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Li, Lin
Chen, Yabin
Jiao, Xiaodong
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Homozygosity Mapping and Genetic Analysis of Autosomal Recessive Retinal Dystrophies in 144 Consanguineous Pakistani Families

Lin Li,[1,2] Yabin Chen,[2] Xiaodong Jiao,[2] Chongfei Jin,[2,3] Dan Jiang,[2] Mukesh Tanwar,[2,4] Zhiwei Ma,[2] Li Huang,[2,5] Xiaoyin Ma,[2,6] Wenmin Sun,[2,5] Jianjun Chen,[2,7] Yan Ma,[2] Oussama M'hamdi,[2] Gowthaman Govindarajan,[2] Patricia E. Cabrera,[2] Jiali Li,[2,5] Nikhil Gupta,[2] Muhammad Asif Naeem,[8] Shaheen N. Khan,[8] Sheikh Riazuddin,[8–10] Javed Akram,[9,10] Radha Ayyagari,[11] Paul A. Sieving,[12] S. Amer Riazuddin,[13,14] and J. Fielding Hejtmancik[2]

[1]Department of Ophthalmology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[2]Ophthalmic Genetics and Visual Function Branch, National Eye Institute, National Institutes of Health, Bethesda, Maryland, United States
[3]Department of Medicine, Brookdale University Hospital and Medical Center, New York, New York, United States
[4]Department of Genetics, Maharshi Dayanand University Rohtak, Haryana, India
[5]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou, Guangdong, China
[6]Laboratory of Developmental Cell Biology and Disease, School of Ophthalmology and Optometry and Eye Hospital, Wenzhou Medical University, Wenzhou, China
[7]Department of Ophthalmology, Shanghai Tenth People's Hospital, and Tongji Eye Institute, Tongji University School of Medicine, Shanghai, China
[8]National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan
[9]Allama Iqbal Medical College, University of Health Sciences, Lahore, Pakistan
[10]National Centre for Genetic Diseases, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan
[11]Shiley Eye Institute, University of California-San Diego, La Jolla, California, United States
[12]National Eye Institute, National Institutes of Health, Bethesda, Maryland, United States
[13]The Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States
[14]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Correspondence: Lin Li, Department of Ophthalmology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. No. 639, Zhizaoju Road, Huangpu District, Shanghai 200011; jannetlee1300@163.com.

LL and YC contributed equally to the work presented here and should therefore be regarded as equivalent first authors.

SAR and JFH contributed equally to the work presented here and should therefore be regarded as equivalent authors.

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Purpose. The Pakistan Punjab population has been a rich source for identifying genes causing or contributing to autosomal recessive retinal degenerations (arRD). This study was carried out to delineate the genetic architecture of arRD in the Pakistani population.

Methods. The genetic origin of arRD in a total of 144 families selected only for having consanguineous marriages and multiple members affected with arRD was examined. Of these, causative mutations had been identified in 62 families while only the locus had been identified for an additional 15. The remaining 67 families were subjected to homozygosity exclusion mapping by screening of closely flanking microsatellite markers at 180 known candidate genes/loci followed by sequencing of the candidate gene for pathogenic changes.

Results. Of these 67 families subjected to homozygosity mapping, 38 showed homozygosity for at least one of the 180 regions, and sequencing of the corresponding genes showed homozygous cosegregating mutations in 27 families. Overall, mutations were detected in approximately 61.8 % (89/144) of arRD families tested, with another 10.4% (15/144) being mapped to a locus but without a gene identified.

Conclusions. These results suggest the involvement of unmapped novel genes in the remaining 27.8% (40/144) of families. In addition, this study demonstrates that homozygosity mapping remains a powerful tool for identifying the genetic defect underlying genetically heterogeneous arRD disorders in consanguineous marriages for both research and clinical applications.

Keywords: homozygosity mapping, genetic analysis, autosomal recessive retinal dystrophies, consanguineous

Hereditary retinal dystrophies (RD) constitute a group of inherited retinal diseases characterized by chronic and progressive visual impairment, genetic heterogeneity, and significant clinical overlap among the different disorders. To date, mutations in more than 200 genes are known to cause the different forms of RD[1] as summarized in RetNet (https://sph.uth.edu/Retnet/; in the public domain). Retinal dystrophies can be inherited as autosomal recessive (ar), autosomal dominant

Table 1. Summary of Microsatellite Markers for Homozygous Mapping

No.	Gene/Locus	Inheritance	Diseases	Location	Mb, GRCh37	Transcript ID	Marker 1	Distance, Mb	Marker 2	Distance, Mb
1	*ABCA4*	AR	SMD, RP, CRD, FF	1p22.1	94.46–94.59	NM_000350.2	D1S2779	0.76		
2	*ABHD12*	AD	US	20p11.21	25.28–25.37	NM_001042472	D20S844	0.83		
3	*ADAM9*	AR	CRD	8p11.22	38.85–38.96	NM_003816.2	D8S1791	0.7		
4	*AIPL1*	AR/AD	LCA, CRD	17p13.2	6.33–6.34	NM_014336.3	D17S796	0.08		
5	*ARL2BP*	AR	RP	16q13	57.28–57.29	NM_012106.3	D16S3057	0.24		
6	*BBIP1*	AR	BBS	10q25.2	112.66–112.68	NM_001195306	D10S597	1.43	D10S1682	0.64
7	*BBS1*	AR	BBS, RP	11q13.1	66.28–66.30	NM_024649.4	D11S913	0.34	D11S1889	1.01
8	*BBS10*	AR	BBS	12q21.2	76.74	NM_024685.3	D12S1684	0.52		
9	*BBS12*	AR	BBS	4q27	123.65–123.67	NM_001178007.1	D4S430	0.04		
10	*BBS2*	AR	BBS, RP	16q21	56.52–56.55	NM_031885.3	D16S3140	0.21		
11	*BBS3*	AR	BBS	3q11.2	97.48–97.52	NM_032146.3	D3S1603	0.94	D3S3619	1.84
12	*BBS4*	AR	BBS	15q22.3-q23	72.98–73.03	NM_033028.4	D15S980	0.08		
13	*BBS5*	AR	BBS	2q31.1	170.34–170.36	NM_152384.2	D2S2284	1.13	D2S2345	1.62
14	*BBS7*	AR	BBS	4q27	122.75–122.79	NM_176824.2	D4S1612	0.43	D4S2985	1.43
15	*BBS8*	AR	RP, BBS	14q31.3	89.29–89.34	NM_144596.2	D14S1058	0.64		
16	*BBS9*	AR	BBS	7p14	33.17–33.65	NM_198428.2	D7S2252	1.1		
17	*BCAMD*	AD	MD	6p12.3-q16	49.18–94.76	N/A	D6S456	0		
18	*BEST1*	AR/AD	RP, MD, ARB	11q12.3	61.72–61.73	NM_004183.3	D11S1765	0.94		
19	*C12orf65*	AD	OA	12q24.31	123.72–123.74	NM_152269.4	D12S1612	1.17		
20	*C1QTNF5*	AD	MD	11q23.3	119.21–119.22	NM_015645.3	D11S4171	0.16	D11S4104	0.57
21	*C21orf2*	AD	CRD	21q22.3	45.75–45.76	NM_004928.2	D21S1890	0.9		
22	*C2orf71*	AR	RP	2p23.2	29.28–29.30	NM_001029883	D2S170	0.07		
23	*C8orf37*	AD	CRD, RP	8q22.1	96.26–96.28	NM_177965.3	D8S1699	0.25		
24	*CA4*	AD	RP	17q23	58.23–58.26	NM_000717.3	D17S1604	0.25		
25	*CABP4*	AR	CSNB, LCA	11q13.2	67.22–67.23	NM_145200.3	D11S1889	0.08		
26	*CACD*	AD	RP, MD, CRD, LCA	17p13	5.13–8.20	N/A	D17S1832	0		
27	*CACNA2D4*	AR	CD	12p13.33	1.90–2.03	NM_172364.4	D12S100	0.15		
28	*CAPN5*	AD	NIV	11q14	76.78–76.84	NM_004055.4	D11S911	0.61		
29	*CDH23*	AR	US	10q23.1	73.16–73.58	NM_022124.5	D10S1650	0		
30	*CDH3*	AR	MD	16q22.1	68.68–68.73	NM_001793.4	D16S496	0.22		
31	*CDHR1*	AR	CRD	10q23.1	85.95–85.98	NM_033100.2	D10S1686	0.38		
32	*CEP290*	AR	SLSN, LCA	12q21.32	88.44–88.54	NM_025114.3	D12S1598	0.99		
33	*CERKL*	AR	RP, CRD	2q31.3	182.40–182.52	NM_001030311.2	D2S2310	0.23		
34	*CFH*	AD	MD	1q32	196.62–196.72	NM_000186.3	D1S2757	1.88		
35	*CIB2*	AD	US	15q24	78.40–78.42	NM_006383.2	D15S1023	0.72		
36	*CLRN1*	AR	US, RP	3q25	150.64–150.69	NM_174878.2	D3S1279	0.34		
37	*CNGA1*	AR	RP	4p12	47.94–48.01	NM_000087.3	D4S3002	0.6	D4S396	1.62
38	*CNGA3*	AR	A, CRD	2q11.2	98.96–99.02	NM_001298.2	D2S2311	0	D2S113	1.68
39	*CNGB1*	AR	RP	16q13	57.92–58.01	NM_001297.4	D16S3057	0.39		
40	*CNGB3*	AR	A, CD	8q21.3	87.59–87.76	NM_019098.4	D8S271	0.76		
41	*CNNM4*	AR	CRD	2q11.2	97.43–97.48	NM_020184.3	D2S113	0.15		
42	*CODA1*	AD	CODA	12q13.13-q14.3	53.89–67.36	N/A	D12S329	0		
43	*CORD17*	AD	CRD	10q26	122.32–129.09	N/A	D10S1757	0		
44	*CRB1*	AR/AD	LCA, RP	1q31.3	197.17–197.45	NM_201253.2	D1S2816	0.52	D1S2840	0.81
45	*CRX*	AR/AD	CRD, LCA, RP	19q13.32	48.33–48.35	NM_000554.4	D19S596	0.9		
46	*CYP4V2*	AR	BCD, RP	4q35.2	187.11–187.13	NM_207352.3	D4S426	1.98		
47	*DFNB31*	AR	US	9q32	117.16–117.27	NM_015404.3	D9S1776	0.69		
48	*DHDDS*	AR	RP	1p36.11	26.76–26.80	NM_024887.3	D1S2885	0.62		
49	*DHX38*	AD	RP	16q22	72.13–72.15	NM_014003.3	D16S3106	0.04		
50	*DTHD1*	AD	LCA	4p14	36.28–36.35	NM_001136536	D4S2950	1.39		
51	*EFEMP1*	AD	MD	2p16	56.09–56.15	NM_001039348	D2S378	1.15		
52	*ELOVL4*	AD	MD	6q14	80.62–80.66	NM_022726.3	D6S460	0.27		
53	*EMC1*	AD	RP	1p36.13	19.54–19.58	NM_015047.2	D1S199	0.38		
54	*EVR3*	AD	FEVR	11p13-p12	25.94–36.78	N/A	D11S1751	0		
55	*EYS*	AR	RP	6q12	64.43–66.42	NM_001142800.1	D6S402	1.46		
56	*FAM161A*	AR	RP	2p15	62.05–62.08	NM_032180.2	D2S2206	0.44		
57	*FSCN2*	AD	RP, MD	17q25	79.5	NM_001077182	D17S928	0.75		
58	*FZD4*	AD	FEVR	11q14.2	86.66–86.67	NM_012193.3	D11S1887	0.27		
59	*GDF6*	AD	LCA, KFS	8q22.1	97.15–97.17	NM_001001557	D8S1822	0.42		
60	*GNAT1*	AD	CSNB	3p21	50.23–50.24	NM_144499.2	D3S3629	0.65		

