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Supplemental Data

Ectopic *GRHL2* Expression Due to Non-coding Mutations Promotes Cell State Transition and Causes Posterior Polymorphous Corneal Dystrophy 4

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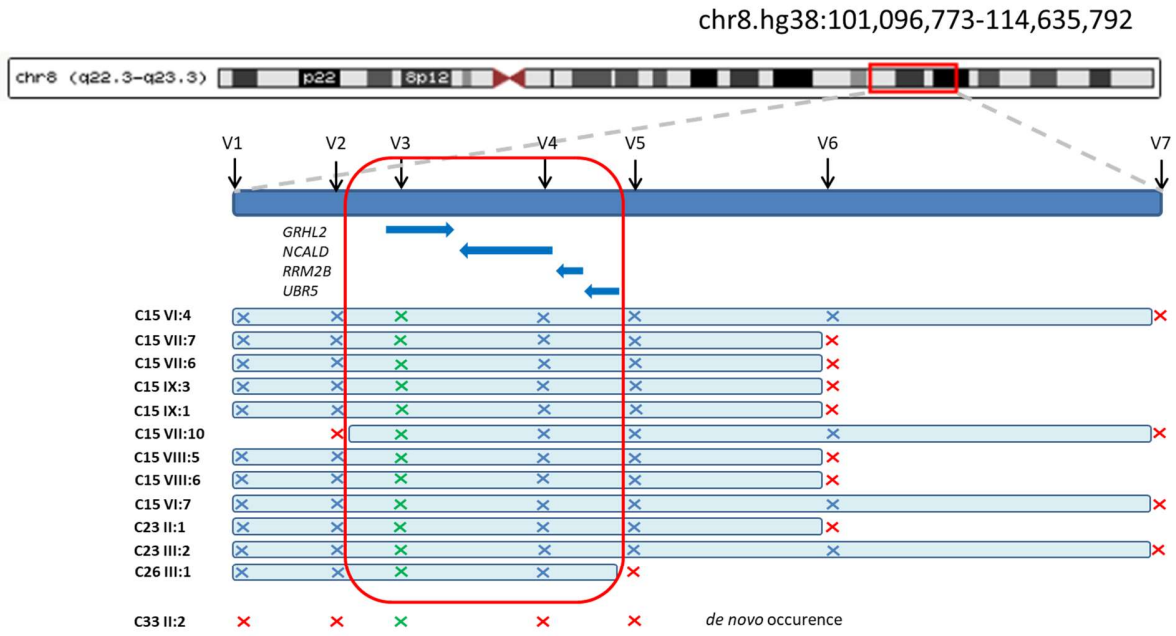
Supplemental Data



	V1	V2	V3	V4	V5	V6	V7
Position (Build GRCh38)	Chr8:101,096,773	Chr8:101,411,163	Chr8:101,493,333	Chr8:101,546,909	Chr8:102,437,115	Chr8:109,214,442	Chr8:114,635,792
Variant	G>A	T>C	G>T	A>G	G>A	AAAACA>TT	A>T
dbSNP	rs554736280	rs564548712	none	rs145339755	rs72683010	rs71303458	none
MAF in 1000G	A: 0.080% (4/5008)	C: 0.060% (3/5008)	unique	G: 0.781% (17/2178)	A: 0.120% (6/5008)	N/A	unique

Figure S1. Ancestral mini-haplotype of 3 families of Czech origin
Rare variants V1, V2, V4-V7 (see table at bottom of schematic haplotype) selected within the PPCD4 linked region (filled blue rectangles) were genotyped in individuals from families C15, C23 and C26 with the same *GRHL2* mutation (V3, green cross). Recombination event (red cross) were identified in family C15 that refined the locus and associated mini-haplotype. The same mini-haplotype was identified in families C23 and C26, with an additional recombination events, that suggests the *GRHL2* mutation in these families arose in a common ancestor. This ancestral mini-haplotype was absent in affected individual II:2 from family C33, who had the same *GRHL2* mutation that occurred *de novo*.

