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CLINICAL UTILITY GENE CARD

Clinical utility gene card 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'.

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'.

1.4 Mutational spectrum

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 ~2% of isolated microphthalmia cases, and are predominantly missense. However, deletion of exon 3 has also been described.[8–10] Autosomal-recessive variants in *RAX* (MCOP3)[10–12] and *ALDH1A3* (MCOP8) can be missense, nonsense or frameshift, with some splice donor variants. A *RAX* gene deletion has also been described in one patient with bilateral anophthalmia, with no other ocular or systemic abnormalities reported.[10] *RAX* mutations account for ~2% of inherited anophthalmia/microphthalmia cases.[11] Only missense variants have been found in *GDF6* (MCOP4)[10,13] and *GDF3* (MCOP7),[14] and are inherited in an autosomal-dominant manner. Homozygous or compound heterozygous variants in *MFRP* (MCOP5)[15–19] or *PRSS56* (MCOP6)[20–23] 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.[18] Missense, nonsense and frameshift variants, plus splice donor variants have been described for both these genes.

COL4A1 (NM_ 001845.5: c.2317G>A (p.Gly773Arg); c.2122G>A (p.Gly708Arg)) variants are often reported in cases of brain small vessel disease with or without ocular anomalies, however, variants have been found in siblings with microphthalmia, other ocular anomalies, but no/few neurologic symptoms.[24] Isolated microphthalmia with coloboma is a heterogeneous condition. Cases without systemic involvement are predominantly associated with autosomal-dominant transmission, although a few recessive cases have been described. MCOPCB1 has been mapped to the X chromosome,[25] and MCOPCB2 to chromosome 15q12-q15.[26] Homozygosity for missense and a splice variant in *VSX2* have been described in MCOPCB3 cases.[27,28] MCOPCB4 is isolated microphthalmia associated with colobomatous cyst and is transmitted as an autosomal-recessive trait. The genetic cause remains unknown. A heterozygous 24 bp deletion in the coding region of *SHH* has been identified in MCOPCB5 patients.[29,30] Heterozygous missense variants in *GDF3*[14] and *ABCB6*[31] have been described in patients with MCO PCB6 and MCOPCB7, respectively. Homozygous splice variant (NM_001080477.3: c.2968-2A>T (p.Val990Cysfs))[32] and frameshift loss of function variant (NM_001080477.3: c.2083dupA (p.Thr695Asnfs))[33] in *TENM3* result in MCOPCB9. A missense

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Table 1 Overview of disease associated with non-syndromic (isolated and complex) microphthalmia

Name of the disease	*OMIM# of the disease*	*Cytogenetic location*	*Associated gene(s)*	*OMIM# of associated gene(s)*
Microphthalmia, isolated 1; MCOP1	251600	14q32	—	—
Microphthalmia, isolated 2; MCOP2	610093	14q24.3	*VSX2*	142993
Microphthalmia, isolated 3; MCOP3	611038	18q21.32	*RAX*	601881
Microphthalmia, isolated 4; MCOP4	613094	8q22.1	*GDF6*	601147
Microphthalmia, isolated 5; MCOP5	611040	11q23.3	*MFRP*	606227
Microphthalmia, isolated 6; MCOP6	613517	2q37.1	*PRSS56*	613858
Microphthalmia, isolated 7; MCOP7	613704	12p13.31	*GDF3*	606522
Microphthalmia, isolated 8; MCOP8	615113	15q26.3	*ALDH1A3*	600463
Microphthalmia, isolated with coloboma 1; MCOPCB1	300345	Chr.X	—	—
Microphthalmia, isolated with coloboma 2; MCOPCB2	605738	15q12-q15	—	—
Microphthalmia, isolated with coloboma 3; MCOPCB3	610092	14q24.3	*VSX2*	142993
Microphthalmia, isolated with coloboma 4; MCOPCB4	251505	—	—	—
Microphthalmia, isolated with coloboma 5; MCOPCB5	611638	7q36.3	*SHH*	600725
Microphthalmia, isolated with coloboma 6, digenic; MCOPCB6	613703	8q22.1	*GDF6*	601147
Microphthalmia, isolated with coloboma 6; MCOPCB6	613703	12p13.31	*GDF3*	606522
Microphthalmia, isolated with coloboma 7; MCOPCB7	614497	2q35	*ABCB6*	605452
Microphthalmia, isolated with coloboma 8; MCOPCB8	601186	15q24.1	*STRA6*	610745
Microphthalmia, isolated with coloboma 9; MCOPCB9	615145	4q34.3-35.1	*TENM3*	610083
Microphthalmia, isolated with coloboma 10; MCOPCB10	616428	10q23.33	*RBP4*	180250
Microphthalmia, isolated with corectopia; MCOPCR	156900	—	—	—
Microphthalmia, isolated with cataract 1; MCOPCT1	156850	16p13.3	—	—
Nanophthalmos 1; NN01	600165	11p	—	—
Nanophthalmos 2; NN02	609549	11q23.3	*MFRP*	606227
Nanophthalmos 3; NN03	611897	2q11-q14	—	—
Nanophthalmos 4; NN04	615972	17q11.2	*TMEM98*	615949

