CUGC for 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 for 'Name of the Disease'

1.2 OMIM# of the Disease:

See Table 1 – column 2 for 'OMIM# of the Disease'

Table 1: Overview of diseases associated with syndromic microphthalmia

Name of the Disease	*OMIM# of disease*	*Cytogenetic location*	*Associated gene(s)*	*OMIM# of associated gene(s)*	*Inheritance*
Aicardi Syndrome; AIC	304050	Xp22	---------	---------	XLD
Alkuraya-Kucinskas Syndrome; ALKKCUS	617822	4q27	*KIAA1109*	611565	AR
Ayme-Gripp Syndrome; AYGRP	601088	16q23.2	*MAF*	177075	AD
Baraitser-Winter Syndrome 1; BRWS1	243310	7p22.1	*ACTB*	102630	AD
Baraitser-Winter Syndrome 2; BRWS2	614583	17q25.3	*ACTG1*	102560	AD
Biemond Syndrome II	210350	---------	---------	---------	Unknown
Blepharophimosis, Ptosis and Epicanthus Inversus; BPES	110100	3q22.3	*FOXL2**	605597	AD
Bosma Arhinia Microphthalmia Syndrome; BAMS	603457	18p11.32	*SMCHD1*	614982	AD
Brain Small Vessel Disease; BSVD	607595	13q34	*COL4A1*	120130	AD
Branchiooculofacial syndrome; BOFS	113620	6p24.3	*TFAP2A*	107580	AD
Cataract 11, Multiple Types; CTRCT11	610623	10q24.32	*PITX3*	602669	AD, AR
Cataract 23, Multiple Types; CTRCT23	610425	22q12.1	*CRYBA4*	123631	AD
Cerebrooculofacioskeletal syndrome 1; COFS1	214150	10q11.23	*ERCC6**	609413	AR
Cerebrooculofacioskeletal syndrome 3; COFS3	616570	13q33.1	*ERCC5**	133530	AR
Cerebrooculofacioskeletal syndrome 4; COFS4	610758	19q13.32	*ERCC1*	126380	AR
CHARGE syndrome	214800	8q12.2	*CHD7*	608892	AD
Chondrodysplasia with platyspondyly, distinctive brachydactyly, hydrocephaly and microphthalmia	300863	Xp11.23	*HDAC6**	300272	XLD
Coloboma-Obesity-Hypogenitalism-Mental Retardation Syndrome	601794	---------	---------	---------	Unknown
Coloboma, Ocular, with or without Hearing Impairment, Cleft Lip/Palate and/or Mental Retardation; COB1	120433	11q22.1	YAP1	606608	AD
Colobomatous microphthalmia, ptosis, nephropathy and syndactyly	----------	4q35.2	*FAT1*	600976	AR
COMMAD Syndrome	617306	3p13	*MITF*	156845	AR
Congenital Disorder of Glycosylation, Type 1q; CDG1q	612379	4q12	*SRD5A3*	611715	AR
Curry-Jones Syndrome; CRJS	601707	7q32.1	*SMO*	601500	Unknown
Dextrocardia with unusual facies and microphthalmia	221950	---------	---------	---------	AD
Duane-Radial Ray Syndrome; DRRS	607323	20q13.2	*SALL4*	607343	AR
Fanconi Anemia, Complementation Group A; FA	227650	16q24.3	*FANCA**	607139	AR
Fanconi Anemia, Complementation Group D2; FANCD2	227646	3p25.3	*FANCD2**	613984	AR
Fanconi Anemia, Complementation Group E; FANCE	600901	6p21.31	*FANCE**	613976	AR

