BMJ Open Ophthalmology

Systematic review of genotype–phenotype associations in *CRX*-associated retinal dystrophies

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To cite: Banjak M, Noureldine J, Mousawi Z, *et al*. Systematic review of genotype–phenotype associations in *CRX*-associated retinal dystrophies. *BMJ Open Ophthalmology* 2025;**10**:e002030. doi:10.1136/bmjophth-2024-002030

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/bmjophth-2024-002030).

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Received 29 October 2024
Accepted 14 March 2025

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ABSTRACT

Background and objectives *CRX*-associated retinal dystrophies (*CRX*-RDs) exhibit significant genotype–phenotype heterogeneity. This study aimed to elucidate the genotype–phenotype associations of *CRX* through a systematic analysis of the reported cases.

Methods 84 studies, including 373 worldwide participants, were reviewed. These studies were checked for quality using Murad's tool for methodological quality and synthesis of case series and case reports. Clinical data, fundus imaging characteristics and genetic pathogenic variants were analysed.

Results The quality analysis revealed an overall good quality of the dataset, with some exceptions that do not detract from this trend. A predominance of cone-rod dystrophy (CRD) and Leber congenital amaurosis (LCA) among *CRX*-RDs (43% and 27%, respectively) was noted. Missense pathogenic variants were significantly associated with macular pigmentation, an absence of peripheral atrophy, an absence of peripheral pigmentation and CRD ($p<0.05$). In contrast, the indels (98% frameshifts) were associated with pale optic discs, attenuated optic vessels, and peripheral bone spicules, and more severe phenotypes, such as LCA ($p<0.05$). Pathogenic variants in the homeodomain were associated with cone and/or CRD; others in the OTX tail were linked to LCA.

Conclusion *CRX* pathogenic variants are associated with specific phenotypic features.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The *CRX* gene plays a crucial role in retinal photoreceptor development and function, and its variants are associated with a spectrum of associated retinal dystrophies (*CRX*-RDs). Previous studies have identified genotypic heterogeneity, with variants in *CRX* resulting in varying disease severity depending on the mutation type.

WHAT THIS STUDY ADDS

⇒ It is the most comprehensive analysis to date of *CRX*-RDs genotype–phenotype correlations from multiethnic affected individuals.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Clinically, it enhances diagnostic accuracy by providing a mutation-based classification system for *CRX*-RDs, which can be integrated into genetic counselling and patient management protocols. In research, it establishes a foundation for variant-targeted gene therapies. At the policy level, it underscores the need for personalised treatment.

INTRODUCTION

Cone-Rod Homeobox (*CRX*) (OMIM: 602225) is 1 of the 300 genes associated with several inherited retinal dystrophies (IRDs); it is located on chromosome 19q13.33 and contains four exons encoding for a 299 amino acid protein that is a photoreceptor transcription factor, responsible for photoreceptor cell differentiation and maintenance of normal cone and rod function.[1] The CRX protein has two domains: the first is known as the Homeobox Domain (amino acids 40-96), a critical DNA-binding domain that allows the binding to specific DNA sequences and regulates the expression of target genes involved in photoreceptor function and development.[2] The second domain is the OTX Tail (amino acids 164-249), which is involved in protein–protein interactions needed for CRX transcriptional activity and aids in CRX regulatory functions.[3] In addition to the WSP motif, which is responsible for the protein–protein interactions necessary for CRX activity as a transcription factor.

