Association of *NR2E3* but Not *NRL* Mutations with Retinitis Pigmentosa in the Chinese Population

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Purpose. Mutations in the *NR2E3* and *NRL* genes have been implicated in both autosomal dominant and autosomal recessive retinitis pigmentosa (RP). In this study, the mutation profiles of these two genes were investigated in Chinese RP patients.

Methods. In 172 RP patients and 360 normal control subjects (180 from Hong Kong and 180 from Beijing), the coding exons and the exon–intron boundaries of *NR2E3* and *NRL* were screened by direct DNA sequencing after PCR. Association analysis was performed for common single-nucleotide polymorphisms (SNPs), whereas in silico programs were used for analysis of rare missense variants.

Results. In *NR2E3*, 14 novel sequence changes have been identified. Two missense variants, p.G56R and p.V118M, were exclusively found in RP patients with frequencies at 1.2% (2/172) and 1.7% (3/172), respectively. All five patients were found to be heterozygous for these two mutations. Computational analysis suggested functional defects on the NR2E3 protein, indicating disease-causing roles. The p.E121K variant of *NR2E3*, which reportedly caused enhanced S-cone syndrome (ESCS) in Caucasians, was found concurrently in RP patients (13.4%) and control subjects from Hong Kong (10.5%) and Beijing (12.8%). In *NRL*, six novel sequence changes were identified, none of them associated with RP.

Conclusions. In this study, *NR2E3* mutations (p.G56R, p.V118M) were found to be responsible for approximately 2.9% of overall RP in Chinese patients, comparable to the contributions of *RHO* and *RP1* mutations. The p.E121K in *NR2E3* is a common SNP in the Chinese, suggesting another genetic or environmental factor is involved in its causative role in ESCS in Caucasians. (*Invest Ophthalmol Vis Sci.* 2010;51: 2229–2235) DOI:10.1167/iovs.09-4299

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Retinitis pigmentosa (RP; MIM 268000; Mendelian Inheritance in Man; National Center for Biotechnology Information, Bethesda, MD) refers to a group of inherited and degenerative retinal diseases due to the death of rod photoreceptors in the retina with high phenotypic and genetic heterogeneity. Affected individuals usually experience early-onset night blindness followed by progressive loss of peripheral vision, decline in the electroretinograph (ERG), atrophy and pigmentary changes to the retinal pigment epithelium (RPE), and eventual loss of central vision or complete blindness. The worldwide prevalence of RP is approximately 1:3000 to 1:5000, affecting more than 1 million individuals.[1–6] RP can follow Mendelian modes of inheritance or present as rare mitochondrial and digenic forms.[7] Major inheritance patterns include autosomal–dominant (15%–20%), autosomal–recessive (20%–25%), and X-linked (10%–15%). The remaining 40% to 55% of cases are classified as simplex RP, because of absent family history or segregation pattern.[8,9] To date, at least 21, 29, and 6 genes or chromosomal loci have been identified for autosomal dominant (AD)RP, autosomal recessive (AR)RP and X-linked (XL)RP, respectively (RetNet, the Retinal Information Network, http://www.sph.uth.tmc.edu/RetNet; provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX, accessed October 1, 2009). Among them, four genes—*RHO* (rhodopsin, MIM 180380), *RP1* (retinitis pigmentosa 1, MIM 603937), *NR2E3* (photoreceptor-specific nuclear receptor, MIM 604485), and *NRL (*neural retina leucine zipper, MIM 162080)— have been reported to be responsible for both ADRP and ARRP. Mutation profiles of *RHO* and *RP1* in RP are documented in different ethnic groups. In Caucasians, *RHO* mutations account for approximately 25% of ADRP and 1% of ARRP, whereas *RP1* mutations account for approximately 5% and less than 1% of ADRP and ARRP, respectively.[10–19] In the Chinese, *RHO* and *RP1* contribute to approximately 2% and 1% of overall RP cases, respectively, much lower than in Caucasians.[20–22]

NR2E3 encodes a member of the nuclear hormone receptor superfamily of ligand-modulated transcription factors that repress cone-specific genes in rods.[23,24] NRL belongs to the basic motif-leucine zipper family of transcription factors and is essential in the regulation of early events leading to the development of rod photoreceptors.[25,26] Both NR2E3 and NRL are expressed predominantly in photoreceptor cells in the retina and act interactively with the cone-rod homeobox transcription factor (*CRX*, MIM 602225), to suppress cone-specific gene expression in mature rods and activate several rod-specific genes, including *RHO*.[23,24,27–30] Lesions in these two genes may lead to dysfunction of the photoreceptors. The genes are thus associated with the pathogenesis of RP and other forms of retinal degeneration. *NR2E3* mutations account for approximately 1.4% of ADRP and 0.25% of ARRP in Caucasians.[13,31] They have also been implicated in the recessively inherited

