

CUGC for Posterior Polymorphous Corneal Dystrophy (PPCD)

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1. Disease characteristics

1.1 Name of the Disease (Synonyms):

Posterior polymorphous corneal dystrophy (PPCD)

1.2 OMIM# of the Disease:

122000; 609141; 618031

1.3 Name of the Analysed Genes or DNA/Chromosome Segments:

OVOL2 (PPCD1); *ZEB1* (PPCD3); *GRHL2* (PPCD4)

1.4 OMIM# of the Gene(s):

616441; 189909; 608576

1.5 Mutational Spectrum:

PPCD is a rare, genetically heterogeneous, autosomal dominant disorder characterised by an abnormality of the corneal endothelium that leads to opacities and lesions (Descemet layer bands and endothelial cell vesicles) of the posterior layers of the cornea. In some individuals there can be secondary corneal oedema, glaucoma and distortion of the iris. To date, three genetically distinct causes of the disease have been identified and further genetic heterogeneity is predicted [1-3].

Currently, three distinct single-nucleotide substitutions and a small heterozygous duplication have been reported within a highly conserved region of the *OVOL2* promoter [2, 4].

Numerous *ZEB1* loss-of-function variants have been reported including; nonsense variants, single-nucleotide substitutions altering conserved splice-site motifs, small deletions, small duplications and structural variants resulting in full or partial deletions of the gene [1, 5]. We have recently catalogued all 49 *ZEB1* PPCD-associated variants reported to date [6]. Two single base pair deletions and one single base pair substitution have also been identified within a non-coding intronic regulatory region of *GRHL2* [3]. *De novo* occurrence of *ZEB1* and *GRHL2* heterozygous variants have been reported and this should be considered as a possibility if the parents of index cases are unaffected [3, 7].

1.6 Analytical Methods:

Genome sequencing is now considered the most comprehensive method for the molecular diagnosis of PPCD given that structural or non-coding variants have been reported for all 3 genetic subtypes [2, 3, 5]. Depending on funding and accessibility of genome sequencing, Sanger sequencing of previously identified mutational hotspots within the *OVOL2* promoter and *GRHL2* regulatory region can be performed with concurrent bi-directional Sanger sequencing of all coding exons and intron-exon boundaries of *ZEB1*. Similarly, exome sequencing can be used to identify coding *ZEB1* variants. However, it must be noted that exome sequencing and targeted Sanger sequencing approaches will not detect all alterations (i.e. structural or non-coding variants and those situated outside the targeted region).

1.7 Analytical Validation

Genomic DNA from a second, independent sample should be used for analytical validation. Point variants and small insertion and deletion variants should be confirmed using bi-directional Sanger sequencing (*OVOL2*; *ZEB1*; *GRHL2*). Breakpoints of deletions or insertions encompassing *ZEB1* should be mapped by PCR and bi-directional Sanger sequencing. Alternatively, qPCR, SNP array and comparative genomic hybridisation assays can all be used as alternative approaches to verify *ZEB1* haploinsufficiency [5].

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 prevalence in the Czech Republic has been estimated to be 1 per 80,000 inhabitants, including two reported founder effects [7, 8]. In most countries the frequency of the disease is unknown.

1.9 Diagnostic Setting:

| | Yes. | No. |
|---------------------------------|----------------------------|--------------------------|
| A. (Differential) diagnostics | X <input type="checkbox"/> | <input type="checkbox"/> |
| B. Predictive Testing | X <input type="checkbox"/> | <input type="checkbox"/> |
| C. Risk assessment in Relatives | X <input type="checkbox"/> | <input type="checkbox"/> |
| D. Prenatal | X <input type="checkbox"/> | <input type="checkbox"/> |

Comment:

PPCD is one of several disorders that primarily affect the corneal endothelium; others include congenital hereditary endothelial dystrophy (CHED) and Fuchs endothelial corneal dystrophy (FECD). CHED, an autosomal recessive disorder caused by bi-allelic *SLC4A11* variants (OMIM# 217700), is the most likely diagnosis for children presenting with bilateral early-onset corneal oedema in the absence of glaucoma. In contrast, typical late-onset FECD should be considered if there is onset of corneal oedema in the 5th to 6th decade; this is commonly associated with expansion of a non-coding triplet repeat situated within an intronic region of *TCF4* (OMIM # 613267) [9]. An additional early-onset form of FECD attributed to heterozygous missense variants in *COL8A2* (OMIM#136800) can present earlier in the 2nd to 3rd decade and should be considered as a differential diagnosis when variants within known PPCD-associated genes have been excluded [10]. Iridocorneal endothelial (ICE) syndrome should be considered as a differential diagnosis if there is unilateral disease [11].