Fanconi Anemia, Complementation Group I; FANCI	609053	15q26.1	*FANCI**	611360	AR
Fanconi Anemia, Complementation Group L; FANCL	614083	2p16.1	*PHF9**	608111	AR
Focal Dermal Hypoplasia; FDH	305600	Xp11.23	*PORCN*	300651	XLD
Fraser Syndrome 1; FRASRS1	219000	4q21.21	*FRAS1*	607830	AR
Fraser Syndrome 2; FRASRS2	617666	13q13.3	*FREM2*	608945	AR
Fraser Syndrome 3; FRASRS3	617667	12q14.3	*GRIP1*	604597	AR
Frontofacionasal Dysplasia	229400	---	---	---	AR
Frontonasal Dysplasia 1; FND1	136760	1p13.3	*ALX3**	606014	AR
Frontonasal Dysplasia 3; FND3	613456	12q21.31	*ALX1*	601527	AR
Fryns Microphthalmia Syndrome	600776	---	---	---	AR
GOMBO Syndrome	233270	---	---	---	IC
Gorlin-Chaudhry-Moss Syndrome; GCMS	612289	1p13.3	*SLC25A24*	608744	AD
Gracile Bone Dysplasia; GCLEB	602361	11q12.1	*FAM111A*	615292	AD
Hallermann-Streiff Syndrome; HSS	234100	---	---	---	Unknown
Heart and Brain Malformation Syndrome: HBMS	616920	19q13.31	*SMG9*	613176	AR
Hemifacial Microsomia; HFM	164210	14q32	---	---	AD
Holoprosencephaly 1; HPE1	236100	21q22.3	---	---	AD, IC
Holoprosencephaly 2; HPE2	157170	2p21	*SIX3*	603714	AD
Holoprosencephaly 3; HPE3	142945	7q36.3	*SHH*	600725	AD
Holoprosencephaly 7; HPE7	610828	9q22.32	*PTCH1*	601309	AD
Holoprosencephaly 9; HPE9	610829	2q14.2	*GLI2*	165230	AD
Incontinentia pigmenti; IP	308300	Xq28	*IKBKG**	300248	XLD
Joubert Syndrome 22; JBTS22	615665	2q37.1	*PDE6D*	602676	AR
Kapur-Toriello Syndrome	244300	---	---	---	IC
Kabuki Syndrome 1; KABUK1	147920	12q13.12	*KMT2D*	602113	AD
Kabuki Syndrome 2; KABUK2	300867	Xp11.3	*KDM6A*	300128	XLD
Kenny-Caffey Syndrome, Type 2; KCS	127000	11q12.1	*FAM111A*	615292	AD
Klippel-Feil Syndrome 1, Autosomal Dominant; KFS1	118100	8q22.1	*GDF6*	601147	AD
Klippel-Feil Syndrome 3. Autosomal Dominant; KFS3	613702	12p13.31	*GDF3*	606522	AD
Macrosomia with Microphthalmia, Lethal	248110	---	---	---	XLD
Manitoba Oculotrichoanal Syndrome;MOTA	248450	9p22.3	*FREM1*	608944	AR
Meckel Syndrome, Type 1; MKS1	249000	17q22	*MKS1**	609883	AR
Meckel Syndrome, Type 2; MKS2	603194	11q12.2	*TMEM216*	613277	AR
Meckel Syndrome, Type 3; MKS3	607361	8q22.1	*TMEM67*	609884	AR
Meckel Syndrome, Type 4; MKS4	611134	12q21.32	*CEP290*	610142	AR
Meckel Syndrome, Type 5; MKS5	611561	16q12.2	*RPGRIP1L*	610937	AR
Microcephaly and chorioretinopathy, autosomal recessive, 3, MCCRP3	616335	15q15.3	*TUBGCP4*	609610	AR
Microcephaly with or without Chorioretinopathy, Lymphedema or Mental Retardation; MCLMR	152950	10q23.33	*KIF11*	148760	AD
Microphthalmia with Hyperopia, Retinal Degeneration, Macrophakia and Dental Anomalies	251700	---	---	---	Unknown
Microphthalmia, Syndromic 1; MCOPS1	309800	Xq28	*NAA10*	300013	XL
Microphthalmia, Syndromic 2; MCOPS2	300166	Xp11.4	*BCOR*	300485	XLD
Microphthalmia, Syndromic 3; MCOPS3	206900	3q26.33	*SOX2*	184229	AD

