

## Dystroglycan Testing via *DAG1* Gene Sequencing (Test #384)

**Brief Description of Clinical Features:** Dystroglycan is the central component of the muscle dystrophin-glycoprotein complex [(DGC) Ervasti et al *Nature* 345:315-319, 1990, Michele and Campbell *J Biol Chem* 278:15457-15460, 2003]. Beta-dystroglycan spans the sarcolemma binding dystrophin intracellularly and alpha-dystroglycan extracellularly. Alpha-dystroglycan is connected to the basal lamina by way of linkage to laminin (Ibraghimov-Beskrovnaya et al. *Nature* 355:696-702, 1992). Alpha-dystroglycan binds additional proteins with laminin G-like domains such as agrin and perlecan in muscle and neurexin in brain (Sugita et al. *J Cell Biol* 154:435-445, 2001). The gene *DAG1* (OMIM #128239) encodes one precursor peptide that is cleaved post translationally to form the alpha and beta subunits of dystroglycan. Both subunits undergo further modification by N- and O-linked glycosylation; however, alpha-dystroglycan undergoes extensive O-linked glycosylation (Ibraghimov-Beskrovnaya et al. *Hum Mol Genet* 2:1651-1657, 1993). The glycosylation status of dystroglycan is critical for ligand binding as well as for pathogenesis (Michele et al. *Nature* 418:417-422, 2002; Muntoni et al. *Curr Opin Neurol* 17:205-209, 2004; Barresi and Campbell *J Cell Sci* 119:199-207, 2006). Although protein components of the DGC are involved in many congenital and limb girdle muscular dystrophies, and although dystroglycan is highly expressed in skeletal and heart muscle as well as brain, mutations in dystroglycan leading to human disease have not yet been identified.

**Genetics:** Alpha and beta dystroglycan are encoded by the *DAG1* gene located on chr 3p21. The 895 residue precursor peptide is directed to the endoplasmic reticulum via a 29 amino acid N-terminal signal peptide. Cleavage of the precursor peptide occurs at p.Ser654 to yield core alpha and beta dystroglycan proteins. A single transmembrane domain (residues 751-774) directs beta-dystroglycan to span the sarcolemma. Interaction between dystrophin and beta-dystroglycan occurs at a PPXY motif (p.Pro828\_p.Tyr831) at the C-terminus of beta dystroglycan. The predicted mass of alpha-dystroglycan core protein and the corresponding protein isolated from tissue differ because of extensive species and tissue specific O-linked glycosylation of the central mucin domain between residues 312 and 485 (Barresi and Campbell, 2006). Studies in the mouse demonstrate that constitutional *DAG1* mutations are lethal in the early embryonic period secondary to disruption of Reichert's membrane (Williamson et al. *Hum Mol Genet* 6:831-841, 1997; Henry and Campbell *Cell* 95:859-870, 1998). Transgenic animals in which expression of the knockout *DAG1* gene construct is limited to the brain show structural malformations like those seen in patients with congenital muscular dystrophies, as well as loss of high affinity binding to laminin (Moore et al. *Nature* 418:422-425).

**Description of This Particular Test:** Testing is accomplished by amplifying the 2 coding exons of *DAG1* and ~50 bp of adjacent noncoding sequence, then determining the nucleotide sequence using standard dideoxy sequencing methods and a capillary electrophoresis instrument.

**Reference Sequences:** Genomic: NC\_000003.10 mRNA and Protein: CCDS 2799.1

**Indication for Testing:** There has not been a human phenotype associated with *DAG1* mutations.

**Sensitivity of Test:** At this time there is no known clinical utility for this test. Analytical and clinical sensitivity are unknown because there are no reported genotype-phenotype correlations for dystroglycan mutations in humans. Findings generated from this test should not alone be considered as diagnostic.

**Turn Around Time:** Maximum of 40 days.

**Specimen Requirements:** See page 4 of the Requisition Form.

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|-------------------------|----------------------------------|-------|--------------------------|-------------|
| <b>Price:</b>           | <b>Sequencing of <i>DAG1</i></b> |       | <b>\$ 640</b>            |             |
| <b>CPT Codes:</b>       |                                  |       |                          |             |
| Sample Ascertainment x1 | 83890                            | \$ 30 | DNA Isolation x1         | 83891 \$ 40 |
| Amplification x 10      | 83898                            | \$170 | Sequencing x10           | 83904 \$260 |
| Separation x1           | 83894                            | \$ 60 | Interpretation/Report x1 | 83912 \$ 80 |

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

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