Microphthalmia, Anophthalmia and Anterior Segment Dysgenesis Panel

Test code: OP0601

The Blueprint Genetics Microphthalmia, Anophthalmia and Anterior Segment Dysgenesis Panel is a 32 gene test for genetic diagnostics of patients with clinical suspicion of anophthalmia or anterior segment dysgenesis disorder.

This panel is specifically designed for differential diagnosis of various developmental eye disorders.

About Microphthalmia, Anophthalmia and Anterior Segment Dysgenesis

Anophthalmia and microphthalmia are rare developmental defects of the globe. Microphthalmia refers to an eye with reduced volume and may be associated with coloboma or with an orbital cyst. Anophthalmia is the absence of one or both eyes. Both anophthalmia and microphthalmia may be unilateral or bilateral, and over 50% may be associated with systemic abnormalities. Anophthalmia and microphthalmia may be inherited as an autosomal dominant, autosomal recessive, or X-linked manner. The major causative gene is SOX2 in which heterozygous, loss of function variants account for 25% of cases. Examples of syndromes associated with anophthalmia/microphthalmia are CHARGE syndrome (CDH7) and COFS syndrome (ERCC2, ERCC5, ERCC6). Anterior segment dysgenesis (ASD) disorders encompass a wide variety of developmental conditions affecting the cornea, iris, and lens. It can be an isolated ocular anomaly or accompanying by systemic defects. Anterior segment anomalies are associated with an approximate 50% risk of glaucoma. Majority of genes associated with ASD show autosomal dominant inheritance. Axenfeld-Rieger syndrome is caused by mutations in PITX2 and FOXC1. The syndrome has an estimated prevalence of 1:200,000.

Availability

Results in 3-4 weeks. We do not offer a maternal cell contamination (MCC) test at the moment. We offer prenatal testing only for cases where the maternal cell contamination studies (MCC) are done by a local genetic laboratory. Read more: http://blueprintgenetics.com/faqs/#prenatal

Gene set description

<table>
<thead>
<tr>
<th>Gene</th>
<th>Associated phenotypes</th>
<th>Inheritance</th>
<th>ClinVar</th>
<th>HGMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB6</td>
<td>Blood group, Langereis system, Pseudohyperkalemia</td>
<td>AD/BG</td>
<td>9</td>
<td>57</td>
</tr>
<tr>
<td>BCOR</td>
<td>Microphthalmia, syndromic, Oculofaciocardiodental syndrome</td>
<td>XL</td>
<td>22</td>
<td>44</td>
</tr>
<tr>
<td>BMP4</td>
<td>Microphthalmia, syndromic, Orofacial cleft</td>
<td>AD</td>
<td>9</td>
<td>42</td>
</tr>
<tr>
<td>CHD7</td>
<td>Isolated gonadotropin-releasing hormone deficiency, CHARGE syndrome</td>
<td>AD</td>
<td>128</td>
<td>746</td>
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<tr>
<td>COL4A1</td>
<td>Schizencephaly, Anterior segment dysgenesis with cerebral involvement, Retinal artery tortuosity, Porencephaly, Angiopathy, hereditary, with nephropathy, aneurysms, and muscle cramps, Brain small vessel disease</td>
<td>AD</td>
<td>27</td>
<td>88</td>
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<tr>
<td>CYP1B1</td>
<td>Glaucoma, primary open angle glaucoma, juvenile-onset, Glaucoma, primary open angle, adult-onset, Glaucoma, primary congenital, Peters anomaly</td>
<td>AR</td>
<td>18</td>
<td>240</td>
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<tr>
<td>ERCC2</td>
<td>Xeroderma pigmentosum, Trichothiodystrophy, photosensitive</td>
<td>AR</td>
<td>18</td>
<td>90</td>
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<tr>
<td>ERCC5</td>
<td>Xeroderma pigmentosum, Xeroderma pigmentosum/Cockayne syndrome</td>
<td>AR</td>
<td>17</td>
<td>51</td>
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<tr>
<td>ERCC6</td>
<td>Xeroderma Pigmentosum-Cockayne Syndrome, De Sanctis-Cacchione syndrome</td>
<td>AD/AR</td>
<td>37</td>
<td>91</td>
</tr>
</tbody>
</table>

