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Short Communication

A CONVENIENT METHOD FOR GENOTYPING OF HUMAN O^6-METHYLGUANINE-DNA METHYLTRANSFERASE POLYMORPHISM

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Summary O^6-methylguanine-DNA methyltransferase (MGMT) is one of the DNA repair enzymes in mammals. We previously screened the variant alleles for the MGMT gene in the general population, and found three variants (V1, V2, V3), two of which caused amino acid substitutions (Leu84Phe for V1, and Trp65Cys for V2). In order to accelerate the ecogenetic and pharmacogenetic studies on MGMT polymorphism, we therefore developed a new PCR-based RFLP method for genotyping. The present method has some advantages over the initial PCR-single strand conformation polymorphism (SSCP) method, particularly regarding its simplicity, rapidity and specificity.

Key Words O^6-methylguanine-DNA methyltransferase, polymorphism, genotyping, PCR-RFLP, ecogenetics

Introduction

O^6-methylguanine-DNA methyltransferase (MGMT) is an enzyme which removes a methyl group from O^6-methylguanine of DNA damaged by alkylating agents. It thus protects DNA from mutations due to replication errors (Coulondre and Miller, 1977). In cultured human fibroblasts, the activity of MGMT showed over a 20-fold inter-individual difference, including individuals with almost no activity (Rüdiger *et al.*, 1989). Based on the assumption that this difference might be derived from genetic polymorphism in the MGMT gene, we previously screened the variant alleles in the general population using a PCR-single strand conformation polymorphism (SSCP) method, and found three variants (V1, V2 and V3), two of which (V1 and V2) cause amino acid substitutions (Otsuka *et al.*, 1996). V1 had C→T transition at nt 262 thus causing Leu84Phe. In addition, V2 had G→C transversion at nt 207, causing Trp65Cys. The allele frequencies of V1 and V2 were

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estimated to be 0.162 and 0.002, respectively.

As a step in establishing the clinical significance of this polymorphism, it is necessary to study the characteristics of these mutant enzymes, and such a study is now in progress at our laboratory. A complementary approach is given by assessing the association of polymorphism with multifactorial diseases, *i.e.* cancers and neurodegenerative disorders. To accomplish this, a simple and rapid genotyping method is thus required.

We herein report a convenient PCR-based RFLP method which can be used instead of the original PCR-SSCP method, and also a few examples of its clinical application.

Materials and Methods

After informed consent, peripheral blood was obtained, and genomic DNA was prepared from leucocytes in 5 ml of blood using the standard method (Sambrook *et al.*, 1989). PCR was performed using 100 ng genomic DNA, the primers, dNTP at 0.2 mM each, 0.45 U of *Taq* DNA polymerase (Wako, Osaka, Japan) in the presence of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100 at a final volume of 10 μl for 30 cycles as follows: 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C. The primers were MGM17 (5′-CTAAGCCCCTGTTCT-CACTTTT-3′) and MGM2 (5′-ACACCGCAGATGGCTTAGTTAC-3′), and final concentration was 0.5 μM. For V1 recognition, 2 U of *Eam*1104I was added to 4 μl of PCR product. Two units of *Mva*I was used to recognize V2. After overnight incubation at 37°C, the product was subjected to 3% agarose gel electrophoresis and then was stained with ethidium bromide.

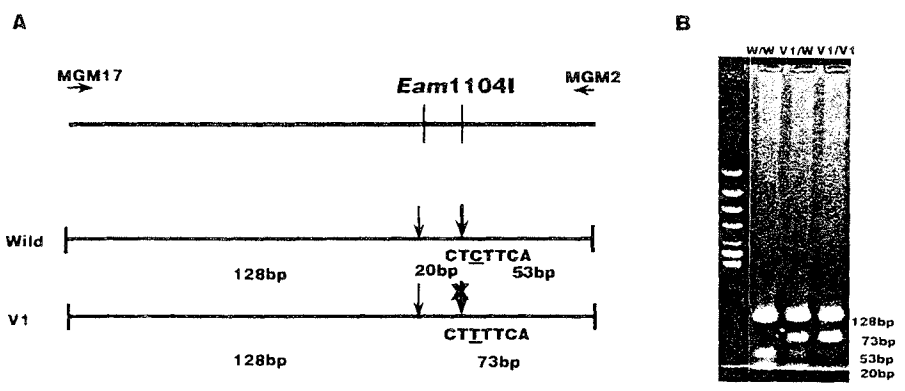

Fig. 1. The detection of a V1 variant. A. The *Eam*1104I recognition sites in an amplified fragment. B. Electrophoretic pattern. Lane 1, marker; lane 2, W/W; lane 3, W/V1; lane 4, V1/V1.

