# Clinical Exome Sequencing Analysis

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|---|---|---|---|
| **Patient name** | **: XXX** | **PIN** | **: XXX** |
| **Gender/ Age** | **: XXX** | **Sample number** | **: XXX** |
| **Referring Clinician** | **: XXX** | **Sample collection date** | **: XXX** |
| **Hospital/Clinic** | **: XXX** | **Sample receipt date** | **: XXX** |
| **Specimen** | **: Peripheral Blood** | **Report date** | **: XXX** |

## Clinical history

XX and XXX are married for 8 years and are planning for pregnancy. XX is presented with severe myopia (-13/-14) and bilateral nystagmus. XX has been evaluated for pathogenic variations.

## Results

**Likely Pathogenic variant was identified in *PAX6* gene**

**List of significant variant identified related to the phenotype:**

| Gene | Region | Variant* | Allele Status | Disease | Classification* | Inheritance pattern |
|---|---|---|---|---|---|---|
| *PAX6* (-) | Exon 7 | c.317G>C **(p.Arg106Pro)** | Heterozygous | Aniridia/ Cataract with late-onset corneal dystrophy **(OMIM#106210)** Anterior segment dysgenesis 5, multiple subtypes **(OMIM#604229)** | **Likely pathogenic** | Autosomal Dominant |

*Genetic test results are based on the recommendation of American college of Medical Genetics [1].
No other variant that warrants to be reported for the given clinical indication was identified.

## Interpretation

***PAX6:* c.317G>C**

**Variant summary:** A heterozygous missense variation in exon 7 of the *PAX6* gene (chr11:g.31801643C>G, NM_001368894.2, Depth: 144x) that results in the amino acid substitution of Proline for Arginine at codon 106 (p.Arg106Pro) was detected.

**Population frequency:** This variant has not been reported in gnomAD database and 1000 genomes database.

**Clinical and Literature evidence:** This variant has been classified as likely pathogenic in ClinVar database [3]. A missense variant (NM_000280.4: c.275G>A ; p. Arg92Gln) has been previously reported in patients affected with congenital aniridia in heterozygous state [4].

***In silico* predictions:** The *in-silico* predictions of the variant are damaging by SIFT, PolyPhen-2 (HumDiv) and LRT. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

**OMIM phenotype:** Aniridia/Cataract with late-onset corneal dystrophy (OMIM#106210), Anterior segment dysgenesis 5, multiple subtypes (OMIM#604229) are caused by heterozygous mutation in the *PAX6* gene (OMIM*607108). Although called aniridia, this disorder is a panocular one taking its name from the noticeable iris hypoplasia seen in most cases. This feature can range from a readily visible, almost complete absence of the iris, through enlargement and irregularity of the pupil mimicking a coloboma, to small slit-like defects in the anterior layer seen only on transillumination with a slit-lamp. The effect on vision is similarly variable.Anterior segment dysgeneses (ASGD or ASMD) are a heterogeneous group of developmental disorders affecting the anterior segment of the eye, including the cornea, iris, lens, trabecular meshwork, and Schlemm canal. The clinical features of ASGD include iris hypoplasia, an enlarged or reduced corneal diameter, corneal vascularization and opacity, posterior embryotoxon, corectopia, polycoria, an abnormal iridocorneal angle, ectopia lentis, and anterior synechiae between the iris and posterior corneal surface These diseases follow autosomal dominant pattern of inheritance [2].

**Variant classification:** Based on the evidence, this variant is classified as a likely pathogenic variant**. In this view, clinical correlation and familial segregation analysis are strongly recommended to establish the significance of the finding. If the results do not correlate, additional testing may be considered based on the phenotype observed.**

## Additional Variant(s)

**The additional variants identified which are significant but may not be related to patient's phenotype. Phenotype – genotype correlation is recommended.**

| Gene | Region | Variant* | Allele Status | Disease | Classification* | Inheritance pattern | Literature evidence |
|---|---|---|---|---|---|---|---|
| *G6PD* (-) | Exon 9 | c.949G>A **(p.Glu317Lys)** | Hemizygous | Hemolytic anemia, G6PD deficient (favism) **(OMIM#300908)** | **Likely Pathogenic** | X-linked | ClinVar: 10401 Pubmed: 32425388 |