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Table 1. Continued

No.	Gene/Locus	Inheritance	Diseases	Location	Mb, GRCh37	Transcript ID	Marker 1	Distance, Mb	Marker 2	Distance, Mb
61	*GNAT2*	AR	A	1p13.1	110.15–110.16	NM_005272.3	D1S2651	0.04		
62	*GPR125*	AD	RP	4p15.2	22.39–22.52	NM_145290.3	D4S3017	0.92		
63	*GPR179*	AD	CSNB	17q21.1	36.48–36.50	NM_001004334	D17S1851	0.41		
64	*GPR98*	AR	US	5q14.3	89.85–90.46	NM_032119.3	D5S618	0.08		
65	*GRK1*	AR	CSNB	13q34	114.32–114.44	NM_002929.2	D13S1295	1.23	D13S293	0.04
66	*GRM6*	AR	CSNB	5q35.3	178.41–178.42	NM_000843.3	D5S408	1.57	D5S2030	0.6
67	*GUCA1A*	AD	CD, CRD	6p21.1	42.12–42.15	NM_000409.3	D6S1582	0.95	D6S1552	0.16
68	*GUCA1B*	AD	RP, MD	6p21.1	42.15–42.16	NM_002098.5	D6S1582	0.94	D6S1552	0.19
69	*GUCY2D*	AR/AD	LCA, CRD	17p13.1	7.91–7.92	NM_000180.3	D17S1353	0.29		
70	*HARS*	AD	US	5q31.3	140.05–140.07	NM_002109.4	D5S500	2.2		
71	*HK1*	AD	RP	10q22	71.03–71.16	NM_000188.2	D10S1647	0.09		
72	*IDH3B*	AR	RP	20p13	2.64	NM_006899.3	D20S842	0.05		
73	*IFT27*	AR	BBS	22q13.1	37.15–37.17	NM_006860.4	D22S283	0.4		
74	*IMPDH1*	AD	RP, LCA	7q31.3-q32	128.03–128.05	NM_000883.3	D7S1875	0.28		
75	*IMPG1*	AD	MD	6q14.2-q15	76.63–76.78	NM_001563.2	D6S456	0.52		
76	*IMPG2*	AR	RP	3q12.2-q12.3	100.94–101.04	NM_016247.3	D3S1271	0.21		
77	*INPP5E*	AD	JS, MORM	9q34.3	139.32–139.33	NM_019892.4	D9S1838	1.31		
78	*IQCB1*	AR	LCA, SLSN	3q13.33	121.49–121.55	NM_001023570	D3S3576	0.7	D3S3513	2.3
79	*ITM2B*	AD	RD	13q14.3	48.81–48.84	NM_021999.4	D13S153	0.05		
80	*KCNJ13*	AR/AD	LCA, SVD	2q37.1	233.63–233.64	NM_002242.4	D2S2348	0.51		
81	*KCNV2*	AR	CD	9p24.2	2.72–2.73	NM_133497.3	D9S1813	1.4	D9S1858	2.02
82	*KIAA1549*	AD	RP	7q34	138.52–138.67	NM_001164665	D7S684	0		
83	*KIZ*	AD	RP	20p11.23	21.11–21.23	NM_018474.4	D20S912	0.25		
84	*KLHL7*	AD	RP	7p15.3	23.15–23.22	NM_001031710	D7S673	0.63		
85	*LCA5*	AR	LCA	6q14.1	80.19–80.25	NM_181714.3	D6S284	0.84		
86	*LRAT*	AR	RP, LCA	4q32.1	155.66–155.67	NM_004744.3	D4S3021	0.72		
87	*LRIT3*	AD	CSNB	4q25	110.77–110.79	NM_198506.3	D4S2945	0.48		
88	*LRP5*	AR/AD	FEVR	11q13.2	68.08–68.22	NM_002335.2	D11S4113	0.55		
89	*LZTFL1*	AR	BBS	3p21.3	45.86–45.96	NM_020347.2	D3S3582	0.47	D3S3640	2.04
90	*MAK*	AR	RP	6p24	10.76–10.84	NM_001242957	D6S470	0.73		
91	*PRDM13*	AD	MD	6q14-q16.2	100.05–100.06	N/A	D6S1717	0.38		
92	*MCDR4*	AD	MD	14q11.2	20.84–21.44	N/A	D14S261	0		
93	*MCDR5*	AD	MD	19q13.31-q13.32	43.81–47.01	N/A	D19S412	0		
94	*MDDC (CYMD)*	AD	MD	7p21-p15	21.81–30.95	N/A	D7S516	0		
95	*MERTK*	AR	RP, CRD	2q14.1	112.66–112.79	NM_006343.2	D2S2269	0.16		
96	*MFN2*	AD	OA	1p36.22	12.04–12.07	NM_014874.3	D1S2667	0.55		
97	*MFRP*	AR	N, M	11q23.3	119.21–119.22	NM_031433.2	D11S4171	0.16		
98	*MKKS*	AR	BBS	20p12	10.39–10.41	NM_018848.3	D20S894	0.29		
99	*MKS1*	AR	BBS	17q22	56.28–56.30	NM_017777.3	D17S1606	0.68		
100	*MVK*	AD	RP	12q24	110.01–110.04	NM_000431.2	D12S1645	0		
101	*MYO7A*	AR	US	11q13.5	76.84–76.93	NM_000260.3	D11S911	0.52		
102	*NEK2*	AD	RP	1q32.2-q41	211.83–211.85	NM_002497.3	D1S425	0.23		
103	*NMNAT1*	AD	LCA	1p36.22	10.00–10.05	NM_022787.3	D1S223	0.11		
104	*NPHP1*	AR	SLSN, BBS	2q13	110.88–110.96	NM_000272.3	D2S1888	0.44		
105	*NR2E3*	AR/AD	ESC, RP	15q22.32	72.10–72.11	NM_014249.2	D15S204	0.19		
106	*NR2F1*	AD	OA	5q14	92.92–92.93	NM_005654.4	D5S2100	0.89		
107	*NRL*	AR/AD	RP	14q11.1-q11.2	24.55	NM_006177.3	D14S64	0.01		
108	*OAT*	AR	Gyrate atrophy	10q26.13	126.09–126.11	NM_000274.3	D10S1723	0.43		
109	*OPA1*	AD	OA	3q28-q29	193.31–193.42	NM_015560.2	D3S3726	1.88		
110	*OPA4*	AD	OA	18q12.2-q12.3	39.25–48.06	N/A	D18S450	0		
111	*OPA5*	AD	OA	22q12.1-q13.1	26.36–36.75	N/A	D22S1162	0		
112	*OPA6*	AD	MD, RP	8q21-q22	83.62–95.58	N/A	D8S270	0		
113	*OPA8*	AD	OA	16q21-q22.3	65.07–74.17	N/A	D16S3066	0		
114	*OPN1SW*	AD	Tritanopia	7q31.3-q32	128.41–128.42	NM_001708.2	D7S530	0.78		
115	*OTX2*	AD	LCA, M	14q21-q22	57.27–57.28	NM_172337.2	D14S980	0.12		
116	*PCDH15*	AR	US	10q21.1	55.56–56.56	NM_033056.3	D10S1788	1.44		
117	*PDE6A*	AR	RP	5q31.2-q34	149.24–149.32	NM_000440.2	D5S640	0.66		
118	*PDE6B*	AR/AD	RP, CSNB	4p16.3	0.62–0.66	NM_000283.3	D4S2936	0.03		
119	*PDE6C*	AR	CD, A	10q23.33	95.37–95.43	NM_006204.3	D10S185	0.18		
120	*PDE6G*	AR	RP	17q25	79.62	NM_002602.3	D17S928	0.63		
121	*PDE6H*	AD	Atrophia areata	12p13	15.13	NM_006205.2	D12S364	1.3		

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Table 1. Continued

No.	Gene/Locus	Inheritance	Diseases	Location	Mb, GRCh37	Transcript ID	Marker 1	Distance, Mb	Marker 2	Distance, Mb
122	*PITPNM3*	AD	CRD	17p13	6.35–6.46	NM_031220.3	D17S1874	0		
123	*PLA2G5*	AD	BFR	1p36-p34	20.40–20.42	NM_000929.2	D1S2843	0.09		
124	*PRCD*	AR	RP	17q25.1	74.54–74.54	NM_001077620.2	D17S801	0.03		
125	*PROM1*	AR/AD	RP, SMD, CRD	4p15.32	15.97–16.09	NM_006017.2	D4S3048	0		
126	*PRPF3*	AD	RP	1q21.1	150.29–150.33	NM_004698.2	D1S498	0.97		
127	*PRPF31*	AD	RP	19q13.42	54.62–54.64	NM_015629.3	D19S572	0.51		
128	*PRPF4*	AD	RP	9q31-q33	116.04–116.06	NM_004697.4	D9S1824	0.83		
129	*PRPF6*	AD	RP	20q13.33	62.61–62.66	NM_012469.3	D20S173	0.73	D20S171	1.8
130	*PRPF8*	AD	RP	17p13.3	1.55–1.59	NM_006445.3	D17S1828	2.22		
131	*PRPH2*	AD	RP, MD, CRD, LCA	6p21.2-p12.3	42.66–42.69	NM_000322.4	D6S1582	0.41	D6S1552	0.7
132	*RAB28*	AD	CRD	4p15.33	13.37–13.49	NM_004249.3	D4S403	0.26		
133	*RAX2*	AR	CRD, MD	19p13.3	3.77–3.77	NM_032753.3	D19S424	0.54		
134	*RB1*	AD	Retinoblastoma	13q14.2	48.88–49.06	NM_000321.2	D13S153	0		
135	*RBP3*	AR	RP	10q11.2	48.38–48.39	NM_002900.2	D10S220	3.96		
136	*RBP4*	AR	RPE degeneration	10q23.33	95.35–95.36	NM_006744.3	D10S583	0.98		
137	*RD3*	AR	LCA	1q32.3	211.65–211.67	NM_183059.2	D1S425	0.41		
138	*RDH12*	AR/AD	LCA, RP	14q24.1	68.17–68.20	NM_152443.2	D14S1065	0.71		
139	*RDH5*	AR	FA, CD	12q13-q14	56.11–56.12	NM_002905.3	D12S1632	0.3	D12S1724	1.24
140	*RGR*	AR/AD	RP	10q23	86.00–86.02	NM_001012720	D10S1717	0.13		
141	*RGS9*	AR	DCA	17q24.1	63.13–63.22	NM_003835.3	D17S807	1.64	D17S1809	0.43
142	*RGS9BP*	AR	DCA	19q13.12	33.17–33.17	NM_207391.2	D19S868	0.28		
143	*RHO*	AR/AD	RP, CSNB	3q21-q24	129.25–129.25	NM_000539.3	D3S1290	1.74	D3S3606	0.05
144	*RIMS1*	AD	CRD	6q12-q13	72.92–73.11	NM_014989.5	D6S1681	0.7		
145	*RLBP1*	AR	RP, CRD	15q26.1	89.75–89.76	NM_000326.4	D15S979	0.92		
146	*RNANC*	AR	CRN	10q21	69.99	N/A	D10S1652	5.58		
147	*RP1*	AR/AD	RP	8q12.1	55.53–55.54	NM_006269.1	D8S1828	1.26		
148	*RP1L1*	AD/AR	MD, RP	8p23	10.46–10.51	NM_178857.5	D8S520	0.05		
149	*RP22*	AR	RP	16p12.1-p12.3	16.85–24.24	N/A	D16S403	0		
150	*RP29*	AR	RP	4q32-q34	176.51–183.72	N/A	D4S415	0		
151	*RP32*	AR	RP	1p21.2-p13.3	101.97–110.88	N/A	D1S2651	0		
152	*RP63*	AD	RP	6q23	102.44–138.54	N/A	D6S457	0		
153	*RP9*	AD	RP	7p14.3	33.13–33.15	NM_203288.1	D7S2252	1.06		
154	*RPE65*	AR/AD	LCA, RP	1p31.2	68.89–68.92	NM_000329.2	D1S219	0.92		
155	*RPGRIP1*	AR	LCA, CRD	14q11.2	21.76–21.82	NM_020366.3	D14S72	0.39		
156	*SAG*	AR	RP, Oguchi disease	2q37.1	234.22–234.26	NM_000541.4	D2S2297	1.85	D2S172	1.05
157	*SDCCAG8*	AR	BBS	1q43	243.42–243.66	NM_006642.3	D1S2811	0.03		
158	*SEMA4A*	AR	RP, CRD	1q22	156.12–156.15	NM_022367.3	D1S305	1.84		
159	*SLC24A1*	AR	CSNB	15q22.31	65.94–65.95	NM_004727.2	D15S153	0.61		
160	*SLC7A14*	AR	RP	3q26.2	170.18–170.30	NM_020949.2	D3S3723	0	D3S1564	0
161	*SNRNP200*	AD	RP	2q11.2	96.94–96.97	NM_014014.4	D2S2159	0.89		
162	*SPATA7*	AR	LCA	14q31.3	88.85–88.90	NM_018418.4	D14S68	0.22		
163	*TEAD1*	AD	Atrophia areata	11p15.2	12.70–12.97	NM_021961.5	D11S1794	0.37		
164	*TIMP3*	AD	SRD	22q12.3	33.20–33.26	NM_000362.4	D22S1162	1.05		
165	*TMEM126A*	AD	OA	11q14.1	85.36–85.37	NM_032273.3	D11S4147	0.83		
166	*TOPORS*	AD	RP	9p21	32.54–32.55	NM_005802.4	D9S1788	0.59		
167	*TRIM32*	AR	BBS	9q33.1	119.45–119.46	NM_012210.3	D9S177	0.99		
168	*TRPM1*	AR	CSNB	15q13.3	31.29–31.45	NM_002420.5	D15S165	0.03		
169	*TSPAN12*	AD	FEVR	7q31.31	120.43–120.50	NM_012338.3	D7S480	0.47		
170	*TTLL5*	AD	CD, CRD	14q24.3	76.13–76.42	NM_015072.4	D14S61	0		
171	*TULP1*	AR	RP, LCA	6p21.31	35.47–35.48	NM_003322.3	D6S1645	0.1	D6S439	0.32
172	*UNC119*	AD	CRD	17q11.2	26.87–26.88	NM_005148.3	D17S1824	0.21		
173	*USH1C*	AR	US	11p15.1	17.52–17.57	NM_005709.3	D11S902	0.03		
174	*USH1E*	AD	US	21q21	20.94–32.43	N/A	D21S1914	0		
175	*USH1G*	AR	US	17q25.1	72.91–72.92	NM_173477.2	D17S1807	0.55		
176	*USH1H*	AD	US	15q22-q23	67.34–70.69	N/A	D15S980	2.42		
177	*USH1K*	AD	US	10p11.21-q21.1	35.89–56.09	N/A	D10S539	0		
178	*USH2A*	AR	US, RP	1q41	216.35-216.60	NM_206933.2	D1S2827	0.21		

Table 1. Continued

No.	Gene/Locus	Inheritance	Diseases	Location	Mb, GRCh37	Transcript ID	Marker 1	Distance, Mb	Marker 2	Distance, Mb
179	*VRD1*	AR	VRD	22q13	45.96–48.35	N/A	D22S1153	0	D22S1170	0
180	*ZNF513*	AR	RP	2p23.3	27.6	NM_144631.5	D2S174	0.76		

Based on the Généthon map in the National Center for Biotechnology Information, a single microsatellite marker with heterozygosity greater than 0.75 or two markers with heterozygosity less than 0.75 but above 0.5 located within 2 Mb of each candidate gene/locus were selected for each disease locus. SVD, snowflake vitreoretinal degeneration; SMD, Stargardt-like macular dystrophy; MD, macular dystrophy; CRD, cone–rod dystrophy; ARB, autosomal recessive bestrophinopathy; FF, fundus flavimaculatus; ESC, enhanced S-cone syndrome; US, Usher syndrome; SLSN, Senior-Loken syndrome; BCD, Bietti's crystalline dystrophy; CD, cone dystrophy; BBS, Bardet-Biedl syndrome; OA, optic atrophy; FEVR, familial exudative vitreoretinopathy; CRN, congenital retinal nonattachment; A, achromatopsia; SFD, Sorsby's fundus dystrophy; N, nanophthalmols; M, microphthalmus; KFS, Klippel Feil syndrome; FA, fundus albipunctatus; JS, Joubert syndrome, MORM, MORM syndrome; NIV, neovascular inflammatory vitreoretinopathy; CODA, cavitary optic disc anomalies; DCA, delayed cone adaptation; VRD, vitreoretinal dystrophy; BFR, benign fleck retina.

(ad), and X-linked (xl), as well as rare mitochondrial and digenic traits.[2] Rod or cone photoreceptor degenerations are two main groups of RDs.

