Supplemental Data

Screening Copy Number Variations in 35 unsolved Inherited Retinal Disease Families

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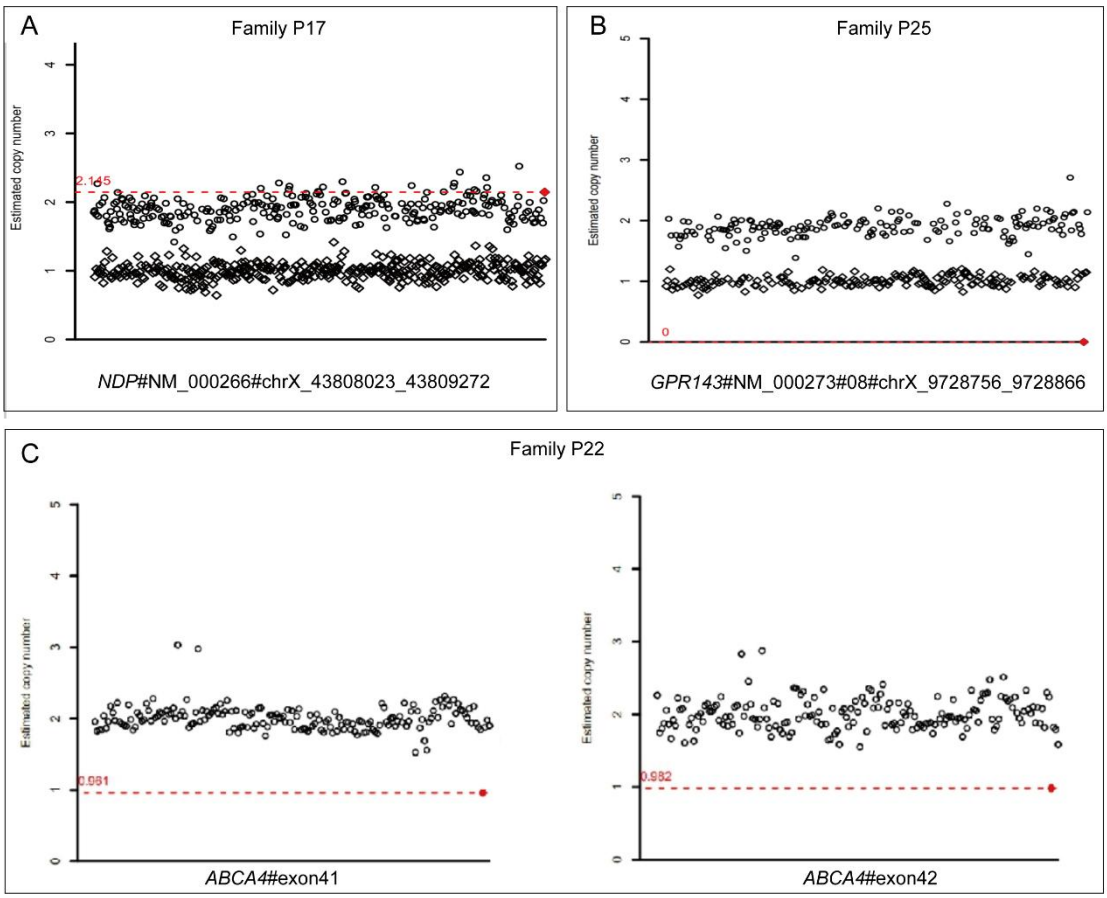

Fig. S1-revised. Copy number variations predicted by HEDEP.

Arrange from left to right on the X-axis by next generation sequencing time.

The Y-axis is the number of copies of IRD genes estimated by the algorithm. Each black point in the figure represents a healthy control sample subjected to HEDEP. Red dotted line represents the patient sample subjected to HEDEP.