https://blueprintgenetics.com/
<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype</th>
<th>Inheritance</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOXC1</td>
<td>Axenfeld-Rieger syndrome, Iridogoniodygenesis, Peters anomaly</td>
<td>AD</td>
<td>21 123</td>
</tr>
<tr>
<td>FOXE3</td>
<td>Aphakia, congenital primary, Anterior segment mesenchymal dysgenesis</td>
<td>AR/AD</td>
<td>3 21</td>
</tr>
<tr>
<td>FOXL2</td>
<td>Premature ovarian failure, Blepharophimosis, epicanthus inversus, and ptosis</td>
<td>AD</td>
<td>69 203</td>
</tr>
<tr>
<td>FRAS1</td>
<td>Fraser syndrome</td>
<td>AR</td>
<td>20 41</td>
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<tr>
<td>FREM1</td>
<td>Bifid nose, Manitoba oculotrichoanal syndrome, Trigonocephaly</td>
<td>AD/AR</td>
<td>8 23</td>
</tr>
<tr>
<td>FOXE3</td>
<td>Oculodentodigital dysplasia mild type, Oculodentodigital dysplasia severe type, Syndactyly type 3</td>
<td>AD</td>
<td>23 103</td>
</tr>
<tr>
<td>HCCS</td>
<td>Linear skin defects with multiple congenital anomalies 1 (MIDAS syndrome)</td>
<td>XL</td>
<td>6 13</td>
</tr>
<tr>
<td>HESX1</td>
<td>Septooptic dysplasia, Pituitary hormone deficiency, combined</td>
<td>AR/AD</td>
<td>11 25</td>
</tr>
<tr>
<td>NDP</td>
<td>Exudative vitreoretinopathy, Norrie disease</td>
<td>XL</td>
<td>25 155</td>
</tr>
<tr>
<td>OCRL</td>
<td>Lowe syndrome, Dent disease</td>
<td>XL</td>
<td>33 251</td>
</tr>
<tr>
<td>OTX2</td>
<td>Microphthalmia, syndromic, Pituitary hormone deficiency, combined, Retinal dystrophy, early-onset, and pituitary dysfunction</td>
<td>AD</td>
<td>16 65</td>
</tr>
<tr>
<td>PAX2</td>
<td>Isolated renal hypoplasia, Papillorenal syndrome</td>
<td>AD</td>
<td>19 82</td>
</tr>
<tr>
<td>PAX6</td>
<td>Aniridia, cerebellar ataxia, and mental retardation (Gillespie syndrome), Keratitis, Coloboma, ocular, Cataract with late-onset corneal dystrophy, Morning glory disc anomaly, Foveal hypoplasia, Aniridia, Optic nerve hypoplasia, Peters anomaly</td>
<td>AD</td>
<td>49 461</td>
</tr>
<tr>
<td>PITX2</td>
<td>Axenfeld-Rieger syndrome, Ring dermoid of cornea, Iridogoniodygenesis, Peters anomaly</td>
<td>AD</td>
<td>13 85</td>
</tr>
<tr>
<td>PQBP1</td>
<td>Renpenning syndrome</td>
<td>XL</td>
<td>8 17</td>
</tr>
<tr>
<td>RAB3GAP1</td>
<td>Warburg micro syndrome</td>
<td>AR</td>
<td>16 58</td>
</tr>
<tr>
<td>SHH</td>
<td>Holoprosencephaly, Microphthalmia with coloboma</td>
<td>AD</td>
<td>29 212</td>
</tr>
<tr>
<td>SIX3</td>
<td>Holoprosencephaly</td>
<td>AD</td>
<td>11 82</td>
</tr>
<tr>
<td>SOX2*</td>
<td>Microphthalmia, syndromic</td>
<td>AD</td>
<td>24 94</td>
</tr>
<tr>
<td>STRA6</td>
<td>Microphthalmia, syndromic, Microphthalmia, isolated, with coloboma</td>
<td>AR</td>
<td>18 31</td>
</tr>
<tr>
<td>TFAP2A</td>
<td>Branchiоoculofacial syndrome</td>
<td>AD</td>
<td>9 42</td>
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<tr>
<td>VPS13B</td>
<td>Cohen syndrome</td>
<td>AR</td>
<td>128 184</td>
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<tr>
<td>ZIC2</td>
<td>Holoprosencephaly</td>
<td>AD</td>
<td>10 112</td>
</tr>
</tbody>
</table>