Among the rod RDs, retinitis pigmentosa (RP, MIM no. 268000), a genetically and clinically heterogeneous retinal degeneration, is the most common worldwide,[3] having a worldwide prevalence estimated to be approximately 1 in 4000 individuals.[4–14] Currently, mutations associated with RP have been identified in more than 82 genes, of which 58 have been shown to be relevant to arRP (RetNet). However, that these 82 genes are responsible only for around 60% of RP[15,16] suggests that the number of currently unidentified genes causing RP might be quite high. In addition, the cone RDs, including cone- or cone–rod dystrophies (CORD) and the macular dystrophies, which mainly affect the central vision, have been associated with more than 30 genes (RetNet).

Pakistan has the highest prevalence of consanguineous marriages in the world, presumably because this practice provides a number of social and economic advantages.[17] In a review of all published retinal degeneration cases in Pakistan, only 4 families with compound heterozygous mutations were identified in 146 (2.7%) genetically resolved arRD families,[18] further supporting the utility of homozygosity mapping in this population. Therefore to identify causative mutations in an ongoing study of large Pakistani arRD families with multiple affected individuals, we carried out homozygosity mapping of known RD loci followed by mutation screening of the genes in homozygous loci in 67 consanguineous families with arRD from Pakistan as a part of an ongoing international collaboration between the National Eye Institute (NEI), National Institutes of Health (NIH), United States, and the National Centre of Excellence in Molecular Biology (NCEMB), Allama Iqbal Medical College, and the National Centre for Genetic Diseases, Shaheed Zulfiqar Ali Bhutto Medical University in Pakistan.

Materials and Methods

Enrollment and Clinical Assessment of arRD Families

This study was approved by the Institutional Review Boards of the National Centre of Excellence in Molecular Biology and the

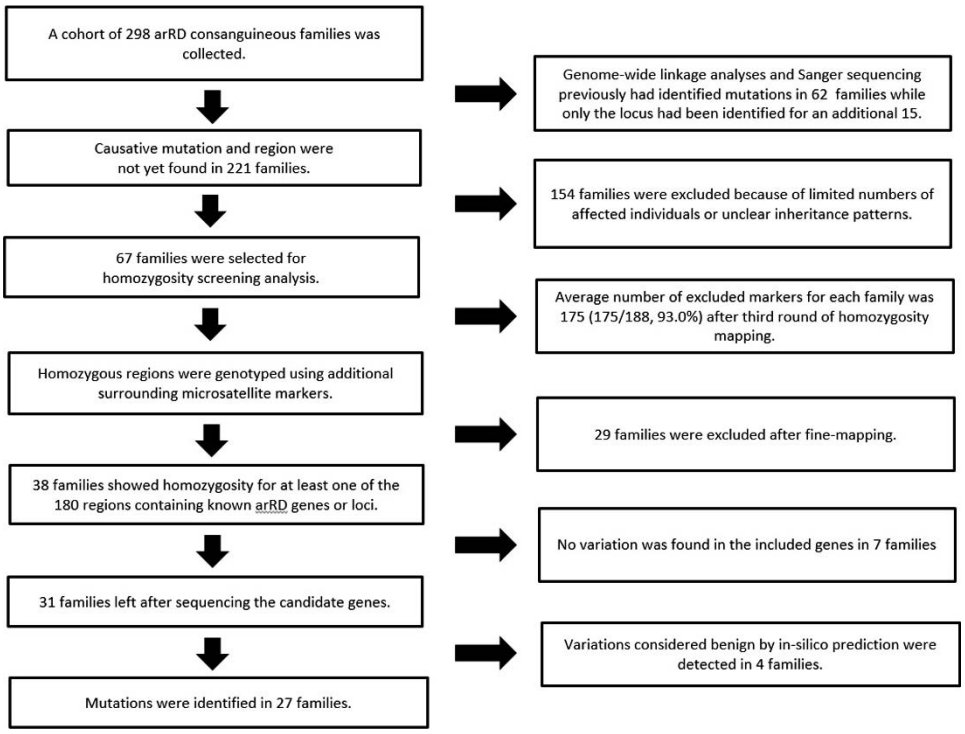

Figure 1. Workflow of this study.

Table 2. Mutations Detected in 67 arRD Families Subjected to Homozygosity Mapping

		Mutation		Pathogenicity			
Fam No.	Gene	Nucleotide	Amino Acid	PP2	S	C	Phen
61220	*CDHR1*	c.1463delG*	p.(G488Afs*20)	N/A	N/A	N/A	RP
61166	*CEP290*	c.148C>T	p.(H50Y)	PoD	DA	D	EORD
61219	*CERKL*	c.847C>T	p.(R283*)	N/A	N/A	N/A	RP
61086	*CNGA3*	c.952G>A	p.(A318T)	PrD	DA	D	RD
61042	*CNGB1*	c.2493-2_2495 delinsGGC*	p.(S831Rfs*2)	N/A	N/A	N/A	RP
61036	*CNGB3*	c.1148delC*	p.(T383Ifs*13)	N/A	N/A	N/A	RD
61192	*EYS*	c.6137G>A*	p.(W2046*)	N/A	N/A	N/A	RP
61016	*EYS*	c.7187G>C	p.(C2396S)	PrD	DA	D	RP
61015	*GRK1*	c.55C>T*	p.(R19*)	N/A	N/A	N/A	RD
61155	*GRM6*	c.824G>A	p.(G275D)	PrD	DA	D	CSNB
61058	*LCA5*	c.652C>G	p.(R218G)	PrD	DA	D	EORD
61076	*LRAT*	c.418G>T*	p.(E140*)	N/A	N/A	N/A	RP
61150	*NR2E3*	c.227G>A	p.(R76Q)	PrD	DA	D	RP
61217	*RBP3*	c.3353_3354delCT*	p.(S1118Cfs*3)	N/A	N/A	N/A	RP
61198	*RDH5*	c.536A>G	p.(K179R)	PrD	DA	D	FA
61199	*RDH5*	c.536A>G	p.(K179R)	PrD	DA	D	FA
61035	*RDH5*	c.758T>G	p.(M253R)	PoD	DA	D	FA
61126	*RDH5*	c.758T>G	p.(M253R)	PoD	DA	D	FA
61065	*RDH12*	c.609C>A	p.(S203R)	PrD	DA	D	RD
61113	*RP1*	c.1126C>T	p.(R376*)	N/A	N/A	N/A	RP
61262	*RP1*	c.787+1G>A	p.(I263Nfs*8)	N/A	N/A	N/A	RP
61231	*RPE65*	c.119G>A	p.(G40D)	PrD	DA	D	EORD
61312	*RPGRIP1*	c.931delA*	p.(N311Ifs*5)	N/A	N/A	N/A	LCA
61206	*TULP1*	c.1138A>G	p.(T380A)	B	DA	N	RP
61301	*TULP1*	c.1466A>G	p.(K489P)	PrD	DA	D	EORP
61309	*TULP1*	c.1466A>G	p.(K489P)	PrD	DA	D	EORD
61191	*USH2A*	c.5740C>T*	p.(Q1914*)	N/A	N/A	N/A	RP/D

* Mutation would be expected to result in nonsense-mediated decay.

Combined NeuroScience Institutional Review Board at the National Institutes of Health. Written informed consent consistent with the tenets of the Declaration of Helsinki was obtained from participating individuals or their guardians before the study. Families segregating arRD with three or more affected individuals were identified by visiting eye hospitals in Pakistan, mostly in the Punjab. Blood samples were drawn from potentially informative family members, and genomic DNA was extracted from leukocytes according to standard protocols.[19] All participants underwent a detailed family, ophthalmic, and medical history, and selected individuals were evaluated by visual acuity, best-corrected visual acuity, slit-lamp biomicroscopy, ophthalmoscopy, fundus photography, and electroretinography (ERG). Previously, as part of this project, 77 families had been mapped by linkage analysis to specific chromosomal locations; the causative gene and mutations had been identified in 62 while for the remaining 15 only the locus had been identified. For the current homozygosity exclusion mapping study, 67 families were selected from the remaining unlinked families based on the availability of DNA samples and consanguineous marriages of the parents of affected individuals. Eight of these families have undergone whole genome linkage analysis without identified causative genes while the remaining 59 families were not screened. Families with possible dominant or X-linked inheritance were excluded, and some families with only two affected offspring of consanguineous matings were included in the early parts of the study.

Homozygosity Mapping and Linkage Analysis

One hundred eighty genes or loci associated with inherited retinal diseases were selected from RetNet (https://sph.uth.edu/Retnet/) and screened by homozygosity exclusion mapping (Table 1). Homozygosity genotyping of 188 microsatellite markers was done in one affected individual of each family. Loci homozygous in the first individual were genotyped in a second affected family member and, if also

Table 3. Variations of Unknown Significance Detected in Three Genes of 67 arRD Families

			Variation		Pathogenicity				
No.	Fam No.	Gene	Nucleotide	Amino Acid	PP2	S	C	Phen	P
1	61221	*CNGB3*	c.1208G>A	p.(R403Q)	PrD	T	N	RD	P
2	61169	*LRP5*	c.4268C>T	p.(P1423L)	B	T	N	RD	P
3	61237	*PROM1*	c.1946C>T	p.(S649L)	B	T	N	RP	P
4	61267	*PROM1*	c.1946C>T	p.(S649L)	B	T	N	RP	P

One homozygous instance of the c.1208G>A, p.(R403Q) variant was identified in 96 healthy individuals. PP2, PolyPhen2; S, SIFT; C, Condel; PrD, probably damaging; B, benign; T, tolerated; N, neutral; Phen, phenotype; P, progressive; PoD, possibly damaging; DA, damaging; D, deleterious; N, neutral; N/A, not applicable.

Table 4. Two-Point LOD Scores of arRD Gene Markers in 31 Families

Fam No.	Marker	Mb	0	0.001	0.01	0.05	0.1	0.2	0.3	0.4	Zmax	θ_{max}
61220	D10S1689	85.67	2.63	2.62	2.56	2.3	1.97	1.3	0.66	0.2	2.63	0
	D10S1717	85.86	2.86	2.85	2.79	2.53	2.2	1.52	0.87	0.33	2.86	0
	CDHR1 c.1463delG, p.(G488Afs*18)	85.95	3.62	3.61	3.55	3.28	2.92	2.18	1.42	0.68	3.62	0
61166	D12S88	86.37	1.91	1.9	1.86	1.69	1.47	1.04	0.62	0.26	1.91	0
	CEP290 c.148C>T p.(H50Y)	88.44	2.53	2.53	2.48	2.29	2.04	1.53	1.02	0.51	2.53	0
	D12S1598	89.52	1.91	1.9	1.86	1.69	1.47	1.04	0.62	0.26	1.91	0
61219	D2S2310	18.22	1.11	1.11	1.08	0.97	0.84	0.57	0.32	0.13	1.11	0
	CERKL c.847C>T, p.(R283*)	18.24	2.41	2.4	2.36	2.19	1.96	1.48	1	0.5	2.41	0
	D2S364	18.3	1.88	1.88	1.84	1.68	1.47	1.05	0.64	0.27	1.88	0
61086	D2S2311	99.02	2.03	2.02	1.98	1.8	1.56	1.08	0.61	0.19	2.03	0
	CNGA3 c.952G>A, p.(A318T)	98.96	2.53	2.53	2.48	2.29	2.04	1.53	1.02	0.51	2.53	0
	D2S2972	102.57	1.12	1.12	1.1	1	0.88	0.62	0.38	0.17	1.12	0
61042	D16S3071	56.66	0.89	0.89	0.86	0.77	0.64	0.4	0.18	0.04	0.89	0
	D16S3057	57.52	1.6	1.6	1.56	1.4	1.2	0.8	0.43	0.13	1.6	0
	CNGB1 c.2493-2_2495delinsGGC	57.91	1.89	1.89	1.85	1.67	1.45	1	0.56	0.19	1.89	0
	D16S3094	59.62	1.6	1.6	1.56	1.4	1.2	0.8	0.43	0.13	1.6	0
	D16S514	62.33	0.58	0.58	0.58	0.57	0.53	0.41	0.25	0.11	0.58	0
61036	*CNGB3* c.1148delC, p.(T383Ifs*13)	87.59	5.21	5.19	5.09	4.64	4.07	2.87	1.67	0.59	5.21	0
	D8S271	88.52	4.72	4.71	4.62	4.18	3.62	2.49	1.37	0.43	4.72	0
	D8S270	93.02	−∞	−0.28	0.69	1.23	1.3	1.06	0.65	0.25	1.3	0.1
61221	*CNGB3* c.1208G>A, p.(R403Q)	87.59	2.53	2.53	2.49	2.31	2.08	1.59	1.08	0.55	2.53	0
	D8S271	88.52	2.21	2.21	2.17	2	1.77	1.31	0.83	0.36	2.21	0
	D8S270	93.02	1.91	1.91	1.87	1.69	1.46	1.02	0.6	0.23	1.91	0
61192	D6S402	62.97	2.7	2.7	2.64	2.38	2.05	1.38	0.77	0.27	2.7	0
	EYS c.6137G>A, p.(W2046*)	64.43	3.61	3.6	3.54	3.26	2.89	2.14	1.38	0.65	3.61	0
	D6S430	67	2.7	2.7	2.64	2.38	2.05	1.38	0.77	0.27	2.7	0
61016	D6S402	62.97	−0.29	−0.29	−0.26	−0.17	−0.11	−0.04	−0.02	−0.01	−0.29	0
	EYS c.7187G>C, p.(C2396S)	64.43	0.51	0.5	0.49	0.41	0.32	0.16	0.06	0.01	0.51	0
	D6S430	67	0.41	0.41	0.39	0.32	0.25	0.12	0.04	0.01	0.41	0
61015	D13S1295	113.09	1.31	1.3	1.27	1.13	0.95	0.61	0.31	0.1	1.31	0
	GRK1 c.55C>T, p.(R19*)	114.32	1.96	1.96	1.92	1.76	1.56	1.13	0.7	0.31	1.96	0
	D13S1825	115.01	0.29	0.29	0.28	0.25	0.2	0.11	0.04	0.01	0.29	0
61155	D5S1960	171.51	−∞	−6.05	−3.32	−1.44	−0.78	−0.33	−0.21	−0.13	−0.13	0.4
	D5S2030	177.81	2.77	2.76	2.7	2.43	2.08	1.4	0.77	0.28	2.77	0
	GRM6 c.824G>A, p.(G275D)	178.41	3.56	3.55	3.49	3.2	2.84	2.09	1.34	0.65	3.56	0
	D5S2073	178.98	0.74	0.74	0.72	0.65	0.54	0.33	0.15	0.04	0.74	0
	D5S408	179.99	−1.19	−0.71	0.1	0.62	0.7	0.56	0.33	0.13	0.7	0.1
61058	D6S402	62.97	0.9	0.9	0.89	0.81	0.72	0.52	0.3	0.09	0.9	0
	D6S284	79.35	0.9	0.9	0.89	0.81	0.72	0.52	0.3	0.09	0.9	0
	LCA5 c.652C>G, p.(R218G)	80.19	0.9	0.9	0.89	0.81	0.72	0.52	0.3	0.09	0.9	0
61076	D4S3021	15.49	1.12	1.12	1.09	0.97	0.81	0.51	0.24	0.06	1.12	0
	LRAT c.418G>T, p. (E140*)	15.57	1.33	1.33	1.29	1.15	0.98	0.63	0.31	0.08	1.33	0
	D4S413	15.84	1.12	1.12	1.09	0.97	0.81	0.51	0.24	0.06	1.12	0
61169	D11S987	67.89	1.62	1.61	1.58	1.43	1.24	0.85	0.47	0.14	1.62	0
	LRP5 c.4268 C>T p.(P1423L)	68.08	1.75	1.75	1.72	1.56	1.35	0.93	0.52	0.16	1.75	0
	D11S1337	68.13	1.62	1.61	1.58	1.43	1.24	0.85	0.47	0.14	1.62	0
61150	D15S1050	71.98	1.56	1.56	1.52	1.38	1.19	0.84	0.5	0.22	1.56	0
	NR2E3 c.417G>A, p.(Q69R)	72.1	2.53	2.53	2.48	2.29	2.04	1.53	1.02	0.51	2.53	0
	D15S204	72.3	1.87	1.87	1.83	1.66	1.44	1.01	0.61	0.25	1.87	0
	D15S1026	73.66	1.91	1.91	1.87	1.69	1.46	1.02	0.6	0.23	1.91	0
61237	D4S2960	15.83	0.28	0.28	0.27	0.22	0.16	0.06	0.01	−0.01	0.28	0
	PROM1 c.1946C>T, p.(S649L)	15.97	1.63	1.63	1.59	1.45	1.27	0.93	0.62	0.32	1.63	0
	D4S1567	16.46	1.56	1.56	1.53	1.39	1.22	0.91	0.61	0.32	1.56	0
61267	D4S2960	15.83	0.3	0.3	0.29	0.26	0.21	0.13	0.06	0.02	0.3	0
	PROM1 c.1946C>T, p.(S649L)	15.97	0.6	0.6	0.58	0.52	0.43	0.27	0.13	0.03	0.6	0
	D4S3048	16.01	0.6	0.6	0.58	0.52	0.43	0.27	0.13	0.03	0.6	0
61217	D10S578	37.04	2.92	2.91	2.86	2.62	2.32	1.7	1.08	0.47	2.92	0
	RBP3 c.3353_3354delCT, p.(S1118Cfs*2)	48.38	3.26	3.25	3.2	2.95	2.64	1.99	1.33	0.67	3.26	0
	D10S196	52.14	2.92	2.91	2.86	2.62	2.32	1.7	1.08	0.47	2.92	0
	D10S220	52.35	2.92	2.91	2.86	2.62	2.32	1.7	1.08	0.47	2.92	0
61198	D12S1724	54.87	1.9	1.89	1.85	1.66	1.41	0.91	0.43	0.1	1.9	0
	RDH5 c.536A>G, p.(K179R)	56.11	3.44	3.43	3.37	3.08	2.71	1.94	1.15	0.45	3.44	0
	D12S1632	56.41	2.25	2.24	2.19	1.96	1.66	1.06	0.51	0.13	2.25	0

