

Supplemental Data

Screening Copy Number Variations in 35 unsolved

Inherited Retinal Disease Families

Xiaozhen Liu, Hehua Dai, Genlin Li, Ruixuan Jia, Xiang Meng, He Huang,

Shicheng, Yu, Liping Yang, Jing Hong

Xiaozhen Liu and Hehua Dai contribute to this article equally.

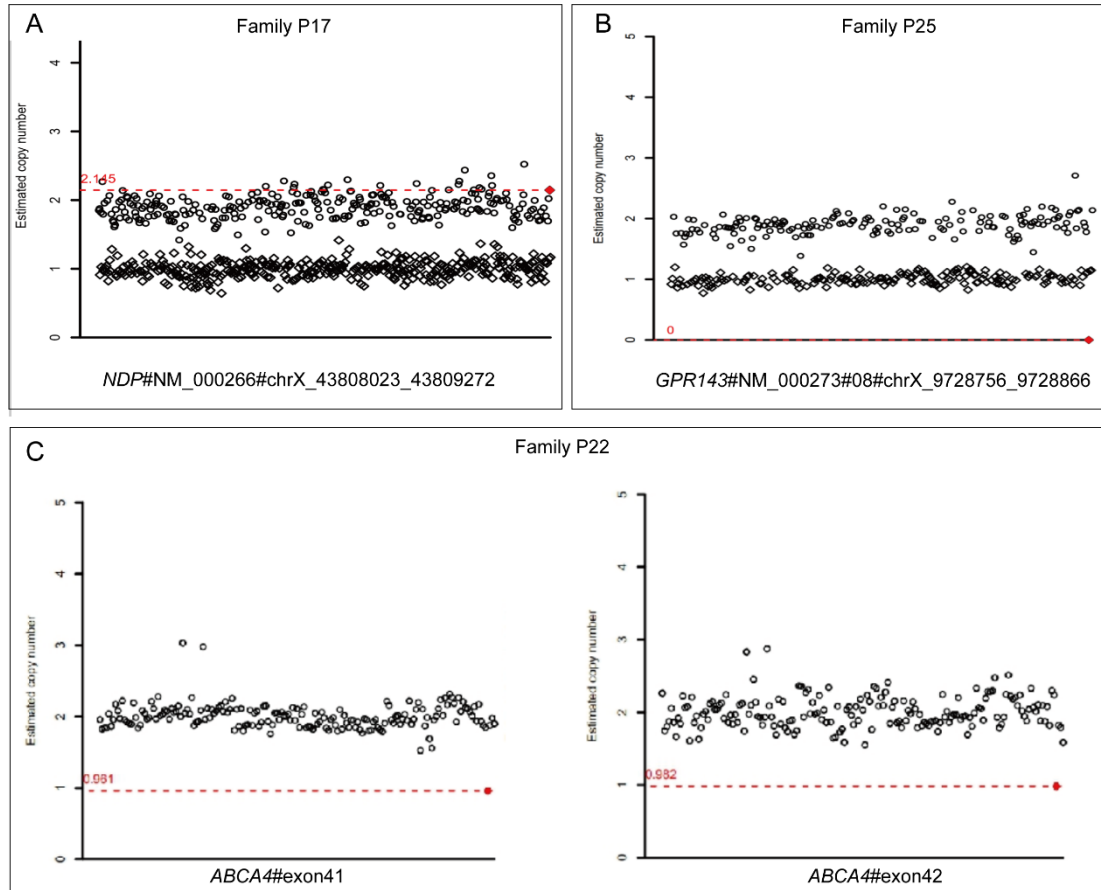


Fig. S1. Copy number variations predicted by HEDEP.

Each point in the figure represents a sample subjected to HEDEP. Arrange from left to right on the X-axis by next generation sequencing time. The Y-axis is the number of copies of *NDP* (#NM_000266#chrX_43808023_43809272, A) and *GPR143* (#NM_000273#chrX_9728756_9728866, B), *ABCA4* (#NM_000350.3#exon 41 and 42, C) genes estimated by the algorithm. Red dotted line represent samples with low data quality. Blue points (