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Table 1. Primer Sequences and Thermal Cycling Conditions for *NR2E3* and *NRL* Screening

Primer	Primer Sequence (5′→3′)	PCR Product Size (bp)	PCR Conditions
NR2E3 1-F	TGTGGAGACAGTAAAGATTAAGAGG	461	2.0 mM $MgCl_2$; 56°C
NR2E3 1-R	AGTTGTTCTGGCTCCTTCCAT		
NR2E3 2/3-F	CGTGGGTTCGTTCAAATGC	670	1.5 mM $MgCl_2$; 56°C
NR2E3 3-R	CAGTGTTGGACTCCATGCTG		
NR2E3 4/5-F	GGCTGAAGAAGTGCCTGCA	756	1.5 mM $MgCl_2$; 56°C
NR2E3 5-R	CCCTGTCTGGTTGACTCGAGT		
NR2E3 6/7/8-F	AGGACAGCACTTCCATTCCTTGG	935	1.5 mM $MgCl_2$; 56°C
NR2E3 8R	TTGTGCTGTCTAATCAGTGAGCTC		
NR2E3 9F	AAATTCCTCCTGACCCACTCTG	365	2.0 mM $MgCl_2$; 56°C
NR2E3 9R	ACACCTACGAGGAATTGCTGG		
NR2E3 P1F	TTCAAGATGTGGCATGAAATGG	286	2.0 mM $MgCl_2$; 56°C
NR2E3 P1R	AACTCTGTCTGAACTCAGGAGCTG		
NRL 1F	CTGGCTTTCCCAAACTCTTG	664	1.5 mM $MgCl_2$; 62°C
NRL 1R	CTTTCAAGGGACCTTCTCCC		
NRL 2F	GAAACAGACTGCGTGGAAGG	567	1.5 mM $MgCl_2$; 62°C TD(−0.2)
NRL 2R	TAACGATGCAGAGAACCGTG		30 cycles + 56°C 10 cycles

F, forward; R, reverse.

enhanced S-cone sensitivity syndrome (ESCS), Goldmann-Favre syndrome (GFS), and clumped pigmentary retinal degeneration (CPRD).[32] However, the *NR2E3* mutations responsible for ESCS, GFS, and CPRD do not overlap with those associated with RP, except for p.R311Q, which is involved in four major types of retinal degenerations in humans.[32] The heterozygous mutation p.G56R, located in the first zinc finger of the DNA-binding domain (DBD), is the most common causative mutation for ADRP in the *NR2E3* sequence.[31,33,34] *NR2E3* mutations have been correlated with specific phenotypes (www.LOVD.nl/eye; Leiden Open Variation Database, provided in the public domain by Leiden University Medical Center, Leiden, The Netherlands).[32] *NRL* mutations contribute to approximately 1% of ADRP and less than 1% of ARRP in Caucasians.[13] Most RP-causative mutations in the *NRL* sequence were missense changes affecting three residues: S50, P51, and G122.[35,36] Loss-of-function mutations in NRL have also been found in patients with CPRD[37] and ESCS.[38] In the Chinese, however, involvement of *NR2E3* and *NRL* mutations in RP has not been investigated. In the present study, we report the mutation patterns of these two genes in Chinese RP patients.

Materials and Methods

Study Subjects

The patients in this study were recruited from the eye clinics of Hong Kong Eye Hospital and Prince of Wales Hospital in Hong Kong. They were given complete ophthalmic examinations, including slit-lamp biomicroscopy, fundus photography and electroretinography. Diagnosis of nonsyndromic RP was based on typical clinical history and features of RP. Patients with syndromic RP, such as Usher's syndrome, Leber congenital amaurosis, and Bardet-Biedl syndrome, were excluded. In total, 172 RP patients were recruited, including 92 males and 80 females, with ages ranging from 6 to 84 years. Based on family history, 18 (10.5%) of the patients were classified as having ADRP, 26 (15.1%) as having ARRP, 2 (1.2%) as having XLRP, and 97 (56.4%) as having simplex RP; 29 (16.8%) patients could not be classified because of absent family information. They therefore had unknown inheritance patterns and might represent a group with nonsyndromic RP of mixed phenotypes. Also recruited were 180 control subjects, older than 60 years. They were confirmed to be free of RP or other major eye diseases, except mild senile cataract, by detailed ophthalmic examination. In addition, a group of 180 normal control subjects from Beijing, which is located in northern China, were also recruited. They were examined at Beijing Tongren Hospital and were described in our previous study.[31] All study subjects were ethnic Chinese.