HEDEP results indicated the proband of (A) Family P17 had a hemizygous duplication CNV (exon 3 dup; #NM_000266#chrX_43808023_43809272) in *NDP*; (B) Family P25 had a hemizygous CNV (exon 3 del; #NM_000273#chrX_9728756_9728866) in *GPR143*; (C) Family P25 had a heterozygous CNV (exon 41-41 del; #NM_000350.3#exon 41 and 42) in *ABCA4*.

Table S1. Primer's list

	Forward primer (5'-3')	Reverse primer (5'-3')	Length
quantitative PCR			
ALB	GGTGTTGATTGCCTTTGCTC	CAGCTGACTCATCAGCAACACAT	113bp
ABCA4_E41	GATTGCCGAGCCCACTAAGGA	GGTTAGTTCATGTAGCCTTAAG	118bp
ABCA4_E42	ATTTATCCAGGCACCTCCAGC	CTCTCCAGGGCGAACTC	63bp
ADGRV1_86	ATTCCAAACGTCTATGCTGCTT	CTGCAGCATTTGTCCTTCCTCT	151bp
PCARE_E1	TGCAGGGTCTCCCCTTATGCT	GAAGTCACCGACTTCCATTCTTCG	136bp
PCARE_E2	ACCCAGAGATGATGGGCAAACC	TTTGAACCCCAAGGCAGAGCAAG	138bp
CRB1_E1	CTGTGAAGGAGCTGTAAGTAGG	GAGAACATCCTCTGGTGTGTTG	136bp
CRB1_E3	GACGAATGTTGGTCCCAGCCTTG	CATGGAGACAAGGTTGACTGGCAC	145bp
GPR143_E2	TTATCCAATTCTCTCTTAGGTATGGTGATC	TTGCTGCTGCTGCGATTTGAGGA	195bp
PROM1_E15	TGACTGCAAAAAAAATAGAGGCAC	CTCATTAATGTTGAGATGTTCAC	85bp
PROM1_E16	GGAAGCATAAGCAGTGAATTG	CTGAGCCAAGTAGCTGTCAT	138bp
PROM1_E17	ACTGGTAAATCCCCCGCAG	CAAACTGTTTGCTTTTGCTTC	72bp
PROM1_E18	CCAGGAAATTTGAGGAACTCC	CAGTGATTGTTCTATAGGAAG	90bp
PROM1_E19	AGCACTCTATACCAAAG	CAACAATCCATTCCCTGTG	54bp
PROM1_E20	GAGAGAGTAACTAGGATTCTAG	CTCAATAATAACAGAGG	81bp
PROM1_E21	GAAACTAAGAAGTATGGGA	AGAGAACTCGATCCACTGCA	69bp
PROM1_E22	ATCAGTGAGAAAGTGGCATCG	CAAGGGGTCGATAATGTAGCT	93bp
PROM1_E23	GGTTTGGCATAGGAAAAGCTA	CATCGTACACGTCCTCCGA	105bp
PROM1_E24	TGTTGAAACTATACCCATGA	GTTAATAACTTAATTTTTAAAG	113bp
PROM1_E25	TATGGAAAATGGTAATAATG	CTTGTCATAACAGGATTGTGAA	69bp
RLBP1_E3	TTTCTAACCCCTGCAGCCCTGA	CTTACCCCTTCTGACATGTTGCC	133bp
RLBP1_E4	CGCATGGTACCTGAAGAGGAACA	TCAGCCACCTCACCTTCTGCAA	130bp
RS1_E1	GCCAAAGACCTAAGAACTAAATGG	AAAGCCTTCTATCTTGCGTGAC	148bp
RS1_E2	CCACATTGGGATTATCGTCT	CCCAGCCAAAATATATTTCA	150bp
RS1_E3	GATGAAGGCGAGGACCCCTGGT	CCTCCTTGGACTGTATACCAG	106bp
RS1_E4	GAATGCCCATATCACAAGCCTC	CTGCTTACCCAAAGCCTTGAC	151bp
RS1_E5	GTGCCTGGCTCTCCAAGTT	CTGGTCCTTGTAGTAAATCCAG	179bp
RS1_E6	GTCTTCTATGGCAACTCGGAC	GCAGGCATCAGGCACACTTG	160bp
NDP_E3	GCCTTTGGTGTCGTTCAGCACT	GTAGGTGGCAGTGAGTCGCAT	133bp
NYX_E1	AAGAAGTGTGGAGGCATGGG	CTCACCATGCAGAAGCAGGA	158bp
USH2A_E22	GTATGCATGCTTGTATCAGGATC	TTGTAGTTACTTCCACTGGTGAC	147bp
USH2A_E23	TGATTGTCTTTGCAGCATCACCTG	TTTACCTCAGTACCAGGCACCTA	157bp
USH2A_E25	GGCAGAGTTCTGAAGAACAAATC	GGTCAAGTTAATCAAACAGGAGAG	180bp
USH2A_E23	GGGTCACCAGTGGAAGTAAC	AACACCAACATATCAACAGGGC	176bp
USH2A_E24	CTCTAGGTTCCTCTGCCATCCT	GATGGTATAACTTCGCGGGAGC	92bp
PCR and Sanger Sequencing			
OPN1LW/MW_E1	CCCAGGCCCAATTAAGAGAT	GGGACGTGCAGAAGAGAGAT	387bp
OPN1LW_E2	TGGACAAAGCTGGAGGGAAA	ATATGGATGTGAGGCGCAGAT	568bp
OPN1MW_E3	TGCAGACGTTTGGGGTCTAA	TCCTATGTTGCAGCCACATT	394bp
OPN1LW_E4	GCCACAGAATTGATCACTTCA	AGTGGACTCATTTGAGGGCA	398bp