Table 4. Continued

Fam No.	Marker	Mb	0	0.001	0.01	0.05	0.1	0.2	0.3	0.4	Zmax	θ_{max}
61199	D12S1724	54.87	−∞	−1.15	−0.17	0.43	0.59	0.55	0.35	0.13	0.59	0.1
	RDH5 c.536A>G, p.(K179R)	56.11	3.17	3.16	3.09	2.81	2.44	1.72	1.02	0.4	3.16	0
	D12S1632	56.41	1.73	1.73	1.72	1.63	1.46	1.03	0.56	0.19	1.73	0
61035	D12S1707	55.03	1.35	1.34	1.34	1.08	0.83	0.35	0.02	−0.06	1.35	0
	RDH5 c.758T>G, p.(M253R)	56.11	1.47	1.47	1.47	1.23	0.99	0.54	0.17	0.01	1.47	0
	D12S90	58.42	0.22	0.22	0.22	0.18	0.14	0.05	−0.01	−0.02	0.22	0
61126	D12S1707	55.03	−∞	0.75	2.26	2.68	2.56	1.96	1.24	0.57	2.68	0.05
	RDH5 c.758T>G, p.(M253R)	56.11	6.42	6.31	6.28	5.72	5.01	3.58	2.21	1.01	6.42	0
	D12S90	58.42	4.58	4.51	4.47	4.04	3.51	2.43	1.43	0.6	4.58	0
61065	D14S1065	68.91	2.93	2.92	2.85	2.54	2.15	1.4	0.73	0.23	2.93	0
	RDH12 c.609C>A, p.(S203R)	68.17	3.59	3.58	3.51	3.2	2.8	1.98	1.16	0.41	3.59	0
61262	D8S509	55.59	2.35	2.34	2.29	2.09	1.83	1.31	0.8	0.36	2.35	0
	RP1 c.787+1G>A	55.53	2.07	2.07	2.02	1.82	1.55	1.03	0.54	0.15	2.07	0
	D8S1828	56.8	2.4	2.4	2.35	2.15	1.89	1.36	0.85	0.37	2.4	0
61113	D8S509	55.59	1.05	1.05	1.04	0.98	0.9	0.7	0.48	0.23	1.05	0
	RP1 c.1126C>T, p.(R376*)	55.53	2.84	2.84	2.79	2.58	2.31	1.74	1.15	0.55	2.84	0
	D8S1828	56.8	2.02	2.02	1.98	1.81	1.59	1.15	0.7	0.3	2.02	0
61231	D1S2829	6.83	1.2	1.2	1.17	1.04	0.88	0.58	0.3	0.1	1.2	0
	RPE65 c.119G>A, p.(G40D)	6.89	1.63	1.63	1.6	1.46	1.28	0.95	0.63	0.33	1.63	0
	D1S219	6.98	1.34	1.33	1.3	1.18	1.02	0.72	0.44	0.2	1.34	0
61312	D14S72	21.37	1.78	1.77	1.734	1.57	1.36	0.94	0.55	0.22	1.78	0
	D14S1070	21.54	2.28	2.27	2.221	2	1.72	1.16	0.61	0.18	2.28	0
	RPGRIP1 c.931delA, p.(N311I*5)	21.76	3.26	3.25	3.201	2.97	2.67	2.05	1.39	0.72	3.26	0
	D14S283	22.69	−0.78	−0.4	0.366	0.88	0.96	0.81	0.53	0.22	0.96	0.1
61206	D6S439	35.15	2.54	2.53	2.48	2.23	1.92	1.31	0.72	0.24	2.54	0
	TULP1 c.1138A>G, p.(T380A)	35.46	3.14	3.13	3.08	2.82	2.5	1.82	1.14	0.5	3.14	0
	D6S1645	35.58	1.31	1.31	1.29	1.21	1.09	0.82	0.53	0.24	1.31	0
61301	D6S1629	33.79	−∞	−2.39	−0.45	0.68	0.94	0.88	0.6	0.25	0.94	0.1
	D6S439	35.15	0.8	0.8	0.78	0.69	0.57	0.36	0.19	0.08	0.8	0
	TULP1 c.1466A>G, p.(K489R)	35.46	4.46	4.45	4.39	4.08	3.69	2.86	1.98	1.03	4.46	0
	D6S1645	35.58	2.35	2.35	2.31	2.1	1.85	1.34	0.83	0.34	2.35	0
	D6S291	36.27	3.62	3.61	3.55	3.26	2.88	2.11	1.32	0.52	3.62	0
	D6S1610	39.26	−∞	−2.26	−0.55	0.59	0.89	0.88	0.59	0.21	0.89	0.1
61309	D6S1629	33.79	2.24	2.24	2.2	2.02	1.78	1.3	0.81	0.34	2.24	0
	D6S439	35.15	1.54	1.53	1.51	1.38	1.23	0.91	0.58	0.26	1.54	0
	TULP1 c.1466A>G, p.(K489R)	35.46	2.83	2.83	2.79	2.59	2.34	1.81	1.25	0.66	2.83	0
	D6S1645	35.58	0.6	0.6	0.58	0.53	0.46	0.32	0.19	0.09	0.6	0
	D6S291	36.27	2.24	2.24	2.2	2.02	1.78	1.3	0.81	0.34	2.24	0
	D6S1610	39.26	−∞	−3.66	−1.89	−0.63	−0.19	0.09	0.11	0.05	0.11	0.3
61191	D1S425	212.08	−∞	−5.67	−2.98	−1.16	0	−0.06	0.06	0.04	0.06	0.3
	D1S2646	214.06	−0.7	−0.37	0.35	0.76	0.76	0.5	0.24	0.07	0.78	0.07
	USH2A c.5740C>T, p.(Q1914*)	215.8	6.27	6.26	6.15	5.67	5.05	3.78	2.47	1.16	6.27	0
	D1S2827	216.14	4.06	4.05	3.96	3.57	3.09	2.11	1.18	0.44	4.06	0
	D1S229	217.09	3.17	3.16	3.12	2.88	2.56	1.85	1.12	0.47	3.17	0
	D1S2860	217.48	4.83	4.82	4.72	4.31	3.78	2.69	1.62	0.69	4.83	0
	D1S213	223.82	−∞	2.55	3.45	3.71	3.44	2.54	1.51	0.57	3.72	0.04

homozygous, in a third affected offspring of consanguineous parents. When when no single marker with a heterozygosity of 75% or greater was available, two markers were tested. We tested two markers within 1 to 2 cM of the candidate gene with heterozygosities over 50%, but approximately 50% of families would be expected to show discordant results for these markers. Thus our assessment was that most discordant homozygosity would be the result of low information content rather than recombination between the two markers. Families uninformative for these markers were genotyped using additional surrounding markers. Families in which homozygosity was shared only by all affected siblings were further investigated by genotyping additional individuals for confirmation of cosegregation. A variant of the multiplexing short tandem repeat with tailed primers (MSTP) approach described by Oetting et. al.,[20] using fluorescently labeled tagged primers homologous to extensions on initial primers in a two-PCR approach, was used to genotype these microsatellite markers. The PCR products were multiplex electrophoresed on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and fragment sizes were determined by GeneMapper version 4.0 (Applied Biosystems). Primer sequences and PCR conditions are shown in Supplementary Table S1.

Two-point linkage analyses were performed using the FASTLINK modification of the MLINK program in the LINKAGE program package.[21,22] Maximum logarithm of the odds (LOD) scores were calculated using ILINK, and LINKMAP was used for multipoint analysis. Autosomal recessive RD was analyzed as a fully penetrant trait with an affected allele frequency of 0.00001. The criteria for establishing linkage have been described previously.[23] The length of the homozygous regions

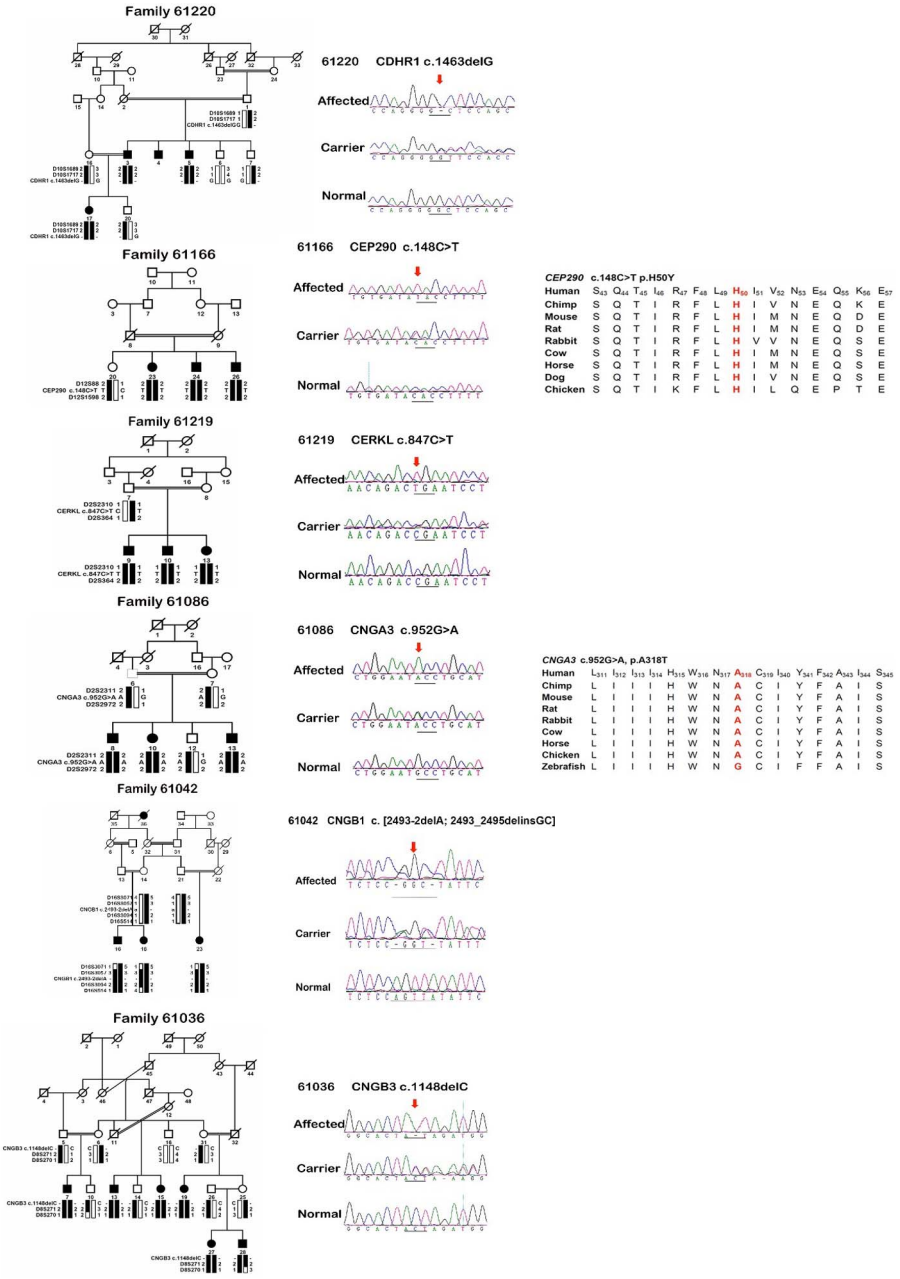

Figure 2. Family 61220, 61166, 61219, 61086, 61042 and 61036 structure and haplotype of flanking markers of loci identified by homozygosity mapping. DNA sequence tracings confirming the mutations are shown adjacent to the pedigrees, and cross-species conservation of amino acids showing missense mutations is shown on the *right*.

was 3 Mb on average. Haplotypes were generated using the Cyrillic 2.1 program (Cyrillic Software, Wallingford, Oxfordshire, UK) and confirmed by inspection.

