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CRX variants in cone–rod dystrophy and mutation overview

Li Huang, Xueshan Xiao, Shiqiang Li, Xiaoyun Jia, Panfeng Wang, Xiangming Guo, Qingjiong Zhang *

State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510060, China

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ABSTRACT

Mutations in the cone–rod homeobox gene (*CRX*) are associated with cone–rod dystrophy (CORD), Leber congenital amaurosis (LCA), and, in rare cases, retinitis pigmentosa (RP). In this study, three variations were detected in 3 of 130 families with CORD, including two novel mutations, c.239A>G (p.Glu80Gly) and c.362C>T (p.Ala121Val). So far, 49 mutations in *CRX* were reported, affecting about 2.35% of LCA, 4.76% of CORD, and 0.80% of RP. These mutations can be classified as missense (38.78%), nonsense (4.08%), deletion (36.73%), insertion (16.33%), and indel (4.08%). They distributed in the three coding exons without mutation hot spots. No clear genotype–phenotype correlation could be established so far.

1. Introduction

Cone–rod dystrophy (CORD, MIM 120970, a prevalence of 1/40,000) are progressive inherited retinal disorders characterized predominantly by cone dysfunction in early stage and subsequent rod degeneration. Clinical manifestation of CORD includes photophobia, reduced visual acuity, color vision defects, and central scotoma. Nystagmus may present in some cases. Absent or severely impaired cone function on electroretinography (ERG) recordings is the typical sign for CORD. Accompanied impairment of rod function is frequently observed soon after significant cone dysfunction [1]. At the advanced stage, CORD might be difficult to differentiate from retinitis pigmentosa based on clinical signs alone.

CORD may be transmitted as an autosomal dominant, autosomal recessive, or X-linked recessive trait with a total of 25 causative genes identified so far. Of these, mutations in 10 genes have been reported to be responsible for autosomal dominant CORD, while 13 genes are held responsible for autosomal recessive CORD. In addition, two genes have been found to be responsible for X-linked CORD (https://sph.uth.tmc.edu/Retnet/).

The cone–rod homeobox gene (*CRX*, MIM 602225, located at 19q13) encodes a protein with 299 residues that is expressed in the inner nuclear layer, where it plays a significant role in the differentiation and maintenance of photoreceptor cells by synergistic interaction with other transcription factors such as NRL and RX [2–4]. The first mutation in *CRX* was detected in a Greek family with autosomal dominant CORD [2]. Thereafter, a number of mutations in *CRX* have been identified as being responsible for CORD as well as Leber congenital amaurosis (LCA) or retinitis pigmentosa (RP) [5,6].

To date, *CRX* mutation in Chinese patients with CORD has not been reported. In this study, the *CRX* gene in probands from 130 unrelated Chinese families with CORD were analyzed by Sanger sequencing. Meanwhile, all *CRX* mutations reported so far were reviewed and summarized.

2. Methods

2.1. Patients samples

One hundred and thirty families with CORD were recruited from our Pediatric and Genetic Eye Clinic, Zhongshan Ophthalmic Center from 1996 to 2011. Written informed consent was obtained from participating individuals or their guardians prior to the study. This study was approved by the Institutional Review Board of Zhongshan Ophthalmic Center. Genomic DNA was prepared from venous leukocytes in a method described previously [7] from the proband of each family as well as 192 unrelated healthy control individuals.

2.2. Mutation identification in CRX

The primers used to amplify each of the three coding exons and their adjacent intronic regions of *CRX* are listed in Supplementary Table 1. Sequences of the amplicons were identified using Sanger sequencing. Each variant was initially confirmed by bidirectional sequencing and then evaluated in 192 control individuals. Mutation description followed the recommendation of the Human Genomic Variation Society (HGVS, http://www.hgvs.org/). Possible

* Corresponding author. Address: Zhongshan Ophthalmic Center, Sun Yat-sen University, 54 Xianlie Road, Guangzhou 510060, China. Fax: +86 20 87333271.
E-mail addresses: qingjiongzhang@yahoo.com, zhangqji@mail.sysu.edu.cn (Q. Zhang).

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Table 1
Information analysis of variations detected in *CRX*.