Microphthalmia, Syndromic 4; MCOPS4	301590	Xq27-q28	---	---	XLR
Microphthalmia, Syndromic 5; MCOPS5	610125	14q22.3	*OTX2*	600037	AD
Microphthalmia, Syndromic 6; MCOPS6	617932	14q22.2	*BMP4*	112262	AD
Microphthalmia, Syndromic 7; MCOPS7	309801	Xp22.2	*HCCS*	300056	XLD
Microphthalmia, Syndromic 8; MCOPS8	601349	6q21	*SNX3**	601349	AD
Microphthalmia, Syndromic 9; MCOPS9	601186	15q24.1	*STRA6*	610745	AR
Microphthalmia, Syndromic 10;MCOPS10	611222	---	---	---	IC
Microphthalmia, Syndromic 11; MCOPS11	614402	10q25.3	*VAX1*	604294	AR
Microphthalmia, Syndromic 12; MCOPS12	615524	3p24.2	*RARB*	180220	AD, AR
Microphthalmia, Syndromic 13; MCOPS13	300915	Xp28	*HMGB3*	300193	XL
Microphthalmia, Syndromic 14; MCOPS14	615877	4q31.3	*MAB21L2*	604357	AD, AR
Microphthalmia with Cyst, Bilateral Face Clefts and Limb Abnormalities	607597	---	---	---	IC
Microphthalmia with Limb Abnormalities; MLA	206920	14q24.2,11p11.2	*SMOC1,FNBP4**	608488, 615265	AR
MOMO Syndrome	157980	---	---	---	Most likely AD
Mowat-Wilson Sydrome; MOWS	235730	2q22.3	*ZEB2*	605802	AR
Muscular Dystrophy-Dystroglycanopathy, Type A; MDDGA1	236670	9q34.13	*POMT1*	607423	AD
Muscular Dystrophy-Dystroglycanopathy, Type A, 2; MDDGA2	613150	14q24.3	*POMT2*	607439	AR
Muscular Dystrophy-Dystroglycanopathy, Type A, 3; MDDGA3	253280	1p34.1	*POMGNT1*	606822	AR
Muscular Dystrophy-Dystroglycanopathy, Type A, 4; MDDGA4	253800	9q31.2	*FKTN*	607440	AR
Muscular Dystrophy-Dystroglycanopathy, Type A, 5; MDDGA5	613153	19q13.32	*FKRP*	606596	AR
Muscular Dystrophy-Dystroglycanopathy, Type A, 7; MDDGA7	614643	7p21.2-p21.1	*ISPD*	614631	AR
Muscular Dystrophy-Dystroglycanopathy, Type A, 8; MDDGA8	614830	3p22.1	*POMGNT2*	614828	AR
Muscular Dystrophy-Dystroglycanopathy, Type A, 9; MDDGA9	616538	3p21.31	*DAG1*	128239	AR
Muscular Dystrophy-Dystroglycanopathy, Type A, 10; MDDGA10	615041	12q14.2	*RXYLT1**	605862	AR
Muscular Dystrophy-Dystroglycanopathy, Type A, 11; MDDGA11	615181	1q42.3	*B3GALNT2*	610194	AR
Nance-Horan Syndrome; NHS	302350	Xp22.13	*NHS*	300457	XLD