**List of significant carrier variants identified:**

| Gene | Region | Variant* | Allele Status | Disease | Classification* | Inheritance pattern | Literature evidence |
|---|---|---|---|---|---|---|---|
| *SLC25A21* (-) | Exon 7 | c.532C>T **(p.Arg178Ter)** | Heterozygous | ?Mitochondrial DNA depletion syndrome 18 **(OMIM#618811)** | **Likely Pathogenic** | Autosomal recessive | ClinVar: 1687162 |
| *TONSL* (-) | Exon 17 | c.2369_2370delCA **(p.Arg790GlyfsTer71)** | Heterozygous | Spondyloepimetaphyseal dysplasia, sponastrime type **(OMIM#271510)** | **Likely Pathogenic** | Autosomal recessive | - |

## Recommendations

- Sequencing the variant(s) in the parents and the other affected and unaffected members of the family is recommended to confirm the significance.
- Alternative test is strongly recommended to rule out the deletion/duplication.
- Genetic counselling is recommended.

## Methodology

DNA extracted from the blood, was used to perform targeted gene capture using a custom capture kit. The targeted libraries were sequenced to a targeted depth of 80 to 100X using Illumina sequencing platform. This kit has deep exonic coverage of all the coding regions including the difficult to cover regions. The sequences obtained are aligned to human reference genome (GRCh38.p13) using Sentieon aligner and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels. Sentieon DNAscope has been used to call the variants. Detected variants were annotated and filtered using the

VarSeq software with the workflow implementing the ACMG guidelines for variant classification. The variants were annotated using 1000 genomes (V2), gnomAD (3.1.2,2.1.1), ClinVar, OMIM, dbSNP, NCBI RefSeq Genes. *In-silico* predictions of the variant was carried out using VS-SIFT, VS-PolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

## Sequence data attributes

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|---|---|
| Total reads generated | 4.63 Gb |
| Data ≥ Q30 | 96.60% |

Genetic test results are reported based on the recommendations of American College of Medical Genetics [1], as described below:

| Classification | Interpretation |
|---|---|
| Pathogenic | A disease-causing variation in a gene which can explain the patients' symptoms has been detected. This usually means that a suspected disorder for which testing had been requested has been confirmed |
| Likely Pathogenic | A variant which is very likely to contribute to the development of disease however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion of pathogenicity. |
| Variant of Uncertain Significance | A variant has been detected, but it is difficult to classify it as either pathogenic (disease causing) or benign (non- disease causing) based on current available scientific evidence. Further testing of the patient or family members as recommended by your clinician may be needed. It is probable that their significance can be assessed only with time, subject to availability of scientific evidence. |

## Disclaimer

- The classification of variants of unknown significance can change over time. Anderson Diagnostics and Labs cannot be held responsible for it.
- Intronic variants, UTR, Promoter region variants and CNV are not assessed using this assay.

- Certain genes may not be covered completely, and few mutations could be missed. Variants not detected by this assay may impact the phenotype.
- The variations have not been validated by Sanger sequencing.
- The above findings and result interpretation was done based on the clinical indication provided at the time of reporting.
- It is also possible that a pathogenic variant is present in a gene that was not selected for analysis and/or interpretation in cases where insufficient phenotypic information is available.
- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity and specificity of variant detection and interpretation due to inability of the data and analysis tools to unambiguously determine the origin of the sequence data in such regions.
- Incidental or secondary findings that meet the ACMG guidelines can be given upon request [5].

## References


1. Richards, S, et al. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine: official journal of the American College of Medical Genetics. 17.5 (2015): 405-424.
2. Amberger J, Bocchini CA, Scott AF, Hamosh A. McKusick's Online Mendelian Inheritance in Man (OMIM). Nucleic Acids Res. 2009 Jan;37(Database issue):D793-6. doi: 10.1093/nar/gkn665. Epub 2008 Oct 8.
3. https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000800417.2
4. Xiao, Ying, et al. "A novel *PAX6* heterozygous mutation found in a Chinese family with congenital aniridia." Genetic Testing and Molecular Biomarkers 23.7 (2019): 495-500. PMID: 31161946.
5. Kalia S.S. et al., Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med., 19(2):249-255, 2017.


### This report has been reviewed and approved by:

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