Screening Candidate Genes

Based upon cosegregation of the risk haplotypes in a family, mutations in the exons and 100 bp of flanking intronic regions of the included known candidate gene associated with inherited retinal diseases were analyzed by Sanger sequencing using ABI PRISM 3130 automated sequencers (Applied Biosystems) and assembled and analyzed with Seqman software (DNAStar Lasergene 8; Madison, WI, USA) and Mutation Surveyor (SoftGenetics, State College, PA, USA). Mutations were submitted to the LOVD (http://databases.lovd.nl/shared/variants; in the public domain).

Assessing Pathogenicity of Identified Variants

A mutation was considered novel if it was not present in the Human Mutation Database Professional Version on Biobase (https://portal.biobase-international.com/cgi-bin/portal/login.cgi; in the public domain) or the National Center for Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/snp/; in the public domain), and sequence changes were considered pathogenic when they segregated with the disease in the family as well as their absence in 192 ethnically matched control chromosomes or at a frequency >

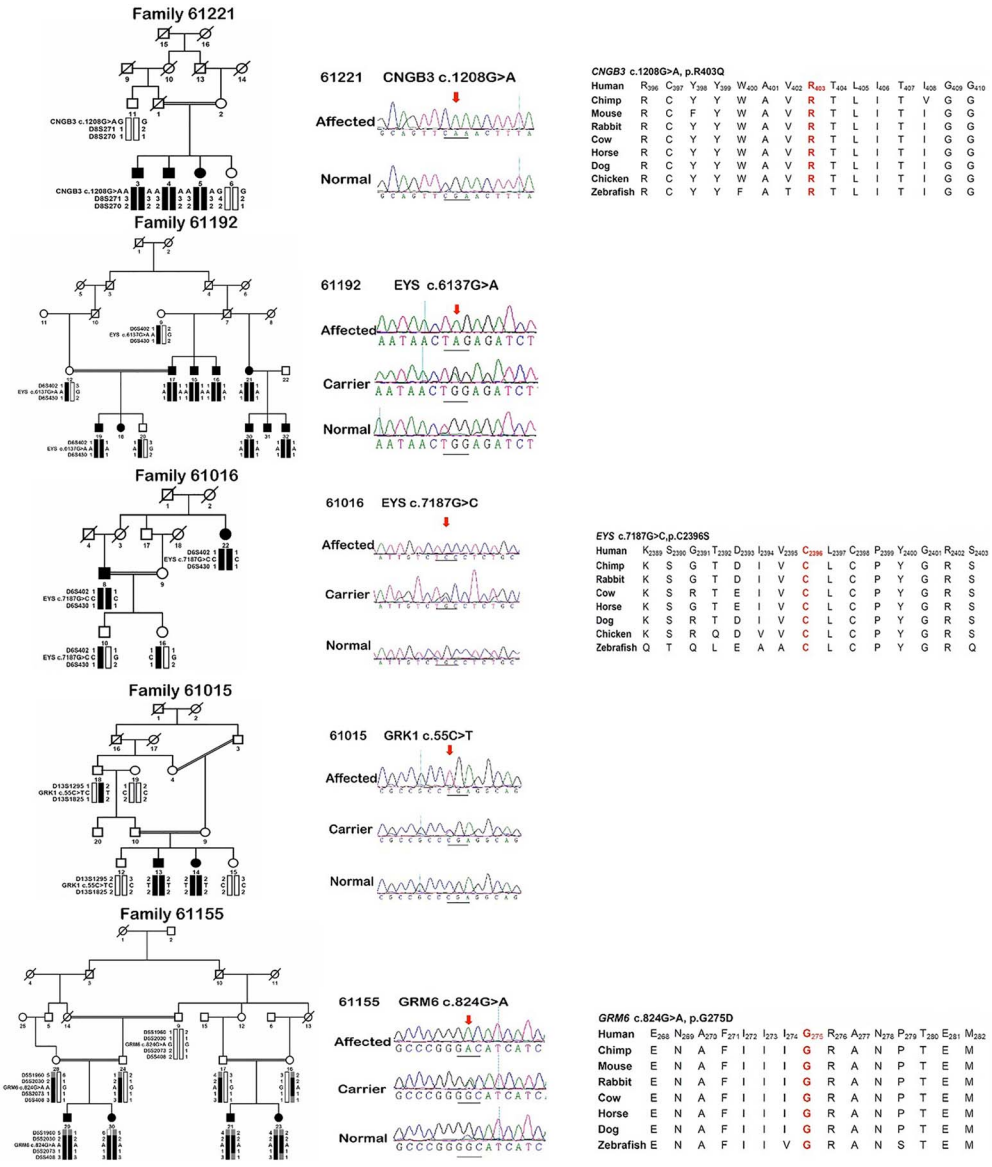

Figure 3. Family 61221, 61192, 61016, 61015 and 61155 structure and haplotype of flanking markers of loci identified by homozygosity mapping. DNA sequence tracings confirming the mutations are shown adjacent to the pedigrees, and cross-species conservation of amino acids showing missense mutations is shown on the *right*.

1% in the ExAC database (http://exac.broadinstitute.org/; in the public domain); and for missense changes were judged pathogenic in a computational test for mutations, including sorting intolerant from tolerant (SIFT and PROVEAN, http://sift.jcvi.org/; in the public domain) analysis, polymorphism phenotyping (PolyPhen2, http://genetics.bwh.harvard.edu/pph2/; in the public domain), and Condel (http://bg.upf.edu/fannsdb/; in the public domain). A SIFT score below the cutoff of 0.05 for a given substitution is classified as damaging while those with scores higher than this value are considered tolerated. In addition, we used Condel[24] (CONsensus DELeteriousness score of missense SNVs), which computes a weighted average of the scores (WAS) of five tools: SIFT, PolyPhen2, MAPP (Multivariate Analysis of Protein Polymorphism), LogR Pfam E-value, and Mutation Assessor. Splicing changes were predicted using Automated Splice Site Analyses (http://www.fruitfly.org/seq_tools/splice.html; in the public domain).

Intragenic Haplotype Analysis for Families Sharing the Same Variation

If the same variation was detected in more than one family, haplotypes of single nucleotide polymorphisms (SNPs) intragenic or within 1 Mb of the mutated gene were genotyped. The frequency of the risk haplotype in the general population was calculated from 96 unrelated Pakistani controls via the CHM algorithm as implemented in the Golden Helix SVS package (Golden Helix, Bozeman, MT, USA).

Results

Patient Cohort and Homozygosity Screening

This cohort included 67 consanguineous families with more than two affected siblings. A total of 67 probands and all affected siblings or ancestors who received a clinical

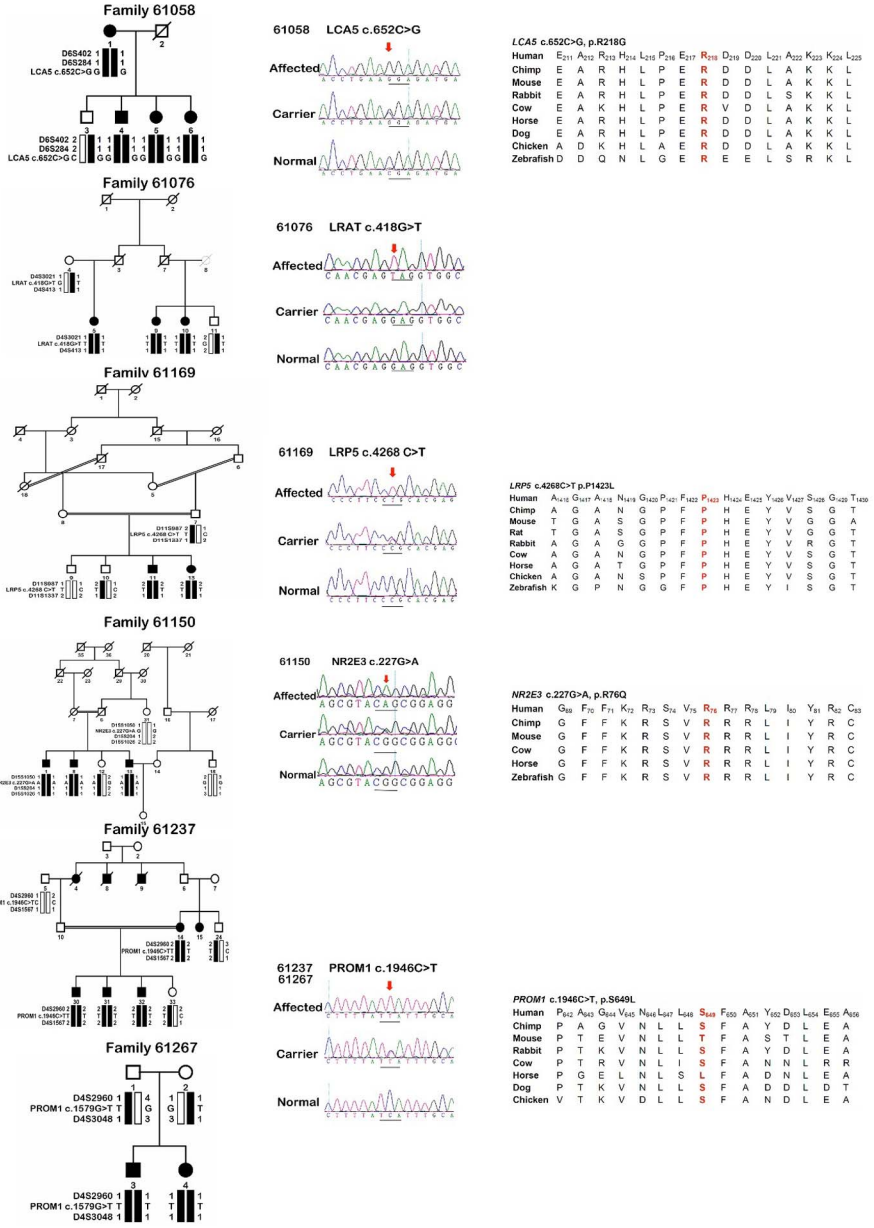

Figure 4. Family 61058, 61076, 61169, 61150, 61237 and 61267 structure and haplotype of flanking markers of loci identified by homozygosity mapping. DNA sequence tracings confirming the mutations are shown adjacent to the pedigrees, and cross-species conservation of amino acids showing missense mutations is shown on the *right*.

diagnosis of arRP were included in this study. Up to three affected individuals in each family were subjected to homozygosity screening in a stepwise manner. After screening the first affected patient with all 188 microsatellite markers, the average number of excluded markers for each family was 126 (126/188 = 67% efficiency), with exclusion rates of 85% after screening a second affected individual and 93.0% a third, so that the average number of markers to be analyzed was reduced from 188 to 13 in each family (Fig. 1). After fine mapping using microsatellite markers, 29 families were excluded from linkage to any known RP gene, making the exclusion rate 43.3% for families undergoing homozygosity screening. The remaining 38 families showed homozygosity and cosegregation in at least 1 of the 180 regions containing known arRD genes or loci.

After sequencing the included candidate genes for which the remaining 38 families showed homozygosity and cosegregation, the underlying pathogenic mutations were revealed in 27 families (Table 2).[25–28] In addition, variations considered benign by in silico prediction were detected in another four families (Table 3). The two-point LOD scores for markers in the homozygous chromosomal segments in these families are shown in Table 4. In seven families, no variations were identified by sequencing the known candidate genes within identified homozygous chromosomal regions, and their significance remains unclear.

