

## **CUGC for congenital primary aphakia**

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## 1. Disease characteristics

### 1.1 Name of the Disease (Synonyms):

Congenital Primary Aphakia; CPAK (Anterior Segment Dysgenesis 2; ASGD2)

### 1.2 OMIM# of the Disease:

610256

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

*FOXE3*

### 1.4 OMIM# of the Gene(s):

601094

### 1.5 Mutational Spectrum:

Congenital primary aphakia (CPAK) is characterised by absence of the lens arising from failure of lens induction from the surface ectoderm and aborted lens development (1). It is caused by mutations in the *FOXE3* gene, located on chromosome 1p33 and consists of a single exon 1981 bp long (NCBI reference sequence NM\_012186.2). *FOXE3* encodes the 319 amino acid *FOXE3* protein, a forkhead-related transcription factor involved in lens formation. Most cases are autosomal recessive and c.720C>A (p.Cys240Ter) is the most common variant associated with CPAK (2–5). There has been one report of a dominant pedigree with an affected family member with a right primary aphakia, sclerocornea, optic disc coloboma and microphthalmia, and left Peters anomaly with congenital cataract and mild microphthalmia resulting from a non-stop mutation c.958T>C (p.Ter320ArgextTer72) (6).

It is important to note that both dominant and recessive mutations in *FOXE3* are associated with a variable mixed phenotype of developmental eye disorders including anterior segment dysgenesis, microphthalmia, Peters anomaly, sclerocornea, early-onset cataract, glaucoma and ocular coloboma (4–7).

### 1.6 Analytical Methods:

Bi-directional fluorescent Sanger sequencing of coding and intron–exon boundaries of *FOXE3* is the mainstay analytical method as an initial analysis. However, *FOXE3* screening is being included on next-generation sequencing (NGS) exome gene panels in some laboratories.

### 1.7 Analytical Validation

Parallel bi-directional fluorescent Sanger sequencing of known controls is required to validate procedures. Diagnostic testing must be carried out within a laboratory environment working to standards compliant with the ISO 15189.

### 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):

CPAK is rare and the prevalence is unknown. Of the 9 families reported, 8 are consanguineous and 7 are of Pakistani origin, suggesting a higher incidence in this ethnic group (<https://databases.lovd.nl/shared/diseases/02923>).

## 1.9 Diagnostic Setting:

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

Comment: Not applicable

## 2. Test characteristics

		genotype or disease		A: true positives	C: false negatives
		present	absent	B: false positives	D: true negatives
test	pos.	A	B	<u>sensitivity:</u> $A/(A+C)$	
	neg.	C	D	<u>specificity:</u> $D/(D+B)$	

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)

The analytical sensitivity of bi-directional Sanger sequencing is estimated to be >98% for the detection of nucleotide base changes, small deletions and insertions in the regions analysed.

### 2.2 Analytical Specificity

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

The analytical specificity of bi-directional Sanger sequencing is >98% given current testing methodologies, based on false positives that may arise due to misinterpretation of rare polymorphic variants that rarely occur in Sanger sequencing.

### 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.

CPAK is a congenital eye defect presenting at birth with no lens formation and variably associated anterior segment dysgenesis. The clinical sensitivity is >98%.

### 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.

A positive test in a patient without signs of this condition is extremely unlikely, and hence the clinical specificity will be high, nearing 100%.

## **2.5 Positive clinical predictive value**

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

Estimated >99% for *FOXE3* variants, as CPAK presents in infancy.

## **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:

Nearly 100% if no CPAK.

Index case in that family had not been tested:

Nearly 100% if no CPAK.

## **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,

clinically.	<input checked="" type="checkbox"/>
imaging	<input checked="" type="checkbox"/>
endoscopy.	<input type="checkbox"/>
biochemistry.	<input type="checkbox"/>
electrophysiology.	<input type="checkbox"/>
other (please describe):	

#### **3.1.2 Describe the burden of alternative diagnostic methods to the patient**

If CPAK is suspected, a clinical diagnosis can be made based on clinical examination and confirmed with ultrasound. Anterior segment ultrasound biomicroscopy may aid detection of co-existent anterior segment dysgenesis.

Due to the association of CPAK and the rubella virus, a TORCH complex evaluation is also recommended (8).

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

Clinical examination provides a cost effective diagnosis.

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

No.

Yes.

Therapy (please describe)

Prognosis (please describe) CPAK is often associated with other ocular anomalies, including microphthalmia, anterior segment dysgenesis, and glaucoma. Early diagnosis will enable prompt supportive treatment.

Management (please describe) CPAK should be managed by specialists with expertise in the condition. Supportive measures for those with sight impairment include involvement of social services. Regular follow-up will be required to monitor progression of associated anterior segment dysgenesis and glaucoma with medical and surgical interventions where needed. Intraocular surgery is not advised unless steps are taken to avoid inflammatory membrane formation and subsequent retinal detachment. Refractive refraction to reduce/prevent amblyopia. Genetic counselling will be offered to the family.

**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) CPAK is a congenital eye abnormality, therefore if it is not present at birth, it will not develop later in life. Identification of an unaffected carrier will inform family planning.

If the test result is **negative** (please describe). The result will inform family planning.

**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)?**

Vision can vary depending on severity of associated ocular anomalies. It can affect schooling and limit professions which require perfect vision. Hence, a clinically confirmed diagnosis can help in providing guidance regarding career choice.

**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.

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

If molecular testing has identified a *FOXE3* mutation in the index patient, clinical examination can identify and exclude disease in relatives. However, further genetic tests are required to

determine the carrier status. It is important to consider that heterozygous variants in *FOXE3* can cause associated ocular anomalies, so patients should be examined to exclude any manifestations. (4–7).

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

Yes

### **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, however, it has to be balanced with the severity of the condition. Primary aphakia is usually accompanied by anterior segment dysgenesis, which may prove extremely difficult to manage. Acting upon a positive result in terms of termination is not always advised as some level of treatment can be provided. Transabdominal ultrasound at 23 weeks gestation has detected CPAK (8). Identification of a causative *FOXE3* mutation allows for pre-implantation diagnosis.

## **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)

Genetic testing for *FOXE3* variants will provide a molecular diagnosis. This yields information regarding carrier status and provide choices that would not otherwise be available to facilitate decision making for the patient and their family. Genetic testing is essential for defining inheritance patterns, carrier status and enabling effective genetic counselling with consequent implications for prenatal or pre-implantation genetic diagnosis.

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## **Conflict of Interest**

The authors declare no conflicts of interest.

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**ABSTRACT:****CUGC for congenital primary aphakia**

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Review of the analytical and clinical validity as well as of the clinical utility of DNA-based testing for mutations in the *FOXE3* gene(s) in

- diagnostic,
- predictive and
- prenatal settings and for
- risk assessment in relatives.