Personalis®

Clinical Report - Neurome™ Neurological Exome

Requested By	
Physician	On file with referring laboratory
Referring Laboratory	Athena Diagnostics, Inc., Marlborough, MA

Patient	
Patient Name	David Jones
Gender	Male
Ethnicity	Caucasian
Birth Date	1/1/2001
MRN	12345678
Family ID	NEU100

Specimen	
Accession #	NEU100A1
Specimen Type	DNA
Lab	Personalis
Collected	3/2/2014 0:00:00
Received	3/3/2014 0:00:00
Limiting Specimen Conditions	None
Specimens	Proband

Case History	
Clinical Diagnosis	Myotonic dystrophy
Clinical features provided	Seizures, muscle weakness, muscle stiffness/cramping, enlarged muscles, speech delay, autism spectrum disorder, hypospadias, ventricular septal defect. Family History: Maternal uncle with muscle cramping/pain
Consanguinity	None reported

Final Result Summary

The following variant(s) has/have been confirmed by capillary electrophoresis testing.

Only the variant(s) with the strongest phenotypic overlap are reported. Please see the Result Details and Neurome™ Assay Information page for additional information regarding methodology and limitations.

1 Variants in Genes Associated with Case History

A pathogenic variant in *CLCN1*, p.Gly230Glu (c.689G>A), has been detected heterozygously in the affected patient.This variant has been previously described in association with disease in the literature.

Clinical Diagnostic Interpretation: Variants in *CLCN1* have been previously reported in the literature in association with both autosomal dominant and autosomal recessive myotonia congenita, diseases with phenotypic overlap with the clinical features described in this patient. This result supports a diagnosis of autosomal dominant myotonia congenita (Thomsen's Disease) and is consistent with the reported family history for this patient. However, because this variant has also been seen in association with recessive disease this result is consistent with this patient being at least a carrier for autosomal recessive myotonia congenita.

The parents were not available to confirm segregation of this variant. Parental testing may clarify the above interpretation and provide information about whether a parent also carries this variant. Testing of additional related individuals may support the current interpretation and/or provide information about who in the family also carries the detected variant.

Recommendation: The results of this test should be interpreted in the context of a clinical presentation, in conjunction with other test results, and in consultation with a physician. Genetic counseling is recommended.

2 Detection of Regions of Homozygosity

No indication of consanguinity or uniparental disomy was observed.

Result Details

1 Variants in Genes Associated with Case History

Details

Gene: *CLCN1*

Neurome™ covered >99.9% of coding bases of this gene at a depth of at least 20x

Summary of disease- and gene-specific literature

"Myotonia congenita is characterized by muscle stiffness present from childhood; all striated muscle groups including the extrinsic eye muscles, the facial muscles, and the tongue may be involved. Men are more severely affected than women. Stiffness is relieved by repeated contractions of the muscle (the "warm-up" phenomenon). Muscles are usually hypertrophic. The autosomal recessive form of myotonia congenita is often associated with more severe stiffness of muscles than the autosomal dominant form. Individuals with the autosomal recessive form may have progressive, minor distal weakness and attacks of transient weakness brought on by movement after rest. The age of onset is variable: in autosomal dominant myotonia congenita, onset of symptoms is usually in infancy or early childhood; in the autosomal recessive form, the average age of onset is slightly older. In both, onset may be as late as the third or fourth decade of life." (Duno et al., 2005)

Classification	Pathogenic	Nucleotide coordinate	c.689G>A (reference: NM_000083.2)
rsID	80356700	Protein coordinate	p.Gly230Glu (reference: NP_000074.2)
Exon	5	Genomic coordinate	chr7:g.143018934G>A (reference: NC_000007.13)
Mutation Type	Missense	Genome Reference	GRCh37 (GCF_000001405.22)

Interpretation for c.689G>A (p.Gly230Glu)

A pathogenic variant in *CLCN1*, p.Gly230Glu (c.689G>A), has been detected heterozygously in the affected patient.This variant has been previously described in association with disease in the literature. Parents were not available to confirm segregation of this variant. Segregation analysis in the parents may aid in the interpretation of this variant. The classification is supported by the following lines of evidence:

The p.Gly230Glu variant has been previously associated with both recessively and dominantly inherited myotonia congenita. The p.Gly230Glu variant has been observed to segregate with dominantly-inherited myotonia congenita, also known as Thomsen's disease, in multiple individuals in three unrelated families affected with milder phenotypic features. Common features of these individuals included EMGs consistent with myotonia, impeded muscle relaxation after exercise, difficulty initiating movement, muscle cramping, stiffness, weakness, pain, and hypertrophy. Disease onset was variable, ranging from childhood through adulthood (Koty et al., 1996).