Identification of Disease-Causing Variants in Homozygous Regions

Overall, 24 causative mutations of 20 genes were identified in 27 families, including 12 missense, 6 nonsense, 4 indel-induced frameshift mutations, and 2 splice-site mutations (the splicing

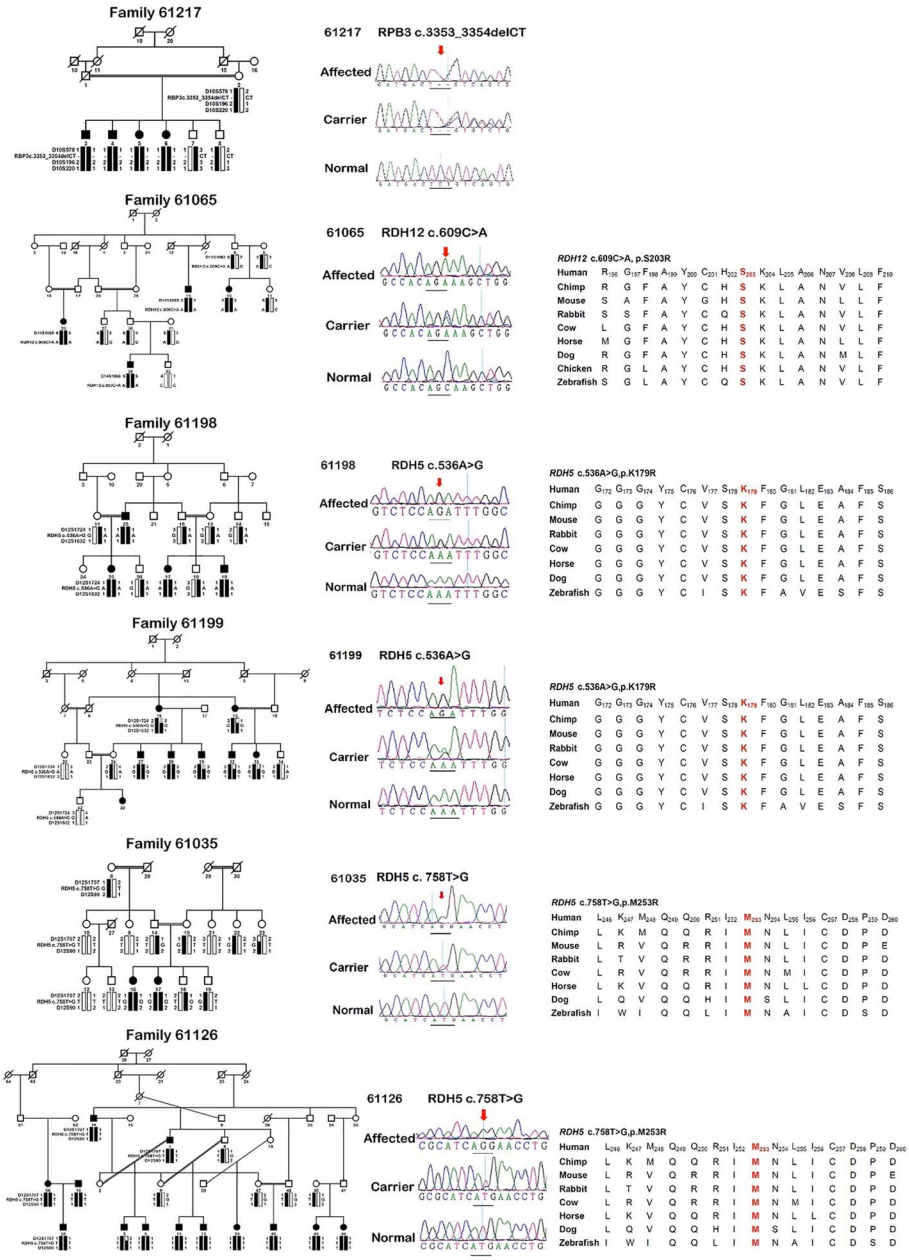

Figure 5. Family 61217, 61065, 61198, 61199,61035 and 61126 structure and haplotype of flanking markers of loci identified by homozygosity mapping. DNA sequence tracings confirming the mutations are shown adjacent to the pedigrees, and cross-species conservation of amino acids showing missense mutations is shown on the *right*.

site change in *CNGB1* was induced by an indel mutation). In all, 11 mutations were novel, and were not found in mutation databases or in 192 ethnically matched control chromosomes, while the remaining 13 mutations had been reported previously. Each of the mutations was located within a homozygous region and cosegregated with the disease. For missense mutations, the substituted amino acid residues are highly conserved across species (Figs. 2–7), and in silico pathogenicity evaluation by PolyPhen2, SIFT, and Condel of the 12 missense mutations predicted these changes to be deleterious. Some variations were detected in two families, including c.536A>G (p.(K179R), family 61198 and 61199) and c.758T>G (p.(M253R), family 61035 and 61126) in *RDH5*, c.1466A>G (p.(K489P), family 61301 and 61309) in *TULP1,* and the probably nonpathogenic c.1946C>T (p.(S649L), family 61237 and 61267) in *PROM1*. Affected families who shared the same variations also shared a common haplotype of alleles at nearby intragenic SNPs, suggesting that the mutant allele was probably derived from a common ancestor (Supplementary Table S2). Although genes or loci previously associated only with autosomal dominant inherited retinal disease were also screened in the study, no mutations were identified in these genes or loci.

Details of Sequence Variations Identified by Homozygosity Mapping

Information regarding the 24 sequence variations felt likely to be causative identified by homozygosity screening, and phenotypes of the 27 families in which they occurred, is

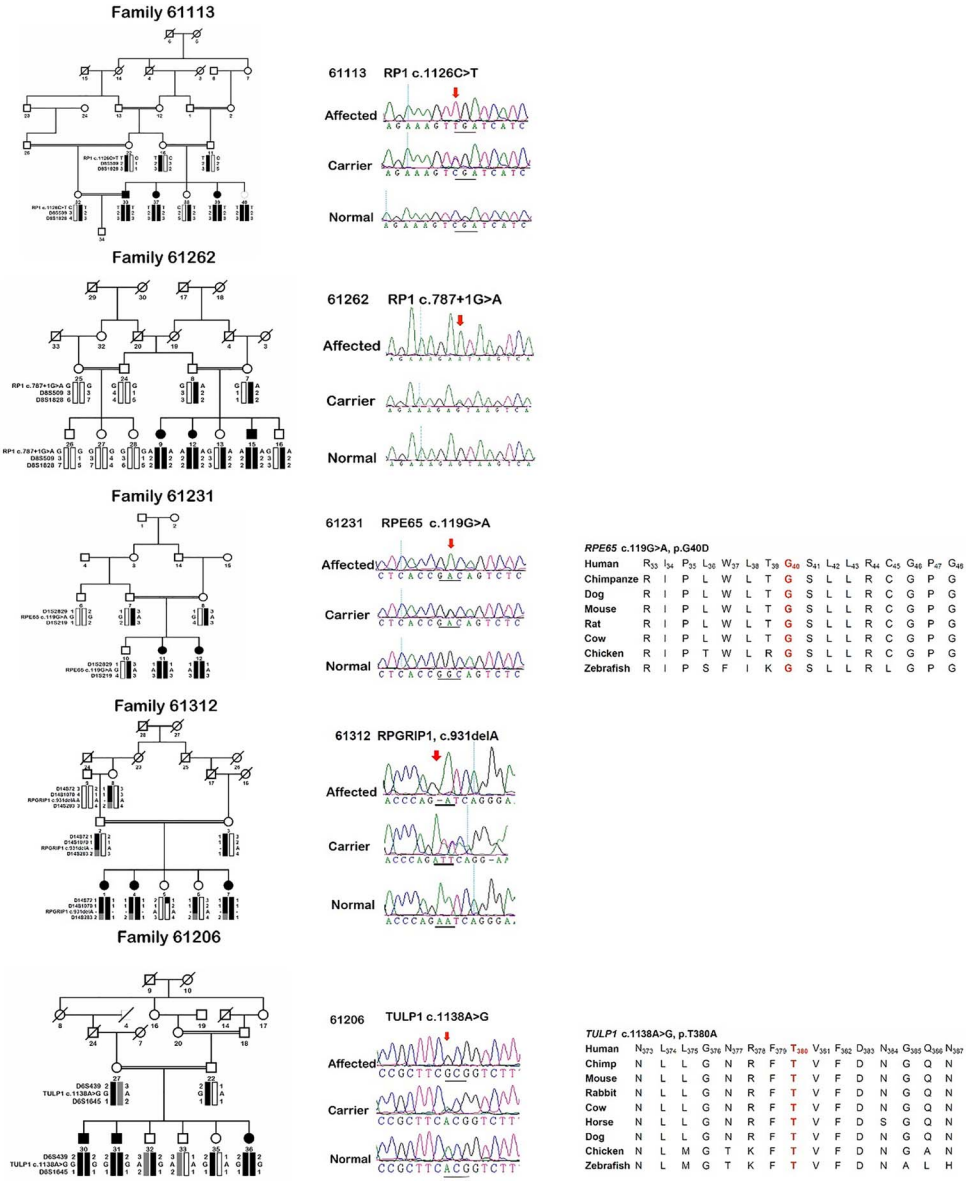

Figure 6. Family 61113, 61262, 61231, 61312 and 61206 structure and haplotype of flanking markers of loci identified by homozygosity mapping. DNA sequence tracings confirming the mutations are shown adjacent to the pedigrees, and cross-species conservation of amino acids showing missense mutations is shown on the *right*.

provided in Table 2, and similar information for variations judged likely to be benign based on in silico predictions or presence in unaffected control individuals is provided in Table 3. The domain structure of the corresponding proteins and locations of the mutations with respect to these are shown in Figures 8 and 9, while the pedigree structure and corresponding haplotypes as well as sequence tracings and cross-species conservation for missense changes are shown in Figures 2 through 7, with the corresponding LOD scores summarized in Table 4.

Details of Sequence Variations Considered Likely to Be Deleterious

The c.847C>T, p.(R283*) mutation in *CERKL* seen in family 61219 is predicted to lead to truncation of the protein through a premature stop codon, and might be expected to lead to nonsense-mediated decay. However, this mutation has been shown to cause accumulation of truncated protein in the nucleus.[29] The c.55C>T, p.(R19*) mutation in *GRK1* seen in family 61015 was predicted to lead to truncation of GRK1 protein and result in nonsense-mediated decay, and seems likely to result in a variant form of Oguchi disease, as described by Zhang et al.,[30] also predicted to result in nonsense-mediated decay; however, detailed clinical data sufficient for this diagnosis could not be obtained, so it is listed in Table 2 as having a stationary RD. Family 61058 (Table 2) contains a c.652C>G, p.(R218G) mutation in *LCA5* segregating in a pseudo-dominant inheritance pattern, but the recessive nature of the mutation is confirmed by the sequencing results (Fig. 4). The c.227G>A p.(R76Q) mutation in the DNA-binding domain (DBD) of NR2E3 (Fig. 9) had been shown to increase dimerization significantly but to abolish DNA binding.[31]

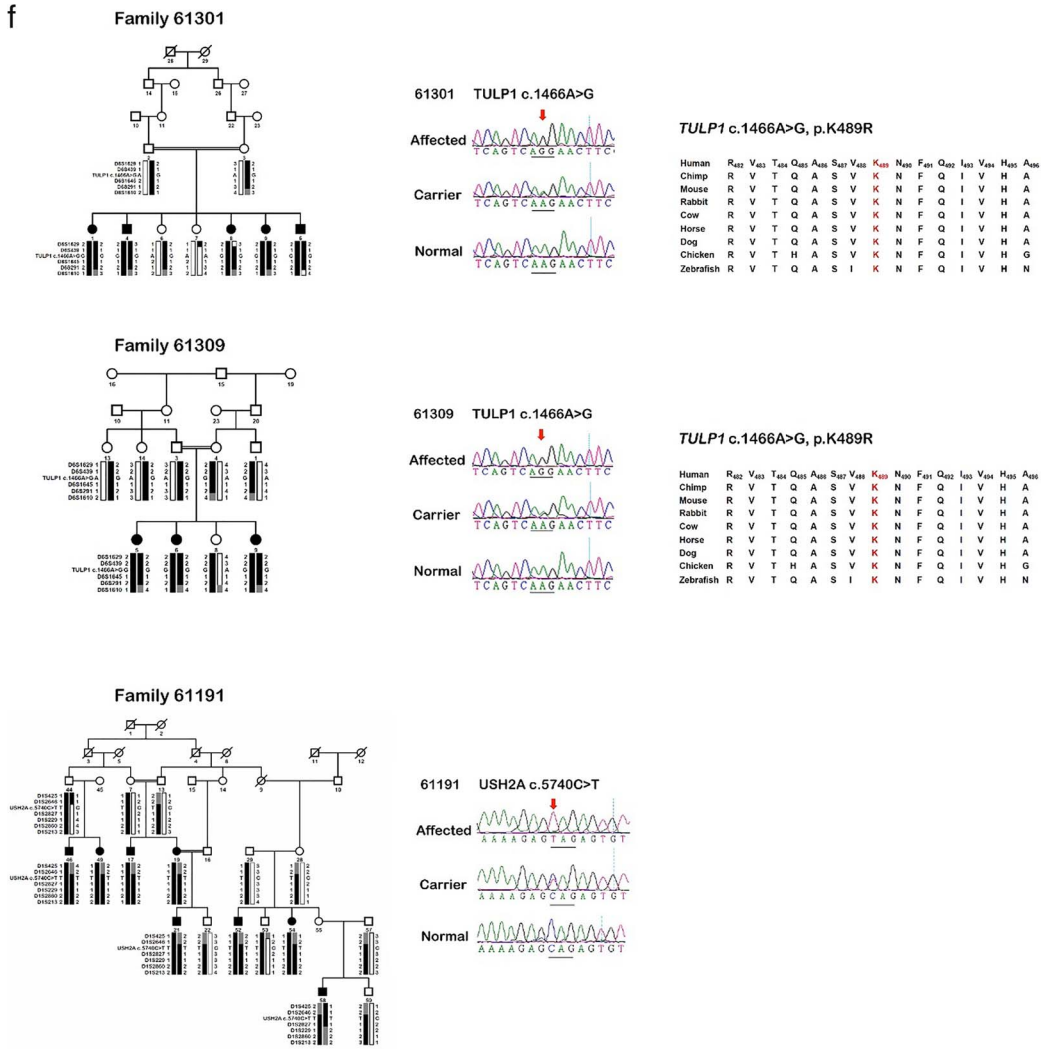

Figure 7. Family 61301, 61309 and 61191 structure and haplotype of flanking markers of loci identified by homozygosity mapping. DNA sequence tracings confirming the mutations are shown adjacent to the pedigrees, and cross-species conservation of amino acids showing missense mutations is shown on the *right*.

Details of Sequence Variations Considered Likely to Be Benign

The previously described missense variation c.1208G>A, p.(R403Q) in *CNGB3*[32] seen in family 61221 (Fig. 3) was identified in a homozygous state in 1 of 96 control individuals. It was predicted to be probably damaging by PolyPhen2 but tolerated by SIFT and neutral by Condel, so it seems most likely only to be a rare variation (Table 3), although a modifying gene affecting the phenotype cannot be excluded. The novel c.4268C>T, p.(P1423L) variation in LRP5 was in an amino acid evolutionarily conserved from humans to zebrafish (Figs. 4, 9), had an allele frequency of 2.529×10^{-5} in the ExAC browser with no homozygotes listed in any population, and was not seen in 96 control individuals of Pakistani ethnic extraction. However, in silico analysis with PolyPhen2 predicted that it would be benign, with SIFT showing that it was tolerated, and Condel that it was neutral (Table 3). Hence, we treated this change as a rare variation even though the possibility that it might be responsible for disease in this family remains, especially since the phenotype in this family was progressive, consistent with a less severe mutation.