The first *CRX* pathogenic variant was reported in 1994 in a Greek family with autosomal dominant cone-rod dystrophy (CRD; OMIM:120970).[4] To date, at least 170 pathogenic variants within *CRX* (according to the Human Gene Mutation Database; last accessed August 2024; http://www.hgmd.cf.ac.uk) have been reported to be associated with different IRDs, including CRD, macular dystrophy (MD), rod-cone dystrophy (RCD) and Leber congenital amaurosis (LCA).[5] *CRX*-associated retinal dystrophies (*CRX*-RDs) are a relatively minor fraction (<2%) of the total cases of LCA,[6] CRD,[7] and non-syndromic RCD cases.[8] Genetic testing is

essential in deducing the aetiology of *CRX*-RDs due to the wide range of observed genotype–phenotype heterogeneity.[9] *CRX* pathogenic variants in most studied families were reported to cause autosomal dominant (ad) inheritance mode. Still, in rare cases, as Ibrahim *et al* show, biallelic pathogenic variants have been associated with autosomal recessive (ar) LCA.[10 11]

The most common missense pathogenic variants within the *CRX* (NM_000554.6) homeodomain were associated with CRD or MD with Bull's eye maculopathy, while truncating pathogenic variants downstream of the homeodomain have led to a broader range of phenotypes, including CRD, MD, LCA and RCD.[5] Some *CRX* pathogenic variants, like p.(Glu80Ala) and p.(Lys88Asn), cause IRDs through gain-of-function mechanisms, leading to the transactivation of the target genes in developing photoreceptors or changing their DNA-binding specificity.[2]

As with many other IRDs, diagnosing *CRX*-RDs at the clinical level is challenging. It necessitates clear genotype–phenotype associations that might facilitate genetic counselling and the development of targeted therapies.

This systematic review aims to assess all the identified *CRX* pathogenic variants from sequencing studies that were reported to be correlated with the *CRX*-RDs. The specific objectives of this study include:

- Summarise the *CRX* pathogenic variants correlated with *CRX*-RDs within the current literature.
- Review the quality of the included studies using Murad's tool for methodological quality and synthesis of case series and case reports.
- Conduct a *CRX* genotype–phenotype analysis to identify specific associations.

METHODS

This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Checklist (online supplemental data).

Eligibility criteria

Journal articles published before 1 August 2024 were included if they were case reports, case series or clinical trials involving *CRX*-RDs diagnosed through standard ophthalmic examinations, even if they also addressed other genetic diseases. Articles had to be written in English with full text available. The review focused on IRDs, and other retinal conditions were excluded, especially if they impacted the eye but were not primarily classified as IRDs.

Information sources and search terms

Four online databases were searched to identify articles: MEDLINE, EMBASE, PubMed and Cochrane Database of Systematic Reviews. Search terms were initially generated for use in PubMed and adapted for the other databases. The Medline database was systematically screened using the following MeSH terms: (CRX protein, human [MeSH] OR Cone-Rod Homeobox Protein [MeSH]) AND (Retinal Diseases [MeSH] OR INHERITED RETINAL DISEASES [MeSH] OR Inherited retinal dystrophy [MeSH]) AND Mutation [MeSH]. Following these selection steps, 84 unique articles published since 1997 were included. The flow chart for identifying eligible articles is shown in figure 1. The population-intervention-comparator-outcome framework was used to develop these search terms: 'population' being *CRX*-RDs of all types, 'intervention' being *CRX* pathogenic variants, no 'comparator' was used and 'outcome' being implications of genetic diagnosis/solving rate for *CRX*-RDs. As there is no registry of rare eye conditions, search terms were curated by referring to the Genetic and Rare Diseases Information Center, the National Eye Institute, Retina International and the Irish Target 5000—Gateway to Vision.

Study selection, data extraction and critical appraisal

Searches and data extraction were performed in duplicate by two independent personnel on 3 September 2024. Duplicate articles were first removed automatically using EndNote V.20. Articles were then screened by publication date, title, abstract and keywords to identify relevant studies, and any remaining duplicates were removed. The remaining articles were reviewed in full text. Reference lists were screened for reverse citations, and a 'cited reference search' was conducted on Web of Science to identify potential forward citations.