OPN1MW_E5	TCCACTCAGGGCTGGAAGAT	CGGGCTTCTTATCAGAGACAT	460bp
OPN1LW_E6	TTCAACCCAGTGTAGTCACCA	TTTTACAGGGATGGAGAAGGA	377bp

Table S2. 35 Chinese IRDs families with (likely) causative copy number variants

Family ID	Clinical diagnosis	Proband's gender	Age(year)		Type	Variant(s) detected by NGS									CNVs validated by an additional method						
			Onset	Examination		Testing method	Gene	Transcript ID	Genotype	exon/intron	Sequence Change		Literature	ACMG classificatio	Testing method	Gene	Transcript ID	Genotype	CNVs	Literature	ACMG classificatio
P01	CORD	F	8	54	Sporadic	HEDEP	*USH2A*	NM_206933.3	Het	exon68	c.14876G>A	p.Gly4959Asp	Liu et al. (2020)	P	MLPA	*USH2A*	NM_206933.3	Het	exon 11-21 del	Liu et al. (2021)	P
P02	CORD	F	10	42	Sporadic	HEDEP	-	-	-	-	-	-	-	-	MLPA	*EYS*	NM_001142800.2	Com.Het	exon 6-7 del	Liu et al. (2021)	P
																			exon 13-15 del	Liu et al. (2021)	P
P03	RP	F	5	45	Sporadic	HEDEP	*EYS*	NM_001142800.2	Het	exon6	c.919G>T	p.Gly307*	Liu et al. (2020)	P	MLPA	*EYS*	NM_001142800.2	Het	exon 31-32 del	Liu et al. (2021)	P
P04	RP	M	Childhood	16	Sporadic	HEDEP	-		-	-	-	-	-	-	MLPA	*PRPF31*	NM_015629	Het	exon 2-14 del	Liu et al. (2021)	P
P05	STGD	F	Childhood	11	Sporadic	HEDEP	*ABCA4*	NM_000350.3	Het	exon19	c.2909C>T	p. Thr970Ile	Stenirri et al. (2008)	P	MLPA	*ABCA4*	NM_000350.3	Het	exon 38-44 dup	Liu et al. (2021)	P
P06	Choroideremia	M	12	49	Sporadic	HEDEP	-		-	-	-	-	-	-	MLPA	*PRPF31*	NM_015629	Het	exon 1-14del	Liu et al. (2021)	P
P07	RP	M	10	44	AD	HEDEP	-		-	-	-	-	-	-	MLPA	*PRPF31*	NM_015629	Het	exon 2-3 del	Liu et al. (2021)	P
P08	RP	M	6	18	Sporadic	HEDEP	-		-	-	-	-	-	-	MLPA	*PRPF31*	NM_015629	Het	exon 2-13 del	Liu et al. (2021)	P
P09	CORD	F	7	24	Sporadic	HEDEP	*EYS*	NM_001142800.2	Het	exon26	c.4655T>A	p.Leu1552*	Liu et al. (2020)	P	MLPA	*EYS*	NM_001142800.2	Het	exon 15 del	Liu et al. (2021)	P
