CUGC for Non-syndromic Microphthalmia Including Next-Generation Sequencing Based Approaches

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1. Disease characteristics

1.1 Name of the Disease (Synonyms):

See table 1, column 1 - ‘Name of the disease’

<table>
<thead>
<tr>
<th>Name of the disease</th>
<th>OMIM# of the disease</th>
<th>Cytogenetic location</th>
<th>Associated gene(s)</th>
<th>OMIM# of associated gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microphthalmia, isolated 1; MCOP1</td>
<td>251600</td>
<td>14q32</td>
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<td></td>
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<tr>
<td>Microphthalmia, isolated 2; MCOP2</td>
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<td>14q24.3</td>
<td>VSX2</td>
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<td>Microphthalmia, isolated 3; MCOP3</td>
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<td>18q21.32</td>
<td>RAX</td>
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<tr>
<td>Microphthalmia, isolated 4; MCOP4</td>
<td>613094</td>
<td>8q22.1</td>
<td>GDF6</td>
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<tr>
<td>Microphthalmia, isolated 5; MCOP5</td>
<td>611040</td>
<td>11q23.3</td>
<td>MFRP</td>
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<td>Microphthalmia, isolated 6; MCOP6</td>
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<td>2q37.1</td>
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<td>15q26.3</td>
<td>ALDH1A3</td>
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<td>Microphthalmia, isolated with coloboma 1; MCOPCB1</td>
<td>300345</td>
<td>Chr.X</td>
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<tr>
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<td>SHH</td>
<td>600725</td>
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<td>10q23.33</td>
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<td>Microphthalmia, isolated with corectopia; MCOPCR</td>
<td>156900</td>
<td>-</td>
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<td>Microphthalmia, isolated with cataract 1; MCOPCT1</td>
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<td>Nanophthalmos 1; NN01</td>
<td>600165</td>
<td>11p</td>
<td></td>
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<td>Nanophthalmos 2; NN02</td>
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<td>MFRP</td>
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<tr>
<td>Nanophthalmos 3; NN03</td>
<td>611897</td>
<td>2q11-q14</td>
<td></td>
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<td>Nanophthalmos 4; NN04</td>
<td>615972</td>
<td>17q11.2</td>
<td>TMEM98</td>
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</table>
Table 1. Overview of disease associated with non-syndromic (isolated and complex) microphthalmia

1.2 OMIM# of the Disease:
See table 1, column 2 - 'OMIM# of the disease'

1.3 Name of the Analysed Genes or DNA/Chromosome Segments and OMIM# of the Gene(s):

1.3.1 Core genes (irrespective if being tested by Sanger sequencing or next-generation sequencing)
See table 1, column 4 - 'Associated gene(s)' and column 5 - 'OMIM# of associated gene(s)'

1.3.2 Additional genes (if tested by next-generation sequencing, including whole exome/genome sequencing and panel sequencing)
See table 2, column 1 - 'Gene' and column 3 - 'OMIM# of gene'