Quality assessment of included studies

Data extraction and methodological appraisal were conducted using *CRX*-customised forms-based on Murad's tool for methodological quality and synthesis of case series and case reports (online supplemental table 1). The first domain, the selection of cases, evaluated whether cases were appropriately selected based on clear inclusion and exclusion criteria to minimise bias and ensure representativeness (online supplemental table 1). The second domain, exposure and outcome, assessed the reliability of genotype and phenotype verification using standardised diagnostic methods to ensure validity and replicability (online supplemental table 1). The third domain, causality, evaluated the adequacy of follow-up and the identification of confounding factors to support causal relationships and control for bias (online supplemental table 1). The fourth domain, reporting clarity, checked if the findings were reported transparently, with limitations and unusual findings detailed, including a clear presentation of genotype-specific effects on the phenotype (online supplemental table 1). The articles that met these criteria were divided among three authors to extract and standardise clinical and genotypic information from the patients. When discrepancies between authors arise, the principal investigator reviews the evidence and rationale provided by the initial authors and makes an independent decision. The extracted data included RefSeq accession numbers for mRNA (NM), country, variant, type of variant, fundus imaging

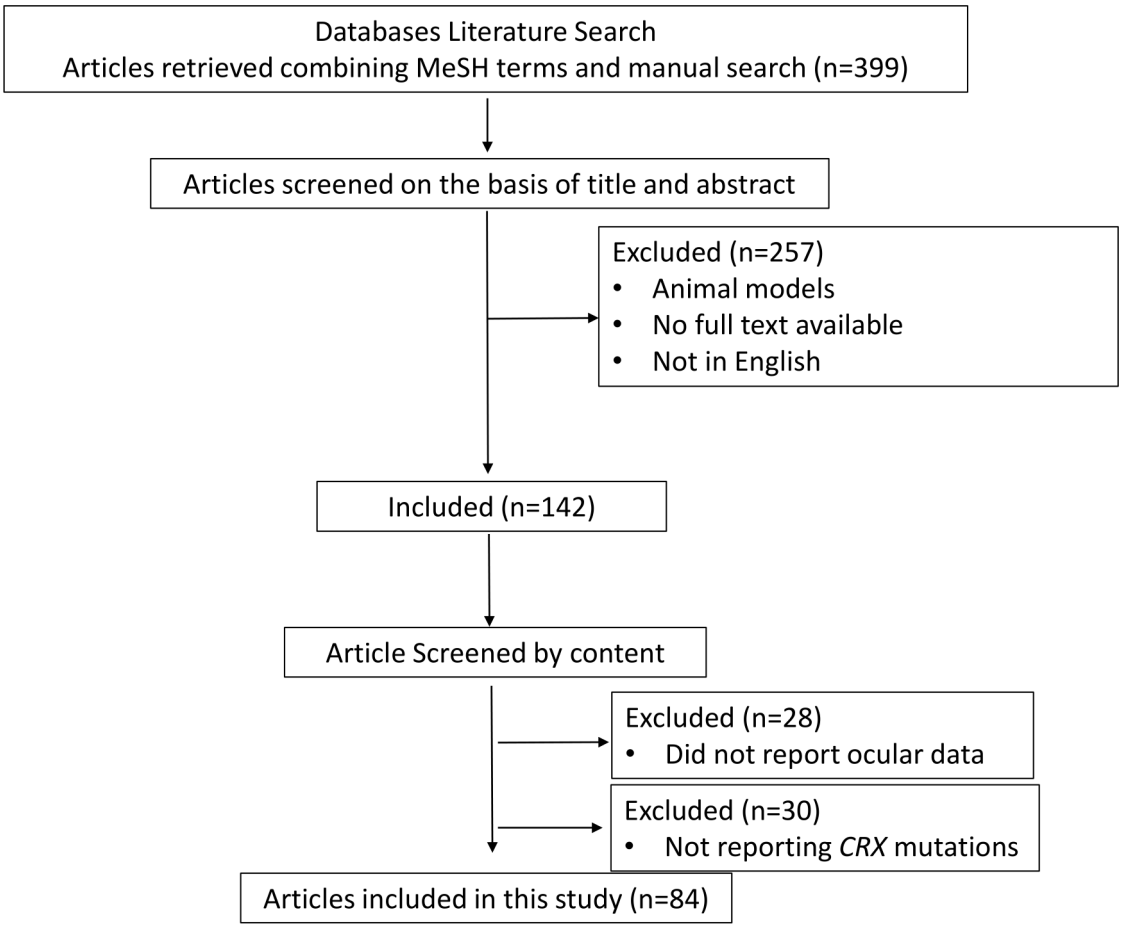

Figure 1 The flow chart for identifying eligible articles. The initial search retrieved 399 papers that were filtered to leave 142 *CRX*-RDs. When screened according to content, 58 more were excluded, leaving 84 articles published.

characteristics, associated phenotype, zygosity, number of patients and year of publication.

Ocular and clinical data

The ophthalmologist (JNeh) evaluated the fundus imaging and categorised them based on the author's description in their original publication. No quantitative measurements were done. The fundus variables assessed were macular conditions, categorised into degeneration, atrophy and other conditions. Macular pigmentation was differentiated as the presence of pigmentation (yes) and others. Optic discs were categorised into normal, pale and other types. Periphery condition was described as having no atrophy, atrophy or other conditions. Periphery pigmentation was listed as no pigmentation, pigmentation, bone spicule and other.

Statistical analysis

