Supplementary material

Title: Phenotypic features of *CRB1* associated early-onset severe retinal dystrophy and the different molecular approaches to identify the disease-causing variants

Journal name: Graefe's Archive for Clinical and Experimental Ophthalmology

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Supplementary Table 1: Primer sequences of *CRB1* gene used in this study (NM_201253.2).

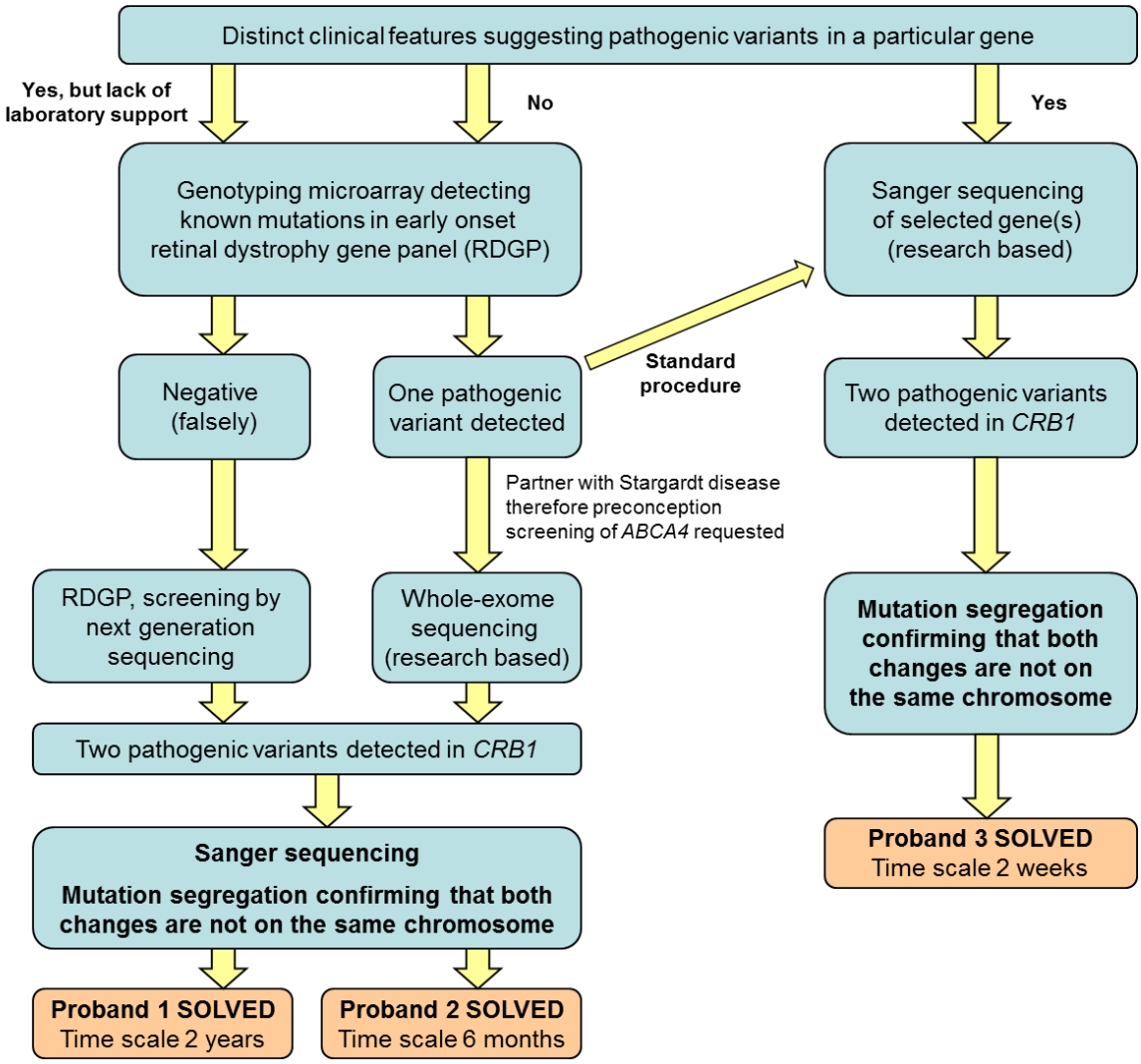
Exon	Primers 5´- 3´ sequence	Tm (°C)	Fragment length (bp)
1	GTGATGCTAAGAAGCACAAAC	60	451
	CTGACTGTTCACATTGACTGG		
2	GAGGCAGCACAAAGGTCACA	60	711
	GAGCTAACTACACCATCTGTG		
3	ACAGAACATTTGACAAGTGCTC	60	399
	GCCGAGAACGTGAGAGCTC		
