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CLINICAL UTILITY GENE CARD

Clinical Utility Gene Card for: autosomal recessive cone-rod dystrophy

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1. DISEASE CHARACTERISTICS

1.1 Name of the Disease (Synonyms)

Autosomal recessive (ar) cone-rod dystrophy (CORD/CRD), ar cone-rod degeneration.

1.2 OMIM# of the Disease

CORD1 600624, CORD3 604116, CORD8 605549, CORD9 612775, CORD12 612657, CORD13 608194, CORD15 613660, CORD16 614500, ESCS 268100, RCD3A 610024, RCD3B 610356.

1.3 Name of the Analysed Genes or DNA/Chromosome Segments

Gene	Name	Locus
ABCA4	ATP-binding cassette, sub-family A (ABC1), member 4	1p21-23
ADAM9	A disintegrin and metalloproteinase domain 9	8p11.23
C8orf37	Chromosome 8 open reading frame 37	8q22.1
CDHR1	Cadherin-related family, member 1	10q23.1
CERKL	Ceramide kinase-like	2q31.3
CNGB3	Cyclic nucleotide-gated channel, beta-3	18q21.3
CORD1	Locus	18q21.1-q21.3
CORD8	Locus	1q12-q24
CORD17	Locus	10q26.1
CRX	Cone-rod homeobox-containing gene	19q13.33
EYS	Eyes shuthomolog (*Drosophila*)	6q12
FSCN2	Fascin homolog 2, actin-bundling protein, retinal	17q25.3
GUCY2D	Guanylatecyclase 2D, membrane	17p13.1
KCNV2	Potassium channel, voltage-gated, subfamily v, member 2	9p24.2
PDE6C	Phosphodiesterase 6C, cGMP-specific, cone, alpha prime	10q24
POC1B	POC1 centriolar protein homolog B	12q21.33
PROM1	Prominin 1	4p15.32
RAB28	RAS-associated protein 28	4p15.33
RPE65	Retinal pigment epithelium-specific protein 65kDa	1p31
RPGRIP1	Retinitis pigmentosa GTPase regulator-interacting protein	14q11.2
TULP1	Tubby-like protein 1	6p21.31

1.4 OMIM# of the Gene(s)

ABCA4 (CORD3) 601691, *ADAM9* (CORD9) 602713, *C8orf37* (CORD16) 614477, *CDHR1* (CORD15) 609502, *CERKL* (RP26) 608381, *CNGB3* (ACHM3) 605080, CORD1 600624, CORD8 605549, CORD17 615163, *CRX* (CORD2) 602225, *EYS* (RP25) 612424, *FSCN2* (RP30) 607643, *GUCY2D* (CORD6) 600179, *KCNV2* (RCD3B) 607604, *PDE6C* (COD4) 600827, *POC1B* 614784, *PROM1* 604365, *RAB28* (CORD18) 612994, *RPE65* (RP20) 180069, *RPGRIP1* (CORD13) 605446, *TULP1* (RP14) 602280.

1.5 Mutational Spectrum

All types of mutations have been reported: nonsense mutations, missense mutations, splice-site mutations, frameshift mutations, and also small deletions, duplications and insertions. Large gene rearrangements are rare; three deletions in *ABCA4* have been identified spanning exon 5, 18 and exon 20–22, respectively.[1–3] In the *KCNV2* gene, several deletions are described ranging from single basepairs deletions to complete gene deletions.[4,5]

1.6 Analytical Methods

Several strategies can be used. (1) Genotyping microarrays with the known cone-rod and cone dystrophy causing mutations (includes recessive, dominant and X-linked causative genes).[6] This approach is relatively inexpensive, although one should consider this chip only contains a subset of the known pathogenic variants. (2) Direct sequencing of the coding regions and their intron–exon boundaries of known CRD genes. (3) When only one mutation in a CRD gene is identified, or no mutations at all, MLPA/quantitative multiplex PCR enables the detection of heterozygous copy number variants (deletions or duplications) affecting one exon or more. (4) Next-generation sequencing (NGS) provides a large-scale sequencing of the exome in a single experiment for an individual and will likely be privileged in diagnostic screening.