Informed consents were obtained from all the subjects participating in this study. The study protocol was approved by the Ethics Committee on Human Research, the Chinese University of Hong Kong, and by the respective human subjects review boards at each participating academic institution. All procedures were performed in accordance with the Declaration of Helsinki.

Mutational Screening of *NR2E3* and *NRL* in Cases and Controls

Peripheral venous blood was collected from all study subjects for extraction of genomic DNA (QIAamp DNA Blood kit; Qiagen, Valencia, CA) according to the supplier's instructions. Polymerase chain reaction (PCR) primers were designed according to sequences from the Ensembl database[39] (Table 1). In total, six amplicons covering coding exons 1 to 9 and part of the promoter sequence of *NR2E3* and two amplicons covering coding exons 1 and 2 of *NRL* were amplified by PCR for direct DNA sequencing with dye-termination chemistry (BigDye Terminator Cycle Sequencing Reaction Kit; ver. 3.1; Applied Biosystems, Inc. [ABI], Foster City, CA) on a DNA sequencer (model 3130XL; ABI), according to the supplier's protocol. The DNA sequences were compared with the human *NR2E3* (ENSG00000031544) and *NRL* (ENSG00000129535) sequences in the Ensembl database. Detected sequence variants in any DNA sample were confirmed by bidirectional sequencing on another stock DNA sample.

Statistical Analysis and Analysis of Variants

For the common polymorphisms, χ^2 analysis was used to test Hardy-Weinberg Equilibrium (HWE) and to compare the genotype frequencies of the single-nucleotide polymorphisms (SNPs) between RP patients and controls using SPSS (ver. 15.0, SPSS Inc., Chicago, IL). The Bonferroni method was used to correct the *P*-values in multiple comparisons. A corrected $P < 0.05$ was considered statistically significant. For the rare missense variants detected exclusively in patients, three Web-based analysis programs—PolyPhen (Polymorphism Phenotyping, http://genetics.bwh.harvard.edu/pph/ provided by the Bork Group and the Sunyaev Lab, Brigham and Women's Hospital, Harvard Medical School, Boston, MA), SIFT (Sorting Intolerant from Tolerant, http://sift.jcvi.org/ provided in the public domain by the J. Craig Venter Institute, Rockville, MD), and Grantham Score (The Single Amino Acid Polymorphism Disease-association Predictor, http://sapred.cbi.pku.edu.cn/ provided by the Sapred Team, Center for Bioinformatics, Peking University, Beijing, China), were used to

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TABLE 2. Sequence Variations Detected in the *NR2E3* and *NRL* Genes among Chinese RP Patients and Control Subjects

Location	Nucleotide Change	Residual Change	Description	Genotype Frequency*	
				Patients (n = 172)	Controls (n = 180)
NR2E3 variants†					
Intron 1	c.119-47C>T	—	Novel	0/3/169	2/4/174‡
Intron 1	c.119-28T>C	—	rs2742318	1/6/164	2/13/165‡
Intron 1	c.119-28_119-13del	—	Novel	0/1/171	0/0/180
Exon 2	c.166G>A	p.G56R	Reported[33]	0/2/170	0/0/180
Exon 2	c.183C>T	p.1611	Novel	0/0/172	0/1/179
Intron 2	c.245+75G>A	—	Novel	0/1/171	0/0/180
Intron 3	c.349+8G>A	—	Novel	1/8/163	0/6/174
Exon 4	c.352G>A	p.V118M	Novel	0/3/169	0/0/180
Exon 4	c.361G>A	p.E121K	Reported[40]	1/22/149	4/14/162‡
Exon 4	c.419A>G	p.E140G	rs1805020	2/37/133	4/37/139
Exon 4	c.488T>C	p.M163T	rs1805021	2/40/130	4/34/142
Exon 6	c.829C>T	p.L277L	Novel	0/1/171	0/0/180
Exon 6	c.843C>T	p.P281P	Novel	0/1/171	0/0/180
Exon 6	c.899C>T	p.T300M	Novel	0/0/172	0/1/179
Exon 6	c.904 G>A	p.V3021	rs1805025	0/13/159	0/6/174
Exon 7	c.963 C>T	p.A321A	Novel	0/5/167	0/4/176
Intron 8	c.1101-87C>T	—	Novel	0/0/172	0/1/179
Intron 8	c.1101-47G>A	—	Novel	0/0/172	0/1/179
Exon 9	c.1124C>T	p.P375L	Novel	0/0/172	0/1/179
3′UTR	c.1230+53C>T	—	Novel	0/1/171	0/0/180
NRL variants†					
Exon 2	c.97G>C	p.G33R	Novel	0/0/172	0/1/179
Exon 2	c.105T>C	p.P35P	Novel	0/0/172	0/1/179
Exon 2	c.108A>C	p.T36T	Novel	0/0/172	0/1/179
Exon 2	c.112T>C	p.S38P	Novel	0/0/172	0/1/179
Exon 2	c.126A>G	p.T42T	Novel	0/1/171	0/0/180
Exon 3	c.450G>C	p.R150R	Novel	0/2/170	0/0/180

