

DMD Gene Analysis in Dystrophinopathies (Duchenne & Becker Muscular Dystrophy)

Clinical Features:

The dystrophinopathies are a group of diseases with overlapping clinical features that are caused by mutations in the X-linked DMD gene. The age-of-onset ranges from early childhood to adulthood. The mild end of the spectrum includes elevated serum creatine phosphokinase (CK) concentration, muscle cramps, quadriceps myopathy, and myoglobinuria. The more severe end of the spectrum includes progressive Duchenne/Becker muscular dystrophy and DMD-associated dilated cardiomyopathy (DCM). Duchenne muscular dystrophy (DMD) can present in early childhood with motor developmental delay, symmetric proximal weakness, waddling gait, difficulty climbing, calf hypertrophy and a highly elevated serum creatine phosphokinase (CK) concentration. As the disorder progresses, respiratory complications or dilated cardiomyopathy (DCM) can occur.⁶ Becker muscular dystrophy (BMD) typically presents as a later-onset disorder with a similar phenotype to DMD including progressive symmetric muscle weakness and atrophy, elevated serum CK, quadriceps weakness and DCM, and it may also cause activity-induced cramping and flexion contractures of the elbow. Females who are heterozygous carriers for a pathogenic variant in the DMD gene causing DMD/BMD are at an increased risk for DCM.⁶ In some individuals with DMD mutations, the heart muscle is primarily affected, resulting in DMD-associated DCM with little or no clinical evidence of skeletal muscle involvement. DMD-associated DCM typically presents in males in young adulthood and in females in the fourth or fifth decade with one or more of the following: i) heart failure with symptoms of congestion, ii) reduced cardiac output resulting in fatigue or dyspnea on exertion, arrhythmias and/or conduction system disease, iii) thromboembolic disease or stroke, iv) elevated serum CK, and/or v) mild skeletal muscle weakness.^{5,6,10} Dystrophinopathies are common neuromuscular disorders with the incidence of DMD reported as 1 in 3,900-4,700 live male births and the incidence of BMD as 1 in 18,450.^{4,8,13}

Genetics:

The dystrophinopathies are inherited in an X-linked manner. Sporadic pathogenic variants occur in approximately 25% of cases, while 75% are maternally inherited.² Penetrance of dystrophinopathies is complete in males; however, penetrance in carrier females can vary and may depend on X-chromosome inactivation.

The DMD gene encodes dystrophin, which is an important component of the dystrophin-associated protein complex (DAPC) and plays a structural role in ensuring membrane stability, regulation of signaling processes and forcing transduction during muscle contraction. Dystrophin is mainly expressed in the skeletal and cardiac muscles and in the brain. Mutations in the DMD gene can lead to reduced or absent dystrophin, which can cause fragility of the muscle fiber and ineffective regeneration of muscle tissue.

Deletions or duplications of one or more exons of the DMD gene account for ~65-70% of all disease causing pathogenic variants.^{1,14} In cases where a child who has a deletion/duplication in DMD is the single occurrence of a dystrophinopathy in the family, the type of deletion/duplication predicts the DMD/BMD phenotype with approximately 90% accuracy; deletions/duplications that alter the reading frame of the protein typically correlate with the more severe DMD phenotype, whereas those that do not alter the reading frame are associated with the milder BMD phenotype.¹ Sequence variants, including single nucleotide changes and small rearrangements, account for ~25-30% of disease causing pathogenic variants.¹¹ Approximately 3-7% of individuals with a dystrophinopathy do not have a pathogenic variant that is identifiable by the combined methods of sequencing and deletion/duplication testing.^{11,12} Deletion/duplication analysis and sequencing of the DMD gene is available at GeneDx. If other neuromuscular disorders are under consideration, the Neuromuscular Disorder Panel is also available at GeneDx.

Test Methods:

Targeted oligoarray comparative genome hybridization (array CGH) analysis with exon-level resolution (ExonArrayDx) to evaluate for a deletion or duplication of one or more exons of the DMD gene. The presence of any potentially disease-associated copy number alteration(s) is confirmed by quantitative PCR, repeat array CGH analysis, or another appropriate method. Sequence analysis of the DMD gene is available as a separate test. Using genomic DNA, coding exons and flanking splice junctions of the evaluated gene are enriched using a proprietary targeted capture method developed by GeneDx. The products are sequenced on an Illumina instrument using paired end reads. The sequence data is aligned to reference sequences based on human genome build GRCh37/UCSC hg19. Sanger sequencing is used to compensate for low coverage and refractory amplifications. The presence of any potentially disease-associated sequence variant(s) is confirmed by dideoxy DNA sequence analysis or by another appropriate method.

Test Sensitivity:

Exon-level array CGH can detect deletions and duplications of the DMD gene greater than 250 bp. The technical sensitivity of the sequencing test is estimated to be 98%. It will not reliably detect deletions, insertions, or rearrangements greater than or equal to ten base pairs. Approximately 65-70% of individuals with a dystrophinopathy have an exon-level deletion or duplication in the DMD gene; the remaining affected individuals are thought to have single point mutations or small rearrangements which may be detectable by sequencing.^{6,7}

References:

1. Aartsma-Rus et al. (2006) *Ann N Y Acad Sci.* 1082:74-6.
2. Barbujani et al. Segregation analysis of 1885 DMD families: significant departure from the expected proportion of sporadic cases. *Hum Genet* 1990; 84: 522-6.

3. Bennett (2004) *Pharmacogenomics*. 5(4):433-8.
4. Bushby et al., (1991) *Lancet* (337):1022-4.
5. Callis et al. (2010). *Expert Rev Mol Diagn* 10:329-351.
6. Darras et al. *Dystrophinopathies*. 2000 Sep 5 [Updated 2011 Nov 23]. *GeneReviews*® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2014. Available at <http://www.genetests.org>.
7. del Gaudio et al., *Hum Mutat* 2008 29:1100-1107. PMID 18752307
8. Dooley et al. (2010) *Clin Pediatr (Phila)* 49:177-9.
9. Flanigan et al. (2003) *Am J Hum Gen* 72: 931-945.
10. Hershberger et al. (Updated [3/2009]). Dilated Cardiomyopathy Overview. In: *GeneReviews at GeneTests Medical Genetics Information Resource* (database online).
11. Flanigan et al. (2014) *Neurologic Clinics* 32 (3):671-688 (PMID: 25037084).
12. Gurvich, et al. (2008) *Ann Neurol* 63(1):81-9. PubMed PMID: 18059005.
13. Tangsrud & Halvorsen (1989) *Acta Paediatr Scand*. 78:100-3.
14. Tuffery-Giraud et al. (2009) *Am J Hum Gen* 30:934-945.