Table 2 Additional genes associated with isolated and complex microphthalmia, often with syndromic features, tested by next-generation sequencing

Gene	*Cytogenetic location*	*OMIM# of gene*	*Associated disease acronym*	*OMIM# of the disease (where applicable)*
BCOR	Xp11.4	300485	Microphthalmia, syndromic 2	300166
BMP4	14q22.2	112262	Microphthalmia, syndromic 6	607932
CHD7	8q12.2	605806	CHARGE syndrome	214800
COL4A1	13q34	120130	Brain small vessel disease with or without ocular anomalies	607595
FREM1	9p22.3	608944	Manitoba oculotrichoanal syndrome	248450
HCCS	Xp22.2	300056	Linear skin defects with multiple congenital anomalies 1	309801
HMGB3	Xq28	300193	Microphthalmia, syndromic 13	300915
MAB21L2	4q31.3	604357	Microphthalmia, syndromic 14	615877
NAA10	Xq28	300013	Microphthalmia, syndromic 1	309800
OTX2	14q22.3	600037	Microphthalmia, syndromic 5	610125
PAX6	11p13	607108	Ocular malformations within the *MAC* spectrum	—
PXDN	2p25.3	605158	Cornea opacification and other ocular anomalies	269400
RARB	3p24.2	180220	Microphthalmia, syndromic 12	615524
SMOC1	14q24.2	608488	Microphthalmia with limb anomalies	206920
SOX2	3q26.33	184429	Microphthalmia, syndromic 3	206900
TMX3	18q22.1	616102	Microphthalmia with coloboma	—
VAX1	10q25.3	604295	Microphthalmia, syndromic 11	614402
YAP1	11q22.1	606608	Ocular coloboma	120433

variant in *RBP4* (NM_006744.3: c.217G>A (p.Ala73Thr)) has been associated with MCOPCB10.[34]

Many specific variants may cause varied phenotypes, for example, 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).[35] Phenotypic findings in patients presenting with microphthalmia and congenital cataract (MCOPCT1) also include mental retardation and an individual with congenital heart disease.[36,37] 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.[11] 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.[38] Furthermore, one patient with a variant in the *ALDH1A3* gene has been described with posterior coloboma and detached retina (NM_000693.2: c.568A>G (p.Lys190Ter)), and another with optic nerve and chiasm hypoplasia (NM_000693.2: c.1165A>T (p.Lys389Ter)) associated with MCOP8.[39] 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 (NM_019022.3: c.322G>A, (p.Asp108Asn)).[40] Consequently, the contribution of *TMX3* variants to MCOPCB1 has been suggested, but remains to be confirmed.

Nanophthalmos is a subtype of simple microphthalmos. Autosomal-recessive nanophthalmos 2 (NNO2) has been associated with homozygosity for a nonsense (NM_031433.3: c.523C>T, (p.Gln175Ter)) or frameshift (NM_031433.3: c.1143insC (p. Gly383Ter)) variant, and compound heterozygosity for a frameshift (NM_031433.3: c.498delC (p.Asn167Thrfs)) or a missense (NM_031433.3: c.545 T>C (p.Ile182Thr)) variant in *MFRP*.[41] Additional complications can develop, including angle-closure glaucoma, cystic oedema and retinal detachment. More recently, two segregating missense variants (NM_015544.2: c.577G>C (p.Ala193Pro); c.587A>C (p.His196Pro)) and a 34 bp heterozygous deletion (NM_015544.2: c.236_263+6del34) in *TMEM98* have been described in autosomal-dominant nanophthalmos (NNO4) pedigrees.[42,43]

Of the monogenic causes of anophthalmia/microphthalmia, *SOX2* has been implicated as a major causative gene, in which variants account for 15–20% of autosomal-dominant cases.[44] However, patients with *SOX2* variants usually present with other systemic malformations; the contribution of *SOX2* variants to isolated microphthalmia specifically remains unknown. The majority of *SOX2* sequence variants are *de novo*; nonsense, missense, frameshift and whole-gene deletions have been reported.[10,45,46] Like *SOX2*, the majority of *OTX2* variants are inherited nonsense and frameshift variants leading to haploinsufficiency, with some reports of whole-gene deletions.[10,47] Patients often present with additional brain abnormalities. In view that variants in the genes listed in Table 2 cause a wide range of ocular phenotypes with different expressivity, their molecular screening must be recommended.