In the future, whole genome sequencing could become available for diagnostic analysis provided that the functional relevance of variants in non-coding regions is established.

1.7 Analytical Validation

Sequence variants have to be confirmed using bidirectional sequencing. In addition, a segregation analysis in the parents and affected and/or unaffected siblings has to be performed to demonstrate 'biallelism' or 'biparental transmission' following a recessive pattern

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of inheritance. The variant should be tested in ethnically matched unaffected control individuals. Variant with known causality may have been entered in the human gene mutation database (HGMD, http://www.hgmd.org/) or gene specific databases Leiden Open Variation Database (LOVD; http://www.lovd.nl/2.0/index.php). The importance of these databases lies in the fact of sharing significant results as well as findings that are debatable due to their low presence or inheritance pattern. These findings might lack support due to their low frequency; however, their contribution in databases may yield to significance by combining data worldwide. Variants should be compared with the presence in the Exome Variant Server (http://evs.gs.washington.edu/EVS/) and in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) to assess the frequency in general and in the unaffected population, respectively. Importantly, the presence of variants in dbSNP does not exclude pathogenicity as mutations may have been typed as rare variant or polymorphism in the past. The Exome Variant Server and dbSNP should serve as a library to consult the variant of interest on the frequency in the healthy population that should be consistent with the frequency of the disease; however, these databases should not be used to systematically discard variants from investigations.

The pathogenicity of the variant can be assessed using several *in silico* predicting online available programs like SIFT (http://www.blocks.fhcrc.org/sift/SIFT.html), polyphen-2 (http://www.bork.embl-heidelberg.de/PolyPhen) and MutPred (http://mutpred.mutdb.org) for missense variants, together with the nucleotide conservation (PhyloP), and score for amino-acid change (Grantham score). These assessments together provide information on the pathogenicity of the variant.

Variants in the canonical splice site region, or close at the boundary within the exon, could alter splicing. To assess the functional effect, one should perform mRNA analysis to show the altered exon structure or the presence of nonsense-mediated decay. *In silico* analysis of splice variants should be performed using MaxEntSplice (http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html), NNSplice (http://www.fruitfly.org/seq_tools/splice.html) and SpliceSiteFinder (http://www.genet.sickkids.on.ca/~ali/splicesitefinder.html).

1.8 Estimated Frequency of the Disease

(Incidence at birth ('birth prevalence') or population prevalence. If known to be variable between ethnic groups, please report):

The exact prevalence of arCRDs is unknown. The estimated prevalence of all CRDs (dominant, recessive or X-linked) is 1/40 000.[7] So far, mutations in *ABCA4* have been shown to be the most common cause,[8,9] with an estimated prevalence of 30–60% of all arCRDs.[10]

The carrier frequency of *ABCA4* mutations in the general population could be estimated around 2%, but the exact evaluation is difficult because it depends on several factors, including ethnic background.[11] Some studies have found that 5–12% of the general population carries heterozygous disease-associated *ABCA4* alleles.[12,13] A recent study by Riveiro-Alvarez *et al*[14] suggested that the *ABCA4* mutation carrier frequency could be considerably higher than previously estimated, with values of identified carrier percentages of about 20% in Spanish population. As suggested by the authors, a hypothesis to explain the discrepancy between observed (genotypic) and estimated (phenotypic) prevalence could be the existence of hypomorphic changes which, when homozygote, do not cause disease.

Ducroq *et al*[15] estimated that there is no significant difference in the frequency of *ABCA4* mutations between sporadic cases of CRD and arCRD.

Of note, a primary role of *ABCA4* in autosomal recessive Stargardt disease (STGD)/fundus flavimaculatus (FFM) and arCRD, together with the gene's involvement in a certain proportion of cases with autosomal recessive retinitis pigmentosa (arRP), strengthens the idea that mutations in *ABCA4* could be the most frequent cause of inherited retinal dystrophy.

