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BRIEF REPORT — POLYMORPHISM REPORT

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Identification of a novel *Tru9* I polymorphism in the human vitamin D receptor gene

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Abstract We found a novel *Tru9* I restriction polymorphism in intron 8 of the vitamin D receptor (*VDR*) gene in healthy French Caucasians. It corresponds to a substitution of A for G at nucleotide +443 bp from the end of exon 8. The allelic frequency of G and A in 151 unrelated subjects was 0.894 and 0.106, respectively. This polymorphism is located in the reverse primer binding site of primers that have been frequently used in the literature to genotype a *Bsm*I restriction polymorphism. The presence of the *Tru9*I A allele may result in allele drop-out when the *Bsm*I restriction fragment length polymorphism (RFLP) is genotyped with the original set of primers. This novel *Tru9*I polymorphism may be useful for analysis of the *VDR* gene.

Key words Vitamin D receptor gene · Polymorphism · Allele drop-out · Primer mismatch

Introduction

The vitamin D receptor (VDR) is a member of the steroid/thyroid hormone nuclear receptor superfamily. The *VDR* gene is located on chromosome 12q12–q14, and restriction fragment length polymorphism (RFLP) analyses for *Bsm*I, *Apa*I, and *Taq*I restriction endonucleases have been used to assess the influence of *VDR* genotypes in physiological and pathophysiological mechanisms (Hitman et al. 1998; McDermott et al. 1997; Morrison et al. 1994; Suarez et al. 1997).

We have genotyped the *Bsm*I variant in intron 8 of the *VDR* gene in healthy French Caucasians, as part of an investigation of loci possibly involved in insulin secretion. An 822-bp fragment was amplified by polymerase chain reaction (PCR), using primers previously described (Morrison et al. 1994). After digestion with *Bsm*I and electrophoresis on 1.2% agarose gels, we observed that, frequently in heterozygous subjects, the b allele containing the *Bsm*I restriction site was poorly amplified (Fig. 1A). This observation was constant for the same DNA sample under several experimental conditions.

Allele drop out by non-amplification may result from a mismatch of a primer and its complementary binding site due to a polymorphism in the binding site (Ellard et al. 1999). To test the hypothesis that inhibition of amplification was due to a polymorphism in one of the primer binding sites, we designed sets of primers flanking each of the original primer binding sites. The primers for the region containing the original reverse primer binding site are OrF 5′-AATACTCAGGCTCTGCTCTT-3′ and OrR 5′-CATCTCCATTCCTTGAGCCT-3′. With these primers, a 331-bp fragment was amplified. PCR conditions were: initial denaturation at 94°C for 5 min, and 35 cycles at 94°C for denaturation, 56°C for annealing, and 72°C for extension, each step lasting 45 s. After this amplification, PCR products from DNA samples that had presented poor (n = 4) or adequate (n = 3) amplification with the original primers were sequenced. Two mismatches were observed in the reverse primer binding site compared with the original reverse primer (R 5′-AACCAGC**GGG**AAGAGGT**C**-AAGGG-3′). The first one was the deletion of a C corresponding to the complementary base of one of the three Gs at position +8bp to +10bp from the 5′ end of the original reverse primer (printed in boldface type and underlined in the sequence above). This deletion was observed in all subjects that we have sequenced, and we wonder whether this variation is indeed a polymorphism or whether it is just due to a misprint in the original description of the primers. Indeed, the *VDR* gene sequence in Genbank (Muzny et al.: accession number AC004466) also lacks a third C in this position. Secondly, we observed a substitution of A for the G that is complementary to the C in position +18 in the original reverse primer (printed in boldface and underlined in the sequence above). This substitution was observed only in the four DNA samples that had been poorly amplified with the original primers. This substitution is located at

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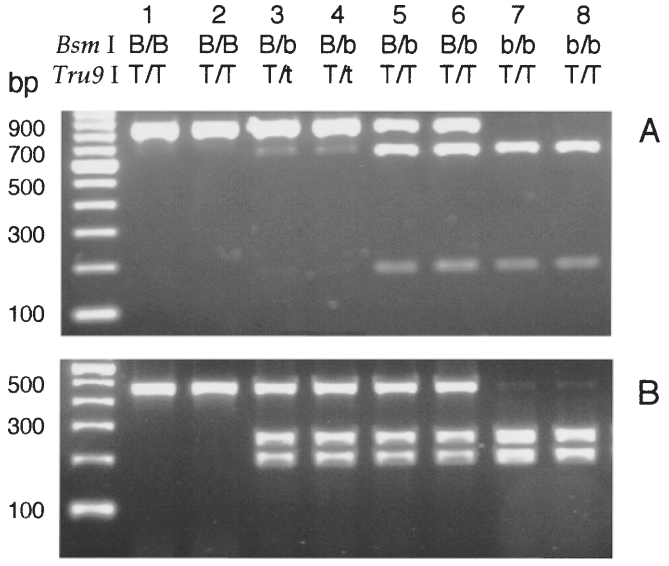