All analyses were performed using SPSS software, V.26 (SPSSs). Variables with a sample size smaller than five patients were excluded for statistical power considerations. Categorical variables, such as *CRX*-RDs, genotypes and clinical data, were presented as percentages. Continuous variables, including age, best-corrected visual acuity (BCVA) for both eyes (oculus uterque, OU) and refraction, were reported as mean±SD. BCVA OU measurements, initially recorded as Snellen fractions, were converted to the LogMAR scale for analysis and further categorised into a binary variable using the median value of 1.3 LogMAR. To evaluate differences in proportions across categorical variables relating to ocular and clinical data, a χ_i^2 test of independence was used. Differences in BCVA OU across various categories were assessed using Kruskal-Wallis one-way analysis of variance and Mann-Whitney U tests. A significance threshold was established at $p<0.05$.

RESULTS

The sources identified from each database were MEDLINE (n=138), PubMed (n=261) and the Cochrane Database of Systematic Reviews (n=0). This screening and selection process is summarised in figure 1; it led to 142 *CRX*-RD. When screened according to content, 58 more were excluded, leaving 84 articles published with 373 *CRX*-RD patients (figure 1).

The quality assessment of the identified studies focused on four methodological aspects: case selection, outcome ascertainment, causality and reporting clarity (online supplemental table 2). The selected cases in the analysed

studies focused on well-characterised cohorts, including familial and sporadic cases. These studies varied in sample size, covering participants from diverse backgrounds and age groups (online supplemental table 2). The selection criteria typically require confirmed MD, CRD, RP or LCA diagnoses linked to *CRX* pathogenic variants. Recruitment was often through tertiary care centers specialising in IRDs, ensuring robust data (online supplemental table 2). Moreover, few case studies did not perform in-depth imaging techniques like the electroretinogram (ERG) or fundus imaging, resulting in less comprehensive phenotypic documentation.

Identification of genetic variants involves various methods, including next-generation sequencing and Sanger sequencing (online supplemental table 2). Ophthalmic evaluations included fundus imaging, optical coherence tomography (OCT) and ERG to confirm phenotypic abnormalities and correlate them with the genotypes (online supplemental table 2). Many studies used appropriate sequencing technologies to identify known and novel *CRX* pathogenic variants, broadening the understanding of *CRX*-RDs (online supplemental table 2).

Causal inferences were drawn through segregation analyses (online supplemental table 2). Inheritance patterns were clarified through familial studies (online supplemental table 2). Pathogenicity was validated using in silico tools and guidelines from the ACMG (online supplemental table 2). Some studies also correlated specific *CRX* variants with clinical outcomes like disease severity, onset age and progression, deepening the understanding of pathogenic variant effects (online supplemental table 2). Some studies lacked full segregation analysis. Some smaller studies, particularly case reports, did not have access to extended family data, which limited the ability to confirm inheritance patterns (online supplemental table 2).