Neurodevelopment Disorder with anomalies of the Brain, Eye and/or Heart; NEDBEH	616975	1p36.23	*RERE*	605226	AD
Norrie Disease; ND	310600	Xp11.3	*NDP*	300658	XLR
Oculoauricular Syndrome; OCACS	612109	4p16.1	*HMX1*	142992	AR
Oculocerebrocutaneous Syndrome	164180	---	---	---	Isolated Cases
Oculodentodigital Dysplasia; ODDD	164200	6q22.31	*GJA1*	121014	AD
Oculodentodigital Dysplasia, autosomal recessive	257850	6q22.31	*GJA1*	121014	AR
Optic disc anomalies with retinal and/or macular dystrophy	212550	14q23.1	*SIX6*	606326	AR
Orofaciodigital Syndrome 6; OFD6	277170	5p13.2	*C5orf42**	614571	AR
Osteoporosis-Pseudoglioma syndrome; OPPG	259770	11q13.2	*LRP5*	603506	AR
Papillorenal Syndrome; PAPRS	120330	10q24.31	*PAX2*	167409	AD
Persistent hyperplasitc primary vitreous, autosomal recessive	221900	10q21.3	*ATOH7*	609875	AR
Popliteal Pterygium Syndrome	263650	21q22.3	*RIPK4*	605706	AR
Renpenning Syndrome; RENS1	309500	Xp11.23	*PQBP1*	300463	XLR
Retinal dystrophy, iris coloboma and comedogenic acne syndrome; RDCCAS	615147	10q23.33	*RBP4*	180250	AR
Rodrigues Blindness	268320	---	---	---	Most likely AR
Steinfeld Syndrome	184705	---	---	---	AD
Single Median Maxillary Central Incisor, SMMCI	147250	7q36.3	*SHH*	600725	Most likely AD
Short Stature, Mental Retardation, Callosal Agenesis, Heminasal Hypoplasia, Microphthalmia and Atypical Clefting	605856	---	---	---	Isolated cases
Skin Creases, Congenital Symmetric Circumferential, Kunze type; CSCSC1/2	616734, 156610	18q21.1-q12.2, 6p21.33	*MAPRE2, TUBB*	605789, 191130	AD
Split-Hand/Foot Malformation; SHFM5	606708	2q31	---	---	AD
Temtamy Syndrome; TEMTYS	218340	12p13.31	*C12ORF57*	615140	AR
Tetraamelia Syndrome 1; TETAMS1	273395	17q21.31-32	*WNT3**		
Townes-Brock Syndrome	107480	16q12.1	*SALL1*	602218	AD
Verheij Syndrome; VRJS	615583	8q24.3	*PUF60*	604819	AD
Waardenburg syndrome, type 2a; WS2A	193510	3p13	*MITF*	156845	AD
Warburg Microsyndrome 1; WARBM1	600118	2q21.3	*RAB3GAP1*	602536	AR
Warburg Microsyndrome 2; WARBM2	614225	1q41	*RAB3GAP2*	609275	AR
Warburg Microsyndrome 3; WARBM3	614222	10p12.1	*RAB18*	602207	AR
Warburg Microsyndrome 4; WARBM4	615663	20p13	*TBC1D20*	611663	AR

Legend: **AD**-Autosomal dominant, **AR**-Autosomal recessive, **XL –** X-linked, **XLD –** X-linked dominant, **XLR –** X-linked recessive, **IC** – Isolated Cases, * little evidence suggesting association with microphthalmia

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

See Table 1, column 4—'Associated gene(s)' and column 5—'OMIM# of associated gene(s) for all genes and related syndromes

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 2: Additional genes associated with syndromic and non-syndromic microphthalmia tested with next-generation sequencing

Gene	*Cytogenetic Location*	*OMIM# of gene*	*Associated disease acronym*	*OMIM# of disease where applicable*
ABCB6	2q35	605452	Microphthalmia, isolated with coloboma 7; MCOPCB7	614497
ADAMTS18	607513	16q23.1	Microcornea, myopic chorioretinal atrophy and telecanthus	615458
ALDH1A3	15q26.3	600463	Microphthalmia, isolated 8; MCOP8	615113
ATOH7	10q21.3	609875	Persistent hyperplasitc primary vitreous, autosomal recessive	221900
BEST1	11q12.3	607854	Microcornea, rod-cone dystrophy, cataract and posterior staphyloma, included; MRCS	193220
	20q13.31			
BMP7	21q22.3	112267	Various ocular abnormalities	---------
CRYAA	22q12.1	123580	Cataract 9, multiple types;CTRCT9	604219
CRYBB1		600929	Cataract 17, multiple types;CTRCT17	611544
	22q11.23			
CRYBB2	2q33.3	123620	Cataract 3, multiple types; CTRCT3	601547
CRYGC	2p22.2	123680	Cataract 2, multiple types; CTRCT2	604307
CYP1B1		601771	Anterior segment dysgenesis 6, mutliple subtypes	617315
	8p21.1			
ESCO2		609353	SC Phocomelia Syndrome	269000
	5q35.1			
FBXW11		605651	Diverse developmental phenotype including brain, eye and digit anomalies	---------
	1p33			
FOXE3	1q21.2	601094	Anterior Segment Dysgenesis 2; ASD2	610256
	1p34.1			
GJA8		600897	Cataract 1, Mutiple Types; CTRCT1	116200
IPO13	11q23.3	610411	Ocular coloboma, microphthalmia and cataract	---------
	11q12.2			
MFRP	19p13.2	606227	Microphthalmia, isolated 5; MCOP5	611040
MYRF		608329	Nanophthalmos	---------
OLFM2	11p13	617492	Bilateral microphthalmia, short stature and facial dysmorphism	---------
PAX6	2q37.1	607108	Ocular malformations within the MAC spectrum	---------
	2p25.3			
PRSS56		613858	Microphthalmia, isolated 6; MCOP	613517
PXDN	18q21.32	605158	Anterior Segment Dysgenesis 7; ASD7	269400
	4q28.2			
RAX	16q23.3	601881	Microphthalmia, isolated 3; MCOP3	611038
SCLT1	6q22.31	611399	Oriofaciodigital Syndrome IX; OFD9	258865
SLC38A8	4q34.3-35.1	615585	Foveal Hypoplasia 2; FVH2	609218
TBC1D32	17q11.2	615867	Oriofaciodigital Syndrome IX; OFD9	258865
TENM3	18q22.1	610083	Microphthalmia, isolated with coloboma 7; MCOPCB7	615145
TMEM98	14q24.3	615949		
TMX3		616102	Nanophthalmos 4	615972
VSX2		142993	---------	---------
			Microphthalmia, isolated 2; MCOP2	610093