P10	USH	F	15	54	Sporadic	HEDEP	-		-	-	-	-	-	-	MLPA	*PRPF31*	NM_015629	Het	exon 14 del	Liu et al. (2021)	P
P11	BCD	M	10	41	AR	HEDEP	*CYP4V2*	NM_207352	Homo	IVS6	c.802-8_810del17bpinsGC	-	Xiao et al. (2011)	P	MLPA	*CYP4V2*	NM_207352	Het	exon 1-11 del	Liu et al. (2021)	P
P12	RP	M	4	26	Sporadic	HEDEP	*CRB1*	NM_201253.3	Het	exon7	c.2234C>T	p.Thr745Met	den Hollander et al. (1999)	P	MLPA	*CRB1*	NM_201253.3	Het	exon 6-8 dup	Liu et al. (2021)	P
P13	RP	M	14	60	Sporadic	HEDEP	-		-	-	-	-	-	-	MLPA	*PRPF31*	NM_015629	Het	exon 14 del	Liu et al. (2021)	P
P14	CORD	M	10	52	AD	HEDEP	*EYS*	NM_001142800.2	Het	exon42	c.8143C>T	p.Arg2715*	Liu et al. (2020)	P	MLPA	*EYS*	NM_001142800.2	Het	exon 14-22 del	Liu et al. (2021)	P
P15	USH	F	4	18	Sporadic	HEDEP	*USH2A*	NM_206933.3	Het	exon61	c.11762_11787 del26bp	p.Ala3922His fs*116	This study	LP	MLPA	*USH2A*	NM_206933.3	Het	exon 47 del	Le Quesne Stabej et al.(2012)	P
P16	RP	M	20	34	AR	HEDEP	*PROM1*	NM_001145848	Het	exon1	c.139delC	p.His47Ilefs*12	Xu et al. (2014)	P	qPCR	*PROM1*	NM_001145848	Het	exon 15-25 dup	Liu et al. (2021)	P
P17	FEVR	M	7	29	XL	HEDEP	-		-	-	-	-	-	-	qPCR	*NDP*	NM_000266	Hemi	exon 3 dup	Liu et al. (2021)	P
P18	Retinoschisis	M	1	4	Sporadic	HEDEP	-		-	-	-	-	-	-	qPCR	*RS1*	NM_000330.4	Hemi	exon 1-3 del	Liu et al. (2021)	P
P19	USH	F	14	39	Sporadic	HEDEP	*USH2A*	NM_206933.3	Het	exon 53	c.10514C>G	p.Pro3505Arg	This study	US	MLPA	*USH2A*	NM_206933.3	Het	exon 61 dup	This study	P
P20	RP	M	10	50	Sporadic	HEDEP	*PCARE*	NM_001029883.3	Homo	exon1	c.644delT	p.Leu215Argfs*41	This study	LP	qPCR	*PCARE*	NM_001029883.3	Het	exon 1-2 del	This study	P
P21	RP	M	22	48	AR	HEDEP	*ADGRV1*	NM_032119.4	Het	exon79	c.17187C>A	p.Cys5729*	Jiao Q et al. (2019)	LP	qPCR	*ADGRV1*	NM_032119.4	Het	exon 86 dup	This study	P
P22	STGD	F	11	15	Sporadic	HEDEP	*ABCA4*	NM_000350.3	Het	exon36	c.5163dupC	p.Thr1722Hisfs*65	This study	LP	qPCR	*ABCA4*	NM_000350.3	Het	exon 41-42 del	This study	P