* Genotype frequency is presented as: homozygous/heterozygous/wild-type.

† Nomenclature of the *NR2E3* and *NRL* variants was referred to the cDNA sequences (ENST00000326995 for *NR2E3* and ENST00000337947 for *NRL*) in the Ensembl database.[39]

‡ Variant that is deviated from Hardy-Weinberg equilibrium in the control group.

evaluate and predict the possible functional effects of the amino acid substitutions on the proteins.

RESULTS

Variants Detected in the *NR2E3* Gene

Among the 172 RP patients and 360 control subjects, a total of 20 sequence changes in the *NR2E3* gene was identified, 14 of them novel (Table 2). Eight variants (c.119-47C>T, c.119-28T>C, c.119-28_119-13del, c.245+75G>A, c.349+8G>A, c.1101-87C>T, c.1101-47G>A, and c.1230+53C>T) were located in noncoding regions, four (p.I61I, p.L277L, p.P281P, and p.A321A) were synonymous changes, and eight (p.G56R, p.V118M, p.E121K, p.E140G, p.M163T, p.T300M, p.V302I, and p.P375L) were missense changes.

Three of the missense variants, p.E140G (rs1805020), p.M163T (rs1805021), and p.V302I (rs1805025), and one intronic variant, c.119-28T>C (rs2742318), were common SNPs registered in the dbSNP database (provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD). They occurred at high frequencies among RP patients and control subjects (Table 2). Besides, three novel variants, c.119-47C>T, c.349+8G>A, and c.963 C>T (p.A321A), were present in more than 1% of both patients and controls and are considered as SNPs. Except for three (c.119-47C>T, c.119-28T>C, and p.E121K), the SNPs showed HWE. However, none of these SNPs was significantly associated with RP ($P_{corr} > 0.05$). Notably, a missense change p.E121K, which was reported as a disease-causing mutation of the autosomal recessive retinopathy ESCS,[40] was found to occur concurrently in the RP patients (Homo/Het/Wt [homologous/heterologous/wild-type] 1 [0.58%]/22 [12.79%]/149 [86.63%]) and control subjects (Hong Kong cohort, 4 [2.33%]/14 [8.14%]/162 [94.19%]; Beijing cohort, 7 [4.07%]/15 [8.72%]/158 (91.86%]). Both heterozygous and homozygous genotypes were detected in patients and controls. A χ^2 analysis showed that SNP p.E121K was not associated with RP ($P > 0.05$), and the genotype and allele frequencies of this SNP were comparable between the control subjects from Hong Kong and Beijing ($P > 0.05$).

Apart from the common polymorphisms, we also identified rare variants in *NR2E3*. Among them, five (p.I61I, p.T300M, c.1101-47G>A, c.1101-87C>T, and p.P375L) were found only in control individuals, whereas another seven (c.119-28_119-13del, c.245+75G>A, p.G56R, p.V118M, p.L277L, p.P281P, and c.1230+53C>T) occurred exclusively in RP patients. Among them, the two missense variants, p.G56R and p.V118M, could be considered disease-causing mutations because (1) they lead to nonsynonymous amino acid changes in the NR2E3 protein; (2) each of them was found in more than one RP patient, with p.G56R in two patients with ADRP and p.V118M in three patients, two of them SRP (simplex RP) and one of unknown inheritance pattern (Table 3); and (3) both variants were absent in the 360 normal control individuals. Patients carrying p.G56R and p.V118M were all heterozygous. They had typical RP features on fundus photography, including optic disc pallor, attenuated retinal vessels, and diffuse pigmentary changes over the retina. Their visual field and electroretinography were also subnormal (data not shown).