1.4 Mutational Spectrum:

An estimated 33-95% of anophthalmia and microphthalmia cases are observed alongside additional non-ocular systemic malformations, with 20-45% of patients diagnosed with a recognised syndrome [2]

Syndromic microphthalmia may be initially difficult to diagnose from birth dependent on the severity of the phenotype and evolution of other signs and symptoms.[3] Only syndromic microphthalmia will be discussed here, but it is important to note for clarity that severe microphthalmia can be used interchangeably with clinical anophthalmia in the literature (see Clinical Utility Gene Card: Non-Syndromic Microphthalmia[1] and Clinical Utility Gene Card: Anophthalmia [4]). Variants in genes such as *ALDH1A3, STRA6, GDF6* and *GDF3* may cause either syndromic or apparent non-syndromic microphthalmia and clear distinctions are hard to make when classifying these genes.

The disease has a complex aetiology with chromosomal, monogenic and environmental causes previously reported.[5,6] Inheritance patterns include autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive, *de novo* sporadic and mosaicism. Mitochondrial disease caused by *HCCS* variants, has also been identified as a cause of syndromic microphthalmia, although inheritance is not mitochondrial but rather X-linked dominant [6]. Similar mitochondrial disorders are caused by *NDUFB11* and *COX7B* variants but microphthalmia was not observed in these patients. [7,8] Precise genetic screening of patients with syndromes historically linked to microphthalmia and now associated with multiple genes, such as mitochondrial disease or CHARGE, will prevent false positives arising where microphthalmia is only associated with one gene. [8,9] The mutational spectrum spans missense, nonsense, deletions, insertions, splice-site variants and chromosomal deletions, duplications and translocations. The more frequently detected variants are described below as syndromic microphthalmia covers a wide range of diseases, some of which are ultra-rare.

SOX2 variants account for 20-40% of autosomal dominant cases and the majority of *SOX2* variants are monoallelic loss-of-function *de novo* sporadic.[10-15] *SOX2* is often screened with *OTX2* in genetic screening of microphthalmia, anophthalmia and coloboma (MAC) and these variants are jointly causal for 60% of all severe bilateral phenotypes [10]. The deletion (NM_003106.3: c.70_89del, p.(Asp24Argfs*65)) is the most frequently detected variant.[11,16] The *SOX2* polyglycine tract between Gly-19 to Gly-23 is a commonly mutated region found in 20% of *SOX2* familial variants.[11] Whole gene deletions have also been found at a rate of 28% in a French patient cohort.[16]

Eye-field transcription factors (EFTFs) are essential for early eye development and account for a large proportion of syndromic microphthalmia cases. All identified *OTX2* variants are heterozygous, with approximately 40% of these *de novo* sporadic.[11, *17-21*] The duplication (NM_172337.3:c.106dup, p.(Arg36Profs*52)) and nonsense variants (NM_172337.1:c.289C>T p.(Gln97*) and NM_172377.1:c.295C>T p.(Gln99*)) have been most frequently reported.[21] *OTX2* whole gene deletions need to be considered when no point variant is found after screening. [3,21] It is important to consider the large phenoptypic variability resulting from *OTX2* variants as these have also been associated with pattern dystrophy of the retinal pigment epithelium, otocephaly-dysgnathia complex, early-onset retinal dystrophy and pituitary dysfunction. [22-24] *OTX2* missense variants are also associated with extreme intrafamilial variability, where observed phenotypes ranged from severe multiple congenital defects including microphthalmia to complete non-pentrance. [20,25]

