Athena Diagnostics

SCN4A (Myotonia) DNA Sequencing Test

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Testing that Makes a Difference.

Patient	Requesting Physician	Accession Number
REPORT, SAMPLE	Not Provided	12345678
Date of Birth / **Sex**: 04/15/1983 / F	Report to: QUEST DIAGNOSTICS	Family Number/Kindred Number:
Specimen Type: WHOLE BLOOD	Address: 200 FOREST STREET	Patient Number: 000000000
Test Category: Not Available	2ND FLOOR	Specimen Collection Date: 05/17/2021
Test Requested: SCN4A (Myotonia) DNA Sequencing Test	MARLBOROUGH, NA 01752	Date Received: 05/18/2021
	Additional Reports to:	Report Date: 06/12/2021

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POSITIVE

This test identified a pathogenic variant in the *SCN4A* gene.

INTERPRETIVE RESULTS TABLE

	Gene/Test	Technical Result	Variant Type	Inheritance	Clinical Relevance	Pub Med ID
Positive	**SCN4A**	**c.3917G>T; p.Gly1306Val**	**Heterozygous Missense**	**Autosomal Dominant**	**Pathogenic**	**33263785 32849172**

No other abnormal variants were detected.

Comments: This test result is consistent with a diagnosis of, or a predisposition to develop, sodium channel myotonic disorder associated with pathogenic variants in the *SCN4A* gene.

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Athena Insight pathogenicity assessment

SCN4A c.3917 G>T is a missense variant classified as pathogenic based on the following information:

Variant: SCN4A c.3917 G>T (p.Gly1306Val)

- This variant has been identified in the general population at a frequency of 0.000 (1 in 249652 chromosomes)
- The frequency of this variant in the general population is consistent with pathogenicity.
- ClinVar contains an entry for this variant (www.ncbi.nlm.nih.gov/clinvar, variant ID: 5903).
- This variant has been identified in multiple unrelated individuals with clinical features associated with this gene and associates with disease in multiple families.
- At least one other missense variant at this codon is considered to be pathogenic or likely pathogenic, suggesting this variant may also cause disease.
- Assessment of experimental evidence suggests this variant results in abnormal protein function. Multiple studies indicate that this variant disrupts normal ion channel properties, resulting in aberrant channel regulation of current and signaling (PMID: 7473241, 16392038).
- SIFT and PolyPhen-2.2.2 (HumVar) predictions of the variant's effect on normal protein function disagree.

Selected Variant References:

Vereb, N, et al. (2021) J Neurol 268: 1708-1720. (PMID: 33263785)
Maggi, L, et al. (2020) Front Neurol 11: 646. (PMID: 32849172)
Sasaki, R, et al. (2020) Neuromuscul Disord 30: 546-553. (PMID: 32660787)
Huang, W, et al. (2017) Protein Cell 8: 401-438. (PMID: 28150151)
Yang, X, et al. (2016) Channels (Austin) 11(1):55-65. (PMID: 27415035)
Park, JH, et al. (2010) Neurologist 16: 203-5. (PMID: 20445432)
Trip, J, et al. (2008) Eur J Hum Genet 16: 921-9. (PMID: 18337730)
Fournier, E, et al. (2006) Ann Neurol 60: 356-65. (PMID: 16786525)
Groome, JR, et al. (2005) Cell Mol Neurobiol 25: 1075-92. (PMID: 16392038)
Lerche, H, et al. (1993) J Physiol 470: 13-22. (PMID: 8308722)
Plassart, E, et al. (1994) Eur J Hum Genet 2: 110-24. (PMID: 8044656)
Mitrovic, N, et al. (1995) J Physiol 487 (Pt 1): 107-14. (PMID: 7473241)
McClatchey, AI, et al. (1992) Cell 68: 769-74. (PMID: 1310898)
Genome Aggregation Database (gnomAD), Cambridge, MA (URL: http://gnomad.broadinstitute.org)

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Recommendations: This individual's family members are at risk for possessing or inheriting this variant. Careful reconciliation of this molecular data with this individual's clinical presentation, family history, and other laboratory results, in conjunction with genetic counseling, is highly recommended.

Health care providers, please contact the Athena Diagnostics Client Services Department at 1-800-394-4493 if you wish to consult with a Laboratory Director or a Genetic Counselor regarding this test result.

Background information: The *SCN4A* gene encodes the voltage-gated sodium channel which functions as an initiator of muscle action potential (1). Mutations in the *SCN4A* gene have been associated with a number of hereditary sodium channel myotonic disorders (1). This group of disorders is predominantly characterized by myotonia which can present in various muscle groups including the face, tongue, neck, arms, and legs to varying degrees depending on the subtype (2, 3, 4). Subtypes include a form of congenital myasthenic syndrome (CMS) and the non-dystrophic disorders of skeletal muscle such as potassium-aggravated myotonia (PAM), paramyotonia congenita (PMC), hyperkalemic periodic paralysis (HyperPP), and hypokalemic periodic paralysis type 2 (HypoPP). Both loss and gain of function mutations have been observed (2, 3, 4).