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TABLE 3. Demographic and Clinical Features of Carriers of *NR2E3* Mutations

Mutation	Subject	Sex	Age at Diagnosis*	Inheritance Pattern
p.G56R	HKRP397	F	54	ADRP
p.G56R	HKRP466	F	56	ADRP
p.V118M	HKRP442	F	51	Unknown
p.V118M	HKRP195	F	52	SRP
p.V118M	HKRP210	M	71	SRP

* Age at disease-onset is not available for these patients. Here shows the age at which the patient was diagnosed and recruited for this study.

To evaluate the functional significance of these variants, we performed multiple protein sequence alignments of NR2E3 to compare the sequences of human, murine, chimp, dog, chicken, and frog by a Web-based program (T-Coffee, ver. 7.71 (http://www.tcoffee.org/ provided in the public domain by the Center for Genomic Regulation, Barcelona, Spain).[41] The results showed that positions 56 (G) and 118 (V) on NR2E3 are conserved across species (Fig. 1). Furthermore, all three in silico programs predicted possible impacts of p.G56R and p.V118M on the NR2E3 protein function (Table 4). The variant p.G56R was predicted as "probably damaging" by PolyPhen with a PSIC (position-specific independent count) score of 2.844, and as "damaging" by SIFT with a score of 0.02. The Grantham score for this substitution was 125. Based on these predictions, the p.G56R variant was likely to be a functional mutation, consistent with the previous prediction by Coppieters et al.[33] The novel variant p.V118M was predicted to be "probably damaging" by PolyPhen with a PSIC score of 2.163, and "damaging" by SIFT with a score of 0.00. The Grantham score for this substitution was 21. Therefore, p.V118M was potentially RP causative.

Variants Detected in the *NRL* Gene

Six sequence changes were identified in the *NRL* gene, all are novel (Table 2). Four of them (p.G33R, p. P35P, p.T36T, and p. S38P) were found only in controls, and two (p.T42T, p.R150R) existed in patients exclusively. Except for the p.R150R, which was found in two patients, each variant was found in one study subject and only the heterozygous genotype was identified.

DISCUSSION

NR2E3 and NRL are both transcription factors and part of a complex pathway that controls the differentiation of postmitotic photoreceptor precursors. NRL is necessary for the precursor cells to differentiate to rod photoreceptors,[25,26] although the NR2E3 may interact with NRL to suppress cone-specific gene expression and to activate a subset of rod-specific genes.[23,28,29] Thus, functional mutations in these two genes are expected to result in improper differentiation of photoreceptors and cause rods and cones dysfunction, which lead to RP or other retinal disorders with rod/cone dystrophy. Mutations in both *NR2E3* and *NRL* have been implicated in ESCS,[38,40,42] CPRD,[37,42,43] ADRP, and ARRP.[31–34,36,44,45] *NR2E3* mutations account for approximately 1.4%[31] ADRP and 0.25%[13] ARRP and *NRL* mutations account for 1% ADRP and less than 1% ARRP.[13] Assuming ADRP and ARRP contribute to 15% to 20% and 20% to 25% of all RP patients, respectively,[8,9] *NR2E3* mutations should account for approximately 0.71% ($0.014 \times 0.15+0.025 \times 0.20$) to 0.91% ($0.014 \times 0.2+0.025 \times 0.25$) of overall RP, and *NRL* for 0.35% ($0.01 \times 0.15+0.01 \times 0.20$) to 0.45% ($0.01 \times 0.2+0.01 \times 0.25$).