Contiguous gene deletions mapped to the locus 14q22-q23 are variable in size and result in a phenotype comparing to MCOPS5 [26,27]. These deletions span *OTX2* and can include important non-EFTF genes like *BMP4 [27].* It is important to also consider the large intrafamilial phenotypical variaiblity linked to 14q22 microdeletions during genetic screening. [25] Whole gene deletion of *BMP4*, and missense and frameshift variants within the gene causes syndromic microphthalmia with complex phenotypes including hypopituitarism and digital anomalies. [5,17,18]

PAX6 is a master regulator of ocular development, and variants can result in complex phenotypes. Although most *PAX6* variants have been identified in aniridia patients, multiple

cases of *PAX6* heterozygous variants have been identified in syndromic bilateral microphthalmia.[11] *PAX6* variants are primarily missense (NM_000280.4:c.767T>C; p.(Val256Ala); c.474C>T p.(Arg38Trp); c.418G>C p.(Arg19Pro)) or compound heterozygous (NM_000280.4, c.[718C>T]; [112C>T] p.[(Arg240*)];[(Arg38Trp)] although biallelic variants are extremely rare and associated with severe microphthalmia, microcephaly and profound CNS defects.[16,28-31]

Biallelic heterozygous variants in *MITF* can cause COMMAD syndrome (**c**oloboma, **o**steopetrosis, **m**icrophthalmia, **m**acrocephaly, **a**lbinism, **d**eafness). An autosomal recessive biallelic combination involving at least one dominant-negative variant (NM_000248.3, c.952A>G, p.(Arg318*)) was associated with the disease. [32]

Other transcription factor variants are responsible for syndromic microphthalmia phenotypes. Variants in *FOXE3* can cause autosomal recessive Anterior Segment Dysgenesis 2 (ASGD2) with bilateral microphthalmia and extraocular manifestations. [11,33] Associated variants are primarily truncations and biallelic, with the most common variant a homozygous nonsense variant (NM012186.3, c.[720C>A]; p.(Cys240*)) [34]

PITX3 variants associated with CTRCT11 are most commonly heterozygous and homozygous deletions and duplications. [35,36] Autosomal dominant heterozygous nonsense, frameshift and missense *SALL4* variants have been most frequently identified, while compound heterozygous and *de novo* variants are less common. [37-40]

Two genes in the retionic acid signalling pathway are associated with syndromic microphthalmia. *STRA6* biallelic variants have a higher incidence of syndromic rather than isolated microphthalmia.[41] Most frequently, homozygous or compound heterozygous nonsense and missense variants have been identified in patients with autosomal recessive inheritance.[42-45] Variants in *RARB* can cause both autosomal dominant and recessive MCOPS12. Compound heterozygous nonsense (NM_000965.4, c.355C>T, p.(Arg119*)), indel frameshift (NM_000965.4:c.1205_1206dupp.(Ile403Serfs*15)) and *de novo* missense variants (NM_000965.4:c.1159C>T, p.(Arg387Cys) and NM_000965.4:c.1159C>A, p.[Arg387Ser]) have been identified. [46]

Variants in *NAA10* cause MCOPS1, also known as Lenz Microphthalmia Syndrome, which is an X-linked recessive disorder. An intronic splice-site variant (NG_0.31987.1 [NM_003491.3]: c.471+2T>A, NC_000023.11 [NM_003491.3]: c.471+2T>A) has been identified in an affected family, but missense variants are more frequently detected. [47, 48] MCOPS2, also known as Oculo-facial-cardio-dental disorder, is an X-linked dominant disorder associated with deletions, insertions, duplications and missense (NM_017745.5:c.254C>T, p.[Pro85Leu]) variants in the *BCOR* gene. [49-51] MCOPS14 is associated with *MAB21L2* heterozygous *de novo* and inherited missense variants. [52-54]

ASGD7 is associated with homozygous frameshift, missense and nonsense *PXDN* variants.[55] Heterozygosity for a missense variant (NM_000394.3:c.346C>Tp.(Arg116Cys)) at a highly conserved residue in *CRYAA* was described in multiple cases of CTRCT9. [56,57]
Variants in *CHD7* and *SEMA3E* can cause CHARGE syndrome although microphthalmia is only associated with *CHD7* variants. [58-60] *CHD7 de novo* nonsense and frameshift variants have been identified.[60,61-62] *SALL4* variants are linked with Duane-Ray Radial Syndrome.