4	GATGATGCCATGGGTCTTGG	60	318
	TCATTTGCTATAAGCGATATGTG		
5	CAGTATAGCAGTCAACCTCC	60	362
	CAGCTCTTCCTGCTAATACAC		
6A	TCCATTACAGTCCTAAACCTG	58	575
	GTAGCCACTTAGCAGCTCC		
6B	CCGAAGCAACAGGGATGTG	58	607
	TGCTCTGCTCTGAGGCATG		
7	CTGTCTTTTGAGCCTTAAGATG	60	732
	CTATACTGGTGGGTCAGTAAC		
8	CTCTCTGCCACCACTCTGCC	60	526
	CAGTCAGTATTAGCCTACTCG		
9A	AGCAACTAGCACAGTATGTAAC	60	384
	CTGACTGCAAACTTGTCAGAC		
9B	CACATTTGGTTTCAGAACAAGG	60	582
	GACCATCCCAAGGGACAGG		
9C	CTCTACCAATTCAGTGGTCAC	60	429
	GACAAGAACAGTGATGCAGAG		
10	CTTGGCATTGACTACATACATG	60	367
	GTTTCATTCTGTCTGAACCTC		
11	GTGCTGTTCCAGAGAGATAAG	60	322
	CAACTGGCTCGTCATTCATAC		
12	TCATTCCTGAGTAGTTCCATTG	60	368
	AGAGATACCTGAAGTACAGTC		

