Towards Routine Screening of Rare Genetic Diseases

The Example of Chronic Granulomatous Disease

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In 1986, when I was a young Ph.D. student working on the signaling pathway of phagocytic NADPH oxidase in Professor Pierre Vignais’s research laboratory, I remember the breakthrough discovery of the first gene involved in chronic granulomatous disease (CGD), a rare genetic disorder (prevalence of 1/200,000). Indeed, Stuart H. Orkin and colleagues very elegantly identified the CYBB gene responsible for X-linked CGD transmission with positional cloning.1 Although a multicenter European evaluation revealed that a cytochrome b with a characteristic heme spectrum was absent in the X-linked form of CGD,2 problems purifying membrane-associated components prevented a clear biochemical characterization of the oxidase system. The discovery of CYBB identified, for the first time, a gene responsible for a human genetic disease based on its chromosomal location without reference to a specific protein product. The three other genes involved in rare autosomal recessive CGDs – NCF1, NCF2, and CYBA – were identified in a more classical manner over the next four years.3–5 In addition, two cases of new genetic subgroups of CGD with autosomal recessive mutations in RAC2 and NCF4, encoding the two NADPH oxidase partners rac2 and p40phox, respectively, have recently been discovered.6,7

Over a 20-year period, several methodological approaches were developed to find mutations in the CYBB gene, which is involved in the most frequent CGD form (60% of CGD cases): single-strand conformation polymorphism analysis, denaturing high-pressure liquid chromatography, and direct DNA sequencing of all 13 exons and adjacent splice sites. Mutations may also be identified in the corresponding mRNA after RT-PCR amplification and sequencing with subsequent confirmation by targeted sequencing in the CYBB gene.

In this issue of the Journal of Molecular Diagnostics, Hill and colleagues, experts in high-resolution melting (HRM) technology, propose for the first time a genetic analysis of the CYBB gene using HRM in XCGD.8 This method has previously been developed for common genetic diseases such as cystic fibrosis.9 Very recently, this method has also been successfully used to detect BRCA1 mutations, prevalence in breast and ovarian cancers,10 or variants in the human mitochondrial genome.11

The first rare X-linked mutations studied using HRM were those involved in ornithine transcarbamylase deficiency.12 However, CYBB appears to be a good candidate for HRM genetic analysis because polymorphisms are rare, the exons’ sizes are compatible with the method, and most of the mutations are missense, nonsense, or small insertions or deletions; only rarely are large mutations such as deletion of the entire gene detected.13 Indeed, the authors validated their approach using blinded validation samples and successfully detected several types of mutations. In addition, index cases and carriers have been easily identified. The reliability of this method has been considerably increased by today’s rapid technological advances, including the development of new DNA dyes, dedicated software to detect subtle changes in melting curve shape, and carousel-based instruments.14 Moreover, this very sensitive approach is attractive because it decreases the cost of CGD genetic analysis (products and working time) after an initial investment in an automated DNA extractor and a high-resolution instrument for HRM performance. In addition, the turnaround time for locating mutations is also reduced compared with other approaches, which is essential to provide the most efficient care possible for the patient. However, despite the authors’ claims that definitive results could be returned within one working day, DNA extraction, PCR, HRM performance, HRM results analysis, targeted sequencing, and sequence analysis are unlikely to be performed in less than two days.

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When the absence of oxidase activity is detected in a patient’s neutrophils, antibiotic treatment must be immediately established even if the mutation has not yet been characterized.

The indisputable advance in this methodology lies in the standardization and traceability of all of the procedures from DNA extraction, sample and plate preparation (drying primers), and finally software curve analysis. Quality assurance including quality controls can now be accurately established for genetic analysis with HRM. Indeed, a diagnostic guideline for HRM analysis by an interlaboratory validation of BRCA1 mutation scanning has been established in Europe. One of the common discussions of molecular pathologists and laboratory diagnosticians concerns test adoption patterns and reimbursement. Diagnostic tests must be robust in terms of analytical performance and practicability, with possible interlaboratory evaluation and validation. HRM methodology gathers all of the parameters necessary for a potential candidate methodology in CGD genetic diagnosis. Quality management and standardization of genetic tests has begun in Europe (http://www.eurogentest.org) and the United States (http://www.rarediseases.info.nih.gov) for rare genetic diseases. HRM diagnostic guidelines could also be established for rarer genetic diseases. Frequently, genetic characterizations of rare diseases are performed first in research laboratories and then the procedures are transferred to clinical laboratories for standardization. However, the ultimate driver for the integration of a new test into routine practice remains demonstration of clinical utility.

CGD is a complex genetic disease involving four to six genes, with two types of transmission caused by diverse types and locations of mutations in the genes. One exception to this diversity is a GT deletion in a GTGT tandem repeat corresponding to the first four bases of exon 2, which is a common mutation (25% of CGD cases) identified in approximately 95% of abnormal alleles analyzed worldwide. Recent studies have demonstrated that the predominance of the ΔGT arises from recombination events between NCF1 and its highly homologous pseudogenes ΦNCF1, which are physically close to the functional gene at the 7q11.23 locus. Because of the presence of these ΦNCF1 and the extreme homology between them and NCF1, it is nearly impossible to detect carriers for A470 CGD by normal PCR and sequencing methods. In addition, the p47 phosphatase protein level and the NADPH oxidase activity in the phagocytes of carriers are indistinguishable from those of normal individuals. At present, carriers for A470 CGD are characterized using a gene-scan method approach based on the presence of the ΔGT to assess the ratio of NCF1 genes to pseudogenes. The application of the HRM method to detect these carriers will be highly valuable, especially as this ACGD is the second most frequent form of the disease. However, HRM may not be useful to analyze the genetic defect of other CGD forms, such as A220 CGD, A670 CGD, and autosomal recessive mutations in RAC2 and NCF4, because of their rarity.

When the clinical diagnosis of CGD has been ascertained by measuring the NADPH oxidase activity in the patient’s phagocytic cells, the application of HRM technology to the genetic analysis of the most frequent forms of CGD (X-linked and A470 CGD) can be immediately established. In genetic counseling, prenatal diagnosis, and familial investigations, HRM will be available to test for the causative mutations. I believe that solid knowledge of the pathophysiology and the clinical expression of the disease and vast experience in biological diagnostic tests are elementary points but are essential to carrying out the genetic analysis of rare diseases whatever method is used.

References