P23	RP	M	4	7	Sporadic	HEDEP	*CRB1*	NM_201253.3	Het	exon7	c.2291G>A	p.Arg764His	Corton M et al. (2013)	LP	qPCR	*CRB1*	NM_201253.3	Het	exon 1 del	This study	P
P24	Macular Retinoschisis	M	4	4	Sporadic	HEDEP	*CRB1*	NM_201253.3	Het	IVS5	c.1171+1G>A	-	This study	LP	qPCR	*CRB1*	NM_201253.3	Het	exon 3 del	This study	P
P25	CN?	M	1	31	Sporadic	HEDEP	-		-	-	-	-	-	-	qPCR	*GPR143*	NM_000273	Hemi	exon 2 del	This study	P
P26	Macular Retinoschisis	F	4	6	Sporadic	WES	*RS1*	NM_000330.4	Homo	exon4	c.214G>T	p.Glu72*	This study	P	qPCR	*RS1*	NM_000330.4	Het	exon 4-6 del	This study	P
P27	Macular Retinoschisis	M	27	27	Sporadic	HEDEP	-		-	-	-	-	-	-	qPCR	*RS1*	NM_000330.4	Hemi	exon 1 del	This study	P
P28	RP	M	15	50	Sporadic	HEDEP	*RLBP1*	NM_000326	Het	IVS7	c.684+1G>C	-	This study	LP	qPCR	*RLBP1*	NM_000326	Het	exon 3-4 dup	This study	P
P29	RP	F	childhood	48	AR	HEDEP	-		-	-	-	-	-	-	qPCR	*USH2A*	NM_206933.3	Homo	exon 22-24 del	This study	P
P30	RP	M	1	2	AD	WES	*ABCA4*	NM_000350.3	Com. Het	exon42	c.5881G>A	p.Gly1961Arg	Riveiro-Alvarez R et al.(2013)	LP	qPCR	*NYX*	NM_022567	Hemi	exon 1 del	This study	P
										exon11	c.1405G>C	p.Gly469Arg	This study	US							
P31	CVD	M	childhood	41	XL	HEDEP	-		-	-	-	-	-	-	Sanger	*OPN1LW*	NM_020061	Hemi	exon 3 del	This study	P
P32	CVD	M	5	5	XL	HEDEP	-		-	-	-	-	-	-	Sanger	*OPN1MW*	NM_000513.2	Hemi	entire gene del	Ayyagari R et al.(2000)	P
P33	CVD	M	childhood	58	XL	HEDEP	-		-	-	-	-	-	-	Sanger	*OPN1MW*	NM_000513.2	Hemi	entire gene del	Ayyagari R et al.(2000)	P
P34	CVD;Deafness	M	childhood	32	Sporadic	HEDEP	*GJB2*	NM_004004.6	Homo		c.235delC	p.Leu79Cysfs*3	Xia H et al. (2019)	P	Sanger/qP	*OPN1LW*	NM_020061	Hemi	exon 3-5 del	This study	P
P35	CVD	M	childhood	57	Sporadic	HEDEP	-		-	-	-	-	-	-	Sanger	*OPN1MW*	NM_000513.2	Het	entire gene del	Ayyagari R et al.(2000)	P