Reporting clarity was generally respected (online supplemental table 2). Most studies transparently documented patient demographics, genetic testing methods and clinical protocols (online supplemental table 2). Visual aids, such as retinal imaging and pathogenic variant schematics, enhanced genotype–phenotype clarity (online supplemental table 2). Commonly noted limitations included the need for larger cohorts or longitudinal follow-up to capture disease progression (online supplemental table 2) fully. Overall, the findings highlighted both strengths and limitations (online supplemental table 2). While most studies were detailed, some had limitations in reporting clarity, particularly regarding patient demographics and follow-up information.

The summary of *CRX*-RDs, the variants identification method and key outcomes for these studies is presented in the Online supplemental data. The complete details were also included (Online supplemental data). The included patients were adults with an average age at baseline of 37.5±22.4 years old, where 47% were females, and the mean age of symptom onset was 28.47±23.4 years (table 1). The majority of the reported cases were from Asia (57%) and Europe (24%), with China encountering the highest number of reported cases (23%, table 1). The most prevalent *CRX* pathogenic variants were p.(Arg41Trp), p.(Ser150Leufs*24) and p.(Leu146del12) (13%, 5% and 4%, respectively, table 1). In addition to that, the majority of variants were missense (41%) and indels (39%), followed by nonsense (10%), copy number variants (8%) and others (2%) (table 1). Only one of the 60 different indels (~2%) was found to be an in-frame variant p.(Leu146del12), making almost all (~98%) of our *CRX* indels frameshifts. Most of the pathogenic variants were located within the homeodomain (90%, online supplemental figure), with fewer pathogenic variants occurring within the WSP motifs (6%) (table 1). *CRX* pathogenic variants were clustered the most in exons 4 and 3 (58% and 33%, respectively).

The clinical observations showed that 43% of the participants were diagnosed with CRD, 27% with LCA and 10% with RCD (table 2). The BCVA average was 1.1 LogMAR (table 2). Fundus images showed that 46% of the patients had macular atrophy, 20% had macular degeneration, more than half showed macular pigmentation, 42% had a normal optic disc and most had attenuated vessels (87%, table 2). The analysis of the patients with tested phenotypic data revealed that 62% did not exhibit atrophy of the retinal periphery, 16% had no pigmentation, 22% had retinal atrophy, 32% had pigmentation, 18% had bone spicules and 10% had RPE changes (table 2).

To screen for novel *CRX* genotype–phenotype associations, we first performed a heatmap analysis that showed significant associations with peripheral pigmentation, peripheral retina condition, optic disc condition, optic vessels' condition, macular pigmentation and macular condition ($p<0.05$, online supplemental figure 1). Our analysis was in line with previous findings stating that *CRX* missense variants were typically associated with CRD (49%, table 3), while frameshift/stopgain variants were more commonly associated with LCA (table 3). Indeed, our analyses went deeper by identifying novel genotype-fundus-associated characteristics. We analysed the possible relation between the variant types and the fundus imaging characteristics ($p<0.05$, table 3). The missense variants were mainly associated with macular pigmentation (79%), normal optic disc (63%), attenuated optic vessels (79%), lack of atrophy at the periphery (77%) and lack of peripheral pigmentation (29%) ($p<0.001$, table 3). On the other hand, the indels (mostly frameshifts) were associated with macular atrophy (52%), macular pigmentation (50%), attenuated blood vessels (94%), lack of atrophy at the periphery (46%) and peripheral pigmentation (37%, table 3). The nonsense variants revealed a tendency towards macular pigments, pale optic disc and bone spicule pigmentation (43%, table 3).

