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Catalog of 46 single-nucleotide polymorphisms (SNPs) in the microsomal glutathione S-transferase 1 (*MGST1*) gene

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Abstract A major goal in our laboratory is to understand the role of common genetic variations among individual patients as regards susceptibility to common diseases and differences in therapeutic efficacy and/or side effects of drugs. As an addition to the high-density SNP (single-nucleotide polymorphism) maps of 12 glutathione S-transferase and related genes reported earlier, we provide here an SNP map of the microsomal glutathione S-transferase 1 (*MGST1*) gene. Among 48 healthy Japanese volunteers examined, we identified a total of 46 SNPs at this locus, 36 of which had not been reported before: 4 in the promoter region, 34 in introns, 3 in the 3' untranslated region, and 5 in the 3' flanking region. No SNP was found in 5' untranslated or coding regions. The ratio of transition to transversion was approximately 1.2:1. Among the 13 insertion–deletion polymorphisms was a 2-bp deletion in the coding region of *MGST1* in DNA from one of the volunteers, which resulted in a frame-shift mutation. Since the gene product encoded by this mutant allele would lack the C-terminal half including the MAPEG (membrane-associated proteins in eicosanoid and glutathione metabolism) domain, *MGST1* activity is likely to be reduced in the carrier's cells. The SNP map presented here adds to the archive of tools for studying complex genetic diseases, population migration patterns, and a variety of pharmacogenetic possibilities.

Key words Single-nucleotide polymorphisms (SNPs) · Highly dense SNP map · Microsomal glutathione S-transferase 1 (*MGST1*) · MAPEG family · Insertion–deletion polymorphisms · Japanese population

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Introduction

Glutathione S-transferases (GSTs, EC 2.5.1.18) constitute a group of phase II detoxification enzymes present in eukaryotic cells and prokaryotic organisms; they play significant roles in defense against carcinogenic, toxic, and pharmacologically active electrophilic compounds (Ketterer and Christodoulides 1994; Primiano et al. 1997). Microsomal glutathione S-transferase 1 (*MGST1*) is a homotrimeric, membrane-bound enzyme that catalyzes conjugation of electrophilic compounds with glutathione and reduction of lipid hydroperoxides (Andersson et al. 1994; Mosialou et al. 1993, 1995). Peroxidase activity may be of importance for protection against lipid peroxidation under conditions of oxidative stress. Although soluble and membrane-bound GSTs are not related with respect to amino acid sequences and immunological properties, they do display extensive functional similarities (Morgenstern et al. 1982; DeJong et al. 1988; Andersson et al. 1994; Mosialou et al. 1995). A number of biochemical approaches have been used to study the properties of *MGST1*, including enzymic activity, sub-cellular distribution, substrate specificity, and proteins structure (Andersson et al. 1994; Weinander et al. 1997; Bannenberg et al. 1999; Schmidt-Krey et al. 2000; Morgenstern et al. 2001). *MGST1* is considered a member of the MAPEG (membrane-associated proteins in eicosanoid and glutathione metabolism) superfamily of structurally and phylogenetically related enzymes, including *MGST2*, *MGST3*, *MGST1L1*, 5-lipoxygenase activating protein, and leukotriene C4 synthase. Enzymes in this superfamily are involved in detoxification, protection from oxidative stress, and synthesis of prostaglandin E and cysteinyl leukotrienes (for reviews, see Jakobsson et al. 1999, 2000).

MGST1, which is abundant in liver microsomes as well as in the outer mitochondrial membrane, has been purified from rat, mouse, and human tissues (Morgenstern et al. 1982, 1984; Andersson et al. 1988; McLellan et al. 1989). The cDNAs of human and rat *MGST1* contain open reading frames encoding 155 amino acid polypeptides, and the

two proteins are 85% identical in amino acid sequence (DeJong et al. 1988). The human *MGST1* gene spans approximately 18 kb of genomic DNA; of its seven exons, three comprise the coding element and four are alternatively spliced first exons (Kelner et al. 1996; Lee and DeJong 1999). Kelner et al. (2000) reported in detail the structural organization of the 5' untranslated region of human *MGST1*, including two functional first exons (exon 1b, 1d) and the promoter region, and mapped *MGST1* to chromosome 12p13.1–p13.2. Simultaneously, they discovered that the promoter region of *MGST1* upstream of the dominant first exon (exon 1b) is involved in the response to oxidative stress as a detoxification mechanism. Using a microarray technique, Chaib et al. (2001) recently established that expression of *MGST1* in human prostate tumors was twice that of normal tissues.