Family	Variations		Computational prediction			Allele frequency in	
ID	DNA	Protein	Blosum 62	PolyPhen-2	SIFT	Patients	Controls
QT470	c.239A>G	p.Glu80Gly	5 → −2	Probably damaging	Damaging	1/260	0/384
QT70	c.362C>T	p.Ala121Val	4 → 0	Penign	Tolerated	1/260	0/384
QT22	c.335C>T	p.Ala112Val	4 → 0	Penign	Tolerated	1/260	1/384

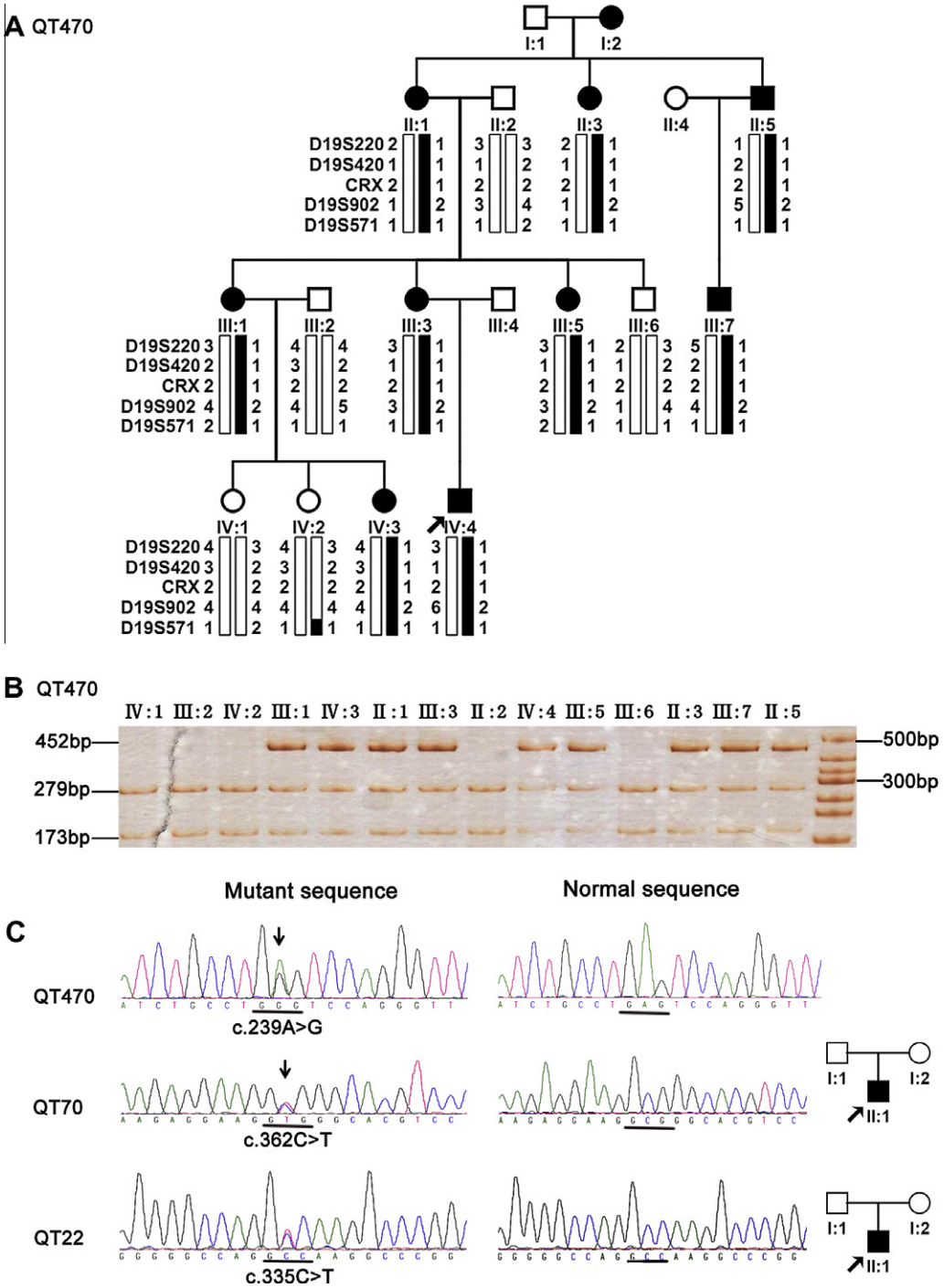

Fig.1. (A) Pedigree and haplotype diagram of QT470 family with CORD. (B) Analysis of restriction fragment length polymorphism. *CRX*-exon 2 PCR products (452 bp) were digested with HinfI. The individual number above each lane is the same as the individual number in A. Amplicons from normal allele were cut into 279 bp and 173 bp fragments while those from mutant allele could not be cut since the mutation erased one HinfI site. Therefore, normal individuals had two bands while patients had three bands on gel electrophoresis. The right lane is a 50 bp ladder marker with 500 bp and 300 bp bands highlighted. (C) Sequence chromatography. Three sequence changes detected in the probands with CORD are shown on the left column while the corresponding normal sequences are shown on the right column.

Table 2
Clinical information of available family members from the three families with *CRX* variations.

ID	Variation	Gender	Age	Age at onset	First symptom	Visual acuity		Fundus changes		Color vision	OCT	
						OD	OS	OD	OS		OD	OS
QT470-II:1	c.239A>G	F	60	12	PV	0.03	FC	NFR,PIG,AV	NFR,PIG,AV	NA	NA	NA
QT470-II:2	No	M	66	No	No	1.00	1.00	Normal	Normal	NA	NA	NA
QT470-II:3	c.239A>G	F	65	63	PV	0.30	0.30	NFR,PIG,AV	NFR,PIG,AV	NA	NA	NA
QT470-II:5	c.239A>G	M	67	60	PV	FC	0.25	NFR,PIG,AV,RA	NFR,PIG,AV,RA	NA	NA	NA
QT470-III:1	c.239A>G	F	39	15	PV	0.10	0.10	NFR,PIG	NFR,PIG	CVD	AM, AP	AM, AP
QT470-III:2	No	M	40	No	No	1.00	1.00	Normal	Normal	NA	NA	NA
QT470-III:3	c.239A>G	F	32	7	PV	0.30	0.25	NFR,PIG,AV,MA	NFR,PIG,AV,MA	CVD	AM, AR, AS	AM, AR,AS