Haplotype analysis indicated that the phenotype and the p.Gly230Glu allele propagated from a common ancestor of these families. Among the 16 individuals with a confirmed p.Gly230Glu variant, 13 individuals were considered affected, 1 individual seemed to be non-penetrant (asymptomatic at the age of 35 with a normal EMG), 1 individual did not report any symptoms and was not evaluated clinically, and 1 individual died before clinical evaluation was carried out (Koty et al., 1996).

The effect of the p.Gly230Glu variant on protein function has been assessed *in vitro*. In comparison to wildtype protein, the p.Gly230Glu variant causes substantial changes in anion and cation selectivity as well as a fundamental change in rectification of the current-voltage relationship. Both ion-binding sites are preserved on the variant protein but the variant protein exhibits different affinities to ions than the wild-type channel. The cation-to-anion permeability ratio of the variant protein is also much greater than that of the wild-type channel (Fahlke et al., 1997).

The variant is predicted to have a deleterious effect on protein function by multiple *in silico* models, including SIFT and PolyPhen-2. The nucleotide guanine and the amino acid residue glycine are conserved at their respective positions across all mammalian species. The variant is absent from healthy genome datasets including 1000 genomes, NHLBI GO-ESP, and the UK10K project.

Capillary electrophoresis testing confirmed that the pathogenic variant is present heterozygously in the affected patient.

Recommendation

The results of this test should be interpreted in the context of a clinical presentation, in conjunction with other test results, and in consultation with a physician. Genetic counseling is recommended. Testing of additional related individuals may support the current Interpretation and/or provide information about who in the family also carries the detected variant. Please contact us to facilitate analysis for family members.

2 Detection of Regions of Homozygosity

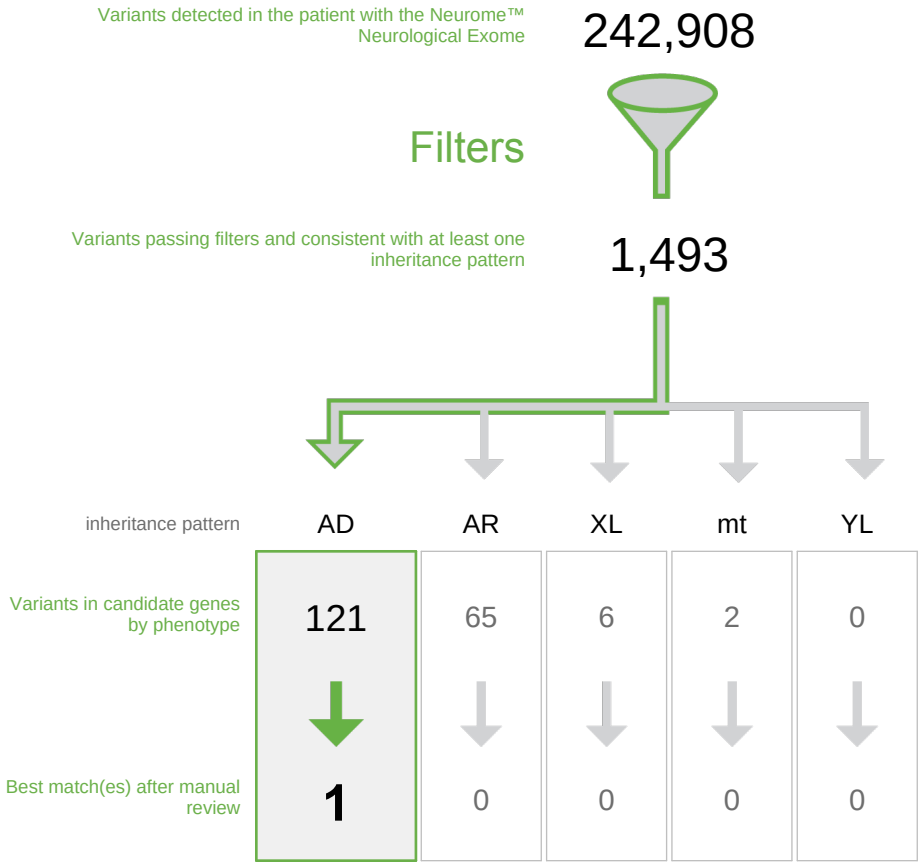
No indication of consanguinity or uniparental disomy was observed.

