Simultaneous detection of the exon 10 polymorphism and a novel intronic single base insertion polymorphism in the XPD gene using single strand conformation polymorphism

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We developed a new method based on the single strand conformational polymorphism (SSCP) technique for the detection of a G23591A (Asp312Asn) polymorphism in exon 10 of the XPD gene. In the process we also identified a novel polymorphism 23623C-ins (IVS10 + 17C-ins) in intron 10 of the same gene. With this newly developed SSCP-based method of genotyping we could detect both polymorphisms in the same assay and thus consequently determine the haplotype. In order to determine the population frequency of the novel polymorphism and the haplotype frequency, 302 healthy individuals were genotyped. The allelic frequency of the 23623C-ins intronic polymorphism was 0.16, whereas the frequency of the variant allele for the G23591A polymorphism was 0.39. Forty-three individuals (14%) were heterozygous for both polymorphisms but none carried polymorphic variants for both G23591A and 23623C-ins on the same allele. The effect of the novel intronic insertion polymorphism, which is located 16 nt downstream of the 3'-end of exon 10 of the XPD gene and involves a mononucleotide C repeat sequence, on expression remains to be determined.

Introduction

The XPD gene encodes an enzyme which has dual roles in the nucleotide excision repair pathway and in basal transcription as a component of the TFIIH factor. Several single nucleotide polymorphisms (SNPs) have been identified in the XPD gene (Shen et al., 1998; Lehmann, 2001). It is postulated that SNPs in DNA repair genes might be responsible for the observed inter-individual differences in DNA repair capabilities (Mohrenweiser and Jones, 1998; Bykov et al., 1999). The two most common SNPs in the XPD gene, which have been the subject of various case-control and functional studies, are G23591A and A35931C (nucleotide numbering according to the XPD gene sequence, GenBank accession no. L47234), causing Asp312Asn and Lys751Gln amino acid changes in exons 10 and 23, respectively (Shen et al., 1998; Sturgis et al., 2000; Hemminki et al., 2001; Seker et al., 2001; Spitz et al., 2001; Hou et al., 2002).

In this communication we describe the development and optimization of a single strand conformational polymorphism (SSCP)-based assay for simultaneous detection of G23591A, a known polymorphism in exon 10, and 23623C-ins (IVS10 + 17C-ins), a novel polymorphic single base insertion in intron 10 of the XPD gene. This novel polymorphic insertion of an additional cytosine changes the length of a run of six cytosines to seven between nucleotide positions 23617 and 23623 in intron 10 of the XPD gene. We genotyped 302 healthy individuals from a Nordic population to validate the newly developed SSCP-based method for genotyping and to determine the population frequency of the novel polymorphism.

Materials and methods

PCR amplification and SSCP analysis for genotyping

A 103 bp fragment containing parts of exon 10 and intron 10 of the XPD gene was amplified using forward primer 5'-GGAGACGGGACGCGCACTCTG (position 23554) and reverse primer 5'-GACGGGGAGCCGGGAAAG (position 23639). PCR reactions were carried out in a 10 µl volume containing 10 ng template DNA, 50 mM KCl, 1 mM MgCl2, 0.11 mM dNTPs, 1 µCi [32P]dCTP, 0.15 µM each primer and 0.3 U Taq DNA polymerase. DMSO (10%) was included as co-solvent. The temperature conditions for PCR were initial denaturation at 94°C for 1 min, followed by three cycles at 95°C (denaturation) for 45 s, 68°C (annealing) for 45 s, 72°C (extension) for 45 s. This was followed by another 32 cycles with an annealing temperature of 67°C and each segment time reduced to 30 s. The final extension was for 5 min at 72°C.

The amplified products were analysed by SSCP on a 10% polyacrylamide gel with electrophoresis carried out overnight at 4°C using 5 W power. Briefly, 1 µl of PCR product was mixed with formamide-dye solution and denatured at 95°C for 3 min, then loaded onto a non-denaturing gel. Electrophoresis under these conditions gave optimal resolution of both polymorphisms and the separate DNA strands containing the G23591A and 23623C-ins polymorphisms migrated differently (Figure 1).

Sequencing analysis

The genotype results obtained by the newly developed SSCP method were confirmed by random sequencing. For sequencing, PCR products were purified through Sephadex microspin columns (Amersham Biosciences) and subjected to 26 cycles of sequencing reaction using a rhodamine dye terminator cycle sequencing kit (Big Dye; Applied Biosystems). The genotypes were confirmed by sequencing both sense and antisense strands using forward and reverse primers in separate reactions. The sequencing reactions were precipitated in ethanol and electrophoresed on a denaturing polyacrylamide gel in an automated sequencer (ABI 377; Applied Biosystems) and analysed using Prism and EditView software. The sequences obtained were aligned with the reference sequence from GenBank (accession no. L47234) using the alignment software in the DNA Star package.

