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COMMENTARY

Commentary on 'Mutation spectrum of the dystrophin gene in 442 Duchenne/Becker muscular dystrophy cases from one Japanese referral center'

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The article 'Mutation spectrum of the dystrophin gene in 442 Duchenne/Becker muscular dystrophy (DMD/BMD) cases from one Japanese referral center' by Takeshima *et al.*[1] describes one of the largest mutation databases, which is composed of details on the mutation data and genotype–phenotype correlations, and proposes strategies for molecular therapies and genetic testing. In this study, pathogenic mutations in the dystrophin gene were identified in all 442 Japanese DMD/BMD cases. This has enabled all Japanese dystrophinopathy cases to receive a proper diagnosis or genetic counseling and will also expedite the application of mutation-specific molecular therapies.

DMD and BMD are allelic X-linked recessive diseases and caused by mutations in the dystrophin gene (MIM#310200, #300376). The dystrophin gene is known by its enormous size, and it has often required a huge effort to identify each patient's mutation. Despite the long history of research into DMD/BMD, however, a foundation treatment is yet to be established. However, research advances in molecular therapy in recent years have been remarkable. Nonsense suppression therapy has been studied, and a compound named PTC124 was in clinical trials to treat DMD cases caused by a nonsense mutation.[2] It has been proposed that induction of exon skipping, which corrects out-of-frame to in-frame, represents a highly plausible method for DMD treatment, and a clinical trial has been conducted in Japan.[3] Rapid and accurate detection of the underlying gene mutation is required to enable decision-making and tailoring of the method of molecular treatment to each patient.

I was interested in two aspects of this article: the details on the mutations and the gene testing strategy.

Regarding the first, in this database, among the 442 mutation events, 270 (61%) and 38 (9%) were large deletions or duplications of one or more exons, respectively. Nonsense mutations were identified in 69 cases (16%). Mutations disrupting splice site consensus sequences were detected in 24 cases (5%). Small deletions/insertions were identified in 34 cases (8%). Deep intron mutations that created pseudoexons with novel splicing consensus sequences were identified in four cases (1%) by dystrophin complementary DNA analysis. Chromosomal abnormalities were detected in two cases (0.5%). A splicing abnormality was identified, although no responsible genomic change was evident (0.2%). Other databases (including the Japanese) have reported missense mutations, but at frequencies much lower than those of nonsense mutations (MIM#300377, Leiden Muscular Dyastrophy database http://www.dmd.nl/).[4] It is therefore mysterious that this large database showed no missense point mutations at all. The missense mutation might not become pathogenic mutation easily in a huge protein similar to the dystrophin.

The second point of interest is the gene testing strategy. From their results, the authors defined a molecular testing strategy for Japanese dystrophinopathy that will be useful in prioritizing cases for various stages of dystrophin gene mutation analysis. This approach involves the following four steps. Multiplex ligation-dependent probe amplification (MLPA) analysis was used to identify deletion and duplication mutations encompassing one or more exons, which account for 70% of dystrophinopathy cases (step 1).[5,6] Sequencing of exons and flanking introns of exons 8, 34, 44 and 74, in which small mutations are detected frequently, revealed mutations in a further 4.5% of dystrophinopathy cases (step 2). After confirmation of the diagnosis as dystrophinopathy, cDNA analysis was performed, which identified mutations in 25% of cases (step 3).[7] After chromosome analysis, mutations were identified in all dystrophinopathy cases (step 4). My concern was in regard to the timing of the muscle biopsy. The authors decided that muscle biopsy should be conducted after step 2 to confirm the diagnosis and obtain dystrophin mRNA for sequence analysis. However, the recent trend is to avoid muscle biopsy as much as possible, due to its invasive nature, and in many cases diagnosis of the gene mutation is attempted using only peripheral blood. However, it seems clear that in cases in which sequencing has been conducted but the mutation remains unidentified, the possibility of misdiagnosis remains. Reading this article, I could not help but think that the muscle biopsy should not be excessively postponed, given its diagnostic speed and benefit.

This article is excellent and profitable for not only muscular dystrophy researchers but also clinicians. I feel that these findings will remain relevant even if next-generation sequencing technology that enables patient genetic information to be obtained more rapidly is more widely used.

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