XomeDx Report
Whole Exome Sequence Analysis

Patient Name:	**GeneDx Accession No:**
Date of Birth:	**Date Specimen Obtained:**
Specimen Type:	**Date Specimen Received:**
Submitters ID No:	**Date Test(s) Started:**
Ordered By:	**Date of Report:**

Test Requested: Diagnostic Testing / XomeDx / Whole Exome Sequence Analysis

Clinical Indication: Seizures

Results: **SEE INTERPRETATION. Three sequence variants with potential clinical relevance identified in disease-related genes.**

Possible Disease-Associated Genes Identified:

Gene	Variant	cDNA	Codon	Zygosity	Maternal Allele	Paternal Allele	Classification
XXXX	p.Ala3716Thr (A3716T)	c.11146 G>A	GCC/ACC	Het	Het	Wildtype	Variant of Unknown Significance
YYYY	p.Arg2699Stop (R2699X)	c.8095 C>T	CGA/TGA	Het	Het	Wildtype	Mutation
ZZZZ	p.Ala69Val (A69V)	c.206 C>T	GCG/GTG	Homoz	Het	Het	Variant of Unknown Significance

Interpretation: **Heterozygous for the A3716T Variant of Unknown Significance in the XXXX gene.**

XXXX p.A3716T: p.Ala3716Thr (GCC>ACC): c.11146 G>A in exon 58 of the XXXX gene (NM_XXXX). This individual's mother is a carrier of the A3716T variant.

Mutations in the XXXX gene are associated with XXXX syndrome, an autosomal recessive disease characterized by distinctive facial features, opthalmologic findings, seizures, non-progressive psychomotor retardation and microcephaly. This individual is heterozygous for one variant of unknown clinical significance in the XXXX gene.

The A3716T missense change represents a non-conservative amino acid substitution, as a non-polar Alanine is replaced by a polar Threonine, at an amino acid residue that is highly evolutionarily conserved. This variant has been observed previously at GeneDx in a patient referred for XXXX gene analysis and in whom another variant of unknown significance was identified. The NHLBI ESP Variant Server reports A3716T was observed in 40/10,712 alleles (0.37%) from Caucasian and African-American individuals with an unknown phenotype, indicating it is not a common benign variant in these populations. However, pathogenic missense changes in the XXXX gene are rare, as the vast majority of disease-causing mutations are nonsense and frameshift mutations. Although XXXX syndrome is an autosomal recessive disorder, approximately 17-40% of patients with XXXX syndrome will have only one identifiable mutation in the XXXX gene (REF1 et al., 2003; REF2 et al., 2004; REF3 et al., 2004). Additionally, no deletion/duplication involving the XXXX gene was detected by the previously completed whole genome microarray analysis (GenomeDx), which has exon-level coverage of this gene.

With the clinical and molecular information available at this time, the clinical significance of the A3716T variant in the XXXX gene is unknown. Clinical correlation and evaluation of this patient's phenotype is recommended to determine whether this genetic disorder is consistent with this patient's condition.

Patient Name:	**GeneDx Accession No:**
Date of Birth:	**Date Specimen Obtained:**
Specimen Type:	**Date Specimen Received:**
Submitters ID No:	**Date Test(s) Started:**
Ordered By:	**Date of Report:**

YYYY p.R2699X:

Heterozygous for the R2699X Mutation in the YYYY gene.

p.Arg2699Stop (CGA>TGA): c.8095 C>T in exon 43 of the YYYY gene (NM_YYYY). This individual's mother is a carrier of the R2699X mutation.

Mutations in the YYYY gene are associated with YYYY syndrome, an autosomal recessive disease characterized by distinctive craniofacial features, ocular findings, seizures, intellectual disability and sensorineural hearing loss (REF4 et al., 2008).

This individual is heterozygous for the R2699X nonsense mutation in the YYYY gene. Although this mutation has not been reported previously to our knowledge, it is expected to result in nonsense-mediated mRNA decay or in protein truncation. As this is an autosomal recessive disorder, a second mutation was not detected in the YYYY gene, although a partial gene deletion or duplication cannot be ruled out.

The finding of a single R2699X mutation in YYYY is not sufficient to establish a diagnosis in this patient without further studies. Clinical correlation and evaluation of this patient's phenotype is recommended to determine whether this genetic disorder is consistent with this patient's condition. Clinical findings and biochemical studies must also be considered in the diagnosis of this patient.

ZZZZ p.A69V :

Homozygous for the A69V Variant of Unknown Significance in the ZZZZ gene.

p.Ala69Val (GCG>GTG): c.206 C>T in exon 1 of the ZZZZ gene (NM_ZZZZ). This individual's mother and father are heterozygous for the A69V variant.

The ZZZZ gene is part of a pathway that is important in _____ (O'Poirier, 2003). Mutations in the ZZZZ gene have not been associated with human clinical disease, to date; however, a missense polymorphism (G160A) in the ZZZZ gene has been reported in the literature to be associated with _____. This gene is activated by _____ through dephosphorylation, which induces translocation of the ZZZZ protein to the nucleus where it associates with XXXX. In addition, the ZZZZ gene interacts with the YYYY gene to activate a pathway that influences _____.

