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SHORT REPORT

Incorrect assignment of N370S mutation status by mismatched PCR/RFLP method in two Gaucher patients

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More than 60 mutations have been described in the β-glucocerebrosidase gene in Gaucher patients with β-glucocerebrosidase (EC 3.2.1.45) deficiency (McKusick 230800, 230900, 231000). N370S is a frequent mutation and is associated exclusively with nonneurological presentation of the disease (Beutler and Gelbart 1996). Here we describe analysis of mutations in the human β-glucocerebrosidase gene by direct sequencing of PCR products, and an incorrect assignment of N370S mutation status by the frequently used mismatched PCR/RFLP method.

The mutation analysis was performed in six Czech β-glucocerebrosidase-deficient type 1 Gaucher patients. All patients were screened for the N370S mutation by the mismatched PCR/RFLP method and later re-evaluated by sequencing. For detection of the N370S mutation, the mismatched PCR/RFLP method (Beutler et al 1990) with modified primers (5′-CTTTGCCTTTGTCCTTACCCTCGA, 5′-GTTACGCACCCAATTGGGTCCTCC) was used. For sequencing, coding parts of the gene were amplified by PCR (Beutler et al 1993). Single-stranded DNA was produced by asymmetric PCR and sequenced using the dideoxy method with T7 polymerase. The sequences were read using an EMBL sequencer.

In two of the patients the genotype established by PCR/RFLP was different from that obtained by sequencing. Patient 3 (N370S/N370S by the PCR/RFLP method) was found to be heteroallelic for the N370S and the 55bp deletion (1263del55) (Beutler et al 1993). Both alleles were cloned and sequenced. In patient 6, PCR/RFLP did not detect the N370S mutation, while the genotype established by sequencing was N370S/D409H. Repeated analysis by the PCR/RFLP method gave the correct result (N370S/wt).

In patient 3 the allele carrying the 55bp deletion was not amplified, because the deleted fragment contained sequence complementary to antisense primer. In patient 6 no obvious reason for failure to detect the mutation was found, but failed digestion by the restriction endonuclease was a possible cause.

When using PCR-based methods a discrepancy between phenotype and apparent genotype may be due to deletions or mutations at the site where one of the amplifying oligonucleotides binds (Beutler et al 1993), or due to multiple mutations. Particularly with such patients, a more detailed investigation including family studies should be undertaken before decisions about disease prognosis or therapy are made. In such cases the reliability

of the techniques used is of great importance. At the very least, a control restriction site should always be present in the PCR products used for PCR/RFLP.

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