control samples which included individuals with known heterozygous or homozygous polymorphic deletion of the *CFHR1* and *CFHR3* genes. Data depict mean +/- standard deviation for affected individuals (n=7, red squares, pedigree numbers: 105, 107, 207, 212, 213, 214 and 223) and unaffected individuals (n=11, blue circles, pedigree numbers: 101, 104, 106, 108, 109, 110, 114, 206, 220, 221, 222). In all affected individuals 3 copies of *CFHR3* exons 1, 2 and 3 and *CFHR1* exons 3, 5 and 6 are present. In contrast in all unaffected individuals two copies of these exons are present.

(B) TaqMan® copy number assays

TaqMan® probes (www.appliedbiosystems.com) were used to further dissect copy number variation (CNV) within the *CFHR3* and *CFHR1* genes. Genomic DNA was processed according to manufacturer's instructions. Assay readings were normalised to control samples and values represent mean +/- standard deviation. All probes were validated using genomic DNA from controls with either heterozygous or homozygous polymorphic deletion of the *CFHR1* and *CFHR3* genes (data not shown). The schematic of the *CFHR3* and *CFHR1* genes depicts the TaqMan® probe locations. Exons are indicated by vertical numbered bars. Probes P2, P3, P5 and P6 were custom designed and supplied by Applied Biosystems. Three copies were detected using probes P1, P2 and P5-7 in every affected individual from the pedigree. In contrast, two copies were detected with these probes in all unaffected individuals examined (n=21, pedigree numbers 1, 101, 203, 206, 104, 115, 110, 215, 106, 217, 218, 114, 220, 221, 222, 108, 112, 224, 225, 226 and 109). For clarity we illustrate probe data from three unaffected (pedigree numbers: 101, 104, 222) and three affected (pedigree numbers: 105, 107, 212) individuals.

(C) *CFHR3-CFHR1* hybrid gene: copy number variation as revealed by sequence coverage discrepancies

Coverage discrepancies between individual 214 (IL1142.tdf, affected, top) and individual 104 (IL1160.tdf, unaffected, bottom) suggest an additional copy of two genomic regions. Although bait density and capture efficiency varies importantly over the extent of the region of interest, the resulting sequence coverage is in general very reproducible from sample to sample. The coverage envelope, depicted at linear scale by the Integrative Genome Viewer, suggests two partial gene duplication events, the first starting at least 10kb upstream of the CFHR3 transcription start site and terminating in intron 3 of CFHR3, the second starting in intron 1 of CFHR1 and terminating at least 13kb downstream of CFHR1.