All data were mined from primary literature or curated genomic and phenotype databases, including GeneReviews (http://www.ncbi.nlm.nih.gov/books/NBK1116/), Online Mendelian Inheritance in Man, OMIM (http://omim.org/) and Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/gene.php?). Novel data should be shared through these databases. They were last accessed on 14th November 2019

1.5 Analytical Validation

The oculome exome gene panel contains a sub panel for microphthalmia, anophthalmia and ocular coloboma which covers the genes involved in syndromic microphthalmia: *ACTB, ACTG1, ALX1, ALX3, ATOH7, B3GALNT2, BCOR, BMP4, CPLANE1, C12ORF57, CHD7, COL4A1, CRYBA4, DAG1, DPYD, ERCC1, ERCC5, ERCC6, ESCO2, FAM111A, FANCA, FANCD2, FANCE, FANCI, FKRP, FKTN, FNBP4, FOXE3, FOXL2, FRAS1, FREM1, FREM2, GDF3, GDF6, GJA1, GLI2, GRIP1, HCCS, HDAC6, HMGB3, HMX1, IKBKG, ISPD, KIF11, KDM6A, KMT2D, LRP5, MAB21L2, MAF, MAPRE2, MCOLN1, MITF, MKS1, NAA10, NDP, NHS, OLFM2, OTX2, PAX2, PAX6, PDE6D, PHF9, PITX3, POMGNT1, POMGNT2, POMT1, POMT2, PORCN, PQBP1, PTCH1, PXDN, RAB3GAP1, RAB3GAP2, RAB18, RARB, RBP4, RERE, RIPK4, RPGRIP1L, RXYLT1, SALL1, SALL4, SCLT1, SEMA3E, SHH, SIX3, SIX6, SLC38A8, SMCHD1, SMG9, SMOC1, SMO, SNX3, SRD5A3, SOX2, STRA6, TBC1D20, TBC1D32, TFAP2A, TMEM216, TMEM67, TMX, TUBB, VAX1, WNT3, ZEB2* (http://www.labs.gosh.nhs.uk/media/764794/oculome_v8.pdf).

The oculome exome gene panel is important as it compensates for the standard exome capture kits that often miss G-C rich genes including those associated with microphthalmia such as *SIX3*, *PITX3* and *SHH.*

Sanger sequencing is less frequently used to screen genes but is used for validation of identified variants using genomic DNA from a new extraction. This is because different sample collection and processing methodologies, sequencing chemistries, instruments, enrichment techniques and data analysis methods between labs can affect NGS assay results. [63]

It is important to look for segregation to determine whether the variant is *de novo* in isolated cases, providing a higher likelihood it affects function . In clinical practice, array comparative genomic hybridisation (aCGH) or multiplex ligation-dependent probe amplification (MLPA) assay may be performed initially to detect copy-number variations (CNVs), such as deletions or duplications. Some molecular service labs also offer fluorescence in situ hybridisation (FISH) to identify or validate structutral variants such as rearrangements or CNV.

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):

A range of studies have estimated the prevalence of microphthalmia between 2-23 per 100,000 births.[11,64-67] An Israeli study investigating early and late onset foetal microphthalmia in caucasian women, reported a prevalence of 41 per 100,000 pregnancies. [68] Microphthalmia accounts for approximately 3-11% of all blind children born globally and there is little evidence of higher prevalence in ethinic group populations. [69,70] However, one prospective study in the UK reported that children of Pakistani descent were at a 3.7 times higher risk of developing a disease on the MAC spectrum than children of white British descent. [70,71]
Between 60-80% of cases of microphthalmia are syndromic, however, lower incidences were found in a Japanese population with only 31% found with systemic features. [66,72,73-74]
Syndromic involvement is expected at 2.7 times higher in bilateral cases of microphthalmia rather then unilateral cases. [73] Epidemiological data suggest risk factors for microphthalmia are maternal age over 40, multiple births, infants of low birthweight and low gestational age. [67,75,76]