QT470-III:5	c.239A>G	F	30	25	PV	0.30	0.30	NFR,PIG	NFR,PIG	CVD	NA	NA
QT470-III:6	No	M	23	No	No	1.20	1.20	Normal	Normal	Normal	Normal	Normal
QT470-III:7	c.239A>G	M	34	15	PV	0.25	0.25	NFR,PIG,AV	NFR,PIG,AV	NA	NA	NA
QT470-IV:1	No	F	18	No	No	1.00	1.00	Normal	Normal	NA	NA	NA
QT470-IV:2	No	F	16	No	No	1.00	1.00	Normal	Normal	NA	NA	NA
QT470-IV:3	c.239A>G	F	13	No	No	1.00	1.00	PIG	PIG	Normal	AM,AP	AM, AP
QT470-IV:4	c.239A>G	M	9	8	PV	0.90	0.90	PIG	PIG	CVD	AM,AP	AM,AP
QT70-II:1	c.362C>T	M	8	FMB	PP	1.00	1.00	PIG	PIG	NA	NA	NA
QT22-II:1	c.335C>T	M	20	FMB	NYS,PV	NA	NA	NA	NA	NA	NA	NA

PV = poor vision; NA = not available; FC = finger counting; NFR = no foveal reflex; AV = attenuation of the retinal vessels; FMB = first few months after birth.
PP = photophobia; RA = retinal atrophy; PIG = Pigmentary deposition; MA = macular atrophy;NYS = Nystagmus;CVD = Color vision defects.
AM = atrophy of the macular area; AP = atrophy of the Photoreceptor cell layer; AR = atrophy of the retinal pigment epithelium; AS = atrophy of the sensory retina.

functional effect of a mutation was predicted using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml) and SIFT (http://sift.jcvi.org/) online tools. Cosegeration analysis of a novel mutation in a large family was confirmed by restriction fragment length polymorphism (RFLP) as well as by genotyping four microsatellite markers around the mutation with the methods as we described previously [8]. For RFLP analysis, genomic fragments harboring the c.239A>G mutation (exon 2 of *CRX*) were amplified by using the same set of primers as for sequencing (Supplementary Table 1). The amplicons of 452 bp length were digested with 1 unit of HinfI at 37 °C overnight. The digested amplicons were separated by electrophoresis on a 10% polyacrylamide gel and the results were visualized with silver staining.