Results

Resolution of the polymorphism in exon 10 of the XPD gene and identification of a novel intronic C insertion polymorphism

In order to develop optimal conditions for the separation of the G23591A polymorphism using the SSCP technique, exon 10 along with adjacent intronic sequence was amplified using different permutations and combinations of primers (data not shown). In the process of optimization using a combination of SSCP conditions and DNA sequencing we identified a novel 23623C-ins polymorphism located in intron 10 of the XPD gene. With a 103 bp amplified fragment we could resolve both the G23591A and 23623 C-ins polymorphisms, which are located 43 bp apart, in the same SSCP assay under the conditions described in Materials and methods (Figure 1). Consequently, using this newly developed SSCP method we could determine the individual haplotype for these two polymorphisms in the XPD gene. A complete concordance was observed in results obtained using SSCP and direct sequencing.

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Determination of population frequency of both the G23591A exon 10 and the novel intronic C-ins polymorphism in the XPD gene

Using the newly developed SSCP-based method we genotyped 302 healthy individuals from an ethnically homogeneous Nordic population (age range 40–55 years), in order to determine the haplotype and population frequencies of the G23591A and 23623C-ins polymorphisms. The frequency of the 23623C-ins allele was 0.16 and the frequency of the A allele for the G23591A polymorphism in exon 10 was 0.39. The genotype distribution was in accordance with the Hardy–Weinberg equilibrium for both polymorphisms (χ² for fitness 0.78 and 0.43, P values 0.37 and 0.51). Forty-three individuals were heterozygous for both the G23591A and 23623C-ins polymorphisms in the XPD gene and none of the homozygotes for either of the polymorphisms carried the other alteration (Table I). None of the heterozygotes for both polymorphisms in the present study carried variants on separate alleles (P < 0.0001).

Discussion

The G23591A (Asp312Asn) polymorphism in exon 10 of the XPD gene has remained less studied, as the variant single base alteration does not result in any gain or loss of restriction site. In theory this polymorphic site is not amenable to genotyping by PCR–RFLP, which is one of the most common methods employed for manual genotyping. Some recent studies have described the use of SmlI for genotyping the G23591A polymorphism (Lunn et al., 2000; Spitz et al., 2001; Xing et al., 2002). However, the genomic sequence of the XPD gene at and near the G23591A polymorphic site lacks the CCWWGG recognition site for SmlI. Further, any error in the reported sequence is ruled out as our sequencing results in this study were in complete conformity with the reported genomic sequence.

In this study we utilized the SSCP method for genotyping the G23591A polymorphism in exon 10 of the XPD gene and a novel 23623C insertion polymorphism located in intron 10.
of the same gene. Despite its obvious limitations, SSCP has remained the most commonly used method for the detection of mutations and polymorphisms in different genes over the years (Hayashi, 1991). It has been estimated that the method is capable of detecting 90% of the base changes in any given DNA sequence (Jaecckel et al., 1998; Nataraj et al., 1999; Berggren et al., 2000). Although the chances of detection of a single base change in any DNA sequence using SSCP are empirical, it seems that sequence length and context play an important role in the mobility shift of separated single strands due to single base changes.

Using the newly developed SSCP-based method we genotyped 302 healthy individuals in order to determine the haplotype and population frequencies of the G23591A and 23623C-ins polymorphisms in the XPD gene. The detected frequency of the variant allele for the G23591A polymorphism in exon 10 of the XPD gene was in conformity with the earlier frequency reported for a Caucasian population (Shen et al., 1998; Mohrenweiser et al., 2002). The two polymorphisms in the XPD gene, a single base change in exon 10 and a single base insertion in the adjacent intron that are 43 bp apart, are likely consequences of two separate events and to have originated through different mechanisms. The observation, at least in this study, that heterozygotes for both polymorphisms carried variants on different alleles suggests that some kind of selection against a haplotype with an A allele at position 23591 (in exon 10) and a C insertion at 23623 cannot be ruled out. Although repeat length polymorphisms in mononucleotide repeat sequences are known, they are more common in repeats with a greater number of nucleotides (Suraweera et al., 2001). Mutations in mononucleotide repeats located in the coding sequences of genes, like MSH2, TGFβII and BAX, involved in cell regulation are common in tumours with microsatellite instability and defective mismatch repair (Zhang et al., 2001; Duval and Hamelin, 2002).

In conclusion, in this communication we report detection of a novel insertion polymorphism (23623C-ins) in intron 10 of the XPD gene, which is located 16nt downstream of the 3’-end of exon 10 and involves a mononucleotide C repeat sequence. The effect of this polymorphism, if any, on the expression of XPD is unknown. We also report a method which can be used for simultaneous detection of both exon 10 and intron 10 polymorphisms and, consequently, for haplotype analysis.

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References


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XPD polymorphisms detected by SSCP