Duchenne muscular dystrophy (DMD) is a neuromuscular disease characterized by progressive muscular weakness and degeneration of skeletal muscle. DMD is the most common X-linked recessive lethal disease, with an incidence of approximately 1 in 3500 newborns (1). Most patients are diagnosed at about 5 years of age, when their physical ability diverges markedly from that of their peers. Weakness and wasting of muscle are progressive and symmetrical, affecting the lower limbs before the upper limbs and the proximal muscles before the distal muscles (2). Affected children are usually wheelchair bound by the age of 12 years. The presence of nonprogressive cognitive impairment is also a common feature in a significant portion of DMD patients. Most patients die in their early 20s as a result of cardiac or respiratory failure.

About 30 years ago my doctoral advisor, Dr. Hanns-Dieter Gruemer, decided that my thesis project would involve carrier testing for DMD using the new molecular testing. Dr. Gruemer’s previous research studies had primarily dealt with membrane alterations and creatine kinase activities in DMD carriers. Although I had little molecular expertise, I am fortunate and grateful that Dr. Gruemer made this very wise thesis project decision, which also had a profound impact on my career.

The molecular breakthrough that occurred at the time I started my project was that DNA probes from the short arm of the X chromosome, which recognized RFLPs linked to the dystrophin (DMD) gene were made available (3). The RFLPs could then be used within affected families to trace the X chromosome carrying the mutant gene using the Southern blot technology. Although the DNA testing was a major improvement, which had previously depended on pedigree analysis and biochemical testing, the RFLP was an indirect linkage-based test and did have several limitations that included a high recombinational error rate across the DMD gene, unavailability of family members, and noninformative meiosis. Localization and the cloning of the DMD gene shortly followed and allowed the performance of direct mutation testing, thus eliminating many of the limitations of the RFLP testing (4). The DMD gene was shown to be an extremely large gene, spanning more than 2000 kb of genomic DNA, composed of 79 exons that encoded a 14-kb transcript which was translated into a protein named dystrophin (4). Intragenic deletions (60%–65% of the cases), which result in the absence of dystrophin, were found to be the major cause of DMD. Rapid and efficient deletion screening became available using the multiplex PCR, which allowed one to simultaneously amplify deletion-prone exons (5). In contrast to Southern blotting, which required several cDNA hybridizations and weeks to obtain results, the PCR could now be completed in 1 day, making it ideal for prenatal testing. Furthermore, the quantitative multiplex PCR allowed for the determination of gene dosage and the accurate identification of DMD deletion carriers (6). Another major breakthrough was the development of multiplex ligation-dependent probe amplification (MLPA) for the determination of gene dosage (7). MLPA is based on an initial probe pair ligation to the DMD exon, followed by a multiplex amplification that produces products of different lengths. The different sized products are then analyzed on a capillary sequencer and the changes in peak heights reflect gene deletions. Finally, with the use of automated high-throughput sequencing technologies, the testing of the nondeletion DMD cases has now become a reality.

In this issue of Clinical Chemistry, Yoo and colleagues report a new tool in DMD diagnostics (8). In this feasibility study, the authors used fetal DNA from maternal plasma and parallel targeted deep sequencing for noninvasive prenatal diagnosis of DMD. The authors first established maternal haplotypes and identified the mutated haplotype from 4 DMD pregnant carrier female carriers and the respective probands. The major challenge of this application was the ability to detect haplotype dosage imbalance in the small amount of fetal DNA in the plasma from the mothers. The authors successfully predicted the fetal genotypes from all 4 families, and most importantly the technique allowed for a
noninvasive prenatal diagnosis within 7 weeks gestation. Although the analysis of cell-free DNA for noninvasive prenatal testing has clearly been demonstrated for the detection of trisomies, the authors showed that it could also be used for a monogenetic disorder like DMD. The novelty here is the testing of fetal DNA, for the actual DMD diagnosis was made using the old linkage method with single-nucleotide variants rather than RFLPs. Thus, the technique does suffer from the same limitations already described and can be used only for families in which a proband has been identified. In fact, the authors even identified a recombinational event in one of their families. Furthermore, the dystrophin gene is not a nice gene, and the presence of new mutations that have been proposed to account for as many as one-third of all cases becomes a major source of errors. For even when the fetal DNA possesses the same haplotype as the proband, the fetus may not be affected because the proband may be the result of an isolated sporadic mutation. Thus it is imperative that the mutation identified in the proband be confirmed in the mother before the prenatal testing, which the authors did in all 4 cases [Table 1 in the article (8)]. However, even when the mutation is not found in the mother, she still has an uncertain risk of carrier status, owing to the possibility of germline mosaicism (9). Cases of germline mosaicism in DMD have been reported, in which a deletion is transmitted to more than one offspring by a mother who shows no evidence of the mutation in her somatic cells. Thus a negative deletion result in a mother does not rule out a recurrence risk for future pregnancies, and prenatal diagnosis should still be offered. Since it depends upon the size of the mutant clone in the mosaic mother, the exact recurrence risk in germ-line carriers is unknown. However, in these cases the risk is significantly increased relative to what had initially been perceived as a new mutation with a low recurrence risk. It has been estimated that mothers of apparently sporadic DMD cases have a 14% recurrence risk (9).

Today several therapies (such as antisense oligonucleotides and antibiotics) are being applied according to the specific dystrophin mutation, thus requiring accurate and complete molecular analysis. Although many exciting and innovative testing strategies will continue to emerge, we are still faced with a huge and complex gene (with a high mutation rate, recombinations, and germline mosaicism) that will continue to provide us interesting challenges.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

References