Among the *NR2E3* sequence variants detected in our study in Chinese individuals, the four reported (rs2742318, rs1805020, rs1805021, and rs1805025) and three novel (c.119-47C>T, c.349+8G>A, and p.A321A) SNPs were not associated with RP. It is noted that a previously reported rare variant in Caucasians, p.E121K, was found at high frequency in our

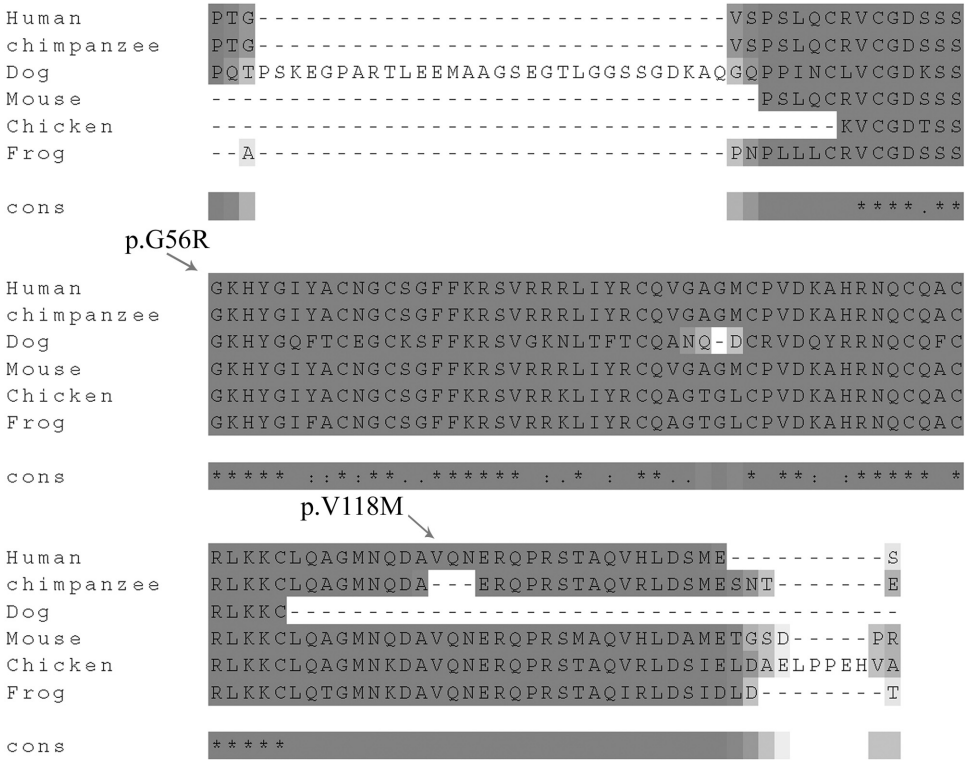

FIGURE 1. Multiple sequence alignment for NR2E3 (partially). Sequences of human (*Homo sapiens* NP_055064.1), chimp (*Pan troglodytes* XP_001175020.1), dog (*Canis familiaris* XP_544754.2), mouse (*Mus musculus* NP_038736.1), chicken (*Gallus gallus* NP_989925.1), and frog (*Xenopus tropicalis* NP_001090633.1).

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TABLE 4. Evaluation of Pathogenic Potential of Missense Mutations of *NR2E3*

Location	Functional Domain	Residual Change	Cases (n = 172)	Controls		PolyPhen		SIFT		Grantham Score
				Hong Kong	Beijing	Prediction	PSIC Score	Prediction	Score	
Exon 2	DBD	p.G56R*	2	0/180	—	Probably damaging	2.844	Intolerant	0.02	125
Exon 4	DBD	p.V118M	3	0/180	0/180	Probably damaging	2.163	Intolerant	0.00	21

The criteria for prediction are: PolyPhen PSIC score: >2.00, probably damaging; 1.50–1.99, possibly damaging; 1.25–1.49, potentially damaging; 1.00–1.24, borderline; 0.00–0.99, benign. SIFT score: 0.00–0.05, intolerant; 0.051–0.10, potentially intolerant; 0.101-0.20, borderline; 0.201–1.00, tolerant. Grantham score: 0–50, conservative; 51–100, moderately conservative; 101–150, moderately radical; >151, radical. DBD, DNA-binding domain.

* Functional prediction of the G56R mutation had been reported by Coppieters et al.,[33] and our results are consistent with that in their report.

study population, evenly distributed in RP patients (13.4%) and control subjects (10.5% in the Hong Kong cohort and 12.8% in the Beijing cohort). In a study by Haider et al.,[40] the p.E121K mutation was detected in one patient with ESCS but not in 500 control individuals. The p.E121K has not been reported by other studies, suggesting that it is a rare variant in Caucasians. However, in this study, it was a common SNP in a Chinese population. This variant has not been identified in the Han Chinese in Beijing (HCB) in the International HapMap project, probably because of the small sample size (n = 45) used in the HapMap project. In our present study, the control subjects who carry either one or two copies of the p.121K allele do not have any identifiable abnormalities at the peripheral retina or the macula. There were no visual dysfunctions in visual acuity or visual field or experience of nyctalopia. p.121K is thus nonpathogenic. In Haider et al.,[40] heterozygous p.E121K was considered a disease-causing mutation for ESCS. However, as ESCS is an autosomal recessive retinopathy, heterozygous p.E121K may be insufficient to cause ESCS. Other genetic risk factors may have been involved. Recently, Fradot et al.[45] reported that *NR2E3* p.E121K, located in the DNA-binding domain, was not defective in transcriptional inhibitory activity. This result is a supportive evidence that p.E121K does not cause functional defects in NR2E3, nor does it cause disease on its own.