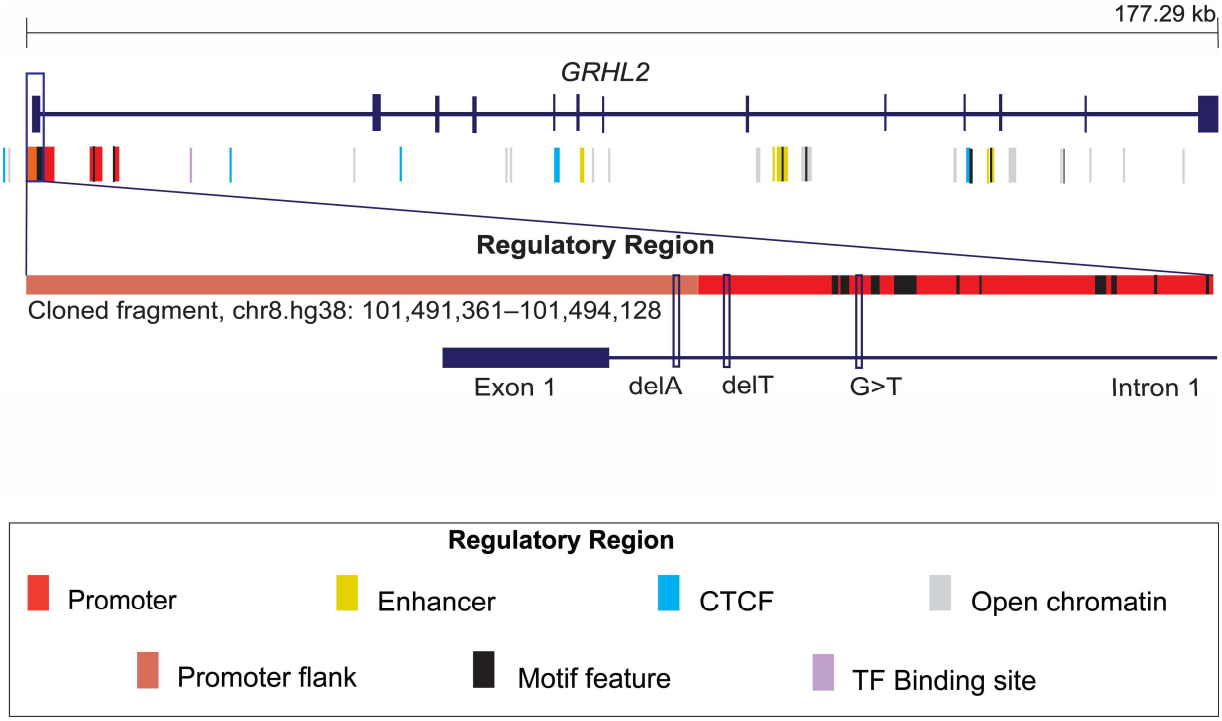
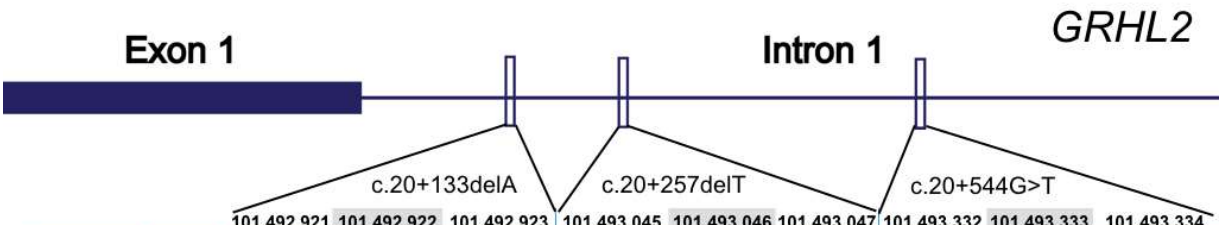


Figure S2. Schematic representation of the regulatory region surrounding the three *GRHL2* promoter mutations

GRHL2 gene structure showing regulatory element annotations from the Ensembl Regulatory Build, colored by feature. Zoom-in view of the intron 1 region encompassing all 3 mutations showing they fall within a predicted promoter region. The image is extracted from Ensembl v90 using GRCh38 assembly and GENCODE v22 gene annotations. G>T represents c.20+544G>T, delT represents c.20+257delT, delA represents c.20+133delA. Mutations are annotated in accordance with the *GRHL2* cDNA sequence (Ensembl: ENST00000251808.7). Red bar represents the regulatory region (2,728 bp) sequenced in unsolved PPCD patients and cloned for the luciferase assay.