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cytogenetic location</th>
<th>OMIM# of gene</th>
<th>Associated disease acronym</th>
<th>OMIM# of the disease (where applicable)</th>
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<td>BCOR</td>
<td>Xp11.4</td>
<td>300485</td>
<td>Microphthalmia, syndromic 2</td>
<td>300166</td>
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<td>BMP4</td>
<td>14q22.2</td>
<td>112262</td>
<td>Microphthalmia, syndromic 6</td>
<td>607932</td>
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<tr>
<td>CHD7</td>
<td>8q12.2</td>
<td>605806</td>
<td>CHARGE syndrome</td>
<td>214800</td>
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<tr>
<td>COL4A1</td>
<td>13q34</td>
<td>120130</td>
<td>Brain small vessel disease with or without ocular anomalies</td>
<td>607595</td>
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<tr>
<td>FREM1</td>
<td>9p22.3</td>
<td>608944</td>
<td>Manitoba oculotrichoanal syndrome</td>
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<td>HCCS</td>
<td>Xp22.2</td>
<td>300056</td>
<td>Linear skin defects with multiple congenital anomalies 1</td>
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<td>HMGB3</td>
<td>Xq28</td>
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<td>MAB21L2</td>
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<td>Microphthalmia, syndromic 1</td>
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<td>OTX2</td>
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<td>Microphthalmia, syndromic 5</td>
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<td>PAX6</td>
<td>11p13</td>
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<td>PXDN</td>
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<td>Cornea opacification and other ocular anomalies</td>
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<td>RARB</td>
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<td>Microphthalmia, syndromic 12</td>
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<td>SMOC1</td>
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<td>Microphthalmia with limb anomalies</td>
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<td>SOX2</td>
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<td>Microphthalmia, syndromic 3</td>
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</table>
Isolated microphthalmia is rare; most patients have associated ocular anomalies (complex), such as ocular coloboma, cataract, and anterior segment dysgenesis. Nearly 80% of cases are associated with multisystemic features forming part of a syndrome (1-4). Only isolated and complex (non-syndromic) microphthalmia will be discussed (see Clinical Utility Gene Card for syndromic microphthalmia). There is a complex aetiology with chromosomal, monogenic, and environmental causes identified. It is clinically and genetically heterogeneous and may be inherited in an autosomal dominant, recessive, or X-linked recessive manner, although most cases of non-syndromic microphthalmia are sporadic. The occurrence of de novo mutations, mosaicism and incomplete penetrance makes prediction of the inheritance pattern difficult. Chromosomal duplications, deletions and translocations have been identified; a locus for autosomal dominant microphthalmia has been mapped to 15q12-15,(5) and for autosomal recessive microphthalmia at 14q32.(6, 7) Autosomal recessive VSX2 variants (causing MCOP2) account for approximately 2% of isolated microphthalmia cases, and are predominantly missense. However deletion of exon 3 has also been described.(8, 9) Autosomal recessive variants in RAX (MCOP3) and ALDH1A3 (MCOP8) can be missense, nonsense or frameshift, with some splice donor variants. Only missense variants have been found in GDF6 (MCOP4) and GDF3 (MCOP7) and are inherited in an autosomal dominant manner. Homozygous or compound heterozygous variants in MFRP (MCOP5) or PRSS56 (MCOP6) are associated with autosomal recessive posterior microphthalmia, which defines a rare distinct phenotype restricted primarily to the posterior segment of the eye. Patients with MFRP variants also develop a progressive rod cone dystrophy. Missense, nonsense and frameshift variants, plus splice donor variants have been described for both these genes.

Many specific variants may cause varied phenotypes e.g. NM_001142617.1: c.1157G>A and c.1156G>A (p.Gly304Lys) in STRA6 causes MCOPCB8 (isolated microphthalmia and coloboma) and Matthew-Wood syndrome (bilateral anophthalmia with pulmonary agenesis and other associated systemic defects).(10) Phenotypic findings in patients presenting with microphthalmia and congenital cataract (MCOPCT1) also include mental retardation and an individual with congenital heart disease. Patients with OTX2 variants have been described with specific hippocampal abnormalities and phenotypic findings in patients affected by RAX variants include developmental delay with autistic features and hypoplastic optic nerve and chiasm. MCOP4 has been reported in cases as isolated, or associated with skeletal anomalies, coloboma or polydactyly. Autism and cardiac anomalies have been described as additional features in a MCOP8-affected Pakistani patient, although these phenotypes may be unrelated to ALDH1A3 variants. Furthermore, one patient with a variant in the ALDH1A3 gene has been described with posterior coloboma and detached retina and another with optic nerve and chiasm hypoplasia associated with MCOP8. This makes the genetic classification system of isolated/complex and syndromic microphthalmia challenging.

A patient with a 2.7 Mb deletion at 18q22.1, incorporating the gene TMX3, presented with microphthalmia. Two additional sequence variants have been identified in unrelated patients; a male with unilateral microphthalmia and retinal coloboma (NM_019022.3: c.116G>A (p.Arg39Gln)); and a female with unilateral microphthalmia and severe micrognathia.
165 Consequenlty, the contribution of TMX3 variants to MCOPCB1 has been suggested but remains to be confirmed.