1.9 Diagnostic Setting

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

A: Genetic testing can be useful to complete the diagnostic procedures in a subject clinically suspected to be affected by arCRD. It may be crucial for the constitution of cohorts of patients eligible for developing therapies.
B: In families with affected persons with a known genetic defect, predictive testing could be performed in presymptomatic persons but has obvious and practical legal limitations.
C: A positive genetic test in the index patient allows a focused analysis opportunity for relatives at the molecular level, which may be useful for genetic risk assessment and for genetic counselling in family members of a diseased person.
D: Prenatal diagnosis is rarely requested and is available for families with known mutations. In families with multiple siblings, indirect analysis of the haplotypes could give information in prenatal testing. Linkage analysis for the family should exceed a logarithm of odd score higher than three.

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives B: False positives	C: False negative D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Posiytive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

2.1 Analytical Sensitivity
(proportion of positive tests if the genotype is present)

The analytical sensitivity is >99%. The false negative rate depends on DNA analysis technologies. In Sanger sequencing, polymorphisms in the region encompassed by the primer sequence may hamper biallelic detection. Polymorphisms with MAF around 1% are now characterized. The risk that a patient carries a rare polymorphism in the sequence covered by primers designed in non-polymorphic regions is <1%. False negative results using NGS may originate from a lack of sequence coverage and allele drop out. Low NGS coverage should not be regarded as a factor to take into account when calculating the false negative rate, as sequencing runs which do not pass the quality thresholds should not be considered. Currently, sequencing of patient cDNAs should not be recommended. Instability and/or degradation of mutant mRNA is not uncommon, especially as approximately 30% of the mutations are nonsense mutations that could be subjected to NMD at mRNA level.[16] The rate of false negative results <1%.

2.2 Analytical Specificity
(proportion of negative tests if the genotype is not present)

Nearly 100%. False positives in sequencing are rare.

2.3 Clinical Sensitivity
(proportion of positive tests if the disease is present)

Because of extensive genetic heterogeneity, genetic testing is rarely carried out for all known CRD genes in patients with clinical diagnosis of CRD. This limitation will probably be overcome with the introduction of NGS into molecular genetic testing. Currently, the overall proportion of positive genetic tests in CRD cases is around 78%;[17] the large majority of causes for the disease can be found in the gene *ABCA4*. Depending on the population, this can range from 24 to 65%. Individually other genes account for few CRD cases compared with *ABCA4*.[15,17–22]

2.4 Clinical Specificity
(proportion of negative tests if the disease is not present)

The clinical specificity can be dependent on variable factors, such as age or family history. In such cases, a general statement should be given, even if a quantification can only be made case by case.

The proportion of biallelic mutations should be 0 if the disease is not present. The proportion of single heterozygote mutations should be the sum of population carrier frequencies of each of the tested gene.

2.5 Positive clinical predictive value
(lifetime risk to develop the disease if the test is positive)

CRD are often characterized by interfamilial and intrafamilial variability. Hence, even in the case of positive test, when two clearly pathogenic alleles are identified, one should consider that the onset of disease or lifetime risk to develop the disease in an asymptomatic subject cannot be established with certainty. In genes with a higher prevalence, however, for example, *ABCA4*, the description of the natural history in large cohorts has offered a better view of the disease history and outcome.

2.6 Negative clinical predictive value
(Probability not to develop the disease if the test is negative)

Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested:

If the subject is negative for the genotype identified in index case, the risk to develop the disease is non-existing. In case of a single heterozygous mutation in the specific gene and no other pathogenic gene defect has been identified, the risk is 0% or close to 0% to develop the disease, as phenocopies formally cannot be excluded.

Index case in that family had not been tested:

Because of the locus heterogeneity, the probability of an individual to be affected has to be calculated based on its rank in the genealogy.

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: The tested person is clinically affected
To be answered if in 1.9 'A' was marked)

3.1.1 Can a diagnosis be made other than through a genetic test?

No	☐	
Yes	☒	
	Clinically	☒
	Imaging	☒
	Endoscopy	☐
	Biochemistry	☐
	Electrophysiology	☒
	Other (please describe)	

3.1.2 Describe the burden of alternative diagnostic methods to the patient

The diagnosis of CRDs, a heterogeneous class of hereditary retinal diseases, is essentially based on clinical data obtained from ophthalmological evaluation, including retinal imaging and electrophysiological recordings. Retinal imaging and electrophysiological test are time-consuming, sometimes stressful for the patient and costs for the health-care system. Moreover, clinical investigations are sometimes incomplete in young children or may require general anaesthesia. Nevertheless, these clinical investigations are mandatory before genetic test as clinical diagnosis and molecular testing cannot be considered alternative diagnostic methods for CRDs.