A novel missense variation c.1946C>T, p.(S649L) in *PROM1* was detected and cosegregated with the disease in families 61237 and 61267 (Table 3; Fig. 4). S649 is only weakly conserved from human to chicken, but the substitutions, from serine to threonine, are relatively conservative (Fig. 4). This mutation does not occur in a transmembrane domain (Fig. 9) and is predicted to be benign by three in silico analyses. Thus, we presumed that this mutation is a rare variation, even though p.(S649L) was not found in 192 control chromosomes from the Pakistani population and had an overall frequency of 5.456×10^{-5} in the ExAC database, with all six variant alleles identified in the South Asian population with no homozygotes. Affected members of the two families share a common haplotype of alleles at 10 consecutive SNPs in and around *PROM1* suggesting that the variant allele is derived from a common ancestor (Supplementary Table S2).

Clinical Features

An overview of the clinical data from affected individuals in each family is shown in Table 2. The clinical symptoms, age of onset, and mode of inheritance in these families are generally

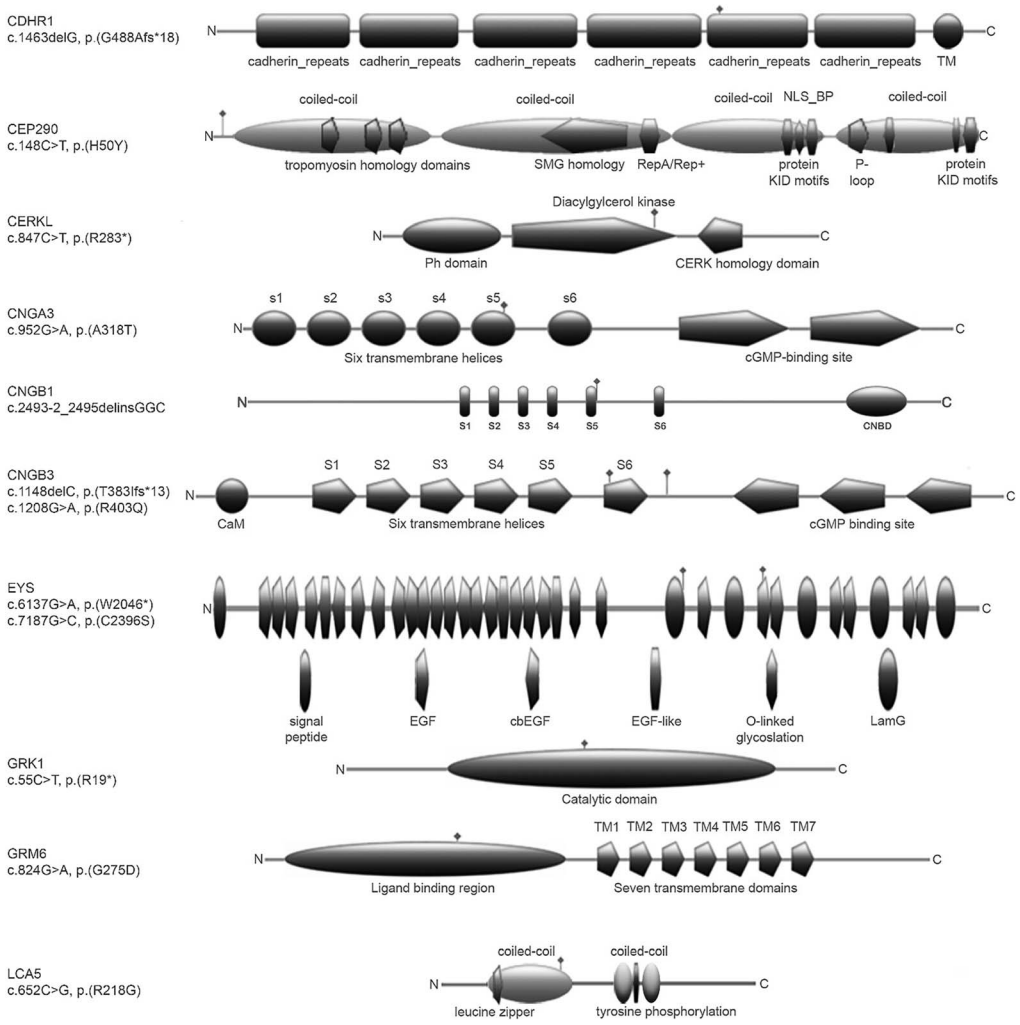

Figure 8. Domain structure and mutations of proteins in which mutations were identified by homozygosity mapping: CDHR1, CEP290, CERKL, CNGA3, CNGB1, CNGB3, EYS, GRK1, GRM6, LCA5.

consistent with the form of retinal degeneration described in the literature and on RetNet. However, the clinical data available for this study are limited to ophthalmic history and examination, fundus photographs, and ERGs, so that it is difficult to distinguish closely related forms of the retinal disease, for example, Leber congenital amaurosis (LCA) or CORD from RP. Thus, we use RD to represent those families in which the clinical findings are consistent with the previously described retinal disease, but were insufficient to distinguish unambiguously among the various diseases previously associated with that gene: RP, LCA, congenital stationary night blindness (CSNB), or fundus albipunctatus, which together account for 8 of 27 (30%) families. Four of these had an early onset, but the differentiation between early-onset arRP and LCA, difficult with the best documentation, could not be made reliably. Thirteen families showed clear signs of RP. One family was consistent with LCA with typical fundoscopy, extinguished ERGs, and a history of a congenital onset accompanied by nystagmus. Four families that carried a homozygous *RDH5* mutation received a diagnosis of fundus albipunctatus by fundus photographs. Of the remaining two families, one showed the typical signs of CSNB without progression, and the second was consistent with Usher syndrome (RP with deafness). Although there was no indication of vestibular dysfunction in any affected individual in this family, suggesting Usher type 2 and consistent with the causative gene, this was simply listed as Usher syndrome because formal vestibular testing was not performed.

Discussion

Consanguineous matings have long been known to increase the risk of recessive diseases by increasing the fraction of the genome that is homozygous and identical by descent, and thus the number of potentially deleterious alleles descended from a common ancestor[33]; and homozygosity mapping provides an efficient means of localizing causative genes for recessive traits in these populations,[34] particularly in populations with high consanguinity rates. Pakistan, with consanguinity rates ranging from 17% to 38%,[35] is an optimal country in which to implement this approach.

In addition, the clinical phenotypes for some families are of particular interest. One such was family 61015, shown in Table 2 as having a stationary form of RD. The phenotype in this family, as far as it could be ascertained, was consistent with mutations in *GRK1* causing Oguchi disease and was similar to that in a previous Pakistani family with a variant of Oguchi disease due to deletion of exon 3,[30] although the degree of recovery from dark adaptation could not be ascertained in this

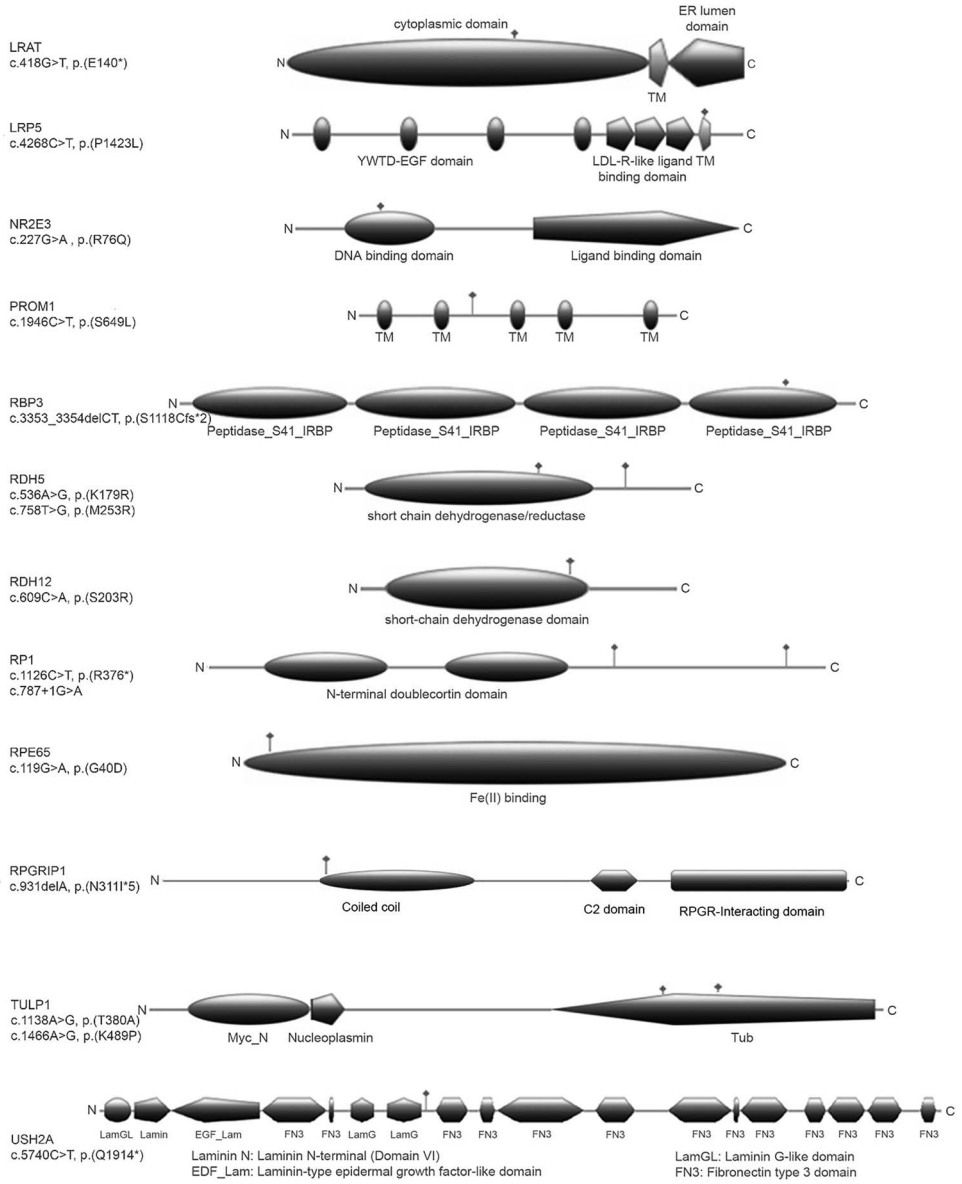

Figure 9. Domain structure and mutations of proteins in which mutations were identified by homozygosity mapping: LRAT, LRP5, NR2E3, PROM1, RBP3, RDH5, RDH12, RP1, RPE65, RPGRIP1, TULP1, USH2A.

family. Thus, it is listed simply as a stationary RD, even though it is very likely to have Oguchi type CSNB. Another was family 61150, with a mutation in NR2E3. This gene primarily has been associated with enhanced S-cone syndrome and Goldmann-Favre syndrome. However, the phenotype in family 61150 is most consistent with progressive arRP, similar to that observed in a family of Portuguese "crypto-Jews" with a mutation in this gene.[36] In addition, Sharon et al.[37] have described NR2E3 mutations in a series of patients with clumped pigmentary retinal degeneration, who also might be consistent with the phenotype observed in this family.

Taken together with our previous characterization of autosomal recessive retinal degenerations (arRD) in Pakistan, we have studied a total of 144 consanguineous arRD families, in addition to 154 families with limited numbers of affected individuals or unclear inheritance patterns. Of these 144 families, we have identified putative causative variations in 40 genes and 11 loci so far, and these genes and loci collectively account for disease in 104 of the 144 families (72.2%) (Table 5; Fig. 10). The percentages of families who had variants in the 40 genes and 11 loci are shown in Figure 10, in decreasing order: *RPE65* 6.9% (10/144), *TULP1* 6.9% (10/144), *RP1* 4.9% (7/144), *PDE6A* and locus 4.9% (7/144), *USH2A* and new locus 3.5% (5/144), *RDH5* 2.8% (4/144), 11p11.2-q13.2 locus 2.1% (3/144), *GRM6* 2.1% (3/144), and 1p13.3 locus 2.1% (3/144). It should be noted that this tabulation counts families sharing an intragenic haplotype as separate families. Other genes or loci were identified in only one or two families in this cohort, respectively, accounting for less than 2% of the arRD population in Pakistan.

The most frequently mutated genes in arRD differ remarkably among the populations of different ethnic origins. *RPE65* and *TULP1* were the genes most frequently mutated in Pakistani patients with arRD, while the genes found to be most frequently mutated in other populations were *RP1* in Saudi Arabians,[38] *RDH12* in Spanish,[39] and *USH2A* worldwide.[15] The overall rate of variant detection was 61.8% (89/144) in this study, which is comparable to the worldwide