Five of the rare *NR2E3* variants detected in this study were found in control individuals only and could be excluded as disease-causing mutations (Table 2). Among the seven variants that were identified exclusively in RP patients, c.119-28_119-13del, c.245+75G>A, p.L277L, p.P281P, and c.1230+53C>T are less likely to be disease causative, because they were either located in the noncoding regions or led to synonymous amino acid changes, although their involvement in the pathogenesis of RP via moderating transcription level or mRNA stability could not be completely excluded. The remaining two variants, p.G56R and p.V118M, are likely to be causative of RP. These two mutations were found in 1.2% (2/172) and 1.7% (3/172) of RP patients. The p.G56R, which is located in the first zinc-finger of the DNA-binding domain of NR2E3, has been implicated in ADRP in Caucasian populations,[31,33,34] contributing to approximately 1% to 2% of the ADRP population.[31] Based on segregation analysis and bioinformatics predictions, Coppieters et al.[33] hypothesized that the ADRP causing mutation, p.G56R, may predominantly influence the terminal differentiation and maintenance of rods through disruption of the transactivation of NR2E3, possibly through loss of interactions with other cofactors, like NRL and CRX.[33] Using functional analysis, Escher et al.[34] demonstrated that dominant negative activity—that is, competition for dimerization by a DNA binding-defective mutant— of the p.G56R mutant protein was involved in the molecular mechanism of ADRP. In this study, in silico programs showed that p.G56R caused deleterious functions of the NR2E3 protein, consistent with the predictions of Coppieters et al.[33] In our study subjects, the two p.G56R carriers were ADRP patients, both with disease diagnosed when they were in their 50s and both with severe visual field loss. Ocular examinations detected a few small spots of clumped pigmentation in peripheral retina, mild atrophic changes of the retinal epithelium in the macula, and typical attenuation of retinal vessels (Fig. 2). By contrast, in Caucasian studies, the phenotypes of the subjects carrying the p.G56R mutation were remarkable for severe rod loss in early childhood.[31]

Apart from p.G56R, we identified in three RP patients a novel *NR2E3* mutation, p.V118M. This variant was absent in the 360 control subjects from Hong Kong or Beijing, suggesting that its occurrence in RP patients is less likely to be due to chance, and it could be pathogenic. The substitution of a valine

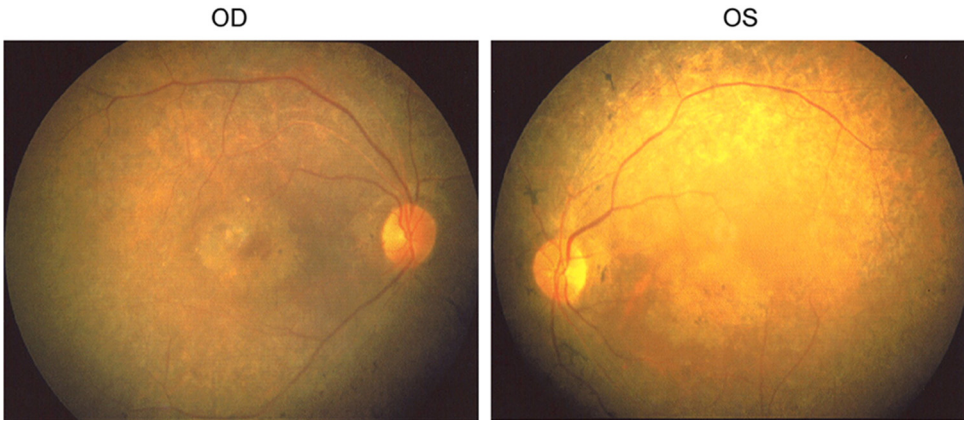

FIGURE 2. Fundus photo of the subject HKRP466, a carrier of the *NR2E3* p.G56R mutation.