Because the clinical appearance of these endothelial dystrophies can be similar, or if advanced corneal oedema prevents an adequate examination, genetic testing is useful to confirm the diagnosis of PPCD. Testing relatives (both affected and unaffected) can further support the likely pathogenicity of variants and inform families of reproductive risks. Non-invasive prenatal diagnosis (NIPD) is an option where one parent is affected, and a disease-associated variant has been identified. Pre-implantation genetic diagnosis can be considered for families presenting with severe early-onset forms of PPCD. Pre-symptomatic testing of at-risk individuals can be informative (e.g. screening for refractive error or secondary glaucoma), but does not offer an accurate prediction of prognosis because of the variable severity, rate of progression and the incomplete penetrance associated with the condition.

2. Test characteristics

| | | genotype or disease | |
|------|------|---------------------|--------|
| | | present | absent |
| test | pos. | A | B |
| | neg. | C | D |

A: true positives C: false negatives
B: false positives D: true negatives

sensitivity: $A/(A+C)$
specificity: $D/(D+B)$
pos. predict. value: $A/(A+B)$
neg. predict. value: $D/(C+D)$

2.1 Analytical Sensitivity

(proportion of positive tests if the genotype is present)

When using the recommended analytical and validation method described above (i.e. genome sequencing followed by Sanger sequencing-based validation methods) analytical sensitivity is high. However, when genome sequencing is not used as the primary detection method some variants could be missed due to allelic dropout, large deletions, insertion and/or duplications that evade detection by PCR-based genotyping methods and/or exome sequencing. In such instances variant-negative individuals displaying typical PPCD-associated phenotypic features should be analysed by genome sequencing. Even when genome sequencing is applied regulatory and/or non-coding variants of uncertain pathogenicity may also be identified.

2.2 Analytical Specificity

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

When using the analytical methods and validation described above, analytical specificity will be high. False positives may potentially arise due to misinterpretation of rare polymorphic variants.

2.3 Clinical Sensitivity

(proportion of positive tests if the disease is present)

The clinical sensitivity 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.

PPCD is genetically heterogeneous and in addition to the three genetic causes described to date, further genetic heterogeneity is likely (unpublished data). There are – geographic variations in the prevalence of the three subtypes reported to date.

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 clinical specificity is reasonably high, however, in rare instances PPCD can be associated with incomplete penetrance. This can result in an individual with a positive family history acquiring a positive genetic result in the absence of a disease phenotype [1, 12].

2.5 Positive clinical predictive value

(life time risk to develop the disease if the test is positive)

PPCD can be associated with incomplete penetrance and at least three asymptomatic individuals with *ZEB1* variants have been reported [1, 12]. Furthermore, affected individuals can be asymptomatic with minimal clinical signs, and therefore can be incorrectly assigned as unaffected.

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 index case is asymptomatic by adulthood and has a negative test result for a known familial variant, it is highly predictive of unaffected status and the negative clinical predictive value will be close to 100%.

Index case in that family had not been tested:

If the index case is asymptomatic and does not have clinical signs it is unlikely they will develop disease later in life. But, given the variable age-of-onset of PPCD and non-penetrance in rare instances, [1, 12] an absence of phenotype does not exclude a positive genetic diagnosis.

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. (continue with 3.1.4)

Yes, X

clinically. X

imaging . X

endoscopy.

biochemistry.

electrophysiology.

other (please describe):

3.1.2 Describe the burden of alternative diagnostic methods to the patient

Phenotypic characteristics of PPCD are detectable by slit-lamp biomicroscopy, particularly in retroillumination. Specular or confocal microscopy are also useful for diagnostic purposes, although peripheral corneal lesions can be difficult to visualize with these techniques.

Histology of tissue excised at the time of corneal transplantation may show characteristic signs of epithelialization and multilayering of the endothelial layer. PPCD subtypes can only be reliably distinguished by genetic testing.