*Some regions of the gene are duplicated in the genome leading to limited sensitivity within the regions. Thus, low-quality variants are filtered out from the duplicated regions and only high-quality variants confirmed by other methods are reported out. Read more.*

Gene, refers to HGNC approved gene symbol; Inheritance to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR) and X-linked (XL); ClinVar, refers to a number of variants in the gene classified as pathogenic or likely pathogenic in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/); HGMD, refers to a number of variants with possible disease association in the gene listed in Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/). The list of associated (gene specific) phenotypes are generated from CDG (http://research.nhgri.nih.gov/CGD/) or Orphanet (http://www.orpha.net/) databases.
Non-coding disease causing variants covered by the panel

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genomic location HG19</th>
<th>HGVS</th>
<th>RefSeq</th>
<th>RS-number</th>
<th>Comment</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>CHD7</td>
<td>Chr8:61763035</td>
<td>c.5405-17G&gt;A</td>
<td>NM_017780.3</td>
<td>rs794727423</td>
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<tr>
<td>ERCC6</td>
<td>Chr10:50681659</td>
<td>c.2599-26A&gt;G</td>
<td>NM_000124.3</td>
<td>rs4253196</td>
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<tr>
<td>FOXC1</td>
<td>Chr6:1613076</td>
<td>c.*734A&gt;T</td>
<td>NM_001453.2</td>
<td>rs3517904</td>
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<tr>
<td>OCRL</td>
<td>ChrX:128687279</td>
<td>c.239-4023A&gt;G</td>
<td>NM_000276.3</td>
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<td></td>
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<tr>
<td>PAX6</td>
<td>Chr11:31832374</td>
<td>c.-129+2T&gt;A</td>
<td>NM_000280.4</td>
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</tbody>
</table>

Test performance

Blueprint Genetics offers a comprehensive Microphthalmia, Anophthalmia and Anterior Segment Dysgenesis Panel that covers classical genes associated with anophthalmia, anterior segment dysgenesis disorder, Axenfeld-Rieger syndrome, CHARGE syndrome, COFS syndrome, isolated anophthalmia – microphthalmia, microphthalmia, Peters anomaly and septo-optic dysplasia. The genes are carefully selected based on the existing scientific evidence, our experience and most current mutation databases. Candidate genes are excluded from this first-line diagnostic test. The test does not recognise balanced translocations or complex inversions, and it may not detect low-level mosaicism. The test should not be used for analysis of sequence repeats or for diagnosis of disorders caused by mutations in the mitochondrial DNA.

Analytical validation is a continuous process at Blueprint Genetics. Our mission is to improve the quality of the sequencing process and each modification is followed by our standardized validation process. Average sensitivity and specificity in Blueprint NGS Panels is 99.3% and 99.9% for detecting SNPs. Sensitivity to for indels vary depending on the size of the alteration: 1-10bps (96.0%), 11-20 bps (88.4%) and 21-30 bps (66.7%). The longest detected indel was 46 bps by sequence analysis. Detection limit for Del/Dup (CNV) analysis varies through the genome depending on exon size, sequencing coverage and sequence content. The sensitivity is 71.5% for single exon deletions and duplications and 99% for three exons’ deletions and duplications. We have validated the assays for different starting materials including EDTA-blood, isolated DNA (no FFPE) and saliva that all provide high-quality results. The diagnostic yield varies substantially depending on the used assay, referring healthcare professional, hospital and country. Blueprint Genetics’ Plus Analysis (Seq+Del/Dup) maximizes the chance to find molecular genetic diagnosis for your patient although Sequence Analysis or Del/Dup Analysis may be cost-effective first line test if your patient’s phenotype is suggestive for a specific mutation profile.

