ABCA4, RDS (PRPH2), and *ELOVL4* Gene Analysis in Stargardt Disease, Stargardt-Like Macular Disease, Other Macular Dystrophies, and Cone Dystrophies

Clinical Features:
Macular dystrophies are the leading cause of visual impairment leading to irreversible blindness in the developed world**.**

1. ABCA4-related disorders:

Stargardt disease (STGD) / Fundus flavimaculatus (FFM) is the most common autosomal recessive macular dystrophy with an estimated prevalence of 1 in 10,000 individuals. STGD manifests in the first or second decade of life with decreased central vision, progressive bilateral atrophy of the retinal pigment epithelium, and the appearance of orange-yellow flecks distributed in the posterior pole, sometimes extending beyond the vascular arcade. A milder form of the same disorder, fundus flavimaculatus, has a later age at onset, slower progression, and more-widespread distribution of the flecks.

Cone-rod dystrophy (CORD) has an estimated prevalence of 1 in 40,000 individuals. Most patients experience visual loss, impaired color vision, and a central scotoma early in life. During the initial stage of disease, the fundus may be normal or show fine macular lesions and pallor of the optic disc. In the later stages, the fundus shows pigmentary deposits resembling bone spicules, frequently in the macular area. Cone-rod dystrophy is characterized by more severe cone degeneration, which in the electroretinogram (ERG), is distinguished by more distinctive reduction of the photopic cone b-wave amplitude than the scotopic (rod b-wave) amplitude, compared to rod degeneration.

Retinitis pigmentosa (RP) is a disorder characterized by progressive peripheral vision loss and night vision difficulties (nyctalopia) that can lead to central vision loss. In many cases, the degeneration tends to be worse in the inferior retina. As RP affects predominantly the rod photoreceptors, the scotopic ERG is more severely reduced than the photopic ERG.

Age-related macular Degeneration (AMD) is the leading cause of severe central visual impairment among the elderly and is associated both with environmental factors, such as smoking, and genetic factors. While few studies suggested that sequence variants in ABCA4 are associated with AMD[1,2,7] several other reports could not replicate these results and disputed the findings.[4,6,7,11] Therefore, AMD is currently not a recommended indication for ABCA4 testing.

2. PRPH2 (RDS)-related disorders**:**
The phenotype spectrum caused by mutations in the RDS gene is characterized by a remarkable intra- and inter-familial variability.

Macular dystrophies: The Pattern dystrophies constitute a group of disorders characterized by deposits of yellow orange or grey pigment, predominantly in the macular area that can resemble the flecks seen in Stargardt disease. Five main categories of pattern dystrophies are discriminated on the basis of the pattern of pigment distribution: adult-onset foveomacular vitelliform dystrophy (AFVD), butterfly-shaped pigment dystrophy, reticular dystrophy of the pigment epithelium, multifocal pattern dystrophy simulating fundus flavimaculatus. In general, these disorders are relatively benign, manifesting usually in midlife with mild-to-moderate disturbance of central vision. However vision loss may occur in up to 50% of the affected individuals after the age of 70.[25]

Cone-rod dystrophy

Retinitis pigmentosa

3. ELOVL4-related disorder:
Autosomal dominant Stargardt-like macular dystrophy is clinically very similar to Stargardt disease. It is a highly penetrant retinal disorder, with typical onset in childhood characterized by progressive loss of central vision followed by a rapid progression to legal blindness. This disorder is characterized by atrophic macular lesions with sharp borders associated with or without yellow fundus flecks. The lesion becomes more advanced over the course of a few years with increased atrophy of the retinal pigment epithelium that resembles the lesions seen in patients with autosomal recessive Stargardt disease. Temporal atrophy of the optic nerve head is present in almost all patients. Age of onset does vary greatly within and between families.[21]

Inheritance Pattern/Genetics:

1. ABCA4 gene – autosomal recessive
Stargardt disease / FFM : The variant spectrum includes missense (71%), nonsense (6%), splice site (10%), in-frame deletion (9%) and frame-shift (4%) variants1,3,5,8,10,12,15-20. In one patient, a partial gene deletion encompassing exons 20-22 was identified.[21] There are at least 8 different recurrent variants, which collectively account for approximately 61% of the identified disease alleles.[8] The variants 5882G>A (Gly1961Glu), 2588G>C, and 3113C>T (Ala1038Val) have each been described in ~10% of STGD patients in distinct populations. The 2588G>C allele is frequent in the U.S. while completely absent in populations from Southern Europe. The IVS35+2T>C splice site variant is frequent in the Italian population (~10% of

disease alleles) and the variant Ala1038Val appears to be a founder variant in the German population.[8]

arCORD : The majority of pathogenic ABCA4 variants causing arCORD result in protein truncation or mRNA decay, and thus are functional null alleles.

2. ELOVL4 gene – autosomal dominant
Two frameshift and one nonsense variant have been reported in ELOVL4.

3. PRPH2 (RDS) gene - autosomal dominant, with incomplete penetrance. Digenic inheritance with the ROM1 gene has been reported in adRP.

The variant spectrum in RDS includes missense (59%), nonsense (6.9%), splice site (2.6%), frameshift variants (30%), and gross deletions (0.8%). The majority of variants in macular dystrophies are missense variants located in the intradiscal D2 loop.[27] An exception is multifocal pattern dystrophy simulating STGD1/fundus flavimaculatus, which appears to be caused mainly by frameshift variants. A specific RDS missense variant, L185P, causes RP only in patients who also carry a variant in the ROM1 gene.[28]

Test Methods:
The coding regions and splice junctions of the 3 genes of this panel are enriched using a proprietary targeted capture system developed by GeneDx. The targeted regions are sequenced simultaneously by massively parallel (NextGen) sequencing on an Illumina platform with paired-end reads. Bi-directional sequence is assembled, aligned to reference gene sequences based on human genome build GRCh37/UCSC hg19, and analyzed for sequence variants. Capillary sequence is used to confirm all potentially pathogenic variants and to obtain sequence for regions where fewer than 19 reads are achieved by NextGen sequencing.

Test Sensitivity:
1. Macular Dystrophies:
Stargardt disease / FFM: It is estimated that the carrier frequency of ABCA4 variants in the general population is 5-10 in 100. Sequencing of all 50 exons of ABCA4 is expected to identify a pathogenic variant on 66-80% of disease alleles.[2,5,8,9,10]

Autosomal dominant Stargardt-like macular dystrophy: Variants in the ELOVL4 gene have been identified in all individuals diagnosed with autosomal dominant Stargardt-like macular dystrophy who were reported in the literature.[23,24,25]

Autosomal dominant macular dystrophy (adMD): RDS variants account for 7-23% of families with adMD26-27, although reduced penetrance has been observed in about 10% of cases with adMD.

Cone-rod dystrophies:

It is estimated that ABCA4 variants account for arCORD in 24–75% of patients.[2,3,13] RDS variants probably account for a small number of cone and cone–rod dystrophy cases.

Retinitis pigmentosa:

ABCA4 variants are only a minor cause (2–5%) of autosomal recessive RP[2,3,13,14] (see information sheet for arRP). RDS variants account for 8–9% of adRP cases (see information sheet for adRP).

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