AD= autosomal dominant; AR= autosomal recessive; BCD= Bietti Crystalline Corneoretinal Dystrophy; CORD= Cone-rod Dystrophy; CN= Congenital nystagmus; CVD= Color Vision Deficiency; F=Female; FEVR= Familial Exudative Vitreoretinopathy; HEDEP= Hereditary Eye Disease Enrichment Panel; Het= heterozygous; Hemi= Hemizygous; Homo= homozygous; Com. Het= Compound heterozygous; LP= P= likely pathogenic; LCA=Leber Congenital Amaurosis; M=Male; MD=Macular Degeneration; MLPA= Multiplex Ligation-Dependent Probe Amplification; NGS= Next generation sequencing; P= pathogenic; qPCR= quantitative Polymerase Chain Reaction; RP= Retinitis Pigmentosa; STGD=Stargardt Disease; XL= X-Linked; US = uncertain significance; WES= the Whole Exome Sequencing

Table S3. The classification of CNVs according to ACMG and the AMP variant interpretation guidelines

Family ID	CNVs validated by an additional method			Evidence of pathogenicity															
	Gene	CNVs	ACMG classification	Very strong	Strong				Moderate						Supporting				
				PVS1	PS1	PS2	PS3	PS4	PM1	PM2	PM3	PM4	PM5	PM6	PP1	PP2	PP3	PP4	PP5
P01	*USH2A*	exon 11-21 del	P	Y	Y							Y			Y		Y		
P02	*EYS*	exon 6-7 del	P	Y	Y							Y					Y		
		exon 13-15 del	P	Y	Y							Y					Y		
P03	*EYS*	exon 31-32 del	P	Y	Y							Y					Y		
P04	*PRPF31*	exon 2-14 del	P		Y	Y						Y					Y		
P05	*ABCA4*	exon 38-44 dup	P	Y	Y										Y		Y		
P06	*PRPF31*	exon 1-14del	P		Y				Y			Y					Y		
P07	*PRPF31*	exon 2-3 del	P		Y				Y			Y			Y		Y		
P08	*PRPF31*	exon 2-13 del	P		Y							Y					Y		
P09	*EYS*	exon 15 del	P	Y	Y							Y			Y		Y		
P10	*PRPF31*	exon 14 del	P		Y				Y			Y					Y		
P11	*CYP4V2*	exon 1-11 del	P	Y	Y							Y			Y		Y		
P12	*CRB1*	exon 6-8 dup	P	Y	Y										Y		Y		
P13	*PRPF31*	exon 14 del	P		Y				Y			Y					Y		
P14	*EYS*	exon 14-22 del	P	Y	Y							Y					Y		
P15	*USH2A*	exon 47 del	P	Y	Y							Y			Y		Y		
P16	*PROM1*	exon 15-25 dup	P	Y	Y										Y		Y		
P17	*NDP*	exon 3 dup	P	Y	Y												Y		
P18	*RS1*	exon 1-3 del	P	Y	Y				Y			Y					Y		
P19	*USH2A*	exon 61 dup	P	Y						Y					Y		Y		
P20	*PCARE*	exon 1-2 del	P	Y						Y		Y		Y	Y		Y		
P21	*ADGRV1*	exon 86 dup	P	Y						Y					Y		Y		
P22	*ABCA4*	exon 41-42 del	P	Y						Y		Y		Y			Y		
P23	*CRB1*	exon 1 del	P	Y						Y		Y			Y		Y		
P24	*CRB1*	exon 3 del	P	Y						Y		Y			Y		Y		
P25	*GPR143*	exon 2 del	P	Y						Y		Y			Y		Y		
P26	*RS1*	exon 4-6 del	P	Y		Y				Y		Y					Y		
P27	*RS1*	exon 1 del	P	Y						Y		Y					Y		
P28	*RLBP1*	exon 3-4 dup	P	Y						Y					Y		Y		
P29	*USH2A*	exon 22-24 del	P	Y						Y		Y			Y		Y		
P30	*NYX*	exon 1 del	P	Y						Y		Y			Y		Y		
P31	*OPN1LW*	exon 3 del	P	Y						Y		Y					Y		
P32	*OPN1MW*	entire gene del	P	Y	Y							Y					Y		
P33	*OPN1MW*	entire gene del	P	Y	Y							Y			Y		Y		
P34	*OPN1LW*	exon 3-5 del	P	Y						Y		Y					Y		
P35	*OPN1MW*	entire gene del	P	Y	Y							Y			Y		Y		

Y indicates the affected family has the evidence of pathogenicity