166 Nanophthalmos is a subtype of simple microphthalmos. Autosomal recessive nanophthalmos-2 (NNO2) has been associated with homozygosity for a nonsense
167 variant (NM_031433.3: c.523C>T, (p.Gln175Ter)) or frameshift (NM_031433.3: c.1143insC (p.
168 Gly383Ter)) variant and compound heterozygosity for a frameshift (NM_031433.3:
169 c.498delC (p.Asn167Thrfs)) or a missense (NM_031433.3: c.545T>C (p.Ile182Thr)) variant
170 in MFRP.(12) Additional complications can develop, including angle closure glaucoma, cystic
171 edema, and retinal detachment. More recently, two segregating missense variants
172 (NM_015544.2: c.577G>C (p.Ala193Pro); c.587A>C (p.His196Pro)) and a 34 bp
173 heterozygous deletion (NM_015544.2: c.236_263+6del34)
174 c.694_721delAGAATGAAGACTGGATCGAAGATGCCTCgtaagg) in TMEM98 have been
175 described in autosomal dominant nanophthalmos (NNO4) pedigrees.(13, 14)
176 Of the monogenic causes of anophthalmia/microphthalmia, SOX2 has been implicated as a
177 major causative gene, in which variants account for 15-20% of autosomal dominant
178 cases.(15) However, patients with SOX2 variants usually present with other systemic
179 malformations; the contribution of SOX2 variants to isolated microphthalmia specifically,
180 remains unknown. The majority of SOX2 sequence variants are de novo; nonsense,
181 missense, frameshift and whole gene deletions have been reported.(16, 17) Like SOX2, the
182 majority of OTX2 variants are inherited nonsense and frameshift variants leading to
183 haploinsufficiency, with some reports of whole gene deletions.(18) Patients often present
184 with additional brain abnormalities. In view that variants in the genes listed in Table 2 cause
185 a wide range of ocular phenotypes with different expressivity, their molecular screening must
186 be recommended.
187 All data was mined from primary literature or curated genomic and phenotype databases,
188 including ClinVar®, public archive of interpretations of clinically relevant variants
190 (http://www.ncbi.nlm.nih.gov/books/NBK1116/); The Human Gene Mutation Database,
191 HGMD® (http://www.hgmd.org/) and Online Mendelian Inheritance in Man, OMIM®
192 (http://omim.org/). Novel data should be shared through these databases. They were last
193 accessed on 15th November 2016.
194 1.5 Analytical Validation
195 Sequencing of both DNA strands. Disease-causing variants should be confirmed using
196 genomic DNA from a new extraction. Causative variants found with next-generation
197 sequencing should be verified using Sanger sequencing or other specific molecular methods
198 (e.g. PCR digest); for further details, see the Eurogentest Guideline. It is important to look for
199 segregation to determine whether the variant is de novo in isolated cases, providing a higher
200 likelihood it is pathogenic. In clinical practise, array comparative genomic hybridisation
201 (aCGH) or multiplex ligation dependent probe amplification assay (MLPA) may be performed
202 initially to detect deletions or duplications. Some molecular service labs also offer
203 fluorescence in situ hybridisation (FISH) to identify rearrangements or copy number
204 variation.
205 1.6 Estimated Frequency of the Disease
206 (Incidence at birth ("birth prevalence") or population prevalence. If known to be variable
207 between ethnic groups, please report):
208 The birth prevalence of microphthalmia ranges from 2 to 17 per 100,000 (19-24). In a
209 prospective UK incidence study over 18 months, 135 confirmed cases of microphthalmia,
anophthalmia and ocular coloboma (MAC) were reported in children under 16 years of age; microphthalmia was present in 66 (48.9%) children; isolated in 31 (23%) and mixed in 35 (25.9%) (25). Microphthalmia was reported in 3.2-11.2% of blind children worldwide in 2006 (4).

Epidemiological data suggests risk factors for microphthalmia are maternal age over 40, multiple births, infants of low birth weight and low gestational age.(4, 23, 26) There is no predilection with regards to race or gender.(23, 26) Isolated microphthalmia is most commonly unilateral.(26)

### 1.7 Diagnostic Setting:

<table>
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<tr>
<th></th>
<th>Yes</th>
<th>No</th>
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<tbody>
<tr>
<td>A. (Differential) diagnostics</td>
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<td></td>
</tr>
<tr>
<td>B. Predictive Testing</td>
<td>☒</td>
<td></td>
</tr>
<tr>
<td>C. Risk assessment in Relatives</td>
<td>☒</td>
<td></td>
</tr>
<tr>
<td>D. Prenatal</td>
<td>☒</td>
<td></td>
</tr>
</tbody>
</table>

Comment: Due to time constraints, such as pregnancy, panel diagnostic or Whole Exome Sequencing or Whole Genome Sequencing (WES/WGS) filtering is preferred if there is a request for prenatal diagnosis (which is rare).
2. Test characteristics

