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Supplemental Data

Null Mutations in *LTBP2* Cause Primary Congenital Glaucoma

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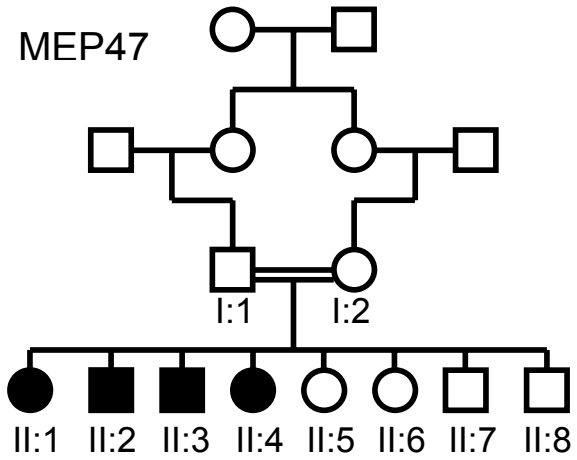
Figure S1. Homozygosity mapping of PCG in family MEP47 to chromosome 14q24.
DNA from the four affected individuals in family MEP47 were mixed in equimolar amounts then analysed as a single sample on an Affymetrix 50k SNP array. The resultant genotypes were examined by eye to look for large regions of homozygosity. Only one obvious region was observed, on chromosome 14q24 (data not shown). Highly informative microsatellite markers were used to confirm linkage. Pedigree and microsatellite haplotype data are depicted. Note the homozygous region only in the affected members of the family with the critical interval spanning a region of ~6.7Mb between the markers D14S258 and D14S42.



Marker	Physical distance (Mb)	DeCODE Genetic distance (cM)	I:1	II:2	II:1	II:2	II:3	II:4	II:5	II:6	II:7	II:8
D14S1038	58.6	60.06	222 224	222 224	222 224	222 224	222 224	222 224	224 222	222 222	222 224	224 222
D14S1065	67.86	66.49	**250** 261	**250** 261	**250 250**	261 261	**250 250**	**250 250**	261 **250**	261 261	261 261	261 **250**
D14S258	69.6	68.5	**175** 171	**175** 169	**175 175**	171 175	**175 175**	**175 175**	171 **175**	171 169	171 169	171 **175**
D14S277	72	71.02	**149** 151	**149** 157	**149 149**	**149 149**	**149 149**	**149 149**	151 **149**	151 **149**	151 157	151 **149**
D14S1047	73.9	72.94	**138** 136	**138** 142	**138 138**	**138 138**	**138 138**	**138 138**	136 **138**	136 **138**	136 142	136 **138**
D14S61	75.4	74.72	**212** 223	**212** 216	**212 212**	**212 212**	**212 212**	**212 212**	223 **212**	223 **212**	223 216	223 **212**
D14S53	75.9	75.71	**147** 141	**147** 147	**147 147**	**147 147**	**147 147**	**147 147**	141 **147**	141 **147**	141 147	141 **147**
D14S42	76.3	76.18	**118** 118	**118** 120	**118 118**	**118 118**	**118 118**	118 120	118 **118**	118 **118**	118 120	118 **118**
D14S983	76.5	77.43	**265** 272	**265** 265	**265 265**	**265 265**	**265 265**	265 265	272 **265**	272 **265**	272 265	272 **265**

Figure S2. Testing the LTBP2 antibody.
The rabbit polyclonal antiserum used in this study was raised against a protein fragment containing the amino-terminal third of cow LTBP2 (LifeSpan Biosciences Inc.). (A) In order to obtain a theoretical value of the molecular weight (MW) in kilodaltons (kDa) for cow LTBP2 and its homologues, LTBP1 and fibrillin1, the corresponding full-length protein sequences where analysed using the compute pI/MW tool at ExPASy. (B) Protein extracts from cow eyes were resolved under reducing conditions by SDS PAGE and analysed by western blotting using the LTBP2 antibody, anti-rabbit immunoglobulin conjugated to horse radish peroxidise as secondary followed by chemilluminescence detection. Lanes 1, 2 and 3 represent 30, 15 and 5 micrograms of total protein extract. The band sizes for the protein ladder are shown. Note that the antibody recognised a single immunoreactive species at ~210 kDa, the expected size for LTBP2, but does not recognise any distinct bands at ~312 or ~147 kDa, the calculated sizes for fibrillin1 and LTBP1 respectively.

