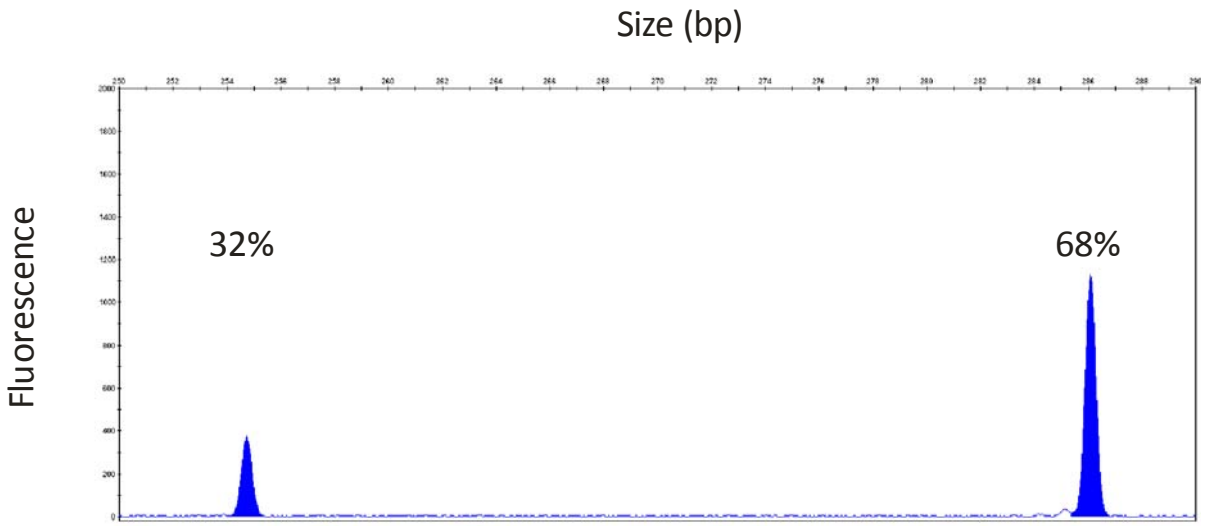
Supplementary Material

Weisschuh et al.

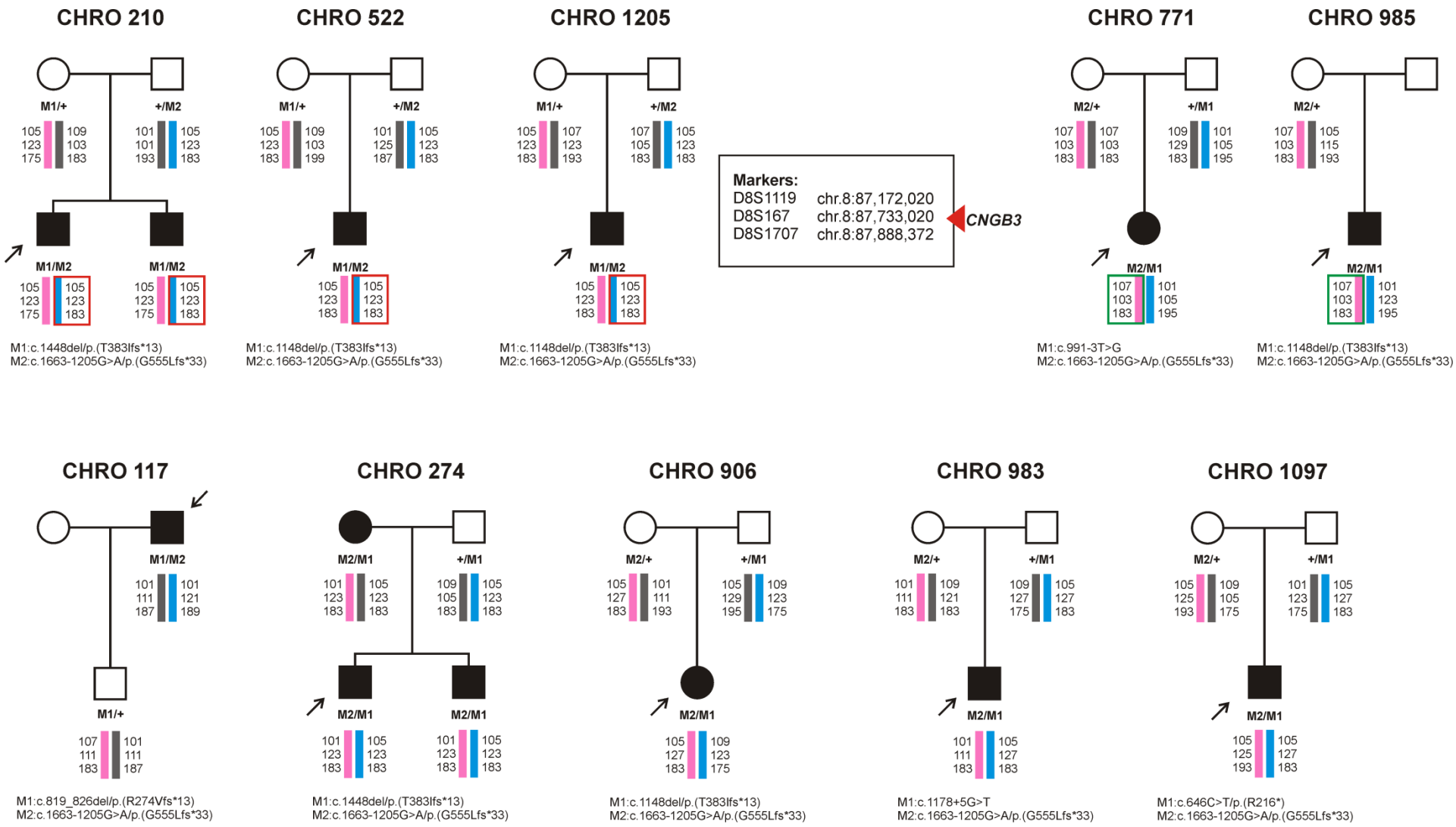
Deep-intronic variants in *CNGB3* cause achromatopsia by pseudoexon activation

