Novogene

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Whole Exome Sequencing Report

Patient Name	DOB	Gender	Sample Type	Specimen ID	Novogene Case Number
Name	**MM/DD/YYYY**	**Male**	**Saliva**	**N/A**	**WES19-1234567**

Ordering Physician: Dr.XX | Test Ordered: WES Trio

Hospital: Hospital name | Department: Department name

MRN: | Collected:- | Received: MM/DD/YYYY | Report Date: MM/DD/YYYY

CLINICAL HISTORY

Proband: Heterochromia iridis, hypertelorism, depressed nasal bridge

Father: Not affected

Mother: Not affected

RESULT SUMMARY

Finding	Count
1. High Impact Findings	**1** variant
2. Possibly High Impact Findings	0 variant
3. Findings of Variants of Uncertain Significance Related to Patient Phenotype	0 variant
4. Medically Actionable Secondary Findings (Opt In)	0 variant
5. Carrier Status Findings (Opt In)	0 variant

RESULTS:

1. High Impact Findings:

Gene	Variant	Transcript	Chromosome Location	Zygosity	Disease	Inheritance	Classification	Maternal Allele	Paternal Allele
PAX3	c.873dupC	NM_181457.3	chr2:223086025	Het	Waardenburg syndrome	AD	Pathogenic (PVS1, PS2, PM2, PP4)	Wild type	Wild type

Variants Interpretation:

Patient is heterozygous for *PAX3* c.873dupC, which is classified as pathogenic. The variant has occurred *de novo*. Disease caused by *PAX3* variants is inherited in an autosomal dominant manner. The variant has occurred de novo, as it was not observed in the parents (data not shown). For disorders caused by *de novo* mutations, there is a low recurrence risk for the possible siblings because of the possibility of germline mosaicism. Genetic counseling is recommended.

Gene Description:

This gene is a member of the paired box (PAX) family of transcription factors. Members of the PAX family typically contain

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a paired box domain and a paired-type homeodomain. These genes play critical roles during fetal development.

Disease Description:

Waardenburg syndrome type 1 is an autosomal dominant auditory-pigmentary syndrome characterized by pigmentary abnormalities of the hair, skin, and eyes; congenital sensorineural hearing loss; and dystopia canthorum, the lateral displacement of the ocular inner canthi.

Reference:

1) Tassabehji M, Read A P, Newton V E , et al. Mutations in the PAX3 gene causing Waardenburg syndrome type 1 and type 2. Nature Genetics, 1993, 3(1):26-30.

2) Hoth CF, Milunsky A, Lipsky N , et al. Mutations in the paired domain of the human PAX3 gene cause Klein-Waardenburg syndrome (WS-III) as well as Waardenburg syndrome type I (WS-I). American Journal of Human Genetics, 1993, 52(3):455-462.

2. Possibly High Impact Findings

Not detected

3. Findings of Variants of Uncertain Significance Related to Patient Phenotype

Not detected

4. Medically Actionable Secondary Findings (Opt In)

Not detected

5. Carrier Status Findings (Opt In)

Not detected

Summary of the test:

Novogene Whole Exome Sequencing (WES): Clinical grade WES is one of the most comprehensive tools for detection of rare disease causing and associated variants in an individual's DNA. Whole Exome includes all the expressed and majority of the transcribed regions in human genome, and it is believed to cover over 85% of known and to be discovered disease causing genetic variants.

In this assay, human genomic DNA is extracted or provided and is then sheared using ultrasound. The fragments are end-repaired and A-tailed in advance of sequencing library preparation. Pre-capture libraries containing dual-indexed sequencing barcodes are enriched using Agilent SureSelect Human All Exon V6 capture baits, targeting >99% of regions in CCDS, RefSeq and Gencode databases, for coding regions and splice junction sites of ~ 20,000 human genes. The post capture libraries are sequenced on a NovaSeq 6000 instrument (Illumina) to obtain an average coverage depth of ~100x. Multiple quality control steps were performed for sample and derivative quality evaluation.

Data Analysis, Interpretation and Reporting

The sequencing data that passed Novogene's quality control criteria for its clinical WES test according to US CLIA regulations are analyzed using Novogene in-house bioinformatics platform, which aligns sequence data to human genome (GRCh37/UCSC hg19) and performs variant calls and annotation. Variant calling files (VCFs) are uploaded to HIPAA compliant Breakthrough Genomics' genomic data analysis platform ENLITER™. The ENLITER™ platform also performs general QC analysis of the sequence data, such as variant coverage, TS/TV ratio, variant quality, allele fraction spectrum to ensure that the reported variant findings were obtained from high quality sequencing data. Evaluation is focused on coding exons along with flanking intronic bases. All pertinent inheritance patterns are considered. All identified variants are evaluated with respect to their pathogenicity and causality, and these are categorized following ACMG guidelines. All variants are verified to have good raw read quality according to CAP/CLIA recommendations before they are reported out according to HGVS nomenclature. Only those variants with evidence for causing or contributing to

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disease are reported as primary findings. Incidental findings that do not correlate with the provided phenotype(s) are reported according to "ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing". (PMID: 23788249, see Appendix Table 1), if elected to be included in the report by the patient or legal guardian.

