Problem with Detection of an Insertion-Type Mutation in the $\text{BCHE}$ Gene in a Patient with Butyrylcholinesterase Deficiency

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Genomic DNA was extracted from EDTA-treated venous blood as described by Kunkel et al. (9). Coding exons of the $\text{BCHE}$ gene were amplified as nine independent fragments by PCR, and each amplified product was analyzed by single-stranded DNA conformation polymorphism analysis (2, 4) and denaturing HPLC (WAVE System; Transgenomic). PCR products with variant migration patterns were sequenced directly with a BigDye Terminator Cycle Sequencing FS Ready Reaction Kit and a PRISM 310 Genetic Analyzer (Applied Biosystems). Bands with different mobilities were excised from the gel and cloned into pDRIVE (Qiagen) for sequencing. The mutation was confirmed by PCR-restriction fragment length polymorphism analysis.

We detected a G-to-C missense mutation at codon 365 (G365R) in exon 2 of $\text{BCHE}$. The proband was homozygous for this mutation, his father was heterozygous, and his mother was homozygous wild type. Representative DHPLC and sequencing results are shown in panels A and B of supplemental Fig. 1, which appears in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol50/issue12/. We confirmed our results with PCR-restriction fragment length polymorphism analysis using TaqI (Fig. 2 in the online Data Supplement). If our results are correct, the pedigree and genotype segregations are not easily understood. Hemizygosity of the region containing the G365R site resulting from a large deletion, an inversion, uniparental disomy (genomic imprinting), or a de novo mutation may explain the pedigree. We first tried long PCR to address the possibility of a large deletion, but the result did not provide a clear explanation for the pedigree. We did, however, notice a faint band that migrated more slowly than the target PCR product for exon 2. We therefore changed the extension time of the amplification conditions. The results obtained by electrophoresis on an agarose gel of PCR products from reactions performed with different extension time is shown in Fig. 1. When longer extension times were used, PCR product was longer. We suspected an insertion mutation and therefore excised the longer fragment from the gel and cloned and sequenced it. We identified an abnormal sequence inserted in exon 2 as an Alu sequence and a direct repeat (300 + 15 bp). This inserted sequence may have caused premature termination of transcription (Fig. 3 in the online Data Supplement). Both the proband and his mother were heterozygous for this insertion. We therefore concluded that the proband was a compound heterozygote for the G365R missense mutation and the Alu insertion mutation.

Alu sequences are short interspersed elements that are distributed widely throughout the human genome (10). Alu sequences can be divided into subfamilies of related sequences in apoB associated with cardiovascular disease. Arterioscler Thromb Vasc Biol 2003;23:872–8.


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elements on the basis of diagnostic mutations that are shared by subfamily members. The family described here is the second reported in which insertion of an Alu has caused BCHE deficiency; the first was described by Muratani et al. (8). Although the Alu sequence in the present study was classified as AluYb8 because of similarities with the AluYb8 reported by Muratani et al. (8), it also differed at several nucleotides.

In the present study, if genetic analysis had been performed only on the proband, he would have been misdiagnosed as homozygous for the G365R mutation. Analysis of the family revealed a discrepancy in genotype segregation, which directed us to additional analyses and allowed us to reach the proper conclusion. Shorter PCR programs are convenient for mutation detection, but fragments that are longer than expected may not be amplified and would therefore be missed. We conclude from our present experience that PCR with short extension times may be a source of pitfalls in mutation detection.

References


Monitoring of Hematopoietic Chimerism by Short tandem Repeats, and the Effect of CD Selection on Its Sensitivity, Kazuyuki Matsuda, Kazuyoshi Yamauchi, Minoru Tsuchiya, Takefumi Suzuki, Mitsutoshi Sugano, Eiko Hidaka, Kenji Sano, and Tsutomu Katsuyama (1) Department of Laboratory Medicine, Shinshu University Hospital, Matsumoto, Japan; 2 Clinical Laboratory Center, The University of Tokyo Hospital, Tokyo, Japan; 3 Department of Laboratory Medicine, Shinshu University School of Medicine, Matsumoto, Japan; 4 address correspondence to this author at: Department of Laboratory Medicine, Shinshu University Hospital, 3-1-1 Asashi, Matsumoto 390-8621, Japan; fax 81-263-34-5316, e-mail yamauchi@hsp.md.shinshu-u.ac.jp

The assessment of chimerism, the ratio of donor to recipient cells, is critical for monitoring the engraftment of donor cells and determining the recurrence of the original disease after allogeneic bone marrow transplantation (BMT). Chimerism in patients after BMT has been assessed by several methods (1). Fluorescence in situ hybridization (FISH) analysis is particularly well established (1). Although FISH analysis is a highly sensitive and quantitative method, its application is restricted to cases with sex-mismatched BMT because the sex chromosome is the common chimerism marker (1).

Many minisatellites, such as short tandem repeats (STRs), are highly polymorphic because of allelic variation in repeat copy numbers (2, 3). A method that makes use of this polymorphism, PCR-based analysis of STRs (PCR-STR), which is a useful tool for human identification in forensic testing (4), has been used to monitor hematopoietic chimerism after BMT (5–13). In recent years, a method using real-time quantitative PCR has been developed (14). This method is more sensitive than PCR-STR, but it requires special instrumentation and expensive reagents and thus is unsuitable for routine assay in the clinical laboratory.

We investigated the assay characteristics of PCR-STR by comparing it with FISH analysis and examining the effect of cell selection (according to the immunophenotype of the original leukemic clone) on the ability of PCR-STR to detect chimerism.

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