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

Slit-lamp biomicroscopy in combination with specular or confocal microscopy are generally used to assess clinical signs of PPCD as part of routine clinical care. Depending on the availability of corneal tissue (i.e. if excised during corneal transplantation) histological confirmation of the diagnosis can also be made (cost will vary based on regional resources). A genetic diagnosis can provide important prognostic information that will aid clinical care. For example, *OVOL2* variant positive patients (PPCD1) are more likely to require corneal graft surgery [2] and develop secondary glaucoma compared to the other PPCD subtypes [13, 14]. *ZEB1*-associated disease (PPCD3) is reported to be associated with significant corneal astigmatism, which must be managed in childhood to prevent amblyopia [7, 15].

A positive genetic test will facilitate accurate genetic counselling and carrier testing for all PPCD subtypes. Rarely, in families with a severe *OVOL2*-associated phenotype a positive genetic test can facilitate a prenatal and/or preimplantation genetic diagnosis. In the future a genetic diagnosis may enable patients to access gene-direct and/or personalised therapeutic treatments.

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

No.

Yes.

Therapy (please describe)

No therapy is currently available

Prognosis (please describe)

Prognosis for PPCD patients is variable. Notably, patient with *OVOL2* variants (PPCD1) have the highest risk of developing corneal oedema and secondary glaucoma.

Management (please describe)

A positive genetic diagnosis should result in the individual being monitored for the development of secondary glaucoma and visual loss from corneal oedema. Evidence suggests that patients with *OVOL2*, followed by *GRHL2* variants, are at greatest risk of developing both secondary glaucoma and corneal oedema [2, 3]. Glaucoma is initially managed medically with drops, but outflow obstruction from anterior synechiae may mean that glaucoma drainage surgery is required. Corneal oedema may require corneal transplantation. Although PPCD is primarily an endothelial disease there is as yet no evidence to support the superiority of endothelial keratoplasty over penetrating keratoplasty for transplant survival.

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)

Affected children should be monitored for visual loss, secondary glaucoma, amblyopia and strabismus. Individuals, particularly with *OVOL2* and *GRHL2* variants, have a life-long risk of secondary glaucoma [2, 3], and the intraocular pressure should be monitored. Secondary corneal oedema may develop in childhood, particularly with the *OVOL2* variant, which may require corneal transplantation. Children with *ZEB1* variants (PPCD3) may have significant refractive error and should be monitored for amblyopia.

If the test result is **negative** (please describe)

No lifestyle interventions are required if there is no evidence of corneal disease. However, not all loci for PPCD have been identified, so if there are clinical changes, individuals should be monitored as above.

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)?

A child at risk should be monitored for visual acuity loss, amblyopia, strabismus, and secondary glaucoma. Adults should be monitored for corneal oedema and secondary glaucoma. Despite treatment, individuals with *OVOL2* variants may become partially sighted and lifestyle must be adjusted accordingly. Individuals with *GRHL2* and *ZEB1* variants are less severely affected but may not achieve the vision standard for driving.

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?

A molecular diagnosis in an affected individual will resolve the mode of inheritance within a family and can facilitate informative genetic testing for family members. However, for some families the genetic cause of disease may remain unresolved, given that further genetic heterogeneity is anticipated for PPCD.

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

Genetic testing is not essential, but still recommended in patients with definitive clinical symptoms and a first-degree family member with a positive genetic test. Family members with characteristic corneal endothelial disease without a confirmed genetic result should still be monitored for amblyopia and secondary glaucoma.

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

Yes, other family members with characteristic clinical signs are likely to have reduced vision, which can lead to amblyopia in children, and to be at risk of secondary glaucoma.

3.4 Prenatal diagnosis

(To be answered if in 1.9 "D" was marked)

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

Yes, although prenatal testing for PPCD is uncommon. There is a discrepancy in opinion among medical professionals and families regarding the use of prenatal diagnosis due to the relatively mild phenotype associated with *GRHL2* and *ZEB1* variants. Pre-implantation genetic diagnosis is normally only considered for families with the severe early-onset form of PPCD, i.e. disease attributed to *OVOL2* variants (PPCD1).

4. If applicable, further consequences of testing

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe)

An accurate molecular diagnosis can facilitate effective genetic counselling. For index patients and their family members it may also alleviate adverse psychological effects associated with misunderstanding of the symptoms. Patients with a genetic diagnosis may be eligible for gene-specific therapies developed in the future.

Acknowledgement

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Conflict of Interest

The authors declare no conflict of interest

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ABSTRACT:

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Review of the analytical and clinical validity as well as of the clinical utility of DNA-based testing for variants in the *OVOL2*, *ZEB1* and *GRHL2* gene(s) in

X diagnostic,

X predictive and

X prenatal settings and for

X risk assessment in relatives.