The CRX domains' analyses revealed that half of the homeodomain variants were associated with cone and/

Table 1 Demographic and genetic characteristics of the *CRX*-RD patients

Characteristics	Participants (N=373) Mean	SD
Age at baseline (n=180)*	37.51	22.4
Age of onset (n=200)†	28.47	23.4
Sex (female %)	146 (47%)	
Continent		
Asia	141 (57%)	
Europe	61 (24%)	
North America	46 (18%)	
South America	2 (1%)	
Not listed/total	123/373	
Country		
China	56 (23%)	
Japan	48 (19%)	
USA	23 (9%)	
Canada	22 (9%)	
UK	18 (7%)	
Korea	17 (7%)	
Germany	11 (4%)	
Denmark	7 (3%)	
South Korea	6 (2%)	
Other countries‡	42 (17%)	
Not listed/total	123/373	
Variant types		
Indel	145 (39%)	
Missense	151 (41%)	
Nonsense	36 (10%)	
CNVs	30 (8%)	
Other types‡	10 (2%)	
Not listed/total	0/373	
Amino Acid change		
p.(Arg41Trp)	50 (13%)	
p.(Ser150Leufs24)*	20 (5%)	
p.(Leu146del12)	15 (4%)	
p.(Arg41Gln)	11 (3%)	
p.(Glu80Gly)	10 (3%)	
p.(Arg40Trp)	8 (2%)	
p.(Ser213Profs6)*	8 (2%)	
Other changes‡	252 (68%)	
Not listed/total	0/373	
Domains		
Homeodomain	143 (90%)	
OTX tail	7 (4%)	
WSP motif	10 (6%)	
Not listed/total	213/373	
Exon		
4	216 (58%)	
3	120 (33%)	
2	5 (1%)	
Spanning more than one exon	30/ (8%)	
Not listed/total	2/373	

Values are arithmetic mean±SD for continuous variables. Categorical variables are shown as numbers (n) and percentages (%). In the country analysis, a frequency threshold of 2% was used; thus, countries with frequencies ≤1% were not shown. n: sample size.
The % calculated for demographic and genetic characteristics did not include the 'not listed' ones.
*Age of diagnosis.
†Age at symptom onset.
‡Include all subcategories with low sample size.
CNVs, copy number variants; CRX-RD, CRX-associated retinal dystrophy.

or CRD (CCRD), compared with 41% in the OTX tail (p<0.001, table 3), indicating a strong correlation between homeodomain variants and the risk of developing CCRD. On the other hand, variants in the OTX tail were more commonly associated with LCA than those in the homeodomain (40% vs 10.5%, respectively, p<0.001, (online supplemental table 3).

DISCUSSION

The current work revealed that CRD is the most prevalent *CRX*-RD, followed by LCA and RCD. Notably, the p.(Arg41Trp) pathogenic variant was shown to be the most frequent, contributing mainly to central poor vision, photophobia, macular degeneration and attenuated vessels. In addition, patients carrying it exhibited low BCVA values and a high incidence of CRD, highlighting its pivotal role in *CRX*-associated CCRD in East Asian populations.[12 13] Of interest, we found that CRD and MD with Bull's eye maculopathy are predominantly caused by variants within the homeodomain, providing further insight into the genetic mechanisms underlying these specific manifestations of *CRX*-RDs.[5] It is known that the homeodomain (and the region below) is a DNA-binding domain that enhances CRX transactivation, vitally regulating other retinal genes.[14] Missense variants within these regions remove all the functionality of CRX, causing haploinsufficiency,[14] where a single normal copy of the gene is insufficient for the normal phenotype[15]; this might explain the Bull's eye maculopathy seen in CRD and MD.