Table 5. Sequence Variations Identified in 144 Unselected Pakistani Families

No.	Fam #	Gene	Nucleotide	AA	Dis	Prog	Reference
1	61004	*IMPDH1*	c.931G>A	p.(D311N)	RP	Prog	50
2	61006	*RP1*	c.4555delA	p.(R1519fs*2)	RP	Prog	51
3	61014	*BBS2*	c.1237C>T	p.(R413*)	RP	Prog	52
4	61015	*GRK1*	c.55C>T	p.(R19*)	RD	Sta	This study
5	61016	*EYS*	c.7187G>C	p.(C2396S)	RP	Prog	43
6	61019	*PDE6A*	c.769C>T	p.(R257*)	RP	Prog	53
7	61020	*RPE65*	c.95-1G>A	N/A	RD	Prog	54
8	61021	*PDE6A*	c.2098dupT	p.(Y700Lfs*21)	RP	Prog	53
9	61029	*GRK1*	c.827+623_883del	p.(Y277Qfs*6).	OD	Sta	30
10	61032	*AIPL1*	c.773G>C	p.(R258P)	RD	Prog	55
11	61035	*RDH5*	c.758T>G	p.(M253R)	FA	Sta	27
12	61036	*CNGB3*	c.1148delC	p.(T383Ifs*13)	RD	Prog	42
13	61037	*PROM1*	c.1726C>T	p.(Q576*)	RP	Prog	56
14	61039	*CNGA1*	c.626_627delTA	p.(I209Sfs*26)	RP	Prog	57
15	61040	*RP1*	c.1458_1461dup	p.(E488*)	RP	Prog	51
16	61042	*CNGB1*	c.2493-2_2495delinsGGC	p.(S831Rfs*2)	RP	Prog	41
17	61043	*RP1*	c.5252delA	p.(N1751fs*4)	RP	Prog	51
18	61049	*BBS3*	c.123+1118del53985	N/A*	RP	Prog	58
19	61058	*LCA5*	c.652C>G	p.(R218G)	EORD	Prog	This study
20	61061	*11p11.2-q13.2*	N/A	N/A	RP	Prog	†
21	61063	*TULP1*	c.1138A>G	p.(T380A)	RP	Prog	59
22	61064	*RLBP1*	c.466C>T	p.(R156*)	FA	Sta	60
23	61065	*RDH12*	c.609C>A	p.(S203R)	RD	Prog	47
24	61070	*SLC24A1*	c.1613_1614delTT	p.(F538Cfs*23)	CSNB	Sta	61
25	61074	*PDE6A*	c.1408-2A>G	p.(K470_L491del)	RP	Prog	53
26	61076	*LRAT*	c.418G>T	p.(E140*)	RP	Prog	This study
27	61077	*GUCY2D*	c.2384G>A	p.(R795Q)	EORD	Prog	41
28	61078	*LCA5*	c.1151delC	p.(P384Qfs*18)	EORD	Prog	41
29	61081	*PDE6A locus*	N/A	N/A	RP	Prog	53
30	61084	*TULP1*	c.1466A>G	p.(K489R)	RP	Prog	59
31	61086	*CNGA3*	c.952G>A	p.(A318T)	RD	Prog	This study
32	61103	*GUCY2D*	c.2189T>C	p.(F730S)	RD	U	41
33	61104	*11p11.2-q13.1*	N/A	N/A	RP	Prog	†
34	61107	*RLBP1*	c.346G>C	p.(G116R)	FA	Sta	60
35	61111	*TULP1*	c.1466A>G	p.(K489R)	RP	Prog	59
36	61113	*RP1*	c.1126C>T	p.(R376*)	RP	Prog	This study
37	61115	*ZNF513*	c.1015T>C	p.(C339R)	RP	Prog	62
38	61116	*RPE65*	c.963T>G, c.782T>C	p.(N321K), p.(L261P)	RD	Prog	†
39	61117	*RP1*	c.3697delT	p.(S1233Pfs*22)	RP	Prog	48
40	61120	*LRAT*	c.538A>T	p.(K180*)	RP	Prog	†
41	61122	*TULP1*	c.1466A>G	p.(K489R)	RP	Prog	59
42	61124	*PDE6A*	c.769C>T	p.(R257*)	RP	Prog	53
43	61125	*OAT locus*	N/A	N/A	RD	Prog	†
44	61126	*RDH5*	c.758T>G	p.(M253R)	FA	Sta	27
45	61129	*SAG locus*	N/A	N/A	RD	Prog	†
46	61130	*GNAT1*	c.386A>G	p.(D129G)	CSNB	Sta	63
47	61133	*PDE6A*	c.2028-1G>A	p.(K677Rfs*24)	RP	Prog	64
48	61138	*USH2A*	c.11473delC	p.(H3825Ifs*10)	RP/D	Prog	41
49	61140	*PDE6A*	c.1408-2A>G	p.(K470_L491del)	RP	Prog	53
50	61141	*USH2A*	c.4645C>T	p.(R1549*)	RD	U	41
51	61142	*CNGB1*	c.2493-2_2495delinsGGC	p.(S831Rfs*2)	RP	Prog	41
52	61147	*TTPA locus*	N/A	N/A	RP	Prog	†
53	61150	*NR2E3*	c.227G>A	p.(R76Q)	RP	Prog	45
54	61151	*USH2A locus*	N/A	N/A	RP/D	Prog	†
55	61155	*GRM6*	c.824G>A	p.(G275D)	CSNB	Sta	44
56	61157	*RP1*	c.6098G>A	p.(C2033Y)	RP	Prog	†
57	61160	*RPE65*	c.179T>C	p.(L60P)	RD	Prog	54
58	61161	*PDE6β*	c.1655G>A	p.(R552Q)	RP	Prog	65
59	61166	*CEP290*	c.148C>T	p.(H50Y)	EORD	Prog	This study
60	61167	*11p11.2-q13.1*	N/A	N/A	RP	Prog	†
61	61170	*GRM6*	c.1336C>T	p.(R446*)	CSNB	Sta	66
62	61171	*TULP1*	c.1466A>G	p.(K489R)	RP	Prog	59
63	61172	*GRM6*	c.2267G>A	p.(G756D)	CSNB	Sta	67
64	61173	*MERTK*	c.718G>T	p.(E240*)	RP	Prog	68

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Table 5. Continued

No.	Fam #	Gene	Nucleotide	AA	Dis	Prog	Reference
65	61176	*FAM161A*	c.1600A>T	p.(R534W	RP	Prog	41
66	61179	*BBS8*	c.115-2A>G	p.(E39_Q48del)	RP	Prog	69
67	61183	*PDE6β*	c.1160C>T	p.(P387L)	RP	Prog	70
68	61185	*USH2A*	c.12523T>G	p.(W4175G)	RP/D	Prog	41
69	61186	*CRB1*	c.433T>C	p.(C145R)	RD	Prog	†
70	61191	*USH2A*	c.5740C>T	p.(Q1914*)	RP/D	Prog	This study
71	61192	*EYS*	c.6137G>A	p.(W2046*)	RP	Prog	This study
72	61198	*RDH5*	c.536A>G	p.(K179R)	FA	Sta	46
73	61199	*RDH5*	c.536A>G	p.(K179R)	FA	Sta	46
74	61206	*TULP1*	c.1138A>G	p.(T380A)	RP	Prog	28
75	61217	*RBP3*	c.3353_3354delCT	p.(S1118Cfs*3)	RP	Prog	This study
76	61219	*CERKL*	c.847C>T	p.(R283*)	RP	Prog	26
77	61220	*CDHR1*	c.1463delG	p.(G488Afs*20)	RD	Prog	25
78	61227	*AIPL1*	c.465G>T	p.(Q155H)	RD	Prog	55
79	61231	*RPE65*	c.119G>A	p.(G40D)	EORD	Prog	This study
80	61235	*RPE65*	c.361delT	p.(S121Lfs*6)	EORD	Prog	54
81	61239	*FAM161A*	c.1139G>T	p.(R380L)	RP	Prog	†
82	61259	*TULP1*	c.1561C>T	p.(P521S)	RP	Prog	71
83	61262	*RP1*	c.787+1G>A	p.(I263Nfs*8)	RP	Prog	48
84	61268	*TULP1*	c.1495+4A>C	p.(P499Rfs*104)	RP	Prog	49
85	61274	*BBS12*	c.1616G>T	p.(G539V)	RP/D	Prog	†
86	61281	*RPE65*	c.1087C>A	p.(P363T)	RD	U	46
87	61282	*RPE65*	c.1087C>A	p.(P363T)	RD	U	41
88	61283	*RPE65*	c.1087C>A	p.(P363T)	RD	U	41
89	61284	*RPE65*	c.1087C>A	p.(P363T)	RD	U	41
90	61285	*RPE65*	c.1087C>A	p.(P363T)	RD	U	41
91	61289	*CDHR1*	c.1463delG	p.(G488Afs*20)	RP	Prog	25
92	61301	*TULP1*	c.1466A>G	p.(K489R)	EORP	Prog	49
93	61309	*TULP1*	c.1466A>G	p.(K489R)	EORP	Prog	49
94	61312	*RPGRIP1*	c.931delA	p.(N311Ifs*5)	LCA	Sta	this study
95	61324	*SAG*	c.874C>T	p.(R292*)	RD	U	72
96	61373	*CERKL*	c.847C>T	p.(R283*)	RD	U	26
97	61376	*PRCD*	c.2T>C	p.(M1T)	RP	Prog	73

AA, amino acid; Dis, disease; OD, Oguchi disease; RP, retinitis pigmentosa; FA, fundus albipunctatus; EORD, early-onset RD; RP/D, RP with deafness; EORP, early-onset RP; N/A, not available; Prog, progressive; Sta, stationary; U, unknown.

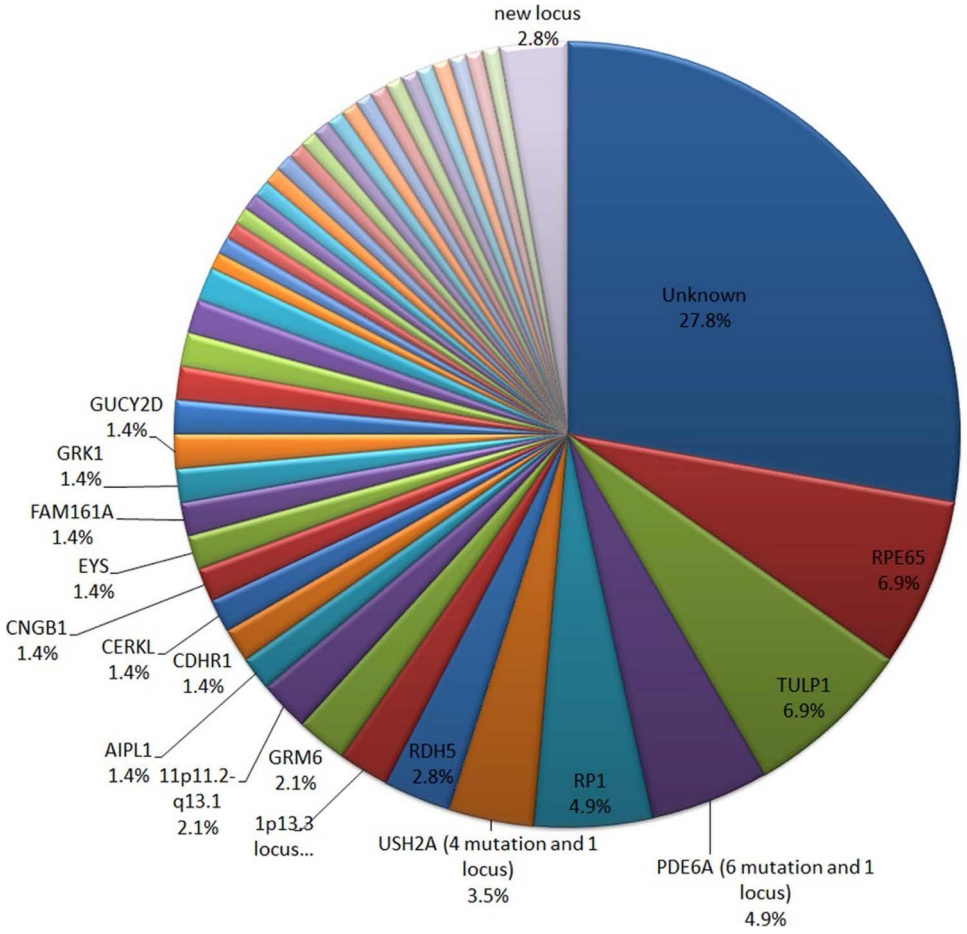
* Deletion beginning in intron 3 and extending beyond end of the BBS3 gene.

† Riazuddin S, written communication, 2017.

variation detection rate of 60%.[15] A recent report that summarized 103 published Pakistani RD families[18] found *AIPL1* and *CRB1* to be the most frequent causative genes, probably because LCA families accounted for approximately 20% of the arRD families, while there were significantly fewer LCA families in our patient cohort. In addition, the previous summary included families screened by sequencing previously identified candidate genes, which might tend to favor those genes identified early and/or widely publicized. In our study, sharing of variations by different families is likely to be the result of the variant allele being derived from a common ancestry, since all of the families that shared the same variation also shared common intragenic SNP haplotypes for the associated gene. In addition, although we included the 79 genes/loci reportedly responsible only for autosomal dominant RD, no mutations were identified in genes previously associated only with adRD.

Taken together, we failed to uncover the pathologic variation in 27.8% of families in our arRD patient cohort. Although 38 families showed homozygosity and cosegregation in at least 1 of the 180 regions containing known arRD genes or loci, no disease-causing mutations were identified in 11 of these, so that they are excellent candidates for identification of new arRD genes residing within linked regions in which no mutations were identified in the known candidate gene. The most promising approach to discover novel genes after such a systematic analysis of many consanguineous families is next-generation sequencing, although familial locus heterogeneity or compound heterozygous mutations might explain the phenotype of a small proportion of the families, as intrafamilial locus heterogeneity was detected in 15.3% of the families studied in a recent report of Pakistani families with hearing impairment.[40] In addition, compound heterozygous mutations were identified in 2.7% of genetically resolved arRD families in a review of all published retinal degeneration cases in Pakistan.[18] Although homozygosity mapping has been proven effective, a major limitation is that this type of analysis will overlook familial locus heterogeneity or compound heterozygous mutations.

In conclusion, homozygosity mapping of known genes is relatively inexpensive while still being accurate and comprehensive. Our results provide a key bridge between bench and bedside and should make genetic diagnosis of arRD in patients more accessible and practical. This should greatly enhance the clinical genetic counseling, diagnosis, and early intervention of arRD in the Pakistani population. These results also highlight the importance of analyzing the causative genes and their exons in different ethnic groups in a systematic and population-specific fashion.



Gene or Locus	Families	%
Total	144	100
Unknown	40	27.8
RPE65	10	6.9
TULP1	10	6.9
PDE6A (6 mutation and 1 locus)	7	4.9
RP1	7	4.9
USH2A (4 mutation and 1 locus)	5	3.5
RDH5	4	2.8
1p13.3 locus	3	2.1
GRM6	3	2.1
11p11.2-q13.1	3	2.1
AIPL1	2	1.4
CDHR1	2	1.4
CERKL	2	1.4
CNGB1	2	1.4
EYS	2	1.4
FAM161A	2	1.4
GRK1	2	1.4
GUCY2D	2	1.4
LCA5	2	1.4
LRAT	2	1.4
PDE6β	2	1.4
RLBP1	2	1.4
SAG (1 mutation and 1 locus)	2	1.4
BBS2	1	0.7
BBS3	1	0.7
BBS8	1	0.7
BBS12	1	0.7
CEP290	1	0.7
CNGA1	1	0.7
CNGA3	1	0.7
CNGB3	1	0.7
CRB1	1	0.7
GNAT1	1	0.7
IMPDH1	1	0.7
MERTK	1	0.7
NR2E3	1	0.7
PRCD	1	0.7
PROM1	1	0.7
RBP3	1	0.7
RDH12	1	0.7
RPGRIP1	1	0.7
SLC24A1	1	0.7
ZNF513	1	0.7
OAT locus	1	0.7
TTPA locus	1	0.7
new locus	4	2.8
Total of known	104	72.2

Figure 10. Contributions of specific genes and loci to Pakistani families with arRP.

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