(A)

	Accession No.	Amino acids	MW (kDa)
Cow LTBP1	NP_001096561	1338	147
Cow LTBP2	NP_776810	1963	212
Cow Fibrillin1	NP_776478	2871	312

(B)

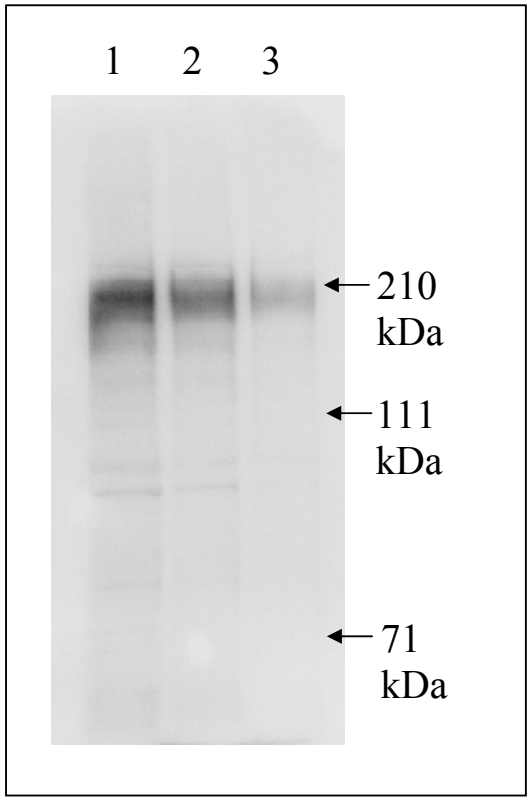


Table S1. Oligonucleotide primers used in this study.

(A) Primer pairs used for the amplification of the coding regions and splice site junctions of the human *LTBP2* gene.

(B) Additional primers required to perform the PCR prior to mutation restriction analysis.

(A)

