AvaGen™
THE GENETIC EYE TEST

PATIENT INFORMATION

Patient ID:20390_P
DOB (MM/DD/YYYY): 01/01/2000
Sex: Male

SAMPLE INFORMATION

Sample ID: Validation20
Sample Type: Buccal Swab
Date Collected: 04/28/2021
Date Received: 05/01/2000
Date Reported: 5/22/2021

ORDERING PROVIDER

Physician Name: Unknown
Clinic Name: 0.0
Test Indication: Yes

FINAL RESULTS

CONDITION TESTED	RESULT	DETAIL	EXPLANATION
Keratoconus (KC)	HIGH genetic risk	95 polygenic risk score	Tested for variants within 75 genes found to be associated with keratoconus.
TGFBI Corneal Dystrophies (CD)	Positive for Lattice Type I	c.1861A>C variant	Tested positive for 1 out of 70 known variants associated with TGFBI corneal dystrophies.

This AvaGen Genetic Test result should be considered with other clinical criteria, the patient's family history and communicated in a setting that includes appropriate genetic counseling.

Keratoconus (KC) Risk Assessment

Based on the polygenic risk score of **95**, this patient's risk for **KC** is **HIGH**.

0 to 33
Low risk

34 to 66
Moderate risk

67 to 100
High risk

THE POLYGENIC KC RISK SCORE: The AvaGen Genetic Eye Test provides a polygenic risk score for individuals tested for their genetic risk for KC. The risk score is the cumulative sum of individual risk contributed by several independent SNPs that were identified in our genetic association study by screening thousands of variants in 75 genes related to corneal structure and function. KC is a complex genetic disease that involves genetic and environmental components as well as their interactions that contribute to the development of the disease. Genetics is an important contributor in KC risk, but it is not the only contributing factor that determines risk for KC.

Avellino Lab USA, Inc. 1505 Adams Drive, Suite B2, Menlo Park, CA 94025 650-396-3741 www.avellino.com
Contact genetic counselor: genetic.counselor@avellino.com ; CLIA ID: 05D2048075 CA ID: CLF-0034331 1* Laboratory Director: Mir Noorbash, PhD

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Keratoconus Polygenic Test Details

Keratoconus risk genes for this patient:

ABCA4, ABCB5, COL2A1, COL4A1, COL5A1, LTBP2

Keratoconus-Related Genes Tested:

ABCA4, ABCB5, ABCC6, ADAMTS18, ADGRV1, AGBL1, ANGPTL7, BEST1, CHST6, COL2A1, COL4A1, COL4A2, COL4A3, COL4A4, COL5A1, COL5A2, COL6A1, COL8A2, COL12A1, COL17A1, CYP4V2, DIAPH1, DOCK9, FOXE3, FYN, GJA8, GSN, HGF, IL1A, IL1RN, IL6, IL10, ITGB1, KERA, KRT3, KRT12, KRT13, KRT15, KRT16, KRT23, KRT24, LCAT, LOX, LRRN1, LTBP2, MAP2K1, MAP3K19, MTOR, MYLK, NLRP1, OVOL2, PAX6, PIK3CG, PIKFYVE, PIK3R1, PRDM5, PTK2, PXDN, PXN, RAF1, RHOA, SFTPD, SHC1, SIX5, SLC4A11, TACSTD2, TCF4, TGFBI, TLN1, UBIAD1, VSX1, WNT9A, WNT9B, ZEB1, ZNF469

Corneal Dystrophy (CD) Test Result

This patient has 1 out of 70 known variants associated with TGFBI corneal dystrophies.

Corneal Dystrophy associated variants within the TGFBI gene in this patient:

POSITIVE for a disease-causing variant, c.1861A>C (p.Tyr621Pro) in TGFBI gene. Heterozygous TGFBI p.Tyr621Pro is a disease-causing variant for Corneal Dystrophy, Lattice Type I.

AvaGen Detects the Following TGFBI Associated Corneal Dystrophies

Granular Type 1	Lattice Type IIIA	Epithelial Basement Membrane
Granular Type 2	Reis-Bucklers	Schnyder's-like
Lattice Type 1	Theil-Behnke	

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Other Variants*:

COL17A1, chr10:g.105816869_105816895del; ZNF469, c.8996G>T, p.G2999V (Pathogenic); ABCA4, c.2971G>C, p.G991R; COL4A3, c.3031C>T, p.R1011C; ADGRV1, c.5239G>A, p.E5429K; AGBL1, c.1408G>A, p.V424I

*Other Variants: These variants were observed in the 75 sequenced genes in this patient. The significance of these variants is currently unknown with respect to Keratoconus. However, they might be relevant to other genetic disorders.
( ) : The term in the parenthesis represents ClinVar classification of these variants(https://www.ncbi.nlm.nih.gov/clinvar/).

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Methodology and Limitations:

After extracting the patient's genomic DNA from a buccal swab sample, a next-generation sequencing (NGS) analysis is carried out. Avellino utilizes a custom panel that primarily targets the coding regions of 75 genes identified to be involved in the structure and function of the eye. Sequence results are aligned with the Genome Reference Consortium Human Genome Build 37 (GRCH37.p13) and a risk score is calculated. The test also screens for 70 known disease-causing TGFBI variants.