No regions of abnormal autozygosity were identified in the patient. Autozygosity can indicate consanguinity or uniparental disomy, depending on its abundance and distribution.

Samples Analyzed

Only the proband (accession#:NEU100A1) was submitted for analysis. Neurome™ sequencing and confirmatory capillary electrophoresis was performed on this sample.

Analysis



Filters
Quality
Eliminates variants failing quality metrics as determined by the Neurome™ Pipeline
Population Frequency
Eliminates common variants (>1% allele frequency in the general population)
Inheritance Pattern
Variants are considered under the inheritance patterns that are consistent with the observed genotype of the variant(s), unless that pattern is impossible given the family history. For analyses in which exome sequencing was performed on the patient only, variation may be inherited or occur as a new mutation (*de novo*) for any of the following categories.
AD: autosomal dominant (including reduced penetrance, unless inappropriate)
AR: autosomal recessive (including homozygous and compound heterozygous states)*
XL: X-linked (including dominant and recessive modes, and reduced penetrance or skewed X-inactivation, unless inappropriate)
mt: mitochondrial (including reduced penetrance, unless inappropriate)
YL: Y-linked (including reduced penetrance, unless inappropriate)

Candidate Genes by Phenotype
Variants are ranked based on the gene's likelihood to explain the patient's reported clinical features. Reported clinical features are compared to the clinical features observed in individuals with disease-causing variants in a gene. All variants in genes above the ranking threshold are included.

Manual Review
High-ranking candidate variants are manually reviewed using available gene and variant-based evidence.

* For samples submitted as patient-only, compound heterozygous variations may be associated or not with the disease based on whether they are located in trans or cis, respectively. When available, targeted parental testing may be used to ascertain phase-transmission of the identified heterozygous variations. In the absence of parental DNA for testing, Personalis conclusions are derived by assuming heterozygous variants with pathogenic interpretation are located on separate alleles (in trans). This assumption may be supported by targeted testing of additional related family members. Homozygous variations interpreted as Pathogenic may actually be the result of hemizygosity. When available, targeted parental testing may rule out hemizygosity.

References

Dunø M, Colding-Jørgensen E. Myotonia Congenita. 2005 Aug 03 [updated 2011 Apr 12]. In: Pagon RA, Adam MP, Ardinger HH, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2014. Available from http://www.ncbi.nlm.nih.gov/books/NBK1355/ PubMed PMID: 20301529.

Koty PP, Pegoraro E, Hobson G, Marks HG, Turel A, Flagler D, Cadaldini M, Angelini C, Hoffman EP. Myotonia and the muscle chloride channel: dominant mutations show variable penetrance and founder effect. Neurology. 1996 Oct;47(4):963-8. PubMed PMID: 8857727.

Fahlke C, Beck CL, George AL Jr. A mutation in autosomal dominant myotonia congenita affects pore properties of the muscle chloride channel. Proc Natl Acad Sci U S A. 1997 Mar 18;94(6):2729-34. PubMed PMID: 9122265; PubMed Central PMCID: PMC20158.

Neurome™ Assay Information

General Information: The Athena Neurome™ is a clinical diagnostics service appropriate when a patient's medical history and physical exam suggest a syndrome of unknown genetic etiology.

Methodology: Genomic DNA is provided to Personalis for analysis. Personalis will create indexed genomic libraries using our proprietary Neurome™ library preparation kits. These libraries will be pooled with other indexed libraries and enriched using the Neurome™ enrichment kit with genome-wide structural variant detection. The resulting enriched pools will be loaded into one or more flowcell lanes and sequenced using a sequencing by synthesis chemistry on Illumina sequencers using paired-end reads. After sequence deconvolution, approximately 9-15 Gigabases of sequence will be obtained per sample. Base calling, alignment, variant calling, annotation and QC reporting is performed using the Neurome™ pipeline. Variants are initially filtered according to a combination of attributes including quality, population frequency, estimated severity of variant on protein function, and ultimately ranked by their likelihood to explain phenotype. High ranking variants are manually reviewed by Personalis' team of geneticists, bioinformaticians, genetic counselors, and laboratory directors who will determine if any of the variants identified are likely to be causative of the presenting phenotype. In cases in which only the patient was submitted for analysis, more stringent variant filters are applied for novel, potentially *de novo* variation. Stricter phenotype ranking thresholds are applied. All variation in genes linked to the reported phenotype that have been previously reported in the literature are manually reviewed. Only the variant(s) with the strongest phenotypic overlap are reported. Other variants that may explain part of the reported phenotype may be present but may not be reported. These "variants in candidate genes by phenotype" may be requested by the ordering provider. Contact Athena for more information.