The most prevalent types of *CRX* variants were *CRX* indels (mostly frameshifts) and missense variants (39%

Table 2 Clinical characteristics of *CRX*-RDs

Clinical characteristics	Participants (N=373)	
	Mean	SD
CRX-RDs		
CRD	144 (43%)	
LCA	94 (27%)	
RCD	37 (10%)	
RD	22 (6%)	
MD	28 (8%)	
Other	21 (6%)	
Not listed/total	21/373	
Vision		
BCVA OU (LogMAR, n=269)	1.1	0.9
Fundus autofluorescence		
Macular condition		
Atrophy	99 (46%)	
Degeneration	44 (20%)	
RPE changes*	16 (7%)	
Bull's eye maculopathy	15 (7%)	
Others†	42 (20%)	
Not listed/total	157/373	
Macular pigmentation		
Yes	22 (65%)	
Granular	5 (15%)	
Others†	7 (20%)	
Not listed/total	339/373	
Optic disc		
Pale	15 (23%)	
Peripapillary atrophy	18 (27%)	
Normal	28 (42%)	
Others†	5 (8%)	
Not listed/total	307/373	
Vessels		
Attenuated	273 (87%)	
No attenuation	39 (13%)	
Not listed/total	61/373	
Peripheral pigmentation		
Pigmentation	34 (32%)	
Bone spicule	19 (18%)	
No pigmentation	17 (16%)	
Granulation	7 (6%	
Others†	29 (28%)	
Not listed/total	267/373	
Peripheral condition		
No atrophy	42 (62%)	
Atrophy	15 (22%)	
RPE changes	7 (10%)	
Others†	4 (6%)	
Not listed/total	305/373	

For continuous variables, values are the arithmetic mean±SD. Categorical variables are shown as numbers (n) and percentages (%). n: sample size.
The % calculated for the clinical features did not include the 'not listed' ones.
RPE changes: Retinal pigment epithelium.
†Others**: Include all subcategories with very low sample size.
BCVA, best-corrected visual acuity; CRD, cone-rod dystrophy; CRX-RDs, CRX-associated retinal dystrophies; LCA, Leber congenital amaurosis; MD, macular dystrophy; OU, oculus uterque (both eyes); RCD, Rod-cone dystrophy; RD, retinal dystrophy.

and 41%, respectively). Additionally, the frequency of missense variants was higher in CRD patients than those with LCA (49% vs 9%, respectively), while most LCA patients (46%) carried indel variants. This observation was supported by Zhen *et al*, who reported *CRX* indels and missense variants as the most prevalent types and are generally associated with LCA.[10] Our study's indels and nonsense variants were associated with BCVA levels above the 1.3 LogMAR threshold, in contrast to most missense variants associated with BCVA levels below the 1.3 LogMAR threshold. In this regard, it is noteworthy to mention that the *CRX* indels and nonsense may probably not be considered as 'null' variants. With two exceptions (p.(Lys88Argfs*99) and p.(Leu57Valfs*13)), all indels are found in exon 4 (last exon). Similarly, all nonsense variants except (p.(Glu104*), p.(Gln99*), p.(Gln106*), p.(Gln105*) and p.(Arg98*) are also in exon 4. This observation contradicts their classification as null variants, as they will probably escape the nonsense-mediated decay and result in truncated proteins with residual or altered functions. Supporting this, patients with whole-gene *CRX* deletion (considered as true null variants) causing haploinsufficiency exhibit a milder late-onset MD phenotype.[16] Therefore, the association of indels and nonsense variants with LCA should not be taken as the consequence of being 'null variants' that cause haploinsufficiency, but, in contrast, it results from the functional presence in the different CRX domains (homeodomain, WSP motif, OTX tail).

Our genotype–phenotype analyses confirmed the previous phenotypic associations (missense associations with CRD and frameshift/nonsense with LCA) and went deeper by identifying novel fundus-associated changes. The missense variants were mainly associated with macular pigmentation, absence of peripheral atrophy, peripheral pigmentation, as well as CRD.[2 17] At the same time, indels frequently led to attenuated optic vessels, pale optic discs and peripheral bone spicules, often associated with more severe phenotypes such as LCA.[2 17]