Test Limitations

Absence of a primary diagnostic finding identified by this test does not exclude the possibility of a genetic basis for the clinical condition for this proband. Variants in the intronic, UTR and promoter regions and other copy number variants are not intended to be detected by this assay. Specifically, detection of abnormal variants depends on the presence of these sequence variants in the targeted region that was sequenced. Some genes have inherent sequence properties (for example: long repetitive sequences, chromosomal rearrangements, tri-nucleotide repeat expansions, high GC content, homology, intronic variants outside the splice-site and epigenetic effects) that may result in suboptimal data, and variants in those regions may not be reliably identified. Therefore, it is possible that the gene region where a disease-causing mutation exists in the patient was not sequenced using the current technologies of this test and therefore was not detected.

It is possible that a particular genetic variant may not be recognized as the underlying cause of the genetic disorder due to incomplete scientific knowledge about the biological function of all genes in the human genome and the impact of variants in those genes. Variants interpretation may change over time as more information about genotype/phenotype correlation in specific genes becomes available. Results should be interpreted in the context of clinical findings, relevant medical history, family history, genealogy, and other laboratory examination data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. The results of this testing, including the benefits and limitations, should be discussed with your healthcare provider.

CLIA Statement

This Whole Exome Sequencing test was developed, and its performance characteristics established by the Novogene Laboratory. This laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing.

<signature>____________________________________ ____________________

<Name>, Ph.D., FACMGG, Clinical Consultant Date

Disclaimer: The technical component was performed at Novogene Laboratory. The professional component was performed by Laura Li, Ph.D. ABMGG, CCG, Breakthrough Genomics, 15375 Barranca Pkwy, B205, Irvine, CA 92618(CLIA# 05D2166862).

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Appendix A

Table 1: Medically Actionable Incidental Findings as Recommended by ACMG

ACTA2	*ACTC1*	*APC*	*APOB*	*LDLR*	*ATP7B*	*BMPR1A*	*BRCA1*	*BRCA2*	*COL3A1*
DSC2	*DSG2*	*DSP*	*FBN1*	*GLA*	*KCNH1*	*KCNQ1*	*LMNA*	*MEN1*	*MLH1*
MSH2	*MSH6*	*PMS2*	*MUTYH*	*MYBPC3*	*MYH11*	*MYH7*	*MYL2*	*MYL3*	*NF2*
OTC	*PCSK9*	*PKP2*	*PRKAG2*	*PTEN*	*RB1*	*RET*	*RYR1*	*CACNA1S*	*RYR2*
SCN5A	*SDHAF2*	*SDHB*	*SDHC*	*SDHD*	*SMAD3*	*SMAD4*	*STK11*	*TGFBR1*	*TGFBR2*
TMEM43	*TNNI3*	*TNNT2*	*TP53*	*TPM1*	*TSC1*	*TSC2*	*VHL*	*WT1*	

Table 2: Genes and Diseases for Carrier Status Reporting as Recommended by ACOG

Disease （N=18）	**Gene (N=21)**
Cystic Fibrosis	*CFTR*
Beta-thalassemia and Sickle Cell	*HBB*
Niemann-Pick Disease	*NPC1, SMPD1*
Gaucher Disease	*GBA*
Tay-Sachs Disease	*HEXA*
Maple Syrup Urine Disease	*DBT, BCKDHA, BCKDHB*
Glycogen Storage Disease: Type Ia	*G6PC*
Bloom Syndrome	*BLM*
Fanconi Anemia	*FANCC*
Familial Dysautonomia	*IKBKAP*
Familial Hyperinsulinism	*ABCC8*
Canavan Disease	*ASPA*
Mucolipidosis IV	*MCOLN1*
Joubert Syndrome	*TMEM216*
Galactosemia	*GALT*
Phenylketonuria	*PAH*
Smith–Lemli–Opitz syndrome	*DHCR7*
Medium-chain acyl-CoA dehydrogenase deficiency	*ACADM*

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Table 3: Report Components Description

Report Sections	Examples of Variant Types
1. High impact finding related to patient phenotype	1. AD, X-linked: pathogenic 2. AR: pathogenic/pathogenic; or pathogenic / likely pathogenic
2. Possibly impact finding related to patient phenotype	1. AD, X-linked: likely pathogenic 2. AR: pathogenic/VUS; or likely pathogenic / likely pathogenic; or likely pathogenic/VUS
3. Findings of variants of uncertain significance related to patient phenotype	1. AD, X-linked: VUS 2. AR: VUS/VUS
4. Medically actionable incidental findings, can be opted in (Table 1)	Pathogenic or likely pathogenic variants
5. Carrier status, can be opted in (Table 2)	Pathogenic or likely pathogenic variants

Demo Report