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with a methionine was predicted to be damaging to protein function by the PolyPhen and SIFT programs. However, the Grantham score of this substitution was 21, suggesting that the substitution is conservative and the impact may be milder. Multiple alignments of protein sequences showed that residue-118 (V) is conserved across species, suggesting a conserved function. The p.V118M is located in the DNA binding domain of the NR2E3 protein (UniProtKB/Swiss-Prot Q9Y5 × 4/ www/ebi.ac.uk/swissprot/ provided in the public domain by the European Bioinformatics Institute, Heidelberg, Germany). It may therefore affect the efficiency of DNA binding, but functional studies are warranted to confirm this property. A nearby mutation, p.R104Q in the DBD, had been found to be causative of a mild form of ESCS in concert with the p.R334G mutation in the ligand-binding domain (LBD) in NR2E3.[46] Therefore, mutations near the terminus of the DBD in NR2E3 may impair the binding efficiency of the protein but the impact on the pathogenesis of ESCS is not decisive. Another variant (p.E121K) flanking the p.V118M was also found to be causative of the recessive-inherited ESCS[40]; but it was found as a common polymorphism in the Chinese individuals in our present study, suggesting the mutations located around this region have milder pathogenicity than that of the p.G56R. Notably, unlike p.G56R, which was found in two ADRP patients, p.V118M was found in two SRP and one patient with unknown inheritance. The disease was diagnosed in the three patients when they were in their middle or late age. They had fundus changes typical of RP and depressed or indistinguishable waveform elicited in electroretinography (data not shown). Such phenotypic features are different from ESCS. These findings may suggest that mutations located at the terminus of the DBD can also result in RP. However, as the patients' phenotypes are not inherited in a monogenic dominant pattern, it is likely that the p.V118M mutation contributes only partially to the RP of these patients. However, we do not have further evidence to exclude it as a de novo mutation. Meanwhile, our screening of another three RP genes, *NRL*, *RP1*, and *RHO*, in the three patients revealed no mutation. It is possible that mutations in as yet unidentified RP genes are involved in the genetic pathogenesis of RP in these patients.

Therefore, *NR2E3* mutations are responsible for 2.9% (5/172; 95% confidence interval [CI], 1.3%–6.7%) of overall RP in the Chinese according to this study, comparable to the contributions of *RHO* (2%) and *RP1* (1%). The latter two genes, especially *RHO*, however, account for a far higher proportion of RP in Caucasians.[7,19]

In the *NRL* gene, six novel variants were detected. So far, at least 10 reported *NRL* mutations (p.S50T, p.S50P, p.S50L, p.P51L, p.P51S, p.P51T, p.P67S, p.A76V, p.G122E, and p.L235F) have been implicated in different subtypes of RP.[35–37,44,47–49] Six of these mutations affect residues 50 or 51, which are located in one of the two highly conserved regions of the transactivation domains of NRL,[44] located at residues 3 to 27 and 41 to 54, respectively. They could thus be mutation hotspots. These amino acid residues can be important structural or functional domains of the protein. In our present study, four (p.G33R, p.P35P, p.T36T and p.S38P) of the six *NRL* variants were located just between the two transactivation domains. However, they were detected only in control subjects, suggesting that mutations within this region of the minimal transactivation domain of *NRL* are nonpathogenic. The two synonymous changes detected in RP patients, p.T42T and p.R150R, are located on the transactivation domain and the extended homology domain, respectively.[50] They do not cause amino acid substitution. Since all known RP-causing mutations in *NRL* are either missense or nonsense changes,[50] these two variants are less likely to be disease-causing. Therefore, the *NRL* makes no contribution to RP risks in the Chinese population according to this study.

In summary, among 172 Chinese RP patients and 360 control subjects, we found two *NR2E3* mutations: the novel p.V118M and the reported p.G56R. Accordingly, *NR2E3* mutations contribute to approximately 2.9% (5/172; 95% CI, 1.3–6.7%) of overall RP cases in Chinese, more than in Caucasians. By contrast, no disease-causing mutation was found in *NRL*. Thus, like *RHO* and *RP1*, the mutation profiles of *NR2E3* and *NRL* are distinctively different between Caucasian and Chinese populations. Besides, the *NR2E3* p.E121K variant, which has been shown to cause ESCS in Caucasians, is presented as a common SNP in Chinese, showing that other genetic or environmental factors are involved in the pathogenesis of ESCS caused by p.E121K.

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