



Investigating the relationship between polymorphism rs406193 and the risk of prostate cancer

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Abstract

In many cancers, an increase in gene expression of DNMT3b has been reported. This enzyme inhibits the expression of many tumor suppressor genes through the methylation of the promoter sequence and plays a key role in the progression of cancer. In this study, the relationship between polymorphism rs406193 of this gene and the risk of prostate cancer in 60 samples was investigated. The results showed a significant association between genotype TT and the risk of prostate cancer ($p=0.048$). It is recommended that this study be repeated in larger populations and that the relationship between this polymorphism and gene expression be investigated.

INTRODUCTION

Gene expression silencing by methylation has been suggested as a major mechanism in the development of prostate cancer (1). DNA methylation is a covalent chemical change in which a group of enzymes called DNA methyl transferase (DNMT) add a methyl group (CH₃) to the carbon position of five cytosine rings in DNA (2). Methylation of cytosine nucleotides in humans is limited to the CG sequence (5'..CG..3'). DNA methylation can be de novo when CPG dinucleotide in both strands are non-methylated, or maintenance when CPG dinucleotides are methylated on one of the strands (3). DNMT1 has protective methyl transferase activity, while DNMT3A and DNMT3b have strong de novo activity. The mutation in the DNMT3b gene is responsible for craniofacial syndrome, which is characterized by features such as facial abnormalities, immune system defects, and centromere instability of chromosomes 1, 9, and 16 (4). This instability is associated with the abnormal hypomethylation of CPG sites in their pericentromeric satellite areas. In addition to point mutations and gene deletions, transcription suppression by hypermethylation of promoter sequences can also cause tumor suppressor genes in cancers (5). Hypermethylation can be caused by an increase in DNMT levels, which be detected in a variety of cancers (6). It has recently been found that T Nucleotide replacement (instead of C Nucleotide) in the DNMT3b promoter area (149 bp pairs after transcription initiation site) is

significantly associated with an increased risk of lung cancer (7). Although the mechanism of this association is not yet clear, it is assumed that the replacement of T instead of C positively regulates the expression of the DNMT3b gene, thereby de-novo causing inappropriate methylation of tumor suppressor genes (8). In this study, the rs406193 DNMT3b polymorphism was genotyped in prostate cancer patients and control subjects.

MATERIALS AND METHODS

Sixty blood samples, including 30 samples from prostate cancer patients and 30 from healthy individuals, were obtained from Imam Reza Hospital in Kermanshah to examine the relationship between DNMT3b gene polymorphism and the risk of prostate cancer. Blood sampling was performed in tubes containing anticoagulant EDTA-K2. QIAamp DNA Blood Mini Kits were used to extract DNA, and the extraction steps were performed according to the kit's protocol. RFLP-PCR was used to evaluate rs406193 genotypes. In order to amplify the target area, the PCR reaction was performed with specific primers. The reaction was performed using Ampliqon MasterMix and 100 pmol of each primer with an annealing temperature of 65 °C for 40 cycles. PCR products were digested with AvrII restriction enzyme at 37 °C and resolved on a 3% agarose gel. A p -value ≤ 0.05 was considered as significant.

RESULTS

The wild-type homozygote CC showed a single band of 380 bp, the variant TT showed two bands of 207 and 173 bp, and the heterozygote CT form was identified by the presence of three bands of 380, 207, and 173 bp on the agarose gel (Fig. 1). Table

1 shows rs406193 polymorphism frequencies in prostate cancer vs. control samples: 23% vs. 30% for CC (wild-type), 40% vs. 53% for CT, and 36% vs. 16% for TT. These results showed a significant association between genotype TT and the risk of prostate cancer ($p=0.048$).

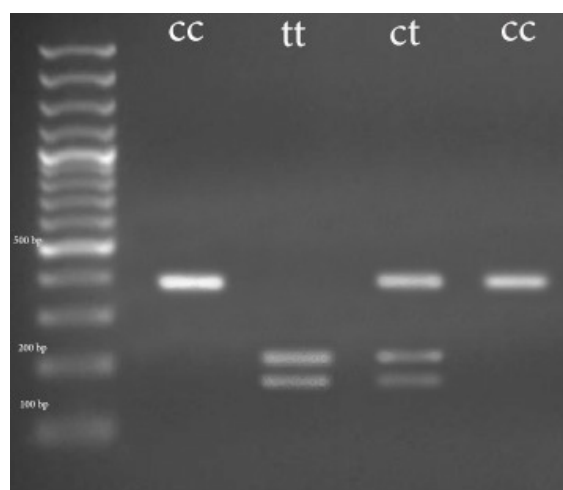


Fig 1. Enzymatic digestion analysis on Agar 2% gel: The wild-type homozygote CC showed a single band of 380 bp, the variant TT showed two bands of 207 and 173 bp, and the heterozygote CT form was identified by the presence of three bands of 380, 207, and 173 bp on the agarose gel

Table1. Genotype frequency of polymorphism rs406193 in patients and control group

Genotype	Prostate sample	Control sample	<i>p</i> -Value
CC	7(23%)	9(30%)	0.148
CT	12(40%)	16(53%)	0.133
TT	11(36%)	5(16%)	0.048

DISCUSSION

Promoter methylation is one of the most common epigenetic changes seen in cancer cells. As a result of these changes which usually occur in the promoter area, the expression of the related gene is silenced (9). In many cancers, an increase in the gene expression of DNMT3b has been reported. This enzyme inhibits the expression of many tumor suppressor genes by methylation of the promoter sequence and plays a key role in the progression of cancer (10). C/T polymorphism in the DNA methyltransferase 3b (DNMT3b) promoter region results in increased activity and has recently been identified as a risk factor for many cancers (7, 11). In this study, the relationship between polymorphism rs406193 of this gene and the risk of prostate cancer was investigated. The results showed a significant association between genotype TT and the risk of prostate cancer ($p=0.048$). Shen et al. reported an association between DNMT3b promoter polymorphisms and lung cancer, in which the combined variant genotype (CT + TT) was associated with a 2-fold increased risk (12). Singal et al. examined the association between polymorphism and prostate cancer in an American population and reported no significant association between this polymorphism and the risk of prostate cancer (13). It

is recommended that this study be repeated in larger populations and that the relationship between this polymorphism and gene expression be investigated.

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