

Patient REPORT, SAMPLE	Requesting Physician Not Provided	Accession Number 12345678
Date of Birth 04/15/1983	Sex F	Report to QUEST DIAGNOSTICS
Specimen Type WHOLE BLOOD	Address 200 FOREST STREER	Family Number/Kindred Number
Test Category Diagnostic (Symptomatic)	2ND FLOOR	Patient Number 000000000
Test Requested CLCN1 DNA Sequencing Test	MARLBOROUGH, MA 01752	Specimen Collection Date 03/08/2022
	Additional Reports to:	Date Received 03/09/2022
		Report Date 03/24/2022

CLCN1 DNA Sequencing Test

NEGATIVE

This test did not identify any variants associated with myotonia.

INTERPRETIVE RESULTS TABLE			
	Test	Technical Result	Clinical Relevance
Negative	CLCN1	No abnormal variants detected	See Limitations of Analysis

Comments: While this analysis did not identify any pathogenic or likely pathogenic variants associated with myotonia, the diagnosis cannot be completely ruled out due to variants not detected by this assay or variants in another gene.

Clinical limitations: The frequency of mutations in the *CLCN1* gene associated with myotonia is not known at this time, however, mutations have been reported in multiple populations (4).

Other testing available: Other disorders may appear clinically similar to those caused by mutations in this gene. Athena Diagnostics recommends additional testing, if not already performed, based on this individual's clinical presentation and family history. Please contact the Athena Diagnostics Client Services Department or visit AthenaDiagnostics.com for information regarding additional testing.

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).

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 congenita, autosomal dominant, MIM ID: #255700 for myotonia congenita, autosomal recessive

Methods:

CLCN1 DNA Sequencing Test



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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,

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