SCN4A gene information: MIM ID: *603967; Chromosome Location: 17q23.3; NCBI Reference Sequence: NM_000334.4; In the cDNA, the initiator codon, ATG (methionine), is designated as codon number 1 and the "A" is designated as nucleotide +1. The initiator codon is located in exon 1 and all subsequent numbering is sequential.

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Phenotype information: MIM ID: #608390 for myotonia congenita, atypical, acetazolamide-responsive, MIM ID: #168300 for paramyotonia congenital, MIM ID: # 170500 for hyperkalemic periodic paralysis, type 2, MIM ID: # 613345 for hypokalemic periodic paralysis, type 2 and MIM ID: # 614198 for myasthenic syndrome, acetazolamide-responsive

Methods:
DNA sequencing was performed using advanced ("next-generation") sequencing technology. Advanced sequencing was performed on sheared genomic DNA libraries enriched for target regions using in-solution hybrid capture. Target regions include coding regions in exons and at least ten non-coding nucleotides adjacent to each of these coding regions. Resulting sequences were then analyzed using bioinformatics tools in a standardized process that includes alignment to the GRCh37/hg19 genomic build and use of the Genome Analysis Toolkit 2.3-9 for variant detection. A mean coverage depth of at least 30 sequencing reads is obtained within each target region, with a minimum coverage depth of at least 20X. Target regions with coverage depths less than the minimum are not reported. Internal testing demonstrated approximately 99% sensitivity and approximately 99% specificity for detecting DNA sequence variation. In some limited circumstances, such as for regions that are difficult to sequence using advanced sequencing, Sanger sequencing of PCR-amplified target regions (using bi-directional sequence or alternate dye chemistries) may be performed in lieu of this process. Benign and likely benign variants, if identified, are considered normal and are not reported, but are available upon request.

Limitations of DNA sequencing analysis: This method does not detect large deletions, insertions, or structural variations, and may not detect variants in patients exhibiting mosaicism. Furthermore, allele dropout cannot be detected in Sanger sequenced regions. Additionally, this test will not detect variants in low coverage regions, which may occur in an analysis of this scope. Regions with exceptionally high or low GC content, repetitive regions or regions having a high degree of similarity with other regions in the genome are more susceptible to low coverage and/or reduced sensitivity. Furthermore, this test does not detect variants in regions of the gene not analyzed (including promoter, 5' and 3' untranslated regions, introns, and certain regions having consistently low coverage).Although rare, false positive or false negative results may occur. All results should be interpreted in the context of clinical findings, relevant history, and other laboratory data.

Limitation of variant analysis: The classification and interpretation of the variant(s) identified reflect the current state of Athena Diagnostics' understanding at the time of this report. Variant classification and interpretation are subject to professional judgment, and may change for a variety of reasons, including but not limited to, updates in classification guidelines and availability of additional scientific and clinical information. This test result should be used in conjunction with the health care provider's clinical evaluation. Inquiry regarding potential changes to the classification of the variant is strongly recommended prior to making any future clinical decision. For questions regarding variant classification updates or our Family Insight Program, please call Athena Diagnostics at 800-394-4493 to speak to a genetic counselor or laboratory director, or visit https://www.athenadiagnostics.com/athenainsight.

For patients who are interested in sharing de-identified genetic and health information to improve understanding of genetics and health, please visit https://GenomeConnect.org. GenomeConnect is an online registry designed by the Clinical Genome Resource (ClinGen) and is not affiliated with Quest Diagnostics.

Background References

1. Graves, TD, et al. (2005) Postgrad Med J 81: 20-32. (PMID: 15640425)
2. Saperstein, DS. (2008) Semin Neurol 28: 260-9. (PMID: 18351527)
3. Trivedi, JR, et al. (2014) Exp Neurol 253: 28-30. (PMID: 24361411)
4. Jurkat-Rott, K, et al. (2010) Pflugers Arch 460: 239-48. (PMID: 20237798)
5. Furby, A, et al. (2014) Neuromuscul Disord:Jul 2. [Epub ahead of print] (PMID: 25088311)

This test was developed and its analytical performance characteristics have been determined by Athena Diagnostics. It has not been cleared or approved by the U.S. Food and Drug Administration. This assay has been validated pursuant to the CLIA regulations and is used for clinical purposes.

Laboratory results and submitted clinical information reviewed by,

Sat Dev Batish, PhD, FACMG, FAAN	Zhenyuan Wang, PhD, FACMG
Chief Director, Genetics	Senior Director, Genetics

Laboratory oversight provided by Vivekananda Datta, M.D., Ph.D., CLIA license holder, Athena Diagnostics (CLIA# 22D0069726)