High-Sensitivity Detection of the A3243G Mutation of Mitochondrial DNA by a Combination of Allele-Specific PCR and Peptide Nucleic Acid-Directed PCR Clamping

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Abstract

Background: The A3243G mutation of mitochondrial DNA (mtDNA) is involved in many common diseases, including diabetes mellitus and mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS). For detection of this mutation, allele-specific PCR is highly sensitive but requires strict control of PCR conditions; it thus is not adequate for a routine clinical test. We aimed to develop a routinely available PCR method for quantitative detection of low-level heteroplasmy of the A3243G mutation.

Methods: Quantitative allele-specific PCR for the A3243G mutation was performed in the presence of peptide nucleic acid (PNA), in which PNA is complementary to the wild-type mtDNA, with one primer having a 3′ end matched to nucleotide position 3243 of the mutant.

Results: With our method, amplification of wild-type mtDNA was suppressed 7000-fold compared with amplification of the mutant mtDNA under a broad range of conditions: DNA, 5–100 ng; annealing temperature, 61–66 °C; and PNA, 1.5–3.5 μmol/L. Hence, 0.1% heteroplasmy of the A3243G mutation can be reliably quantified by this method. Blood samples from 40 healthy volunteers showed <0.06% heteroplasmy, suggesting that 0.1% is diagnostically significant.

Conclusions: PNA maintains the specificity of allele-specific PCR over a wide range of conditions, which is important for routine clinical testing.

Abbreviations: mtDNA, mitochondrial DNA; np, nucleotide position; MELAS, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; DM, diabetes mellitus; RFLP, restriction fragment length polymorphism; LMPCR, ligation-mediated PCR; PNA, peptide nucleic acid; Tm, melting temperature; and SNP, single-nucleotide polymorphism.

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