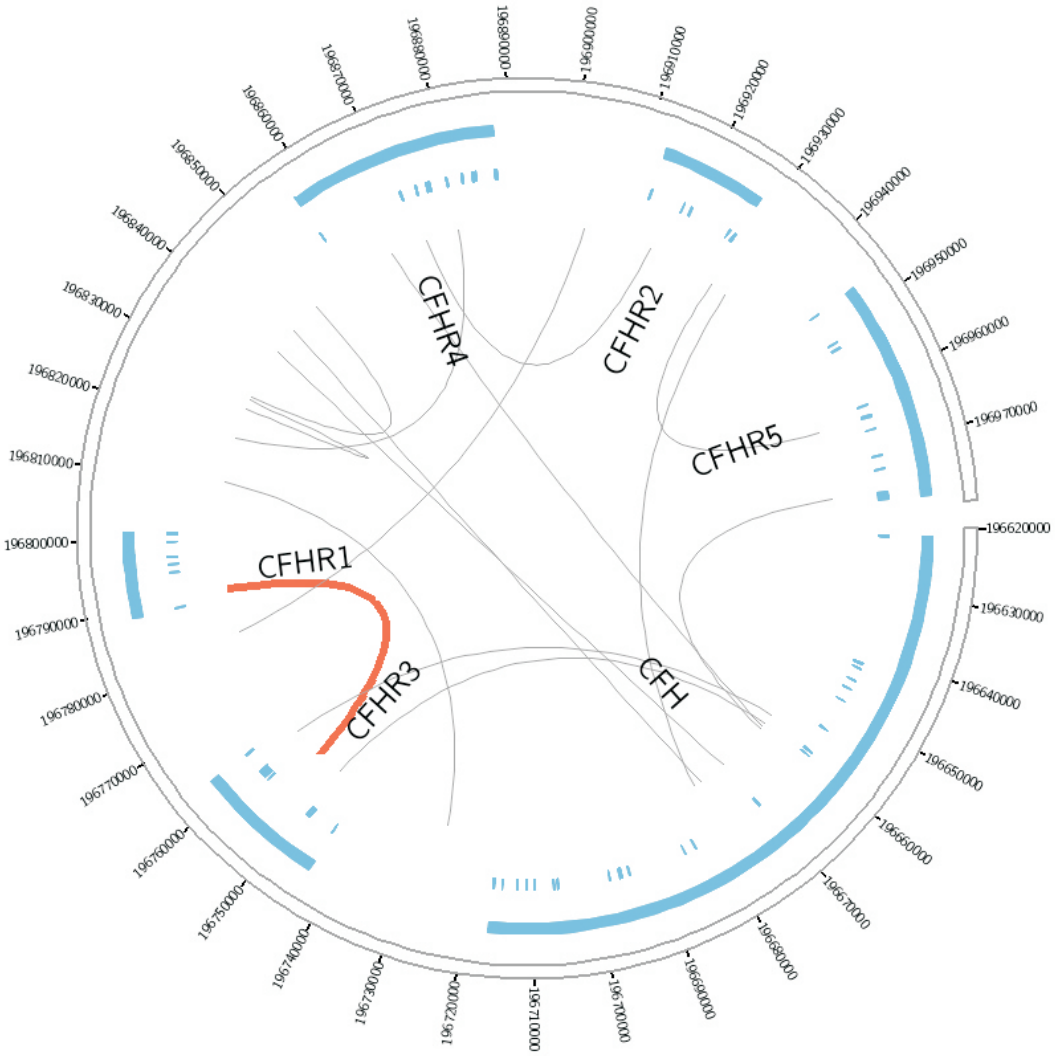


Malik et al., Supplemental Figure 2

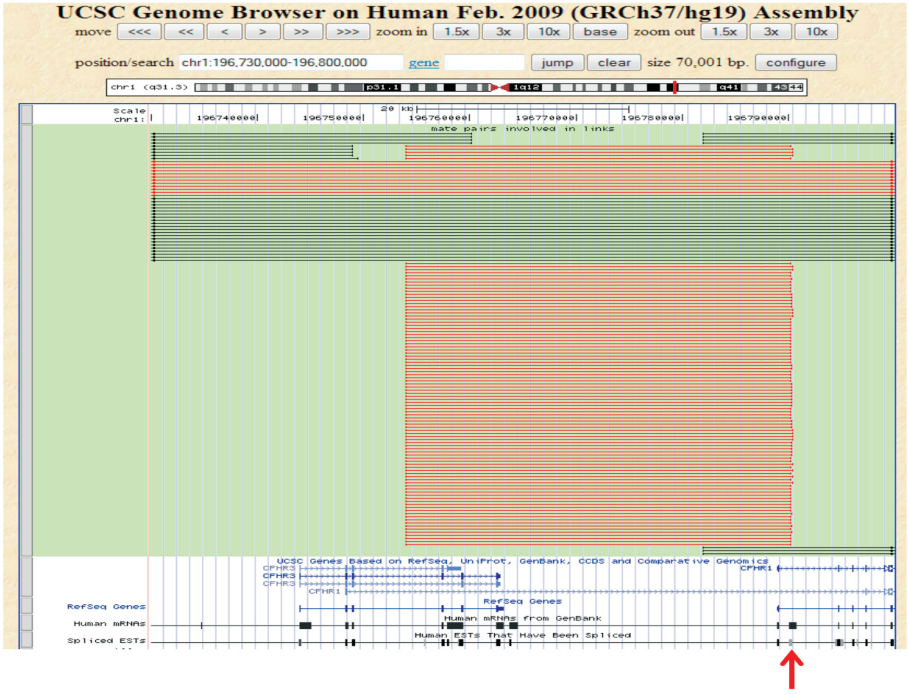
Supplemental figure 2. **Breakpoint analysis using paired-end reads and atypical mapping.**

Breakpoint analysis was performed with SV detect. Individual 214 (affected) is represented in the upper panel and individual 104 (unaffected) is in the lower panel. Atypical mapping occurred when the upstream end of the paired end sequence mapped >1kb away from where it was predicted to map. Paired end reads with atypical mapping are represented by red lines in the lower two panels. The green rectangle shows atypical mapping which only occurs in individual 214 and represents the breakpoint at *CFHR3* intron 3 (NCBI37/hg19:chr1:196754062) .