<table>
<thead>
<tr>
<th>genotype or disease present</th>
<th>absence</th>
</tr>
</thead>
<tbody>
<tr>
<td>pos. test</td>
<td>A</td>
</tr>
<tr>
<td>neg. test</td>
<td>C</td>
</tr>
</tbody>
</table>

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

- sensitivity: \( \frac{A}{A+C} \)
- specificity: \( \frac{D}{D+B} \)
- pos. predict. value: \( \frac{A}{A+B} \)
- neg. predict. value: \( \frac{D}{C+D} \)

2.1 Analytical Sensitivity

2.1.1 if tested by conventional Sanger sequencing

Less than 100%. The proportion is likely <100%, because primers may be localised on sequences containing SNVs or rare variants, which results in a preferential amplification of one allele (allele drop out). A supplementary deletion/duplication diagnostic test should be performed for genes with a known proportion of large genomic deletions/duplications as outlined in section 1.5.

2.1.2 if tested by Next-generation sequencing

Less than 100%. The proportion is likely <100%, because there might be disease-causing variants in regions that could not be enriched and/or sequenced by next-generation sequencing due to suboptimal coverage of some regions of interest with this technology but depending on next-generation sequencing strategy. If amplicon-based enrichment strategies are being used, primers may be localized on SNVs or rare variants, which results in preferential amplification of one allele. In patients with a highly suggestive phenotype in whom testing for specific gene alterations proves negative, a supplementary deletion/duplication diagnostic test should be performed for genes with a known proportion of large genomic deletions/duplications as outlined in section 1.5.

2.2 Analytical Specificity

2.2.1 if tested by conventional Sanger sequencing

Nearly 100%. False positives may at the most arise due to misinterpretation of rare polymorphic variants.

2.2.2 if tested by Next-generation sequencing

Less than 100%. The risk of false positives due to misinterpretation of rare polymorphic variants may be higher compared with Sanger sequencing because of greater number of analysed genes.

2.3 Clinical Sensitivity

2.3.1 if tested by conventional Sanger sequencing

Of those patients that undergo genetic testing of known causative genes with Sanger sequencing, less than 10% of patients with isolated microphthalmia receive a molecular
diagnosis and these are predominantly bilateral severe cases.

Most studies group microphthalmia with MAC and therefore the most common causative genes are SOX2, OTX2, PAX6 and GDF6 contributing up to 10%, 3%, 2.5% and 8%, respectively. These are often syndromic cases and so the actual contribution to isolated microphthalmia is likely to be much lower.

2.3.2 if tested by Next-generation sequencing

See 2.3.1. Mutation detection rates are higher when combined WES with array aCGH and high resolution analysis of intragenic microdeletions and microduplications are performed. WGS may aid in the detection of pathogenic variants in the promoter region, introns and other non-coding regulatory elements, and provide better coverage than exome sequencing. Regulatory element disruption in microphthalmia remains largely uncharacterised.

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.

2.4.1 if tested by conventional Sanger sequencing

Unknown, however, if microphthalmia is not present it is unlikely that a positive test will be detected.

2.4.2 if tested by Next-generation sequencing

See 2.4.1.

2.5 Positive clinical predictive value

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

This is a congenital anomaly of the eye, therefore patients will be born with this defect, therefore nearly 100%, however variable expressivity has been noted.

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 the non-affected relative is not a carrier of an identified disease-causing mutation, they have no increased risk, except a small risk related to the prevalence in the general population.

Index case in that family had not been tested:

Unknown

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?
3.1.2 Describe the burden of alternative diagnostic methods to the patient

The definition of microphthalmia is heterogeneous, however an axial length (AL) of <21 mm in adults and <19 mm in a 1 year old is most widely accepted as it represents a reduction of 2 SD or more below normal. Microphthalmia can be detected using ultrasound during the second trimester, or after birth in conjunction with clinical examination. Microphthalmia can be associated with microcornea, which is defined as a horizontal diameter <9mm in a newborn and <10mm in children 2 years and older. Posterior microphthalmia is a rare subset of microphthalmia in which the total axial length of the eyeball is reduced whilst anterior segment dimensions including corneal diameter, anterior chamber depth and anteroposterior length of the lens are normal, also detected by ultrasound. Nanophthalmia, a second rare subset of microphthalmia, is classically distinguished from posterior microphthalmia based on the presence of decreased anterior chamber dimensions.