Analytical Range: Neurome™ performance over the whole exome: Approximately 90% of bases are expected to have quality scores of Q20 or higher. Neurome™ performance over the augmented clinical genes: The Neurome™ additionally augments coverage in over 7,500 additional genes currently known to be associated with human disease.

Expected (Normal) Value: Diagnostic: 0, 1, or more variants detected.

Result Reports: Personalis provides a core summary clinical report detailing variants in the patient's DNA sequence in genes potentially causative of the patient's condition. These reports may exclude genes that have significant associated intellectual property restrictions. Personalis will not report on variants that are not associated with disease, variants commonly seen in many healthy people, variants associated with risk for common diseases or changes in drug metabolism, variants in genes that are known to cause disease but are unrelated to the presenting disorder in the patient, variants that do not put the patient at risk of developing disease but may confer increased risk in his or her offspring (i.e. diseases for which the patient is a "carrier").

Detection of consanguinity/uniparental disomy: Regions at least 4Mb in length where the percentage of heterozygous variants (pass quality filtered, with dbSNP ID) falls below 10% are identified and considered as Regions of Homozygosity (ROH) for results generation and interpretation. This 10% cutoff is not fixed and is subject to technical improvements. Suspicion of uniparental disomy (UPD) is reported if a single ROH region >10Mb on one chromosome is identified, especially if telomerically located. Presence of multiple large ROH located on different chromosomes may be indicative of parental blood relationship. Suspicion of consanguinity is reported if >10% of the genome falls into homozygous stretches. This may be consistent with a first- or second-degree parental relationship and will be reported as such. In these cases the physician should consult her/his institution's ethics board to take eventual additional steps (see reference PMID: 23328890). The calculation of the percentage of the genome falling into homozygous stretches, as well as the number of homozygous stretches detected, is subject to technical limitations that represent both known and unknown factors. Due to the targeting of this assay to exomic content, the consanguinity calculations performed may substantially under- or over-estimate the degree of consanguinity. There may be variability between laboratories and platforms in the consanguinity metrics and absolute values reported.

Disclaimer: This clinical service is intended to detect the following mutations: single nucleotide variants and small indels. The Neurome™ is not intended to comprehensively analyze the following types of mutations: large deletions and insertions, gross duplications, gross rearrangements, deep intronic variants, long repeat sequences, trinucleotide repeat sequences, epigenetic effects, variants involved in multigenic inheritance, some X-linked recessive mutations in females who manifest disease due to skewed X-inactivation and other unknown abnormalities. The services provided by Personalis and the results of those services are intended as a clinical summary of findings. Results should not be used to replace or overrule a qualified, licensed health care provider's judgment, clinical diagnosis, or monitoring of cases, and should not be used as the sole means of tracking information related to a patient. Accuracy of results cannot be guaranteed. The manner in which the services generate results, reports, and other information is complex, dependent upon operator accuracy, pre- and post-analytical factors, and that the possibility of software or other error cannot be eliminated. In addition, interpretation is dependent on reported clinical phenotype and family history. Whether caused by computer error, human error, or because of some other reason, results, reports, and other information may be mis-delivered or undelivered and/or include incorrect data or interpretations. Even if results are correct they might not lead to identification of a patient's disease or condition by a medical professional. In addition, a negative result does not rule out the possibility that the individual carries a variant in a region that has poor sequencing coverage. Furthermore, because of technological limitations, variants in some cases may not be detected by the Neurome™ even if the gene was well covered. Personalis does not provide any express or implied warranty as to the accuracy of results provided, or the outcomes from their use.

This test was developed and its performance characteristics determined by the Personalis Clinical Laboratory. It has not been cleared or approved by the FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. Pursuant to the requirements of CLIA '88, this laboratory has verified its accuracy and precision. Genetic testing using the Methods applied by the Personalis Clinical Laboratory is in accordance with applicable regulatory requirements, test accuracy and Methodology Performance Specifications (see Methodology and Limitations above, visit www.personalis.com or contact us for further information regarding the Methodology and Limitations of this test). The chance of a false positive or false negative result, due to laboratory errors incurred during any phase of the testing (pre-analytical, analytical, or postanalytical), cannot be excluded (CLIA # 05D2053444; State of California, Clinical Laboratory License, Lb ID number CLF00343226; CAP# 8662734).

Approved By

Massimo Morra, MD, PhD, FACMG, Clinical Laboratory Director

Date