Fig. 1A,B. *Bsm*I restriction fragment length polymorphism in intron 8 of the *VDR* gene. **A** Polymerase chain reaction (PCR) amplification with the original set of primers. Note that in heterozygous subjects who are also heterozygous for the *Tru9*I variant (*lanes 3 and 4*) the *Bsm*I b allele is poorly amplified, while it is adequately amplified in heterozygous subjects who are homozygous for the *Tru9*I T allele (*lanes 5 and 6*). **B** PCR amplification with the new set of primers. In all heterozygous subjects the *Bsm*I b allele is now adequately amplified

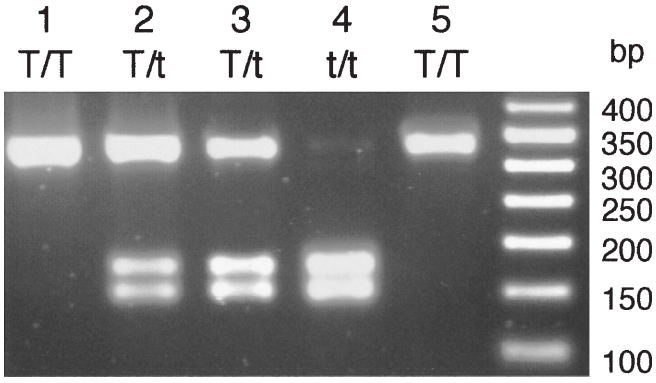

Fig. 2. Ethidium-bromide stained 2% agarose gel showing homo and heterozygous carries of the T and t alleles of the *True9*I restriction fragment length polymorphism in intron 8 of the VDR gene

+443 bp from the end of exon 8 (Muzny et al.: Genbank accession number AC004466).

This nucleotide substitution created a *Tru9*I restriction site (T | T**A**A), and the polymorphism was then tested by restriction site assay in 151 unrelated healthy Caucasian subjects to estimate its frequency. Genotypes were designated conventionally by the first letter of the name of the enzyme, with a capital letter for the absence of the cut site and a lower-case letter for the presence. Allele T (sequence G) yielded a 331-bp fragment and allele t (sequence A) two fragments, of 153 and 178 bp. The allelic frequency of T and t in this group of subjects was 0.894 and 0.106, respectively. The genotype frequency was in Hardy-Weinberg equilibrium: 80.1% TT, 18.6% Tt, and 1.3% tt.

To confirm that inhibition of amplification in the original *BsmI* experiments was due to the polymorphisms in the reverse primer binding site, we designed primers with the forward binding site internal to the original forward binding site and the reverse binding site external to the original reverse binding site. The new primers (NwF 5′-GGCAACCTGAAGGGAGACGTA-3′ and NwR 5′-CTCTTTGGACCTCATCACCGAC-3′) yielded a 461-bp fragment. PCR conditions were: initial denaturation at 94°C for 5 min, and 35 cycles at 94°C for denaturation, 57°C for annealing, and 72°C for extension, each step lasting 45 s. After digestion with *Bsm*I we observed that all alleles had been correctly amplified in the DNA samples in which poor amplification of the b allele had been observed with the original primers (Fig. 1B).

In conclusion, we have observed a novel *Tru9*I polymorphism in intron 8 of the *VDR* gene, in the reverse primer binding site of primers that have been frequently used in the literature to genotype the *Bsm*I RFLP. The presence of the *Tru9*I t allele can result in allele drop-out when the *Bsm*I RFLP is genotyped with the original set of primers. Under some PCR and digestion conditions, subjects who are heterozygous for the *Bsm*I variant who also carry the *Tru9*I t allele can be mistaken for homozygous carriers of the *Bsm*I only amplified allele. This novel *Tru9*I polymorphism could be useful for analysis of the *VDR* gene.

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