Although a diagnosis of microphthalmia can be made relatively easily and cost-effectively, if this anomaly is seen, children should be investigated within a multi-disciplinary team, including Paediatricians and Clinical Geneticists, to ensure this is not part of a syndrome. Further monitoring may be required as syndromic manifestations may present later in childhood.

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

Clinical examination and ultrasound imaging 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) Yes if a variant in a gene is associated with a syndrome, it may lead to search for systemic involvement to prevent morbidity and maximise function e.g. patients with SOX2 anophthalmia syndrome suffer from a range of multisystem abnormalities including seizures and sensorineural deafness, hence early diagnosis will lead to prompt supportive treatment, having long-term health economic benefits.

Management (please describe) Microphthalmia should be managed by specialists with expertise in this condition. If visual function is present, this must be maximised by correcting refractive error and preventing amblyopia. Those with poor vision must be supported by low visual aids and training. MRI imaging of the brain is required to rule out any associated midline neurological or
pituitary defects. Referral to neurology and endocrinology may be indicated. If a child has a non-seeing eye, cosmesis can be addressed by fitting cosmetic shells or contact lenses. Socket expansion in severe microphthalmia may be indicated using enlarging conformers. Although genetic counselling can be challenging due to the extensive range of disease-associated genes and variable expressivity, appropriate counselling can be applied if the mode of inheritance is identified and should be offered to the family.

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

3.2.1 Will the result of a genetic test influence lifestyle and prevention?
If the test result is positive (please describe)
Microphthalmia is a congenital eye anomaly therefore if it is not clinically present at birth then this will not develop later in life. However if an individual is clinically unaffected but is a carrier, this information will inform family planning if the mode of inheritance can be identified.

If the test result is negative (please describe)
If the clinically unaffected person has a negative test result, no further follow-up is required. 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 be variably affected in microphthalmic patients depending on the severity of the anomaly and the other complex features. This may limit schooling and professions that require perfect vision. Hence, a clinically confirmed diagnosis can help to provide guidance on career choice.

3.3 Genetic risk assessment in family members of a diseased person

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?
Yes, although there may be variable expressivity, non-penetrance and germ-line mosaicism, which will complicate the advice that can be given.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?
If a disease-causing mutation is identified in the index patient, family members can be tested but ophthalmic examination is also helpful. Test negative family members, who are clinically unaffected, do not need any further investigation or monitoring.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?
Yes, if the variant is known.
3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?

Yes. Germline mosaicism and/or variable penetrance render the prediction of recurrence risk difficult in monogenic microphthalmic individuals, however molecular genetic studies for known variants are possible on amniotic fluid foetal cells withdrawn after 14 weeks of gestation or on chronic villus sampling at 10 to 12 weeks gestation and can facilitate the diagnosis of microphthalmia. Additionally, trans-vaginal ultrasonography enables the detection of microphthalmia from 12 weeks gestation (28); the maximal coronal or axial planes of the orbit are measured and compared to established eye growth charts (29).

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)

Beyond potentially defining recurrence risk information dependent on the cause and mode of inheritance, identifying the genetic aetiology may guide genetic counselling. It also contributes to the classification of syndromic or non-syndromic microphthalmia, thereby guiding any subsequent investigations for affected patients.

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Conflict of Interest

The authors declare no conflict of interest.

References


CUGC for Non-syndromic Microphthalmia Including Next-Generation Sequencing Based Approaches

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1. Name of the Disease (Synonyms):
Non-syndromic (isolated and complex) microphthalmia; MCOP

2. OMIM# of the Disease:
251600; 610093; 611038; 613094; 611040; 613517; 613704; 615113; 300345; 605738; 600165; 609549; 611897; 615972

3. Name of the Analysed Genes or DNA/Chromosome Segments:
VSX2; RAX; GDF6; MFRP; PRSS56; GDF3; ALDH1A3; SHH; GDF6; ABCB6; STRA6; TENM3; RBP4; MFRP; TMEM98

4. OMIM# of the Gene(s):
142993; 601881; 601147; 606227; 613858; 606522; 600463; 142993; 600725; 601147; 605452; 610745; 610083; 180250; 606227; 615949
Review of the analytical and clinical validity as well as of the clinical utility of DNA-based testing for mutations in the VSX2, RAX, GDF6, MFRP, PRSS56, GDF3, ALDH1A3, SHH, GDF6, ABCB6, STRA6, TENM3, RBP4, MFRP and TMEM98 genes in diagnostic, predictive and prenatal settings and for risk assessment in relatives.