The A69V missense change represents a conservative amino acid substitution as both Alanine and Valine are neutral, non-polar residues. The A69V missense change occurs at a residue that is conserved across species. The A69V variant has been described previously as a single mutation detected in a patient with unusual postering, global developmental delay, hypotonia, dysmorphic facial features, seizures and high urine malonate levels with a normal opthalmological examination (REF5 et al., 2003). However, the A69V variant was found to result in only mildly reduced mRNA expression, and enzyme activity levels were not determined by REF5 et al. The A69V variant was observed in 27/7,7511 alleles (0.34%) from Caucasian and African-American individuals with unknown phenotype, indicating it is not a common sequence change in these populations.

With the information available at this time, the clinical significance of the A69V variant in the ZZZZ gene is unknown. Caution is advised in the interpretation of variants in genes previously unknown to be associated with clinical disease.

Patient Name:	**GeneDx Accession No:**
Date of Birth:	**Date Specimen Obtained:**
Specimen Type:	**Date Specimen Received:**
Submitters ID No:	**Date Test(s) Started:**
Ordered By:	**Date of Report:**

Follow-up Testing: Whole genome microarray analysis (GenomeDx) and the comprehensive epilepsy panel have been completed and results were reported separately.

Recommendations: As only a single mutation or variant was identified in each of the XXXX and YYYY genes in this patient, clinical correlation and evaluation of this patient's phenotype, including biochemical studies, is recommended to determine whether any genetic disorder described here is consistent with the patient's condition. It is possible that this patient has a pathogenic mutation outside of the coding regions analyzed, or in a regulatory region or deep intronic region that would not be detected by whole exome sequencing. Clinical whole genome sequencing, which may be able to determine the presence of any additional pathogenic mutations that could contribute to this patient's phenotype, is recommended. Referral to research programs focusing on genes in the ZZZZ pathway may be helpful to evaluate the significance of the variant identified in ZZZZ in this patient. Genetic counseling is recommended to discuss the implications of this test report.

Methods:

Genomic DNA was extracted from the submitted specimen and additional familial specimens (Mother, GeneDx ID; Father, GeneDx ID; and Brother, GeneDx ID) and the Agilent SureSelect XT2 v4 kit was used to target the exon regions of their genomes. These targeted regions were sequenced using the Illumina HiSeq 2000 sequencing system with 100bp paired-end reads. The DNA sequence was mapped to and analyzed in comparison with the published human genome build UCSC hg19 reference sequence. The targeted coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage and data quality threshold values*. The GeneDx XomeAnalyzer was used to evaluate sequence changes in this individual compared to the other provided family members. The complete coding sequence was analyzed for the XXXX and ZZZZ genes and 99% of the coding sequence of the YYYY gene was analyzed. All reportable sequence variants in the proband and parental samples were confirmed by conventional di-deoxy DNA sequence analysis using a new DNA preparation.

Genomic DNA from this specimen was also examined by array-based comparative genomic hybridization (aCGH) using the current version of ExonArrayDx for the ZZZZ gene. The array contains multiple oligonucleotide probes in all exons and/or their flanking intronic regions in the ZZZZ gene. Hybridization data were analyzed with Genomic Workbench v5 software (Agilent Technologies) to evaluate the copy number at the exon level. The ExonArray is designed to detect most single- exon deletions and duplications. Probe sequences and locations are based on Genome Reference Consortium build 37 (GRCh37)/UCSC hg19.

**Quality Metrics:*

Mean Depth of Coverage[1]	121X
Exome targeted region covered[2]	99.8%
Quality threshold[3]	97.2%

The above values represent metrics from this XomeDx evaluation. 1Mean depth of coverage refers to the sequence mean read depth across the XomeDx targeted region, defined as coding exons and splice junctions of Agilent SureSelect XT2 v4 kit targeted protein coding RefSeq genes. 2The total XomeDx target region covered at 1x. 3The quality threshold refers to the percentage of the XomeDx defined target region where read depth was at least 10x coverage to permit high quality exome variant base calling, annotation and evaluation. Average quality thresholds may range from >90-95% of the XomeDx targeted region, indicating a small portion of the target region may not be covered with sufficient depth or quality to confidently call variant positions.

Absence of a definitive disease-causing mutation identified with the XomeDx test does not exclude the possibility of a genetic basis for the genetic disorder in the proband. Some types of genetic abnormalities may not be detectable with the technologies performed with the XomeDx test. It is possible that the genomic region where a disease causing mutation exists in the proband was not captured using the current technologies and therefore was not detected. Additionally, it is possible that a particular genetic

Patient Name:	**GeneDx Accession No:**
Date of Birth:	**Date Specimen Obtained:**
Specimen Type:	**Date Specimen Received:**
Submitters ID No:	**Date Test(s) Started:**
Ordered By:	**Date of Report:**

Limitations: abnormality may not be recognized as the underlying cause of the genetic disorder due to incomplete scientific knowledge about the function of all genes in the human genome. Only variations in genes associated with the medical condition, or thought to potentially be clinically relevant to the proband's medical condition are reported here. The clinical implications of some variations may not be known at the time of this report.

A medical provider can request reanalysis of the exome data generated here on an annual basis. Current data can be reassessed for the presence of any variants that may be linked to newly characterized genes and/or disorders identified since the date of this report and could be associated with the patient's phenotype, based on available scientific information.

Report Electronically Signed by: Report Electronically Signed By:

References:……
Exome Variant Server, NHLBI Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) [3/2012].