Results and Discussion

As shown in Figs. 1 and 2, the primers MGM17 and MGM2 produce a 201 bp fragment which contains the entire exon 3 of the MGMT gene. The PCR product derived from wild type gene (W) was cleaved into smaller fragments with either *Eam*1104I or *Mva*I: three (128, 20 and 53 bp) fragments with *Eam*1104I, and two (91 and 110 bp) fragments with *Mva*I. On the other hand, the mutations in V1 and V2 eliminate one of two *Eam*1104I sites and the *Mva*I site, respectively. Therefore, two (128 and 73 bp) bands for V1 and an undigested 201 bp band for V2 were

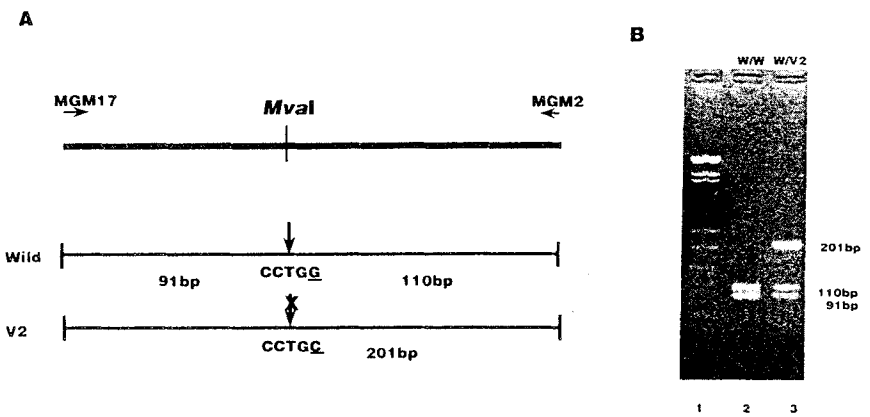

Fig. 2. The detection of a V2 variant. A. The *Mva*I recognition site in the amplified fragment. B. Electrophoretic pattern. Lane 1, marker; lane 2, W/W; lane 3, W/V2.

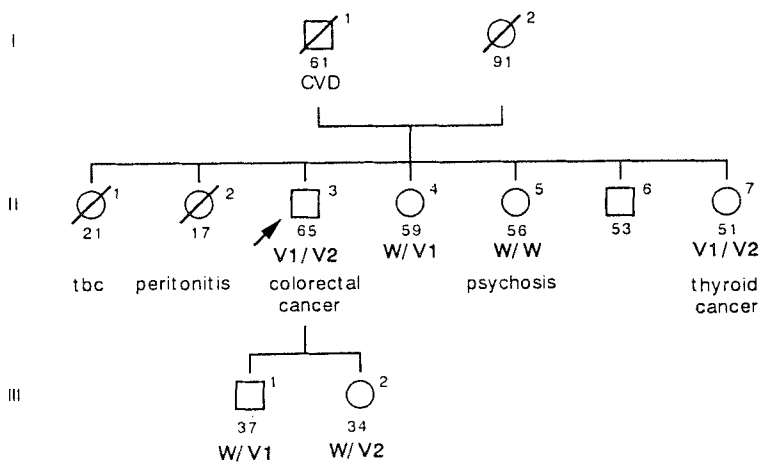

Fig. 3. Genotypes of MGMT polymorphism in a Japanese family. Note that the two members with genotype V1/V2 (II-3, II-7) both developed cancer.

Table 1. The distribution of the genotypes for MGMT polymorphism in normal subjects and in patients with colorectal cancer from "cancer families."

Genotype	Number of patients (%)	Number of normal subjects (%)	R.R.	χ^2 test
W/W	24 (80.0)	160 (71.1)	0.62	N.S.
W/V1	6 (20.0)	55 (24.4)	0.77	N.S.
V1/V1	0 (0)	9 (4.0)		
W/V2	0 (0)	1 (0.4)		
Total	30 (100.0)	225 (99.9)		

R.R.: relative risk, N.S.: not significant.

visualized on agarose gel electrophoresis. The genotypes of MGMT polymorphism in over 100 individuals determined by this method were completely consistent with those screened by the original PCR-SSCP method. The present method seems to be simple and specific, and particularly suitable for the mass screening of a large number of subjects.

Figure 3 illustrates an example of its application for analyzing a family whose propositus (II-3, genotype V1/V2) suffered from colorectal cancer. It is considered noteworthy that one of his siblings (II-7, genotype V1/V2) also suffered from cancer of the thyroid. Another example is shown in Table 1. It shows the distribution of the genotypes for MGMT polymorphism in normal subjects and 30 patients with colorectal cancer from such "cancer families." No significant difference was observed in the distribution of the genotypes between the two groups. In this survey, none of the 30 patients carried genotypes V1/V1, V1/V2, W/V2 or V2/V2. We should thus further investigate patients with cancers of various organs, such as the brain, lung, urinary bladder and female reproductive organs. This newly developed method for genotyping MGMT polymorphism is thus considered to accelerate both the ecogenetic and pharmacogenetic studies on MGMT polymorphism.

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REFERENCES

Coulondre C, Miller JH (1977): Genetic studies of the *lac* repressor, IV. Mutagenic specificity in the *lac*I gene of *Escherichia coli*. J Mol Biol **117**: 577-606

Otsuka M, Abe M, Nakabeppu Y, Sekiguchi M, Suzuki T (1996): Polymorphism in the human O^6-methylguanine-DNA methyltransferase gene detected by PCR-SSCP analysis. Pharmacogenetics **6**: 361-363

Rüdiger HW, Schwartz U, Serrand E, Stief M, Krause T, Nowak D (1989): Reduced O^6-methylguanine repair in fibroblast cultures from patients with lung cancer. Cancer Res **49**: 5623-5626

Sambrook J, Fritsch EF, Maniatis T (1989): Isolation of DNA from mammalian cells: Protocol I. In: Ford N, Nolan C, Ferguson M (eds). Molecular cloning. A laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, New York, pp 9.16-9.19