Table S1. Primer's list

	Forward primer(5'-3')	Reverse primer(5'-3')	Length
quantitative PCR			
ALB	GGTGTGATTGCCTTTGCTC	CAGCTGACTCATCAGCAACACAT	113bp
ABCA4_E41	GATTGCCGAGCCCACTAAGGA	GGTTAGTTCATGTAGCCTTAAG	118bp
ABCA4_E42	ATTTATCCAGGCACCTCCAGC	CTCTCCAGGGCGAACTC	63bp
ADGRV1_86	ATTCCAAACGTCTATGCTGCTT	CTGCAGCATTTGTCCTTCCTCT	151bp
PCARE_E1	TGCAGGGTCTCCCTTATGCT	GAAGTCACCGACTTCCATTCTCG	136bp
PCARE_E2	ACCCAGAGATGATGGGCAAACC	TTTGAACCCCAAGGCAGAGCAAG	138bp
CRB1_E1	CTGTGAAGGAGCTGTAAGTAGG	GAGAACATCCTCTGGTGTGTTG	136bp
CRB1_E3	GACGAATGTTGGTCCCAGCCTTG	CATGGAGACAAGGTTGACTGGCAC	145bp
GPR143_E2	TTATCCAATTCTCTCTTAGGTATGGTGATC	TTGCTGCTGCTGCGATTTGAGGA	195bp
PROM1_E15	TGACTGCAAAAAAATAGAGGCAC	CTCATTAATGTTGAGATGTTTAC	85bp
PROM1_E16	GGAAGCATAAGCAGTGAATTG	CTGAGCCAAGTAGCTGTCAT	138bp
PROM1_E17	ACTGGTAAATCCCCGCAG	CAAAGTGTGCTTTTGTCTTC	72bp
PROM1_E18	CCAGGAAATTTGAGGAACTCC	CAGTGATTGTTCTATAGGAAG	90bp
PROM1_E19	AGCACTCTATACCAAAG	CAACAATCCATTCCCTGTG	54bp
PROM1_E20	GAGAGAGTAAGTAGGATTCTAG	CTCAATAATAACAGAGG	81bp
PROM1_E21	GAAACTAAGAAGTATGGGA	AGAGAACTCGATCCACTGCA	69bp
PROM1_E22	ATCAGTGAGAAAGTGGCATCG	CAAGGGGTCGATAATGTAGCT	93bp
PROM1_E23	GGTTTGGCATAGGAAAAGCTA	CATCGTACACGTCTCCGA	105bp
PROM1_E24	TGTTGAAACTATACCATGA	GTTAATAACTTAATTTTAAAG	113bp
PROM1_E25	TATGGAAAATGGTAATAATG	CTTGTCATAACAGGATTGTGAA	69bp
RLBP1_E3	TTTCTAACCCTGCAGCCCTGA	CTTACCCTTCTGACATGTTGCC	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	CTGGTCCTTGTAATAATCCAG	179bp
RS1_E6	GTCTTCTATGGCAACTCGGAC	GCAGGCATCAGGCACACTTG	160bp
NDP_E3	GCCTTTGGTGTGCTTCAGCACT	GTAGGTGGCAGTGAGTCGCAT	133bp
NYX_E1	AAGAAGTGTGGAGGCATGGG	CTCACCATGCAGAAGCAGGA	158bp
USH2A_E22	GTATGCATGCTTGTATCAGGATC	TTGTAGTTACTTCCACTGGTGAC	147bp
USH2A_E23	TGATTGTCTTTCAGCATCACCTG	TTTACCTCAGTACCAGGCACCTA	157bp
USH2A_E25	GGCAGAGTTCTGAAGAACAAATC	GGTCAAGTTAATCAAACAGGAGAG	180bp
USH2A_E23	GGGTCACCAGTGGAAGTAAC	AACACCAACATATCAACAGGGC	176bp
USH2A_E24	CTCTAGGTTCTCTGCCATCCT	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	TTTACAGGGATGGAGAAGGA	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 exon 13-15 del	Liu et al. (2021) Liu et al. (2021)	P 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.His471Ilefs*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	LP		
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	LP		
P25	Macular CN?	M	1	31	Sporadic	HEDEP	-	-	-	-	-	-	-	-	qPCR	GPR143	NM_000273	Hemi	exon 2 del	This study	LP		
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	LP		
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 exon11	c.5881G>A c.1405G>C	p.Gly1961Arg p.Gly469Arg	Riveiro-Alvarez R et al.(2013) This study	LP US	qPCR	NYX	NM_022567	Hemi	exon 1 del	This study	P		
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	Unaffected	F	-	33	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/qF	OPN1LW	NM_020061	Hemi	exon 3-5 del	This study	P		
P35	Unaffected	F	-	31	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; WES= the Whole Exome Sequencing