Cell type	Marks	101,492,921 G	101,492,922 A / -	101,492,923 T	101,493,045 T	101,493,046 T / -	101,493,047 A	101,493,332 C	101,493,333 G / T	101,493,334 C
A549	CTCF	0.33	0.33	0	0.33	0.33	0.33	0.33	0.33	0.33
	DNAse1	0	0	0.33				0.33	0	0
	H3K9ac									
	H3K36me	0.33	0.33	0.33				0.33	0.66	0.66
	H3K4me1	0.66	0.66	0.66				1	0.99	0.99
	H3K4me3	0	0.33	0.33						
H1ESC	CTCF	0.99	0.99	0.99				0.33	0.33	0.99
	DNAse1	0.66	0.99	0.99	0.66	0.66	0.66	3.29	3.29	3.29
	H3K9ac	0	0	0	1	1	1	0	0	0
	H3K36me									
	H3K4me1	11.17	11.17	11.17	12.82	12.82	12.82	14.79	14.79	14.79
	H3K4me3	0	0	0	1	1	1	0	0	0
NHDF-AD	CTCF				0	0	0	0.1	0.1	0.1
	DNAse1	1	1	1.64	0.15	0.15	0.33			
	H3K9ac	0.33	0.33	0.33	2.30	2.30	2.30			
	H3K36me	0	0.33	0.33	0	0	0			
	H3K4me1							0.33	0.33	0.33
	H3K4me3									
NHEK	CTCF	0.66	0.66	0.66	0.99	0.99	0.99	0.99	0.99	0.99
	DNAse1	1.31	1.31	1.31	0	0	0	1.31	1.31	1.31
	H3K9ac	1	1	1	1	1	1	1	1	1
	H3K36me									
	H3K4me1	21.69	21.69	21.69	31.22	31.22	31.22			
	H3K4me3							16.11	16.11	16.11

Figure S3. Promoter features at the positions of the three intron 1 *GRHL2* mutations
Zoomed-in view of promoter features for each of the 3 mutation positions within intron 1 of *GRHL2*. PCHi-C data from 4 cell types (A549 - epithelial lung carcinoma; H1ES - human embryonic stem cells; NHDF-AD - adult dermal fibroblasts; NHEK - normal human epidermal keratinocytes), and chromatin features based on ChromHMM segmentations of BLUEPRINT histone modification data are shown. PCHi-C data displays features such as DNase1 or CTCF binding sites. ChromHMM histone states for: H3 lysine 4 trimethylation (H3K4me3), H3 lysine4monomethylation (H3K4me1), H3 lysine 36 trimethylation (H3K36me3) for actively transcribed regions are shown. H3 lysine 9 acetylation is associated with promoter regions. The image is based on Ensembl v90 using GRCh38 assembly. Mutations are annotated in accordance with the *GRHL2* cDNA sequence (Ensembl: ENST00000251808.7). Brightness of colors (red and green, brighter = stronger) and numbers in boxes represent scores of relative strength of features.

