Is a muscle biopsy in Duchenne dystrophy really necessary?

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The advances in genetics have improved considerably our abilities to diagnose genetic diseases, and these advances are influencing our diagnostic approach to neuromuscular disorders. In spinal muscular atrophy, a rapid gene test identifies 98% of affected cases and therefore a diagnostic muscle biopsy is not needed anymore.

Things are more complex regarding Duchenne dystrophy (DD). The gene is very large (at least 85 exons), and genetic studies using widely available techniques can identify mutations in only 60 to 70% of children.[1,2] These are frequently deletions (~60%) and, more rarely, duplications (~10%). Their detection is facilitated by two mutational hot spots, allowing a multiplex PCR test, based on 19 exons, to identify the great majority (98%) of deletions/duplications.[1,2] Southern blot analysis can identify further unusual deletions, whereas the remaining cases are thought to be due to a combination of small mutations (including point mutations) and intronic rearrangements. Specialized techniques such as the protein truncation test can be applied to muscle RNA for detection of these less common mutations[3]; however, the protein truncation test is technically demanding and not easily applicable on a large scale. Over the years, mutation detection together with the study of dystrophin expression on muscle biopsy has formed the basis for arriving at the diagnosis of this devastating disorder.[4,5]

However, a muscle biopsy has some constraints: it is performed under general anesthesia in some centers, and is expensive. One may thus question if it is necessary to obtain a muscle biopsy in these children.

Muscle biopsy: The "NO" camp. In a comprehensive study published in this issue of *Neurology*, Mendell et al.[6] used a very sensitive technique for identifying small mutations and deletions of the dystrophin gene from DNA obtained from blood. They studied 93 patients with a clinical diagnosis of DD, confirmed on muscle biopsy, and in whom the standard multiplex PCR analysis had failed to recognize mutations. By this novel approach, a mutation could be identified in 73 of these 93 patients, therefore improving the ability to detect a mutation in DD from ~65 to 70% to ~90% of cases. Interestingly, neither this nor other studies using different approaches have identified mutations in 100% of DD cases studied, and this remains unexplained at the moment.

This study is excellent news for the genetic counseling of DD families. The assignment of carrier status is very precise if the mutation is known. Moreover, the ability to screen all exons allows the mapping of the end points of each deletion; this is not always possible using the PCR approach. Finally, this novel technique, which is capable of detecting the small mutations that escape detection with most of the currently used techniques, appears to be relatively easily applicable to large-scale studies. So, if a mutation is found in most of the patients, why bother with a biopsy?

Muscle biopsy: The "YES" camp. Dystrophin levels on the muscle biopsy correlate better with the phenotype than the "genetic prediction." Various studies have reported figures in excess of 10% for exceptions to the reading frame rule.[7] Classical examples are out-of-frame deletions, deletions in the 5' of the gene[8] or of exon 44 or 45, often associated with an "unexpectedly" mild phenotype.[9] Similarly, several patients with the milder allelic variant Becker dystrophy (BD) carrying truncation mutations have been described.[9] All these cases are able to produce dystrophin by various mechanisms, including in-frame translational reinitiation or exon skipping via alternative splicing. Finally, the effect of splice site mutations is not always predictable but needs to be

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confirmed in the muscle. Many of these BD cases could erroneously be diagnosed as affected by DD if only genetic testing is used. The fact that all patients assessed in the current study had dystrophin levels determined on a muscle biopsy explains why no exceptions to the rule were found.

What are the benefits of muscle biopsy? A precise diagnosis is important for the family for setting a realistic plan of intervention for each child and for the therapeutic trials that will characterize the near future. This can be best achieved by 1) clinical observation of the patient's strength and functional abilities; 2) levels of dystrophin on the muscle biopsy; and 3) knowledge of the genetic defect. Despite the availability of a rapid genetic diagnostic test, both an accurate clinical assessment and a muscle biopsy will still be required in order to have a robust diagnosis of DD and other dystrophinopathies. Needle biopsy under sedation is well tolerated in this age group and represents a valid alternative to an open biopsy under general anesthesia.[10]

Although there is no question that undergoing a muscle biopsy is not pleasant, what patients and their families often find even more painful is not having an accurate diagnosis. Needless to say, I belong to the "YES" camp.

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