	Yes.	No.
A. (Differential) diagnostics	▬	▭
B. Predictive Testing	▭	▬
C. Risk assessment in Relatives	▬	▭
D. Prenatal	▬	▭

Comment: Because of time constraints such as pregnancy, panel diagnostic, 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: present	genotype or disease: 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 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 owing to suboptimal coverage of some regions of interest depending on enrichment or 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)

2.3.1 if tested by conventional Sanger sequencing

Of those patients that undergo genetic testing of known causative genes with Sanger sequencing, those with bilateral severe cases will have a 75% diagnostic rate if aCGH and the coding regions of the following genes are screened; *SOX2, OTX2, STRA6, , ALDH1A3, PAX6, BMP4.* [77]

2.3.2 if tested by Next-generation sequencing

Variant detection rates are higher when combined WES with aCGH and high-resolution analysis of intragenic microdeletions and microduplications are performed. WGS may aid in the detection of variants affecting function 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 and the severity of the phenotype may lead to a delay in clinical diagnosis. Visual acuity may be unaffected, or only slightly affected in patients with less severe forms of disease.

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 variant, 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?

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 <21mm in adults and <19mm 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, or less frequently through fetal MRI, 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.

This diagnosis can depend on the phenotypic severity, but it can be made relatively easily and cost-effectively, confirmed by axial length measures through ultrasound biomicroscopy. MRI brain and orbit imaging is recommended to delineate severe microphthalmia from clinical anophthalmia, determine integrity of the globe, optic nerve, optic chiasm and any associated brain anomalies. [69,78] If this anomaly is found, children should be investigated within a multidisciplinary team, including paediatricians and clinical geneticists, to ensure it is not syndromic. Further monitoring may be required as systemic 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 a search for systemic involvement to prevent co-morbidity and maximise function, for example, patients with CHARGE syndrome (*CHD7)* suffer from a range of multisystem abnormalities including heart defects, endocrine deficiencies and sensorineural deafness, hence early diagnosis will lead to prompt supportive treatment, having longterm 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 1.9 "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): 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 other complex ocular features. This may limit schooling and professions that require perfect vision. Hence, a clinically confirmed diagnosis can help to provide guidance on career choice.

Syndromic microphthalmia is phenotypically heterogenous yet can affect almost all systems in the human body. As such, this may severely impact the quality of life of a patient and their ability to participate in society without the need for both physical and medical assistance. These syndromes can impact on education and career choice, personal relationship development and participation in many activities, including basic human functions. Infant mortality is also an unfortunate circumstance of many syndromes.

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, 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 variant is identified in the index patient, family members can be tested, but complete clinical 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 1.9 "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[79]; the maximal coronal or axial planes of the orbit are measured, and compared with established eye growth charts. [68]

Non-invasive prenatal diagnosis of aneuploidies and some monogenic disorders can be achieved by molecular testing of cell-free foetal DNA (cffDNA) from maternal plasma [80-85]. While non-invasive prenatal diagnosis of microphthalmia is not currently available, the reduced risk of non-invasive, early screening (7-9 weeks), makes cffDNA a valuable emerging tool for diagnosis of genetic disorders, particularly for patients with known risk. [83,85]

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. Preimplantation diagnosis may be an option for bilateral severe microphthalmia.

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Conflict of Interest

The authors declare no conflict of interest

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ABSTRACT:

CUGC for Syndromic Microphthalmia

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1. Name of the Disease (Synonyms):

See Table 1 – column 1 for 'Name of the Disease'

2. OMIM# of the Disease:

See Table 1 – column 2 for 'OMIM# of the Disease'

3. Name of the Analysed Genes or DNA/Chromosome Segments:

See Table 1, column 4—'Associated gene(s)' for all genes and related syndromes

4. OMIM# of the Gene(s):

See Table 1, column 5—'OMIM# of associated gene(s) for all genes and related syndromes

Review of the analytical and clinical validity as well as of the clinical utility of DNA-based testing for variants in the gene(s) in
- ☐ diagnostic,
- ☐ predictive and
- ☐ prenatal settings and for

- risk assessment in relatives