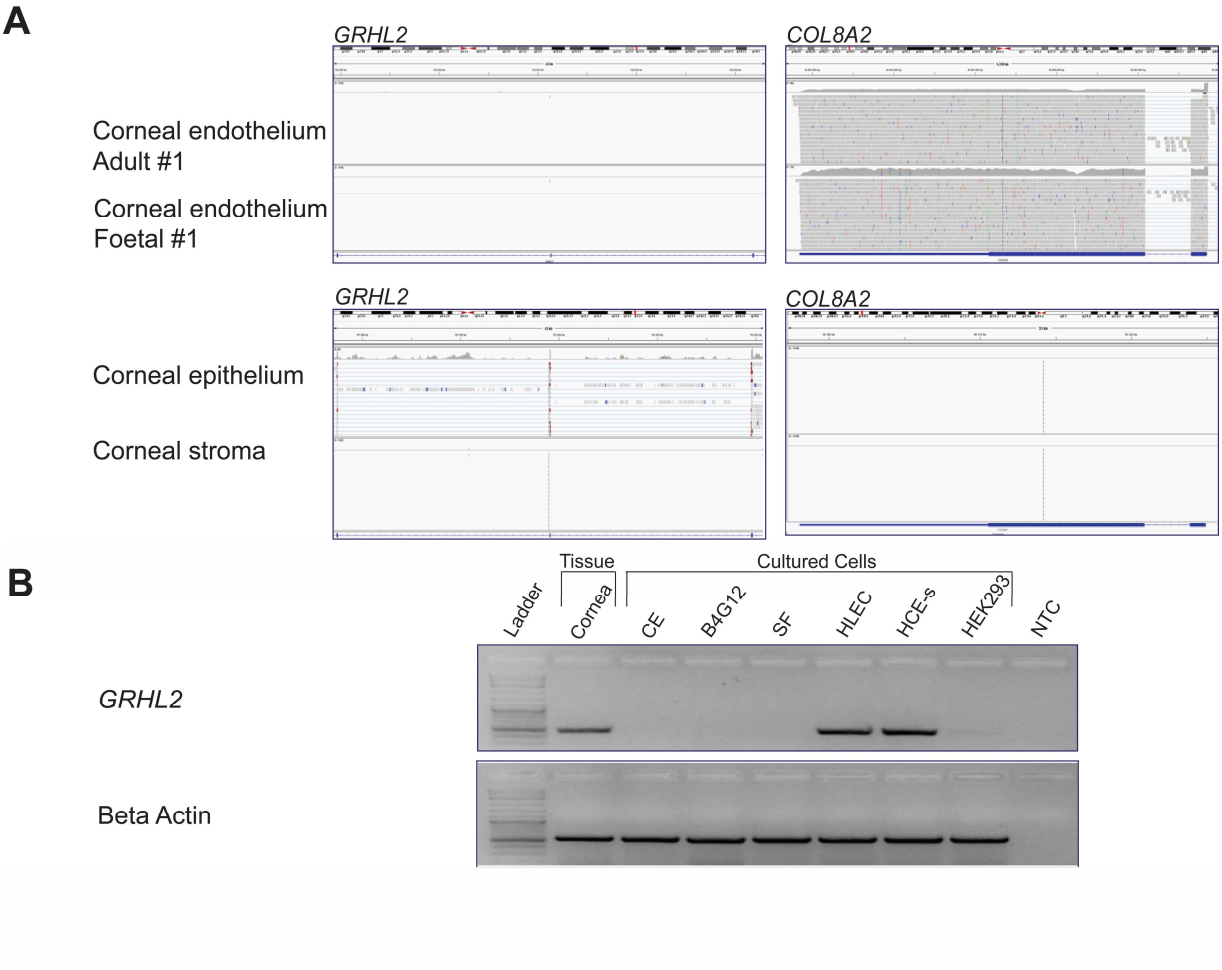


Figure S4. Expression of *GRHL2* in different layers of the human cornea
(A) Interrogation of RNA-seq data derived from adult and fetal human corneal endothelial samples revealed no evidence of *GRHL2* expression. *COL8A2* is highly expressed in corneal endothelium. *GRHL2* is expressed only in the corneal epithelium and not expressed in stroma. **(B)** RT-PCR of corneal tissue and cell types indicates *GRHL2* is expressed in full thickness cornea, human corneal epithelial cells (HCE-s), human limbal epithelial cells (HLEC). No expression was detected in cultured endothelial cells (CE and B4G12) or stromal cells (SF). NTC = no template control.

Table S1. *In silico* predictions of the consequence of *GRHL2* variants on transcription factor binding

AliBaba[1] 2.1 predicts transcription factor binding sites in an unknown DNA sequence utilizing the TRANSFAC public database. MatInspector[2] utilizes a large library of matrix descriptions for transcription factor binding sites to locate matches in DNA sequences. *RKPM values also encompass flanking transcripts. All 3 mutations were predicted to alter transcription factor binding (loss and gain).

Transcription factor	C15, C23, C26, C33 c.20+544G>T	B4 c.20+257delT	B5 c.20+133delA	Expression in endothelium (RKPM)	
				Adult	Fetal
SP1	Site lost[1]			12.52	17.53
NRF1*	4 sites lost[2]			9.31	12.78
ESRRA*	Site gained[2]			41.52	8.74
GLIS1	Site gained[2]			42.75	3.19
AHR	Site lost[2]			32.84	28.87
MYC	Site lost[2]			25.00	5.98
E2F2	Site lost[2]			0.07	1.44
E2F3	Site gained[2]			0.17	0.02
EBF1		Site lost[2]		0.05	5.98
POZ/zinc finger proteins	Site lost[2]	Site gained[2]		N/A	N/A
STAT6		Site gained[2]		27.53	21.94
ZNF354C			Site lost[2]	3.53	5.61
GLI3			Site gained[2]	20.15	14.68
ZBTB7A			Site gained[2]	17.91	7.01