



Patient Information and Requesting Institution

Patient Information

Patient ID: 1234

Date of Birth (MM/DD/YY): 01-09-1993

Sex: Male

Sample Information

Sample ID: 56789

Sample Type: Buccal Swab

Sample Collection Date(MM/DD/YY): 03-27-2019

Date Received(MM/DD/YY): 04-21-2019

Date Reported(MM/DD/YY): 2019-05-19

Ordering Provider

Physician Name: PhysicianName

Clinic Name: ClinicName

Physician Notes (Fill out by physician if needed for age, topography, corneal thickness, etc.):

Indication for Test

HP:0001131 TGFB1 Corneal Dystrophy: An abnormality of the cornea that is characterized by opacity of one or parts of the cornea.

HP:0000563 Keratoconus: A cone-shaped deformity of the cornea characterized by the presence of corneal distortion secondary to thinning of the apex.

HP:0001119 Keratoglobus: Limbus-to-limbus corneal thinning, often greatest in the periphery, with globular protrusion of the cornea.

Pellucid Marginal Degeneration (PMD): a bilateral, noninflammatory, peripheral corneal thinning disorder characterized by a peripheral band of thinning of the inferior cornea

Post Refractive Surgery Ectasia: a condition similar to keratoconus where the cornea starts to bulge forwards at a variable time after LASIK, PRK, or SMILE corneal laser eye surgery.

Glossary

Phenotype- The set of observable characteristics of an individual resulting from the interaction of the individual's genotype with the environment.

Genotype- The genetic constitution of an individual organism.



Allele- One of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome.

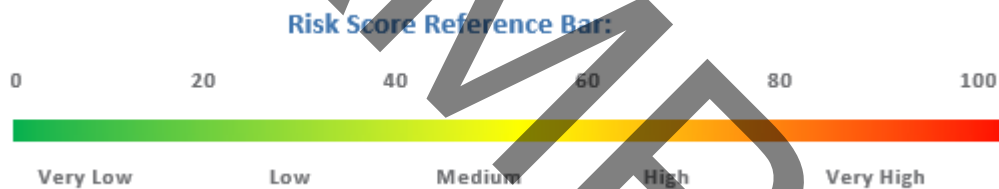
Next Generation Sequencing (NGS) Analysis-The process of determining the nucleic acid sequence of an individual's genome.

Variant of Unknown Significance (VUS)- A sequence variant that, as of the testing date, has not been classified based on a significance to the function or health of an organism.

Report Variant for Keratoconus and *TGFBI* Corneal Dystrophy

Keratoconus

An Integrative Weighted Scoring (IW-Scoring) model¹ was implemented to calculate risk scores from the NGS whole exome and targeted sequencing results of a patient cohort. The patient cohort consisted of 337 keratoconus cases and 147 controls. Using IW-scoring and applying information from the following annotation sources, PhyloP (conservation scores), Polyphen, Mutation Taster, SIFT, LRT, Mutation Assessor and FATTHM, variants for Keratoconus were classified as very low, low, medium, high, and very high risk. The Risk Score Reference Bar below represents the Relative Risk Score ranges. Design Studio Software (Illumina Inc.; San Diego, CA) found our targeted panel design to be 98% accurate for calling variants within the regions of the genome that are tested.



Variants indicating a risk for Keratoconus:

Gene	Transcript	cDNA	Zygoty	AA Change	RiskScore [†]	Relative Risk
AGBL1	NM_152336.2	c.C668T	Heterozygous	p.P223L	45.86	Medium Risk
ZNF469	NM_001127464.1	c.C10804T	Heterozygous	p.R3602C	40.08	Medium Risk

[†]Risk Scores were calculated from an IW Scoring Model

TGFBI Corneal Dystrophy

Variants for *TGFBI* Corneal Dystrophy:

Gene	Transcript	cDNA	Zygoty	AA Change	Reported phenotype
Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected

Variant Interpretation



Keratoconus

AGBL1 ; NM_152336.2 ; c.C668T ; p.P223L ; 45.86 :- ATP/GTP binding protein like 1: Polyglutamylation is a reversible posttranslational modification catalyzed by polyglutamylases that results in the addition of glutamate side chains on the modified protein. Mutations in this gene result in dominant late-onset Fuchs corneal dystrophy. In an Avellino study cohort, this variant was present in 2 out of 337 patients diagnosed with keratoconus.

ZNF469 ; NM_001127464.1 ; c.C10804T ; p.R3602C ; 40.08 :- ZNF469 is expressed in human corneas and a recent study shows that ZNF469 protein shares 30% homology with the helical regions of collagen I and collagen IV suggesting a role in collagen homeostasis and corneal structure. ZNF469 has been found in several studies to contribute to the development of keratoconus. Additionally, the ZNF469 protein may also function as a transcription factor or extranuclear regulator factor in the human cornea. ZNF469 may also be involved in the TGFβ pathway, whose disturbance would lead to the disarray of collagens in human cornea. In an Avellino study cohort, this variant was present in 3 out of 337 patients diagnosed with keratoconus.

All variants are benign or VUS unless otherwise indicated.

