Athena Diagnostics

CLCN1 DNA Sequencing Test

Testing that Makes a Difference.

Patient REPORT, SAMPLE	Requesting Physician Not Provided	Accession Number 12345678
Date of Birth 04/15/1983 Sex M	Report to QUEST DIAGNOSTICS	Family Number/Kindred Number
Specimen Type WHOLE BLOOD	Address 200 FOREST STREET	Patient Number 000000000
Test Category Diagnostic (Symptomatic)	2ND FLOOR	Specimen Collection Date 03/24/2022
Test Requested CLCN1 DNA Sequencing Test	MARLBOROUGH, MA 01752	Date Received 03/25/2022
	Additional Reports to:	Report Date 04/11/2022

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POSITIVE

The most significant finding in this panel of tests is a pathogenic variant in the *CLCN1* gene. This result is consistent with a diagnosis of, or a predisposition to develop, myotonia congenita. Please see the Comments section for further information.

INTERPRETIVE RESULTS TABLE

	Gene/Test	Technical Result	Variant Type	Inheritance	Clinical Relevance	Pub Med ID
Positive	**CLCN1**	**c.689G>A; p.Gly230Glu**	**Heterozygous Missense**	**Autosomal Dominant**	**Pathogenic**	**18220014 10533075**

No other abnormal variants were detected.

Comments: Myotonia congenita associated with pathogenic or likely pathogenic variants in the *CLCN1* gene can be inherited in both an autosomal recessive and an autosomal dominant manner. Additionally, the same variant can exhibit different modes of inheritance between families and within a single family. Therefore, the presence of a pathogenic or likely pathogenic *CLCN1* variant must be interpreted with caution - since mode of inheritance is uncertain for each individual - and carefully reconciled with each patient's clinical symptoms and family history. Please see the pathogenicity assessment(s) below for more information regarding the variant(s) found in this individual.

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Athena Insight pathogenicity assessment

CLCN1 c.689 G>A is a missense variant classified as pathogenic based on the following information:

Variant: CLCN1 c.689 G>A (p.Gly230Glu)

- This variant has been identified in the general population at a frequency of 0.000 (3 in 251566 chromosomes)
- This variant is statistically more frequent in affected individuals than in the general population and/or healthy controls; therefore, the frequency is consistent with pathogenicity.
- Pathogenic variants in the CLCN1 gene (OMIM * 118425) have been associated with autosomal dominant and recessive myotonia congenita.
- This variant is associated with autosomal dominant myotonia congenita in most families (PMID 8857727, 7981750, 18220014), however, it has been associated with autosomal recessive myotonia congenita in at least one family (PMID: 8857733).
- ClinVar contains an entry for this variant (URL: www.ncbi.nlm.nih.gov/clinvar; Variation ID: 17532).
- Assessment of experimental evidence suggests this variant results in abnormal protein function. Experiments show this variant results in altered ion selectivity and is critical for normal ion conductance (PMID: 9122265).
- Both SIFT and PolyPhen-2.2.2 (HumVar) predict that this variant is pathogenic.

Selected Variant References:

Chang, TY, et al. (2007) Acta Neurol Taiwan 16: 214-20. (PMID: 18220014)
Brugnoni, R, et al. (1999) Hum Mutat 14: 447. (PMID: 10533075)
Fahlke, C, et al. (1997) Proc Natl Acad Sci U S A 94: 2729-34. (PMID: 9122265)
Zhang, J, et al. (1996) Neurology 47: 993-8. (PMID: 8857733)
Koty, PP, et al. (1996) Neurology 47: 963-8. (PMID: 8857727)
Steinmeyer, K, et al. (1994) EMBO J 13: 737-43. (PMID: 8112288)
George, AL, et al. (1993) Nat Genet 3: 305-10. (PMID: 7981750)
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: Non-dystrophic myotonic disorders are a group of disorders predominantly characterized by myotonia which can present in various muscle groups including the face, tongue, neck, arms, and legs to varying degrees (1, 2). Skeletal muscle weakness may also be present (3). Age of onset and clinical presentation vary depending on the specific sub type (1). Non-dystrophic myotonic disorders have an overall prevalence of less than 1:100,000 (3).

Subtypes of non-dystrophic myotonic disorders include two forms of myotonia congenita (MC), Thomsen's disease and Becker's disease. MC is characterized by excitability of the skeletal muscle membrane, a muscular appearance, and action myotonia (3, 4). Additionally, affected individuals present with the "warm up" phenomenon which is characterized by percussion myotonia with muscle stiffness after initiating movement with improvement after repeated activity (3, 4, 5,). The most common site of stiffness associated with MC is the legs while the face is less common (3). Schwartz-Jampel syndrome, rippling muscle disease, and Brody disease are also different forms of non-dystrophic myotonic disorders.

Mutations in the *CLCN1* gene have been associated with both forms of MC (5). The *CLCN1* gene encodes the skeletal muscle chloride channel voltage-sensitive 1 protein which important for the normal repolarization of muscle action potential (5). Thomsen's disease resulting from mutations in the *CLCN1* gene is inherited in an autosomal dominant manner and has a typical age of onset within the first 2 years of life (1). Becker's disease resulting from mutations in the *CLCN1* gene is inherited in an autosomal recessive manner and typically has a more severe phenotype (3, 4, 5). Age of onset for Becker's disease is generally later than that of Thomsen's disease with severity of clinical features increasing within the first 2 decades of life (1).

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CLCN1 gene information: MIM ID: *118425; Chromosome Location: 7q34; NCBI Reference Sequence: NM_000083.2; 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.

Phenotype information: MIM ID: #160800 for myotonia congenetia, autosomal dominant, MIM ID: #255700 for myotonia congenetia, autosomal recessive

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. Saperstein, DS. (2008) Semin Neurol 28: 260-9. (PMID: 18351527)
2. Graves, TD, et al. (2005) Postgrad Med J 81: 20-32. (PMID: 15640425)
3. Trivedi, JR, et al. (2014) Exp Neurol 253: 28-30. (PMID: 24361411)
4. Sun, C, et al. (2001) Eur J Hum Genet 9: 903-9. (PMID: 11840191)
5. Brugnoni, R, et al. (2013) J Hum Genet 58(9): 581-7. (PMID: 23739125)

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,

Zhenyuan Wang, PhD, FACMG

Senior Director, Genetics

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