2.3. Review of CRX mutations

CRX mutations detected in this study and those mutations reported previously as well as the associated phenotypes were reviewed and summarized based on previous reports [2,5,6,9–42].

3. Results

3.1. Mutation detection

Sequencing analysis identified three heterozygous variants in *CRX* in 3 of 130 probands with CORD, respectively, including c.239A>G (p.Glu80Gly) in family QT470, c.362C>T (p.Ala121Val) in family QT70, and c.335C>T (p.Ala112Val) in family QT22 (Table 1 and Fig. 1). The first two variants were novel and were not present in the 192 normal individuals. However, the variation c.335C>T (p.Ala112Val), was also detected in 1 of 192 normal controls. Protein sequence alignment of 11 *CRX* orthologs showed that the region with the novel p.Glu80Gly variant was well conserved while the region with the p.Ala121Val variant was conserved in mammals except for *Monodelphis domestica*. The novel p.Glu80Gly change was predicted to be probably damaging by Polyphen-2 and SIFT (Table 1).

Of the three variants, two were detected in two singleton cases, respectively. The c.239A>G variant was identified in a large family

Fig. 2. Fundus changes in the patient with the heterozygous c.239A>G mutation in Family QT470 (III:3). Macular degeneration with pigment deposits on the central macular area and attenuated retinal arteries.