All data were mined from primary literature or curated genomic and phenotype databases, including ClinVar, public archive of interpretations of clinically relevant variants (http://www.ncbi.nlm.nih.gov/clinvar/); GeneReviews (http://www.ncbi.nlm.nih.gov/books/NBK1116/); The Human Gene Mutation Database, HGMD (http://www.hgmd.org/) and Online Mendelian Inheritance in Man, OMIM (http://omim.org/). Novel data should be shared through these databases. They were last accessed on 21 November 2016.

1.5 Analytical validation

Sequencing of both DNA strands. Disease-causing variants should be confirmed using genomic DNA from a new extraction. Causative variants found with next-generation sequencing should be verified using Sanger sequencing or other specific molecular methods (eg, PCR digest); for further details, see the Eurogentest Guideline. It is important to look for segregation to determine whether the variant is *de novo* in isolated cases, providing a higher likelihood it is pathogenic. In clinical practice, array comparative genomic hybridisation (aCGH) or multiplex ligation-dependent probe amplification assay may be performed initially to detect deletions or duplications. Some molecular service labs also offer fluorescence *in situ* hybridisation to identify rearrangements or copy-number variation.

1.6 Estimated frequency of the disease (incidence at birth ('birth prevalence') or population prevalence)

If known to be variable between ethnic groups, please report:

The birth prevalence of microphthalmos ranges from 2 to 17 per 100 000.[48–53] In a 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%).[54] Microphthalmia was reported in 3.2–11.2% of blind children worldwide in 2006.[4]

Epidemiological data suggest risk factors for microphthalmia are maternal age over 40, multiple births, infants of low birthweight and low gestational age.[4,52,55] There is no predilection with regard to race or gender.[52,55] Isolated microphthalmia is most commonly unilateral.[55]

1.7 Diagnostic setting

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

Comment: Because of the 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

	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	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

2.1 Analytical sensitivity (proportion of positive tests if the genotype is present in the analyte)

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 dropout). A supplementary deletion/duplication diagnostic test should be

performed for genes with a known proportion of large genomic deletions/duplications as outlined in the section 'Analytical validation'.

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 owing 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 localised 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 the section 'Analytical validation'.

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

2.2.1 If tested by conventional Sanger sequencing
Nearly 100%. False positives may at the most arise owing to misinterpretation of rare polymorphic variants.

2.2.2 If tested by next-generation sequencing
Less than 100%. The risk of false positives owing to misinterpretation of rare polymorphic variants may be higher compared with Sanger sequencing because of greater number of analysed genes.

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.

2.3.1 If tested by conventional Sanger sequencing
Of those patients that undergo genetic testing of known causative genes with Sanger sequencing, <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.[56] 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 section 'If tested by conventional Sanger sequencing'. 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 promotor 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 section 'If tested by conventional Sanger sequencing'.

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 of not developing 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 1.7 '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	☒
	Imaging	☒
	Endoscopy	☐
	Biochemistry	☐
	Electrophysiology	☐
	Other (please describe)	

3.1.2 Describe the burden of alternative diagnostic methods to the patient
The definition of microphthalmia is heterogenous, 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 <9 mm in a newborn and <10 mm in children 2 years and older. Posterior microphthalmia is a rare subset of microphthalmia in which the total AL of the eyeball is reduced although 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 multidisciplinary 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, for example, 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 owing 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
(To be answered if in 1.7 '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)	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
(To be answered if in 1.7 'C' was marked)

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 germline 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 Prenatal diagnosis
(To be answered if in 1.7 'D' was marked)

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 10–12 weeks gestation, and can facilitate the diagnosis of microphthalmia. In addition, transvaginal ultrasonography enables the detection of microphthalmia from 12 weeks gestation;[57] the maximal coronal or axial planes of the orbit are measured, and compared with established eye growth charts.[58]

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.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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