To avoid expensive and useless genetic analysis, the correct differential diagnosis must be established based on evaluation of the inheritance pattern, history, symptoms, fundus examination, fluorescein angiography, autofluorescence imaging, optical coherence tomography (OCT) and electrophysiology.

The typical clinical presentation of CRD includes visual acuity impairment with reduced central visual acuity, photophobia and loss of colour vision in childhood and early adulthood. The disease progresses over decades to loss first of central and then peripheral visual function (night blindness and peripheral visual field constriction), due to progressive loss first of cone and then rod photoreceptors. Perifoveal atrophy of the outer retina and retinal pigment epithelium ('bull's eye' appearance) is typically observed on fundus examination and OCT, with slightly attenuated arterioles and variable pigmentary retinopathy (diffuse pigmentary changes (both hypopigmentation and pigment clumping) in the fundus – type 1 Fishman; no pigmentary changes or only a mild degree of peripheral pigment clumping – type 2 Fishman).[20] Fluorescein angiography and fundus autofluorescence show heterogeneity in the fluorescence at the posterior pole (from weak speckled fluorescence to a distinct ring of increased/decreased fluorescence) and to a lesser extent in the peripheral retina. In the early phases of the disease, cone dysfunction affects the electroretinogram (ERG) with a relatively preserved rod function (this is a hallmark of the disease, necessary for differential diagnosis).

The differential diagnosis with syndromic CRD, cone dystrophies (COD), autosomal recessive retinitis pigmentosa and autosomal recessive Stargardt disease may be difficult.

CRDs are mostly non-syndromic, whereas they may also be part of syndromes such as Bardet–Biedl syndrome (OMIM #615993) and spinocerebellar ataxia type 7 (OMIM #164500), most of which are accounted by mutations in different genes.[10,23]

The differentiation between pure cone dystrophies and CRDs remains controversial; differential diagnosis is difficult because both diseases share a common clinical presentation (which includes photophobia, visual acuity impairment with reduced central visual acuity and loss of colour vision and the macular appearance). In cone dystrophies, the optic disc may show a variable degree of temporal pallor. The peripheral involvement of visual field, the pigmentary alterations of the peripheral retina and the vascular attenuation are typically absent. Most importantly, ERG shows reduced/absent cone responses, while the rod function is initially normal and should be relatively preserved also in the latest disease stages.[17,24] In the late stage arRP patients, the typical ERG changes are undetectable; however, the clinical course of CRD reflects the opposite sequence of the events in RP (night blindness, progressive impairment of the visual field earlier than visual acuity reduction).

The early stage of CRD may be similar to STGD; however, the presence of yellowish-white, irregular shaped flecks in the posterior pole or in the entire fundus (FFM), the dark choroid on fluorescein angiography and the normal or nearly normal full-field ERG, which are typical of STGD, are very helpful features to differentiate the diseases.

It is important to note that molecular testing is not useful for differentiating retinal degenerations as STGD, CODs and CRDs, most of which are caused by mutations in the same genes, particularly in the *ABCA4* gene.

Several genes have been linked to CRDs with the protein products of these genes being involved in multiple aspects of photoreceptor structure and function, but several genes remain to be discovered. The results of molecular testing should be considered together with the clinical picture, because the identification of a mutation in a given gene does not make a specific diagnosis nor allows differential diagnosis of retinal dystrophies.[17]

In arCRDs, the genotype–phenotype correlation is difficult due to genetic heterogeneity; moreover, phenotypes are similar despite different genes involved, and mutations in some genes (as *ABCA4*) have been reported to cause different phenotypes as CRD, RP and STGD. In the near future with new genetic techniques (ie, NGS), exome sequencing may become cheaper to determine the genetic diagnosis.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?

CRDs, although clinically heterogeneous, are often associated with early-onset, severe and progressive visual disability and blindness.