Table 3 Association of *CRX* variant types with retinal and *CRX*-RDs

Fundus imaging characteristics	Variant types						Xi^2	P value
	Missense		Indel		Nonsense			
Macular condition								
Degeneration	20	(21%)	16	(23%)	5	(24%)	136.5	0.004
Atrophy	45	(46%)	36	(52%)	10	(48%)		
Others*	32	(33%)	17	(25%)	6	(28%)		
Macular pigmentation								
Yes	15	(79%)	3	(50%)	3	(75%)	33	<0.001
Others*	4	(21%)	3	(50%)	1	(25%)		
Optic disc								
Normal	17	(63%)	7	(32%)	0			
Pale	5	(18%)	7	(32%)	2	(67%)	96	<0.01
Others*	5	(19%)	8	(36%)	1	(33%)		
Optic vessels								
Attenuated	99	(79%)	132	(94%)	26	(96%)		
No attenuation	26	(21%)	9	(6%)	1	(4%)	16.78	0.03
Periphery condition								
No atrophy	27	(77%)	10	(46%)	1	(20%)	43	<0.001
Atrophy	7	(20%)	6	(27%)	0			
Others*	1	(3%)	6	(27%)	4	(80%)		
Periphery pigmentation								
No pigmentation	13	(29%)	4	(9%)	0		63.3	0.008
Pigmentation	11	(24%)	16	(37%)	3	(43%)		
Bone spicule	4	(9%)	10	(23%)	3	(43%)		
Others*	17	(38%)	13	(30%)	1	(14%)		
CRX-RDs								
CRD	70	(49%)	63	(44%)	9	(27%)	187	<0.001
LCA	13	(9%)	65	(46%)	11	(33%)		
RCD	26	(18%)	6	(4%)	3	(9%)		
Others†	34	(24%)	8	(6%)	10	(31%)		

χi^2: χ^2 independence test.
*Include all subcategories with very low sample size.
†Others include Stargardt Disease, Nummular macular pigmentation, and Bull's eye maculopathy.
CRD, cone-rod dystrophy; CRX-RDs, CRX-associated retinal dystrophies; LCA, Leber congenital amaurosis; RP, retinitis pigmentosa.

The current study is the largest review of multiethnic *CRX* individuals, highlighting specific associations with fundus characteristics. Nevertheless, several limitations can be considered: (1) In our study, we selected *CRX* patients with both genotypic and clinical data, thus excluding any individual(s) with exclusively genetic or phenotypic information. This selection bias could change the overall prevalence of some *CRX* variants. (2) Our investigation could not validate the clinical features, including IRDs and other fundus conditions. (3) Duplicate individuals might be included if authors named them differently across their studies. (4) No associations with OCT were made due to the limited number of individuals with sufficient measurements.

In conclusion, this work helps understand *CRX* genotype–phenotype correlations' role in improving the diagnosis, prognosis and future treatment of *CRX*-RDs. It comprehensively explains how specific *CRX* pathogenic variants contribute to the associated retinal dystrophies such as LCA, CRD, MD and RCD. These insights have direct clinical implications, enhancing genetic counselling by improving *CRX* genotype–phenotype prognosis and refining patient stratification for future clinical trials. It also contributes to gene therapy development, helping to differentiate between haploinsufficiency-driven and dominant-negative variants, which may require distinct therapeutic approaches, such as AAV-based gene augmentation or variant-specific therapies (eg, antisense

oligonucleotides and CRISPR-based interventions). This systematic review helps understand CRX genotype–phenotype correlations' role in improving the diagnosis, prognosis, and future treatment of CRX-RDs.

Contributors Conceptualisation: SES; Methodology: MB, JNou, JNe and SES; Software: MB and JNou; Validation:, SES and JNou; Formal analysis: MB and JNou; Investigation: MB, JNou, JNeh, ZM and SES; Resources: SES; Data curation: MB and JNou; Writing—original draft preparation: MB, JNou and LJ; Writing—review and editing: SES, LJ; Visualisation: LJ; Supervision: SES and LJ; Project administration: SES. All authors have read and agreed to the published version of the manuscript. Both MB and JNou contributed equally. SES is the guarantor.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information.

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