A



B

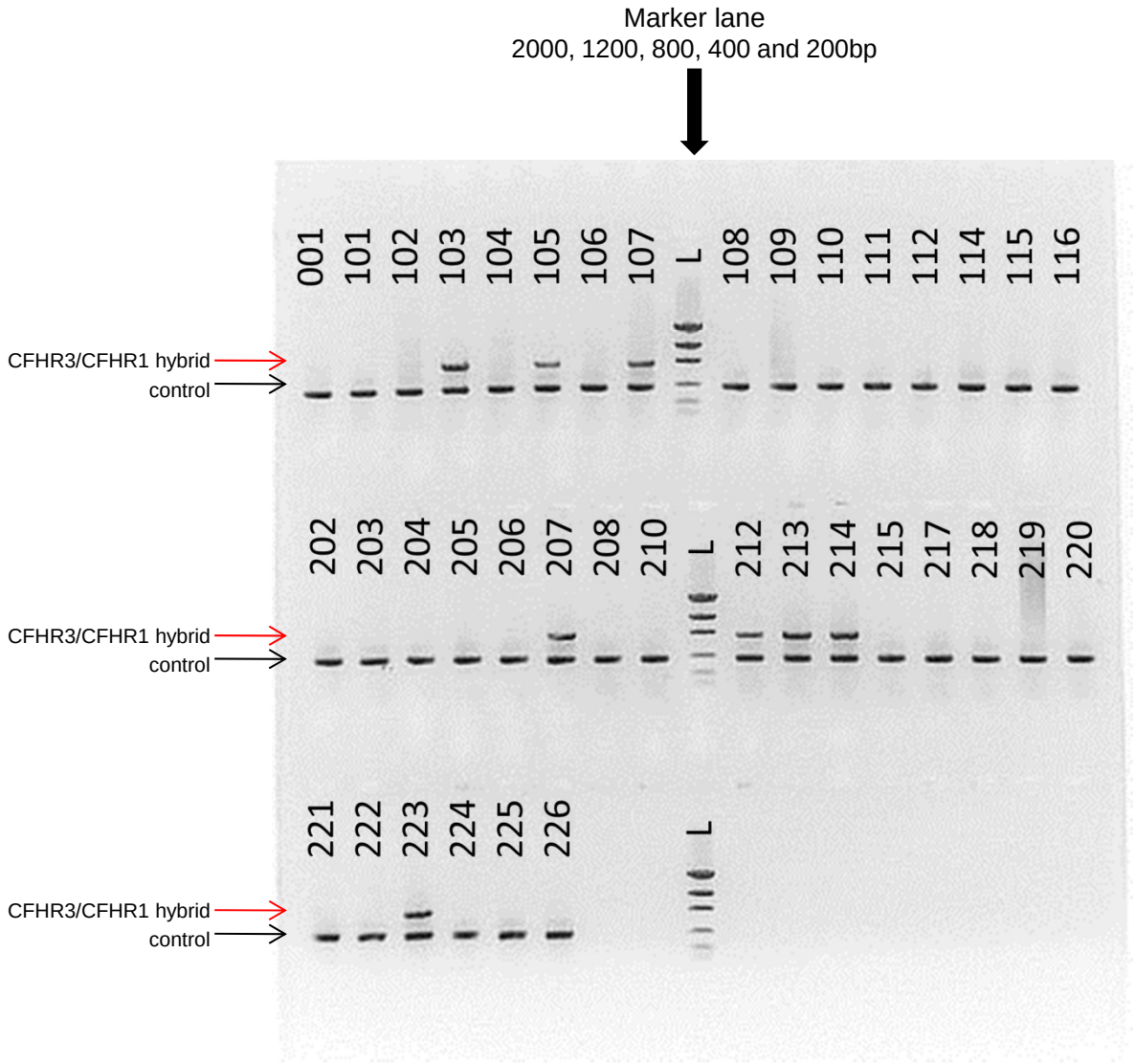


Malik et al., Supplemental figure 3

Supplemental figure 3. ***CFHR3-CFHR1*** **hybrid gene: fusion between** ***CFHR3*** **intron 3 and** ***CFHR1*** **Intron 1.**

(A) This Circos diagram represents the fusion breakpoint. The heavy red line represents 97 paired-end reads with atypical mapping and spanning *CFHR3* intron 3 to *CFHR1* intron 1. The numerous thin grey lines in the diagram represent paired-end reads with atypical mapping with much lower numbers of atypical reads (range 3-12). This is likely due to the high number of Long Interspersed Elements (LINES) and repetitive elements in this region and have not been confirmed by alternative methods.

(B) Fusion breakpoint stands out on the UCSC browser. Sequences from genomic fragments that were caught by baits which were designed to map *CFHR1* actually map to *CFHR3* at their 5' end where no baits were designed. This represents an approximately 35kb deletion.



