

**N-Acetylglucosaminyltransferase-Like Protein (*LARGE*) Gene Sequencing (Test #346)
Walker-Warburg Syndrome (OMIM 236670)
Congenital Muscular Dystrophy, Type 1D (OMIM 608840)**

Brief Description of Clinical Features: Mutations in the *LARGE* gene (OMIM 603590) cause muscular dystrophies in the dystroglycanopathy spectrum. Walker-Warburg syndrome (WWS), a severe congenital muscular dystrophy with defective neuronal migration and associated structural brain and eye abnormalities, is the most severe manifestation (Godfrey et al. *Brain* 130:2725-2735, 2007; van Reeuwijk et al. *Hum Genet* 121:685-690, 2007; Mercuri et al. *Neurology* 72:1802-1809, 2009). Patients with the congenital muscular dystrophy, structural brain abnormalities, and severe mental retardation have also been confirmed to have mutations in the *LARGE* gene (Longman et al. *Hum Mol Genet* 12:2853-2861, 2003; Clement et al. *Ann Neurol* 64:573-582, 2008). The enzyme encoded by *LARGE*, N-acetylglucosaminyltransferase, is necessary for proper post-translational processing of the protein, alpha dystroglycan (ADG). In the absence of this enzyme, ADG remains hypoglycosylated and diverse pathologies follow (Barresi and Campbell, *J Cell Science* 119:199-207, 2006). Molecular diagnosis (and classification) of the dystroglycanopathy subtypes is complex because extensive locus heterogeneity exists for each disorder (Godfrey et al. *Brain* 130:2725-2735, 2007), and because the phenotypes caused by the six demonstrated and putative glycosyltransferase genes continue to expand (eg. van Reeuwijk et al. *Hum Mutat* 27:453-459, 2006).

Genetics: The dystroglycanopathies are inherited in an autosomal recessive manner. It should be noted that five other genes (*POMT1*, *POMT2*, *POMGNT1*, *FKRP*, *FKTN*) encode proteins required for processing of ADG. The small numbers of *LARGE* gene mutations thus far reported include missense, single nucleotide duplication, nonsense, and partial gene deletions.

Description of This Particular Test: Testing is accomplished by amplifying all coding exons (3-16) and ~50 bp of adjacent noncoding sequence, then determining the nucleotide sequence using standard dideoxy sequencing methods and a capillary electrophoresis instrument. As indicated, we will also sequence one (Test #100, \$190) or two (Test #200, \$340) exons in family members of patients with known mutations or to confirm research results.

Reference Sequences: Genomic: NC_000022.10 mRNA: NM_004737.4
Protein: NP_004728.1 mRNA and Protein: CCDS 13912.1

Indication for Testing: Individuals with severe congenital muscular dystrophy and immunofluorescence results demonstrating hypoglycosylation of ADG in muscle.

Sensitivity of test: *LARGE* mutations are an infrequent finding in patients with congenital muscular dystrophy and ADG hypoglycosylation. In a study including 91 patients with demonstrated ADG hypoglycosylation, one patient was found to have *LARGE* mutations (Godfrey et al. *Brain* 130:2725-2735, 2007). In a second cohort of 81 patients with congenital muscular dystrophy and ADG hypoglycosylation, one patient was found to have *LARGE* mutations (Mercuri et al. *Neurology* 72:1802-1809, 2009). Similarly, within a cohort of 36 patients with clinical signs of congenital muscular dystrophy, one patient with *LARGE* mutations was found (Longman et al. *Hum Mol Genet* 12:2853-2861, 2003). Because dystroglycanopathies exhibit extensive locus and allelic heterogeneity a negative *LARGE* sequence result does not rule out a diagnosis of a one of these disorders when classic clinical findings are present. Evaluation of a patient's muscle biopsy by immunofluorescence can detect abnormal glycosylation of ADG and can, therefore, help direct a diagnostic evaluation.

Turnaround Time: Maximum of 40 days, although many tests are completed in 2-3 weeks.

Specimen Requirements: See page 4 of the Requisition Form.

Price: **Sequencing of *LARGE*** **\$ 890**

CPT Codes:

Sample Ascertainment	83890	\$	30	DNA Isolation	83891	\$	40
Amplification x14	83898	\$	230	Sequencing x14	83904	\$	500
Separation	83894	\$	30	Interpretation/Report	83912	\$	60

Accreditation: CLIA ID:52D1027685 (expires 1/18/13) CAP ID:7185561, AU ID:1407125 (expires 12/20/12)

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