NOTES: Genetic counseling is recommended for all variants that show a risk for Keratoconus.

TGFBI Corneal Dystrophy

Not Detected

NOTES: If listed, the patient is positive for the TGFBI Corneal Dystrophy Phenotype.

Data Transfer:

Avellino Lab USA, Inc. (Avellino) recommends that DNA sequence information from this test be stored in the patient's electronic medical record (subject to all applicable laws). This accessibility will facilitate reinterpretation of the sequence in the future in order to best benefit the patient and the patient's family members. Upon request, the company will be pleased to transfer the sequence data, again, subject to all applicable laws.

Methodology

Approach:

After extracting the patient's genomic DNA from a buccal swab sample, a NGS analysis is carried out utilizing 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 to the Human Reference Genome, a risk score is calculated for each rare and deleterious variant and an overall risk assessment for Keratoconus is provided. The test also screens for all known TGFBI corneal dystrophies. If a mutation is found for any of the TGFBI dystrophies it will be indicated in this test report.

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

Next Generation Sequencing Keratoconus and *TGFBI* Corneal Dystrophy

The availability of an individual's unique genomic information can assist in diagnosing disease, contribute to preventative health care strategies and allow therapies to begin at an earlier stage. Further, including genomic information into the patient's family's health history (FHH) may be valuable in determining personal health risk factors.² 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 diseases³ and *TGFBI* corneal dystrophy. Table 1 lists a group of publications emphasizing family-based linkage studies where at least 17 different loci were associated with KC. Additionally, a meta-analysis carried out with a genome wide association study (GWAS) of more than 20,000 individuals in European and Asian populations pointed to a total of 27 different loci linking central corneal thickness (CCT) to KC.⁴



Keratoconus

Table 1

A family based linkage studies for Keratoconus

Chromosome/loci	Study participants	Geographic location	Ref.
5, 9, 14, 15, 16, 18	133 individuals from 25 families	Southern Italy	Bisceglia L, et al. ⁵
15p22.33-p24.2	KC with cataract/familial	Northern Ireland	Hughes AE, et al. ⁶
13q32	15 families	Ecuador	Czugala M, et al. ⁷ Gajecka M, et al. ⁸
16q22.3-q23.1	20 families	Northern Finland	Tyynismaa H, et al. ⁹
3p14-q13	2 generation family	Italy	Brancati F, et al. ¹⁰
5q14.3-q21.1	4 generation Caucasian family	United States	Tang YG, et al. ¹¹
1p36.23-36.21 8q13.1-q21.11	A large Australian pedigree	Australia	Burdon KP, et al. ¹²
4,5,9,12,14,17	Multi ethnic study - 351 individuals from 67 sib pair families	United States	Li X, et al. ¹³
14q24.3	Multi ethnic study - 6 families	England	Liskova P, et al. ¹⁴

TGFBI Corneal Dystrophy

The most studied corneal dystrophies are the ones induced by the *TGFBI* gene, located on chromosome 5q31.1.¹⁵⁻¹⁸ Over 70 different *TGFBI* mutations have been reported worldwide leading to epithelial and stromal corneal dystrophies.¹⁹ Mutations in two hotspots at *TGFBI* amino acid locations, 124 and 555 have been reported to cause the most common *TGFBI* - related corneal dystrophies. These mutations cause a variety of different epithelial and stromal corneal dystrophies resulting in corneal amyloid and non-amyloid deposits.

Limitations and Other Test Notes

Interpretation of the test results is limited by the information that is currently available. More comprehensive interpretation may be possible in the future as more data and knowledge about the pathomechanisms of keratoconus and the *TGFBI* corneal dystrophies are accumulated. Occasionally, a patient may carry an allele which does not capture or



amplify, due to a large deletion or insertion. If so, the report would contain no information about the allele that has not been captured or amplified. The Avellino NGS test is generally not capable of detecting Copy Number Variants (CNVs).

The laboratory sequences all coding exons for each given transcript. Unless specifically indicated, test reports contain no information about other portions of the gene, such as regulatory domains, deep intronic regions or any currently uncharacterized alternative exons.

In most cases, the laboratory is unable to determine the phase of sequence variants. For example, in the event that two likely causative mutations are identified, the laboratory cannot determine whether the mutations are on different chromosomes.

Runs of mononucleotide repeats (eg (A)ⁿ or (T)ⁿ) with n >8 in the reference sequence are generally not analyzed because of strand slippage during amplification.

Unless otherwise indicated, DNA sequence data is obtained from a specific cell-type (epithelial cells from buccal swabs).

Genome build hg19, GRCh37 (Feb2009) is used as Avellino's reference. 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 reproductive options is recommended.

FDA Notes

These results should be used in the context of available clinical findings and should not be used as the sole basis for treatment. This test was developed, and its performance characteristics determined by Avellino. US Food and Drug Administration (FDA) does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing.

Annotation Sources

The Genome Reference Consortium Human Genome Build 37 (GRCH37.p13), 1000Genomes, ExAC, PhyloP (conservation scores), Polyphen, Mutation Taster, SIFT, LRT, Mutation Assessor and FATTHM.

Literature References

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