Supplementary information includes two figure and four tables.

Figures



Supp. Figure S1: Capillary electrophoresis and quantitative analysis of fluorescent labeled RT-PCR products derived from HEK293T cells transfected with plasmids harboring the mutant c.1663-1205A-allele. The fragment size scale is given on the x-axis and fluorescence intensity (in arbitrary units) on the y-axis. Relative amounts of each fragment are given for the corresponding peak as determined by Gene Mapper. The smaller product corresponds to the correctly spliced transcript while the larger product is the aberrant transcript with the pseudoexon.



Supp. Figure S2: Haplotype analysis. Three annotated microsatellites in the vicinity of *CNGB3* were analyzed that span a physical region of 716 kb and a genetic map distance of 0.96 cM on chromosome 8q21.3. Index patients are highlighted by arrows. Shared haplotypes for the c.1663-1205A-allele are indicated by red or green colored boxes. In total, seven haplotypes were identified in ten families.

Tables

Supp. Table S1: Target information and primer sequences used for amplification of *CNGB3*

Oligo Name	Sequence 5´-3´	Amplicon length (bp)	Oligo Name	Sequence 5´-3´	Amplicon length (bp)
CNGB3_LD_A-f	aacaatgaggctacaacatttgcattcttc	9277	CNGB3_LD_K-f	gaagacgtggcaccagaaggaatacat	13197
CNGB3_LD_A-r	acaagtaagtatgagagtggggccatttgt		CNGB3_LD_K-r	tgctttgtccattatagactctaggtgatga	
CNGB3_LD_B-f	cacttccttttctttccgatctcagcaatac	10265	CNGB3_LD_L1-f	aatcttcttttcaaagccccatagtccatt	6404
CNGB3_LD_B-r	ccttctgtacaggcacatgtggtacactaa		CNGB3_LD_L1-r	caatttataggtcaccaggaccatcacaag	
CNGB3_LD_C-f	atagaggcaggatcaatttagaaaggcttg	7067	CNGB3_LD_L2-f	cctaaaataaatctcaaagcatcagggcag	3219
CNGB3_LD_C-r	tgacaagataatgaattgtttgcttttgga		CNGB3_LD_L2-r	gcattttgtagcctctggtttaaatgacct	
CNGB3_LD_D-f	catgtagaactgaccaaagctttctgttcc	10307	CNGB3_LD_M-f	ttctcccaagaatagtggtctttcgatgat	6917
CNGB3_LD_D-r	caaaggtttatgatctgctcatgcctagaa		CNGB3_LD_M-r	ccgtagttggtagggtcttaagcaaatcag	
CNGB3_LD_E-f	atcttctccacaatccccatcaattcattt	10295	CNGB3_LD_N-f	aaaatgctccagagtgcaaattacaatgaa	10293
CNGB3_LD_E-r	actattgcctaagtggaagtggatcatcag		CNGB3_LD_N-r	tctccatttctaggctcatttctgctttct	
CNGB3_LD_F-f	ggtgaacacagtaaaggaccaaacaaaact	7009	CNGB3_LD_O-f	catgttcaagaacttccaagcatatttcaag	5559
CNGB3_LD_F-r	ttttcatcagggacaaataacagatttgaa		CNGB3_LD_O-r	atgtgacttgtcattctagcgtctccttgt	
CNGB3_LD_H-f	ttttgaaggttgcctgataattccttttctt	9600	CNGB3_LD_P-f	tatgaaatgcagagtagatgcagccttttc	15615
CNGB3_LD_H-r	ttcacttgtgcattatggtattgtttgcac		CNGB3_LD_P-r	tgttttgttttctctctccttcgaggtctt	
CNGB3_LD_I-f	gtctcctgtgactgttttgaatgatggttt	13257	CNGB3_LD_Q-f	atccctctcagggtgaaggtgacagt	10333
CNGB3_LD_I-r	gcaagtggtgaaatccaaatgacatagaac		CNGB3_LD_Q-r	ggatgagtctgtttcattcacattcctgtt	
CNGB3_LD_J-f	cttccatggcttcacattgttgattatt	10617	CNGB3_LD_R-f	ggaatggatgactgaccagggactatctta	12517
CNGB3_LD_J-r	tgaaggaaaagttgcttagtgagtcagg		CNGB3_LD_R-r	ataggacagaggtaagtgacggaggagaga	