TGFBI Corneal Dystrophy Variants Tested: c.337G>A, c.367G>C, c.370C>T, c.371G>A, c.371G>T, c.370C>A, c.393G>T, c.535C>T, c.1209T>G, c.1486C>T, c.1501C>A, c.1504A>G, c.1514T>A, c.1526T>G, c.1526T>C, c.1541G>C, c.1545T>A, c.1548C>G, c.1553T>G, c.1553T>C, c.1565T>A, c.1580T>G, c.1603G>T, c.1613C>G, c.1612A>C, c.1616T>A, c.1619T>C, c.1625C>G, c.1631A>G, c.1637C>A, c.1636G>A, c.1640T>G, c.1640T>C, c.1643G>C, c.1649T>C, c.1652C>A, c.1664G>A, c.1663C>T, c.1673T>G, c.1673T>C, c.1675T>G, c.1694T>C, c.1706T>G, c.1706T>A, c.1715A>G, c.1781G>T, c.1838T>G, c.1856T>A, c.1859C>A, c.1858G>C, c.1861A>C, c.1864A>C, c.1866T>A, c.1866T>G, c.1867G>C, c.1868G>A, c.1870G>A, c.1874T>A, c.1877A>G, c.1877A>C, c.1892T>A, c.1998G>C, c.310_311delTC, c.1618_1620delTTT, c.1714_1716delCAC, c.1838_1849del12, c.1870_1875delGTGGTC, c.1879delG, c.1886_1894dupCCAATGTTC, c.371_378delGCACGGAGinsTC

Other Variants: The AvaGen2 panel is composed of 75 genes that have different levels evidence to cause various Mendelian(monogenic) and non-Mendelian(polygenic) eye disorders like corneal dystrophies and keratoconus. Suspicious variants found in these 75 genes in sequenced patients will be compared against pathogenic and likely pathogenic variants in their respective genes in the publicly available ClinVar database and matches will be reported in the 'Other Variants' grey box. Some of the variants reported in the grey box might have been reported in the ClinVar database as variants of uncertain significance (VUS), likely benign or benign variants as relevant to Mendelian(monogenic) diseases for which they were assessed for originally based upon American College of Medical Genetics (ACMG) variant classification guidelines (Richards S et al 2015).

A patient cohort consisting of 739 cases and 368 controls was used to calculate an Odds Ratio (OR) for each of the 2,335 variants found in the 75 genes tested. A Polygenic Risk Score (PRS) was calculated from the sum of the log for these ORs to determine a risk for developing keratoconus. The PRS is specific to the patient's genome and every individual has a unique PRS.

The availability of an individual's unique genomic information can contribute to preventative health strategies and allows therapy to begin at an earlier stage.

Furthermore, including genomic information into the patient's family's health history (FHH) may be valuable in determining personal health risk factors. NGS has the power to detect very rare variants that often are integral to understanding the etiology of complex, multifactorial diseases, such as

keratoconus, related corneal diseases, and TGFBI corneal dystrophy. Design Studio Software (Illumina Inc.; San Diego, CA) found our targeted panel design to be 97% accurate for calling variants within the test's targeted regions of the genome.

As knowledge of genetic information improves over time, new data may become available in the future that could potentially impact the interpretation of your test results. However, Interpretation of the test results is limited by the information that is currently available. More comprehensive interpretation of test results may be possible in the future as more data and knowledge about the molecular mechanism of keratoconus and TGFBI corneal dystrophies is accumulated.

The laboratory sequences all coding exons for each given transcript. Any variants that do not meet internal quality standards are not reported. This test may not provide detection of certain variants or portions of certain genes due to local sequence characteristics, high/low genomic complexity, or the presence of closely related pseudogenes. Analytically difficult features of the genome, such as deletions and duplications >20bp may not be detected in this assay. Rarely, novel sequence variants may interfere with NGS read creation, sequence alignment, and variant calling. Large deletion and/or mosaic variants may not be detected with this technology. Gross deletions, duplications, and changes from repetitive sequences may not be accurately identified by this methodology.

The laboratory has confidence in the test's ability to track a specimen once it has been received. However, the laboratory takes no responsibility for any specimen labeling errors that occur before the sample arrives at Avellino. Genetic counseling to help to explain test results to the patients and to discuss treatment options is recommended.

Literature References

1. Wang, J. *Nucleic Acids Research* 2018, 46 (8), e47-e47. **2.** Chan, I. S. *Annual Review of Genomics and Human Genetics* 2011, 12, 217-244. **3.** Shendure, *J. Nat Rev Genet* 2004, 5 (5), 335-44. **4.** Lu, Y. *Nat Genet* 2013, 45 (2), 155-63. **5.** Bisceglia, L. *Invest Ophthalmol Vis Sci* 2009, 50 (3), 1081-6. **6.** Hughes, A. E. *Investigative Ophthalmology & Visual Science* 2003, 44 (12). **7.** Czugala, M. *Eur J Hum Genet* 2012, 20 (4), 389-97. **8.** Gajecka, M. *Invest Ophthalmol Vis Sci* 2009, 50 (4), 1531-9. **9.** Tyynismaa, H. *Investigative Ophthalmology & Visual Science* 2002, 43 (10), 3160-3164. **10.** Brancati, F. *Journal of Medical Genetics* 2004, 41 (3), 188-192. **11.** Tang, Y. G. *Genetics in Medicine* 2005, 7 (6), 397-405. **12.** Burdon, K. P. *Human Genetics* 2008, 124 (4), 379-386. **13.** Li, X.;

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