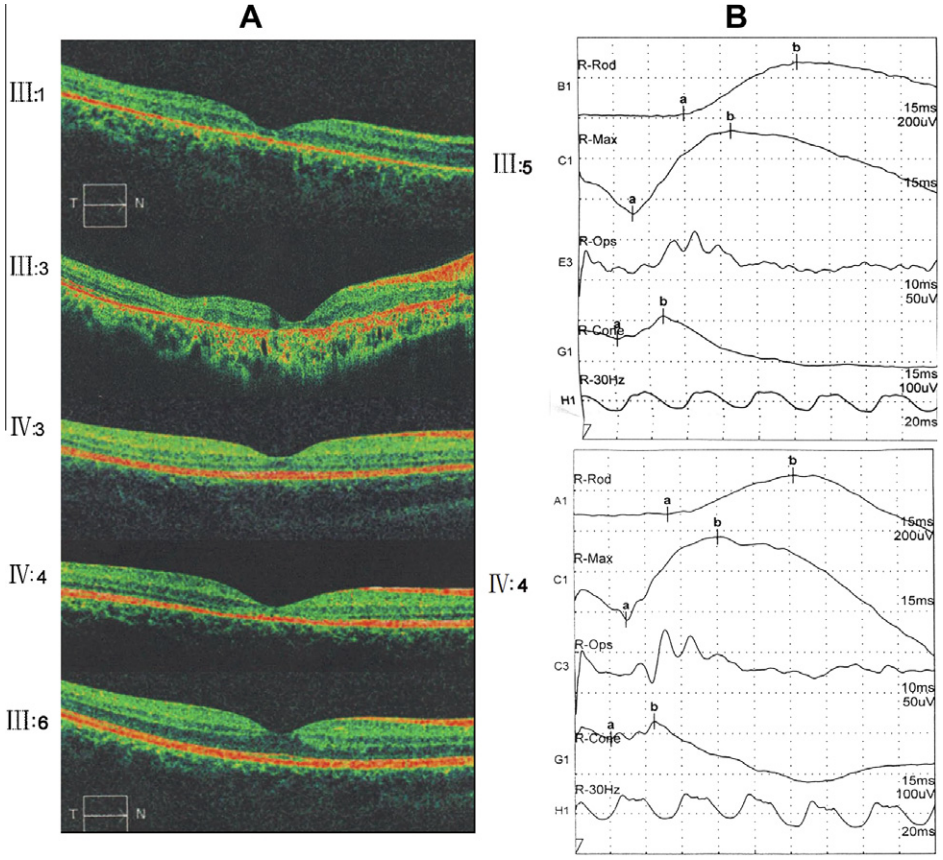

Fig. 3. (A) OCT of QT470 family. The numbers beside the diagram stand for the individuals of QT470 family. III1, III3, IV3, and IV4 show the macular area thinning and the photoreceptor connection part of the inner and outer segment unclear, and, except these, III3 also shows construction of the photoreceptor unclear, retinal pigment epithelium disorder, reflection of Choroid enhanced. Individual III6 is a normal person in this family showing normal OCT. (B) ERG of family members III5 and IV4, show normal rod responses and moderately reduced cone responses. All the images above come from the right eyes, and the expression of left eyes in this family are the same as those of the right eyes.

(QT470) with CORD, where it transmitted across four generations with 10 affected individuals (Fig. 1). Nine of the ten patients and five unaffected individuals in the family participated in this study. RFLP analysis and haplotype analysis demonstrated that the c.239A>G variant cosegregated with the disease in the family (Fig. 1).

Clinical information for the large family with the c.239A>G (p.Glu80Gly) mutation is summarized in Table 2. The ages of onset were from 7 years old to 60 years old. Patients complained of progressive poor vision, photophobia, and color vision defects. All patients except two children in the family showed reduced visual acuity (counting fingers to 0.3) and obvious fundus changes, including macular atrophy with pigment deposit, temporal pale of optic discolor, and attenuation of retinal artery (Fig. 2). Optical coherence tomography (OCT) revealed thinning retina in the macular region (Fig. 3). For the two affected children, IV:3 at 13 years old and IV:4 at 9 years old, they were found to be possibly affected by fundus examination (mildly pigmented macula) but without any visual symptoms. Electroretinography (ERG) revealed mildly reduced cone responses and normal rod responses in these two children (Fig. 3), suggesting CORD in early stage. A summary of clinical information for participating individuals from the three families with *CRX* variants is listed in Table 2.

3.2. CRX mutation spectrum in three different phenotypes

Since the identification of *CRX* [3], 49 mutations in *CRX* have been identified [2,5,6,9–38], including 19 missense (38.78%), two nonsense (4.08%), 18 deletion (36.73%), eight insertion (16.33%), and two indel (4.08%) (Supplementary Table 2). The 49 mutations involved 86 alleles, including 34 alleles with missense mutations (39.53%), four alleles with nonsense mutations (4.65%), 40 alleles with deletion mutations (46.51%), six alleles with insertion mutations (6.98%), and two alleles with indel mutations (2.33%) (Supplementary Table 2). Clinical phenotypes of these mutations varied from LCA to CORD or RP. The c.472G>A and c.789del1 mutations are the two most common variants, presenting in six patients (Supplementary Table 2). Overall, mutations in *CRX* account for approximately 2.35% of LCA cases, 4.76% of CORD cases, and 0.80% of RP cases (Supplementary Table 3) [2,5,6,9–42].

Although most mutations in *CRX* are heterozygous, a few homozygous or compound heterozygous variants have been reported, including a homozygous c.268C>T in an LCA patient [19], a homozygous c.193G>C in an RP patient [15], and a compound heterozygous c.[472G>A];[724G>A] in a CORD patient [22].

4. Discussion

In this study, three heterozygous variations, including one known and two novel, were identified in three patients, respectively. The mutation frequency of *CRX* in this study is 1.54% (2/130), showing no significant difference ($p = 0.112$, Chi-square test) from the frequency of previous reports (Supplementary Table 3). Previously in East Asians, screening for *CRX* mutations in the CORD case series has not been performed, and only two mutations in *CRX* have been reported in two Japanese families with CORD, i.e., c.615del1 and c.122G>A [33,43].