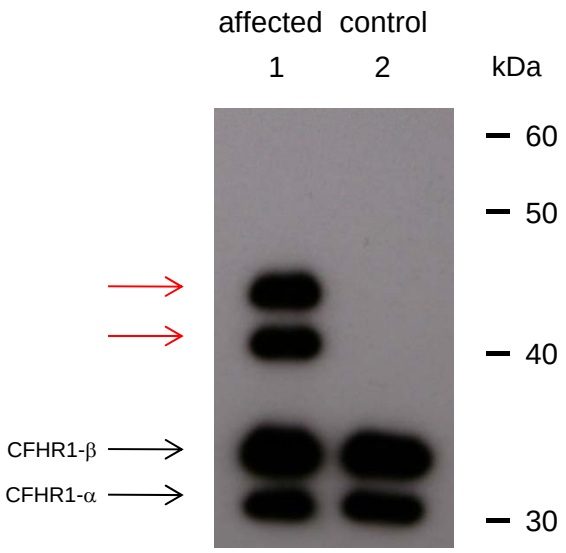
Affected individuals in the pedigree: 103, 105, 107, 207, 212, 213, 214, 223

Malik et al, Supplemental figure 4

Supplemental figure 4. **Genomic polymerase chain reaction (PCR) identifying the *CFHR3/CFHR1* breakpoint within the pedigree.**

We designed a genomic PCR which would generate a single 366bp amplicon in individuals with at least one copy of the *CFHR3* and *CFHR1* genes. In the presence of the *CFHR3-1* hybrid gene an additional 757bp amplicon is generated. Primers used: 5'CCTTGTTGACTTTCCATCTCG3', 5'GGTGGCTTATGCCTGCAA3' and 5'AGGAAACCCATCTCATGTGC3'. In all affected members of the pedigree (n=8, pedigree numbers: 103, 105, 107, 207, 212, 213, 214, 223) the 757bp amplicon is present. In contrast in all unaffected members examined only the 366bp control amplicon is seen.

A



B

CFHR3

MLLLINVILTLWVSCANGQVKPCDFPDIK**HGGLFHENMRRPYFPVAVGKY**YSYYCDE
HFETPSGSYWDYIHCTQNGWSPAVPCLRK**CYFPYLENGYNQNYGRK**FVQGNSTEV
ACHPGYGLPKAQTTVTCTEKGWSPTPRCIR**VT**

CFHR1

TFCDFPKINHGILYDEEKYKPFSQVPTGEVFYYSCEYNFVSPSKSFWTRITCTEEGW
SPTPKCLRLCFFPFVENGHSESSGQTHLEGDTVQIICNTGYR**LQNNENNISCVER**GW
STPPKCR**STDTSCVNPPTVQNAHILSR**QMSKYPSGERVRYECRSPYEMFGDEEVM
CLNGNWTEPPQCKDSTGKCGPPPPIDNGDITSFPLSVYAPASSVEYQCQNLYQLEG
NKRITCRNGQWSEPPK**CLHPCVISREIMENYNIALR**WTAKQKLYLR**TGESAEFVCKR**
GYRLSSRSHTLRTTCWDGKLEYPTCAKR

C

	CFHR3 exon 3				CFHR1 exon 2			
CODON	ATC	CGT	GTC	A	CA	ACA	TTT	TGT
AMINOACID	I	R	V		= T	T	F	C

GA = R in CFHR3 exon 4

Malik et al, Supplemental figure 5

Supplemental figure 5. **Proteomic analysis of the *CFHR3-1* hybrid gene product.**

(A) Western blot for complement factor H-related (CFHR) proteins using heparin-purified sera from healthy control and an affected individual. In control sera (lane 2) the two isoforms of CFHR1 are evident. Using sera from an affected individual (lane 1), two additional bands are seen running above CFHR1-β (red arrows). After electrophoresis a replicate gel was stained with Coomassie Blue and the aberrant bands were manually excised and the gel fragment subjected to peptide mass fingerprinting.

(B) and (C) Protein identification by mass spectrophotometry peptide mapping and sequencing analysis.

Seven peptides matched the predicted CFHR3-1 protein sequence (underlined in bold). Importantly, one peptide (VTTFCDFPK) spanned the amino acid junction between CFHR3 and CFHR1. This peptide included the unique amino acid (the initial T in the sequence VTTFCDFPK) generated by the abnormal splicing between CFHR3 exon 3 and CFHR1 exon 2 (C).