

Reassessing carrier status for dystrophinopathies

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The cloning of the *DMD* gene, and the identifications of mutations in it as the cause of Duchenne muscular dystrophy (DMD), makes a compelling story that is aptly told elsewhere.¹ The locus—the largest in the human genome—consists of 79 exons, distributed over 2.5 million nucleotides on the X chromosome, which are assembled into a complementary DNA (cDNA) of around 14 kb encoding the predominant muscle isoform of the dystrophin protein.² The size of the gene, and the number of exons, had historically made mutation analysis challenging. For more than a decade, the standard clinical assay was a multiplex PCR test that amplified sequences from a limited number of exons; nevertheless, because it included exons within the deletion hotspots of the gene, this method could confirm the presence of mutations in up to 98% of boys with exonic deletions.^{3,4}

Although robust and economical, multiplex PCR testing was less than ideal. First, it left many patient mutations unidentified, as deletion mutations themselves only make about 65% of patients with the dystrophinopathies DMD or the milder Becker muscular dystrophy.⁵ Second, it did not assess all exons, leaving the extent of deletions undefined in many patients. Without the flanking exons defined, one cannot predict the reading frame of the resultant mRNA, which has implications for both prognosis⁶ and the prospects for enrollment in therapeutic trials of exon skipping directed at restoring the reading frame.⁷

The other major limitation of this test was the inability to detect mutations in carriers, who are heterozygous for exon copy number variation. Prior to the molecular era, determination of carrier status in nonobligate females depended on muscle histopathology⁸; elevations of serum creatine kinase (CK), which is age-dependent and has a high false-negative rate⁹; or pedigree analysis of the *DMD* locus using restriction fragment length polymorphisms¹⁰ or short tandem repeat polymorphisms,¹¹ which may be inaccurate due to recombination within the large locus.

In their article in this issue,¹² Bogue and Ramchandren describe the reassessment of carrier status in a large kindred affected by DMD after the detection of a mutation in the proband via a modern molecular method. The c.8660-2A>T mutation, which disrupts the exon 59 splice acceptor site, was detected in the proband by a method that allows direct sequencing of all 79 exons and their flanking intronic sequences.¹³ The proband's maternal grandmother had been told that she was not a carrier in the past, apparently based on serum CK levels, but she identified as a third cousin of the proband another patient in the clinic who was found to have the same mutation. Obligate carrier status could then be inferred for several other women who have been told that they either were not or were unlikely to be carriers.

The story of this pedigree is informative to the practicing clinician, as it highlights the need to be aware of continual improvements and methods of diagnosis, and to assess the era in which a patient's family received genetic counseling. Modern mutational analysis for DMD requires first an exon test that accurately assesses exon copy number of all 79 exons, allowing accurate characterization of the extent of both deletions and duplications, whether in probands or in related females. Adequate methods in current use include both multiplex ligation-dependent probe amplification¹⁴ and comparative genomic hybridization array¹⁵ techniques, but the important thing is the principle of complete quantitative characterization, and other methods may supplant these. In the absence of exon copy number variation, sequencing of all exons should occur by any of several methods.¹⁶ Using this approach, around 95% of all *DMD* mutations can be detected, although the clinician should keep in mind that deep intronic mutations that result in mRNA alterations may require sequencing of muscle-derived cDNA to identify.¹⁷

As Bogue and Ramchandren point out,¹² the “Duty to Reassess” is a topic of current discussion in the molecular genetics and genetic counseling fields.¹⁸ While many genetics providers are in favor

See article

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of recontacting patients, there are significant barriers to implementing a routine process to recontact patients with updates to their genetic testing results. Tracking patients, their results, and their desire to receive new information takes significant time and effort and is unrealistic in most clinical practices. It can also present an ethical challenge when updates to interpretation or technology become available yet the patient is no longer actively followed by the ordering provider, or if the patient's desire for updated information has not been documented.

This case provides an excellent example of the importance of a detailed family history and careful assessment of previous genetic testing in revising the advice provided to a family. Not only was the proband provided with an accurate diagnosis with the help of family history, but additional family members were also provided with more accurate information about their own carrier status. Their report demonstrates a real-world example of appropriate reassessment and reminds the clinician of the need for careful attention to the basis of past genetic counseling advice.

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