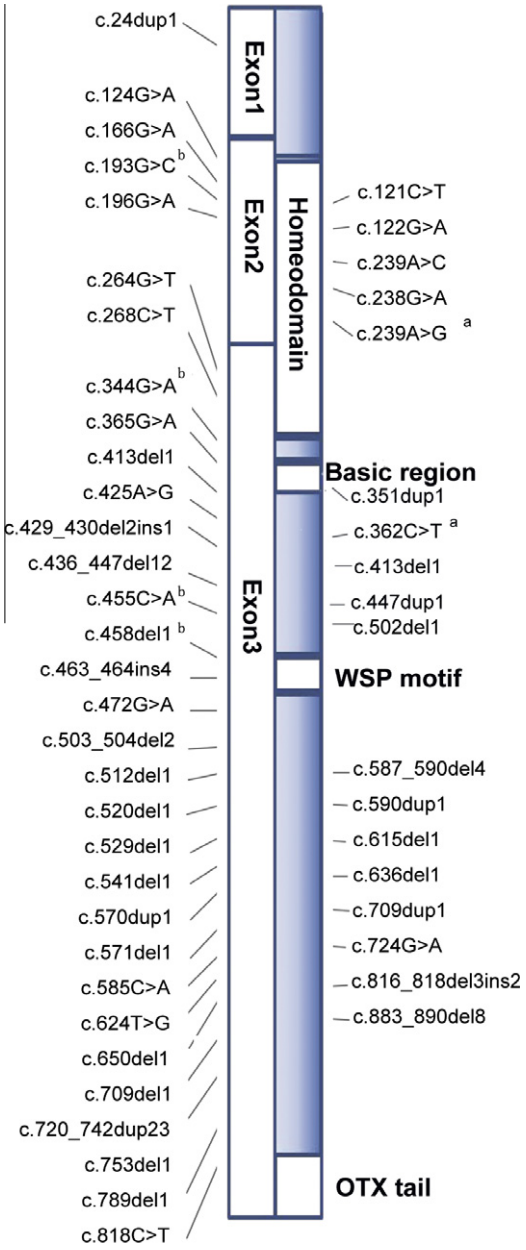

Fig. 4. Mutations in the *CRX* gene. Mutations included in CORD are on the right. Mutations previously identified in LCA and RP are on the left. (A) Mutations detected in this study and (B) mutations detected in the RP primarily.

The c.239A>G (p.Glu80Gly) was detected in a large family with typical CORD. Previously, two other mutations at the same codon, c.239A>C (p.Glu80Ala) [2] and c.238G>A (p.Glu80Lys), were detected in three and one families with CORD, respectively [2,6,10,17]. All patients in the five families with mutations at codon 80 had a phenotype of CORD but not LCA or RP, suggesting a specific cone-related role of this region. Another mutation, c.362C>T (p.Ala121Val), was detected in a singleton case and was predicted benign by PolyPhen-2 and tolerated by SIFT, but was absent in the 192 normal controls (384 chromosomes). Analysis of the patient's parents was not available so that its pathogenicity is not clear. The other variant, c.335C>T, was detected in 1 of 130 patients with CORD as well as in 1 of 192 normal individuals. This variant was described in two other patients previously, one with macular choroid atrophy and the other with high myopia [44], suggesting the likelihood of being benign variant.

So far, 49 mutations in *CRX* involving 86 alleles have been reported. These mutations are responsible for 4.76% of CORD, 2.35% of LCA, and 0.80% of RP, respectively. Unlike *CYP4V2* where several common variants were found in most patients [45], the most common variants in *CRX* were only presented in a few cases (Supplementary Table 2), suggesting mutation hot spots in this gene are highly unlikely. This is further supported by the even distribution of the mutations on all three exons (Fig. 4). From Supplementary Table 2 and Fig. 4, it is obvious that the mutation types as well as the mutation locations in *CRX* are not associated with the phenotypic differences (CORD vs. LCA vs. RP), indicating a lack of genotype–phenotype correlation.

In summary, we detected two novel *CRX* mutations in 2 of 130 (1.54%) families with CORD, which is the first such kind of mutation survey for CORD and *CRX* in an East Asian population. We also reviewed all mutations identified in *CRX* of patients with CORD, LCA, or RP. Mutations in *CRX* are only responsible for a small percentage of CORD, LCA, and RP. Current data do not suggest any genotype–phenotype correlation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.08.110.

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