Exon	Primer Sequence (F)	Primer Sequence (R)	Product Size (bp)
1A	GCCGACCACAAAGCTCTTC	GAGTGCTTCTCCGGGTCTG	434
1B	TGCAGCCAAGGTGTACAGTC	CCCCTCTGTACCCTCCAAAC	342
2	GATGTGCAGAGAATGGCAGA	GCGGAGTGTCTGCTACTGGT	286
3	AGAGTGGCTTCCTGCTTGAG	CTTCACCAAACGGTCCAAAG	476
4	GCAGCCAGAGAGCATTTTTC	AACTCAGCCCCTCTGTGAGA	403
5	AATGCCCTTGAGATGAATGC	CTGGCTCTCTGGCCATCTAC	349
6	GCCTGTTTCTCTGTGGTGGT	CAGCTTCCCTATCCCTGTCA	386
7	TGGTGGATACCCTTCAGAGG	GAGGAGGAGAAGGGCAGACT	441
8	AATGTGGGGAGTGAGCTCTG	AAGGCAGGTCTGGGAAGTCT	351
9	AGGTGGGCTGAGAGGAGTCT	TAGTCCCCTGGAATCAGCAG	415
10	CGGGCACTTGGTCATCTCCT	GTGATCAGGTCTGGGGAAAA	262
11	GCTCCAAACTTCCCAACTGA	GGTTGGGATAAGCACGTGAG	444
12	TCACGTGCTTATCCCAACCT	AGGGACCCAGGATTAACACC	447
13	CAGAGGAGCCAAAAGTGACC	TCCTTCTCACCCTCCTCTGA	206
14	GTCTGAGCACCAGGGAAGAG	GAGGGACCCTGTGTTCTTTG	370
15	CACCCTGCCATAACCTCTGT	TGCTTGGACCTTCTGCTTCT	271
16	GGCTGACTTTATGGCTTCCA	CAGGCTGGAGTTCTGGTCTC	457
17	ATCCTTTGTCCTTGGCCTCT	GAATGTCACTGAGGGGATGG	406
18	AGGGACAAGGATTTGCTGTG	ACCTCTTTCCCTTTCCGTGT	287
19	CCCTGGCCTCATAACTGAGA	GGATGTGTTGGGTCAGTGTG	350
20	CTGCAGAGTCCCACACAGAA	TATTCTGTCCCCTTCCACCA	202
21	TCCCCAAGTTCAGAGTGAGG	AGCTTGTGAGCGACTCTTGG	466
22_23	GGAGACTTCCCCCTTGACTC	GCTGGCTTCCCATGCTCCTG	475
24	GCCCAGAGGAAGCTACACAG	GAGCTAAGGACCAGGCAGTG	273
25	GGAAATCGTCCTGACCTTGA	TGAAAAGCAGCCTCTCAACC	492
26	GTGCATGCGTGTGAGAGAGT	CAGGACCAGTTGAGGAGGAG	298
27	AAGGCCTAGCCTGCTTCTTT	CCTGTAGCTCCTGGTTTTGC	447
28_29	CCTTAGAGGGTCATGAACAGACA	CCCACTCAGGTGAAGGAGTT	489
30	CCCTCATACTGCCTCTCACC	TCCTGGGGACAATCTCTGAC	356
31_32	TGATAGGCAAACACCCTTCC	CCTGCAGGGTATCCCCTTTG	499
33	CAGAGGGTACCAGTCCTTGC	CTAGCAGGTGGAGGAGATGG	455

(B)

Primer	Sequence
1BIIR	GGTCTGCGCAGGTGGCTGGACACGCAGCGACT
4IIF	CCCAGCAGCACGTGGGGTTGTCCCGCAGTGTC
6IIR	CAGCTTCCCTATCCCTGTCA

34	AGTCTGGACACAGCCCTCAG	TCTTCCAGCCTTCCTGAGTT	279
35	CTTGGGCATGGTATGAGCTT	AACCTCTGGCCTGATGTCAC	450

Table S2. The origin of the Pakistani families.
The families, which belong to different clans, were enrolled from different cities in the Punjab province of Pakistan.

FAMILY ID	CLAN	CITY	PROVINCE
MEP47	Khokhar	Khushab	Punjab
PKGL005	Jatt	Muzaffargarh	
PKGL010	Macchi	Gujranwala	
PKGL025	Lohar	Mianwali	

Table S3. Additional features in the PCG families.
The individuals ID (from Figure 2C) and their age at the time of the investigation are shown. Bone mineral density was measured using a DEXA scan. A T-score between -1.00 and -2.50 indicates osteopenia whereas a score of less than -2.50 indicates osteoporosis. In echocardiography, the normal range for aortic valve measurements (AV area) is between 3.0 and 4.0 cm^2. A clinical assessment for Marfan syndrome was also performed on the patients.

	Individuals ID	Gender	Age at time of study (years)	DEXA scan (T-score)		Echocardiogram	Clinical examination	Additional comment
				Spine	Femur neck			
PKGL005								
PCG affected	31	F	23	-2.41	-2.80	Normal	Not suggestive of Marfans	High arched palate
Normal	24	M	23	-1.49	-0.34	-	-	-
PKGL010								
PCG affected	10	M	18	-1.15	-2.69	AV area: 1.8cm^2	Not suggestive of Marfans	High arched palate
Carrier	12	M	13	-2.12	-2.37	AV area: 2.5cm^2	-	-
PKGL025								
PCG affected	24	F	18	-1.30	-1.44	Normal	Not suggestive of Marfans	-
Carrier	22	M	6	-3.56	-3.16	-	-	-