bp, base pairs

Supp. Table S2: Initially known alleles in patient cohort

Supplementary Table S2: Initially known alleles in patient cohort

Patient ID	Clinical diagnosis	Previously known variant	Patient ID	Clinical diagnosis	Previously known variant
frameshift deletions			***in frame deletion***		
CHRO 117	ACHM	c.819_826del/p.(R274Vfs*13)	CHRO 1069	ACHM	c.1190_1192del/p.(C397del)
ZD 54	Cone dystrophy	c.886_896delinsT/p.(T296Yfs*9)	***nonsense***		
MDS 155	Macular dystrophy	c.886_896delinsT/p.(T296Yfs*9)	CHRO 480	ACHM	c.265C>T/p.(Q89*)
CHRO 99	ACHM	c.886_896delinsT/p.(T296Yfs*9)	CHRO 1097	ACHM	c.646C>T/p.(R216*)
CHRO 26	ACHM	c.1148del/p.(T383Ifs*13)	***splice site***		
CHRO 31	ACHM	c.1148del/p.(T383Ifs*13)	CHRO 771	ACHM	c.991-3T>G/p.?
CHRO 210	ACHM	c.1148del/p.(T383Ifs*13)	CHRO 425	ACHM	c.1578+1G>A/p.?
CHRO 274	ACHM	c.1148del/p.(T383Ifs*13)	***startloss***		
ZD 216	Cone dystrophy	c.1148del/p.(T383Ifs*13)	CHRO 667	ACHM	c.2T>C/p.?
CHRO 483	ACHM	c.1148del/p.(T383Ifs*13)	***missense***		
CHRO 522	ACHM	c.1148del/p.(T383Ifs*13)	RCD 246	Cone-rod dystrophy	c.1208G>A/p.(R403Q)
CHRO 216	ACHM	c.1148del/p.(T383Ifs*13)	MST 137	Stargardt disease	c.1208G>A/p.(R403Q)
CHRO 915	ACHM	c.1148del/p.(T383Ifs*13)	ZD 203	Cone dystropy	c.1208G>A/p.(R403Q)
CHRO 1205	ACHM	c.1148del/p.(T383Ifs*13)	CHRO 559	ACHM	c.1208G>A/p.(R403Q)
CHRO 100	ACHM	c.1148del/p.(T383Ifs*13)	CHRO 675	ACHM	c.1397T>C/p.(M466T)
CHRO 262	ACHM	c.1148del/p.(T383Ifs*13)	CHRO 959	ACHM	c.1405T>G/p.(Y469D)
CHRO 1076	ACHM	c.1148del/p.(T383Ifs*13)	CHRO 691	ACHM	c.1781G>C/p.(S594T)
CHRO 906	ACHM	c.1148del/p.(T383Ifs*13)			
CHRO 985	ACHM	c.1148del/p.(T383Ifs*13)			
CHRO 378	ACHM	c.1148del/p.(T383Ifs*13)			

Variant designation based on NCBI Reference Sequence NM_019098.4. ACHM, achromatopsia.

Supp. Table S3: Patients potentially solved with a second coding variant

Supplementary Table S3: Patients potentially solved with a second coding variant

ID	Allele 1	Allele 2	Segregation
CHRO 691	c.1781G>C/p.S594T	c.1271T>G/p.L424R	yes
CHRO 483	c.1148del/p.T383Ifs*13	c.886_896del/p.T296Yfs*9	na
CHRO 99	c.886_896del/p.T296Yfs*9	c.607C>T/p.R203*	yes
CHRO 480	c.265C>T/p.Q89*	c.1633T>G/p.Y545D	yes
CHRO 378	c.1148del/p.T383Ifs*13	c.1430_1431delinsC/p.K477Tfs*17	na

Variant designation based on NCBI Reference Sequence NM_019098.4. na, not analyzed.

Supp. Table S4: *In silico* assessment of intronic variants

Supp. Table S4 is provided as a separate file (.xlsx file).