Supplementary Table 2. *In silico* analysis of *CRB1* missense variants identified in the current study and evaluated as pathogenic. Six different algorithms were used; tolerated and neutral scores are indicated in green as benign; yellow indicates a possibly damaging variant, and red a probably damaging, deleterious, disease-causing mutation. NM_201253.2 and NP_957705 were taken as the reference sequences.

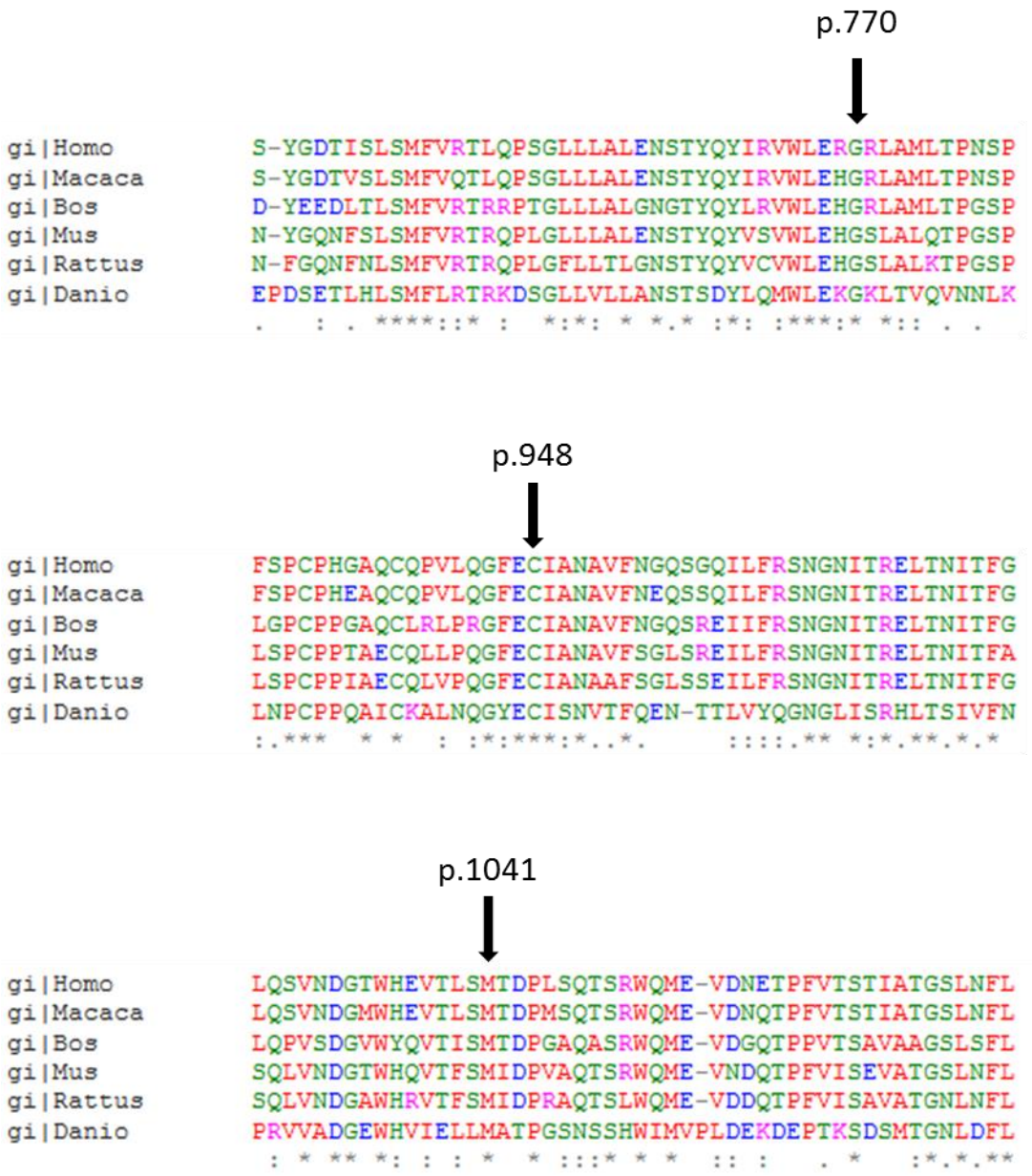
DNA level	Protein level	SNP&GO	MutPred	PROVEAN	SIFT	PolyPhen-2	MutationTaster
c.2308G>A	p.(Gly770Ser)	Neutral	Disease	Disease	Neutral	Disease	Disease
c.2843G>A*	p.(Cys948Tyr)	Disease	Disease	Disease	Disease	Disease	Disease
c.3121A>G	p.(Met1041Val)	Neutral	Possibly damaging	Disease	Neutral	Possibly damaging	Disease

*Previously reported as disease-causing, summarized in Bujakowska K., et al., 2013.

Using MutPred an overall probability score > 0.5 was considered as probably disease-causing and a score > 0.75 was considered as disease-causing.



Supplementary Figure 1. Diagrammatic representation of research approach and workflow applied in the search for the molecular genetic cause in Czech probands with early-onset severe retinal dystrophies. Time scale indicates the total amount of time spent on the analysis including associated administration to fulfill the national health care system requirements for payment in laboratories located outside the country in probands 1 and 2.



Supplementary Figure 2. Evolutionary conservation of the CRB1 protein. T-Coffee multiple sequence alignment result. Amino acids at position 770, 948 and 1041 in the human CRB1 protein sequence (UniProtKB - P82279) are indicated by an arrow.