Bioinformatics

The sequencing data generated in our laboratory is analyzed with our proprietary data analysis and annotation pipeline, integrating state-of-the art algorithms and industry-standard software solutions. Incorporation of rigorous quality control steps throughout the workflow of the pipeline ensures the consistency, validity and accuracy of results. The highest relevance in the reported variants is achieved through elimination of false positive findings based on variability data for thousands of publicly available human reference sequences and validation against our in-house curated mutation database as well as the most current and relevant human mutation databases. Reference databases currently used are the 1000 Genomes Project (http://www.1000genomes.org), the NHLBI GO Exome Sequencing Project (ESP; http://evs.gs.washington.edu/EVS), the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org), ClinVar database of genotype-phenotype associations (http://www.ncbi.nlm.nih.gov/clinvar) and the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk). The consequence of variants in coding and splice regions are estimated using the following in silico variant prediction tools: SIFT (http://sift.jcvi.org), Polyphen (http://genetics.bwh.harvard.edu/pph2/), and Mutation Taster (http://www.mutationtaster.org).

Through our online ordering and statement reporting system, Nucleus, the customer can access specific details of the analysis of the patient. This includes coverage and quality specifications and other relevant information on the analysis. This represents our mission to build fully transparent diagnostics where the customer gains easy access to crucial details of the analysis process.
Clinical interpretation

In addition to our cutting-edge patented sequencing technology and proprietary bioinformatics pipeline, we also provide the customers with the best-informed clinical report on the market. Clinical interpretation requires fundamental clinical and genetic understanding. At Blueprint Genetics our geneticists and clinicians, who together evaluate the results from the sequence analysis pipeline in the context of phenotype information provided in the requisition form, prepare the clinical statement. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals, even without training in genetics.

Variants reported in the statement are always classified using the Blueprint Genetics Variant Classification Scheme modified from the ACMG guidelines (Richards et al. 2015), which has been developed by evaluating existing literature, databases and with thousands of clinical cases analyzed in our laboratory. Variant classification forms the corner stone of clinical interpretation and following patient management decisions. Our statement also includes allele frequencies in reference populations and in silico predictions. We also provide PubMed IDs to the articles or submission numbers to public databases that have been used in the interpretation of the detected variants. In our conclusion, we summarize all the existing information and provide our rationale for the classification of the variant.

A final component of the analysis is the Sanger confirmation of the variants classified as likely pathogenic or pathogenic. This does not only bring confidence to the results obtained by our NGS solution but establishes the mutation specific test for family members. Sanger sequencing is also used occasionally with other variants reported in the statement. In the case of variant of uncertain significance (VUS) we do not recommend risk stratification based on the genetic finding. Furthermore, in the case VUS we do not recommend use of genetic information in patient management or genetic counseling. For some cases Blueprint Genetics offers a special free of charge service to investigate the role of identified VUS.

We constantly follow genetic literature adapting new relevant information and findings to our diagnostics. Relevant novel discoveries can be rapidly translated and adopted into our diagnostics without delay. These processes ensure that our diagnostic panels and clinical statements remain the most up-to-date on the market.

CPT codes

SEQ 81479
DEL/DUP 81479

ICD codes

Commonly used ICD-10 codes when ordering the Microphthalmia, Anophthalmia and Anterior Segment Dysgenesis Panel

<table>
<thead>
<tr>
<th>ICD-10</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q11.0</td>
<td>Anophthalmia</td>
</tr>
<tr>
<td>Q11.2</td>
<td>Microphthalmia</td>
</tr>
</tbody>
</table>

Accepted sample types

- EDTA blood, min. 1 ml
- Purified DNA, min. 5μg
- Saliva (Oragene DNA OG-500 kit)

Label the sample tube with your patient’s name, date of birth and the date of sample collection.

Note that we do not accept DNA samples isolated from formalin-fixed paraffin-embedded (FFPE) tissue.
Resources

- Scottish Sensory Centre
- CHARGE Syndrome Foundation
- American Academy of Ophthalmology
- The Magic Foundation
- NORD - CHARGE Syndrome
- NORD - COFS Syndrome
- Gene Reviews - Microphthalmia
- Gene Reviews - CHARGE Syndrome
- Gene Reviews - Peters Anomaly