The diagnosis is clinical by ophthalmic evaluation, imaging and electrophysiology with good cost effectiveness for the first, while imaging and electrophysiology are more expensive. Genetic diagnostic testing must be performed only after the diagnosis of arCRD has been made upon comprehensive clinical evaluation, because clinical diagnosis and molecular testing cannot be considered alternative diagnostic methods for CRDs. Genetic testing is likely to be significantly more expensive than phenotypic analysis. The molecular diagnosis is useful in confirming the inheritance pattern, and in genetic counselling, provides information regarding disease-associated risks and carrier status.

3.1.4 Will disease management be influenced by the result of a genetic test?

No	☐	
Yes	☒	
	Therapy	Currently, there is no therapy that stops the evolution of the disease or restores the vision, although human gene therapy trials are in progress for *ABCA4*-related disease.
	Prognosis	Although there is not a definite genotype–phenotype correlation and it is not possible to predict the clinical outcomes based on the genotype (gene testing alone does not often determine prognosis and there is a considerable individual variability), it is currently believed that homozygous null mutation of *ABCA4* cause the most severe phenotype of arRP, combinations of a null mutation with a moderate missense mutation result in arCRD and combination of null/mild missense or two moderate missense mutations cause STGD/FFM.[25] The visual prognosis in CRD is poor and the precise prognosis regarding the progression remains difficult, because even intrafamilial variability can often be observed.
	Management (please describe)	The clinical diagnosis may be sufficient to make decisions that are independent of the genotype, because at this moment there is no therapy available. Management aims at treating the complications (eg, cystoid macular oedema, cataract) and helping patients to deal with the social and psychological impact of blindness (evaluation for visual aids and support services for visually impaired subjects). For patients positive for *ABCA4* mutations, avoiding supplementations of vitamin A has been suggested based on findings in animal studies.[26]

3.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history

(To be answered if in 1.9 'B' was marked)

3.2.1 Will the result of a genetic test influence lifestyle and prevention?

If the test result is positive (please describe):

No change in lifestyle (except avoiding vitamin A supplementation in patients positive for *ABCA4* mutations), only guidance regarding career choices (especially for profession requiring perfect vision), evaluation of the risk of having affected children (genetic counselling) and regular ophthalmological follow-up examination.

If the test result is negative (please describe):

No change in lifestyle, informed family planning and regular ophthalmological follow-up examination.

3.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?

No specific options (prevention is not possible).

3.3 Genetic risk assessment in family members of a diseased person

(To be answered if in 1.9 'C' was marked)

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?

Yes. It will likely confirm the autosomal recessive pattern of inheritance, the aetiology of the disease and the recurrence risk.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?

Yes. Positive testing for certain genes in the index patient can reduce the need for testing for other genes in family members by providing a molecular diagnosis. However, testing for the involved gene may still be required in other clinically affected family members, that is, in pregnancy counselling.

If the result in the index patient is negative, molecular testing of family members for their genetic counselling is not recommended.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?

Positive test in the index patient allows pointing the other individuals with biallelic mutations who will develop disease although the age of onset, the rhythm of progression of the disease and its severity cannot be predicted.

Of note, the predictive test may be gene dependent. The possibility to estimate the onset or progression of disease, even though limited by interfamilial and intrafamilial variability, is easier for genes with a higher prevalence, such as *ABCA4*, rather than for rare gene defects.

3.4 Prenatal diagnosis

A prenatal test for CRD is rarely requested, but it could be optional to parents when the gene defect is known or when the family size allows linkage analysis and subsequent analysis on fetal material. Prenatal diagnosis can be discussed in selected cases, after genetic counselling (ie, couples requesting it because of familial occurrence of blindness due to CRD). Testing for two known familial mutations in one gene can be performed on DNA from a fetus obtained by chorion villus sampling or amniocentesis.

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?

Yes.

4. IF APPLICABLE, FURTHER CONSEQUENCES OF TESTING

The genetic diagnosis of CRD has clinical validity for both index cases and their relatives. The identification of a disease-causing mutation can support the clinical diagnosis for index cases and may influence family planning. It allows patients and relatives to choose the most appropriate options, including access to future genetic therapies.

Additional benefits may regard lifestyle planning, such as avoiding vitamin A supplementation in *ABCA4* mutations carriers, and choice of professional orientation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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