We have been focusing on variations in genomic regions that contain genes encoding enzymes involved in metabolism of drugs, and have described more than 1200 SNPs and other variations (Iida et al. 2001a,b,c; Saito et al. 2001a,b; Sekine et al. 2001). Regarding *GST* and related genes, we have already constructed high-density SNP maps of 12 genomic segments that included *GSTM1*, *GSTM2*, *GSTM3*, *GSTM4*, *GSTZ1*, *GSTP1*, *GSTT1*, *MGST1L1*, *MGST2*, *MGST3*, *GSTA1*, and *GSTA4* (Iida et al. 2001b), providing a total of 127 SNPs for these loci. In the study reported here, we screened a Japanese population sample for SNPs at the *MGST1* locus, as a 13th gene member of the *GST* family, by means of direct sequencing combined with a polymerase chain reaction (PCR) method for genomic DNA, and characterized 46 SNPs and 13 insertion–deletion polymorphisms.

Subjects and methods

Blood samples were obtained with written informed consent from 48 healthy Japanese volunteers for this study, which was approved by the ethical committee of the RIKEN SNP Research Center. On the basis of the *MGST1* DNA sequence (accession number AC007528.5) from the GenBank database and published reports (DeJong et al. 1988; Kelner et al. 1996, 2000; Lee and DeJong 1999), we designed primers to amplify the *MGST1* gene in its entirety, as well as 2 kb upstream from the first exon and downstream from the last exon. However, we excluded most of the regions corresponding to repetitive sequences predicted by the RepeatMasker program from the screening protocol. DNA extraction, PCR experiments, and DNA sequencing were performed according to methods described previously (Ohnishi et al. 2000). Each PCR was carried out using 20 ng of DNA pooled from three individuals. All SNPs detected by the PolyPhred Computer Program (Nickerson et al. 1997) were confirmed by sequencing both strands of each PCR product.

Results and discussion

The highly dense SNP map of the 21-kb genomic region presented here contains the entire human *MGST1* gene. The RepeatMasker program predicted the presence of nine copies of Alu sequence, six copies of mammalian interspersed repetitive element (MIR) sequence, three copies of L1 sequence, one L2 sequence, and one mammalian-wide apparent long terminal repeat transposon (MaLR) sequence, comprising in total approximately 24% of this 21 kb of genomic DNA. Nineteen partially overlapping PCR fragments were selected and sequenced using a total of 76 sequence primers. The exon organization of the *MGST1* gene and locations of identified SNPs are illustrated schematically in Figure 1. We documented 46 SNPs in the *MGST1* locus: 4 in the promoter region, 34 in introns, 3 in 3' untranslated regions, and 5 in the 3' flanking region (Table 1, also see Figure 1). No SNP was detected in 5' untranslated or coding regions. Since we had found no SNPs in the 5' untranslated or coding regions of other members of the MAPEG family examined in a previous study [*MGST1L1*, *MGST2*, and *MGST3* (Iida et al. 2001b)], we suspect that there might be strong selective pressure to conserve sequences in these regions. As regards the four SNPs upstream of the dominant first exon 1b (Kelner et al. 2000), three were adjacent to transcription-factor binding sites: i-MGST-2 and i-MGST-3 were located 7 bp upstream of the binding site of EpRE/EBP40 and 8 bp downstream of the HNF3 binding site, respectively; both of those sites appear to have some association with hepatocyte-specific gene expression (Kelner et al. 2000) and i-MGST-4 was located 2 bp upstream of an Sp1 binding site. These three SNPs might affect the quantity of gene product in response to oxidative stress.

After comparing our data with the National Center for Biotechnology Information (NCBI) dbSNP database, we considered 36 (78.2%) of the SNPs listed in Table 1 to be novel. Their overall genomic distribution was 1/364 bp in the 16.7 kb of DNA we sequenced. The frequencies of nucleotide substitutions were counted as 34.8% for C/T, 19.6% for A/G, 19.6% for G/T, 15.2% for C/G, 6.5% for A/T, and 4.3% for A/C. The ratio of transitions to transversions was approximately 1.2:1. This ratio in other MAPEG gene loci (*MGST1L1*, *MGST2*, and *MGST3*) had been established as 1.5:1, 3:1, and 0.9:1 respectively.

We also identified 13 novel insertion–deletion polymorphisms within introns 1C, 2, and 3; exon 3; and the 3' flanking region of the *MGST1* gene (Table 2). One of these was a 2 bp deletion in exon 3 that would cause a frame-shift mutation; the altered allele would generate a gene product lacking the C-terminal half that contains the MAPEG domain. Hence this result suggests that *MGST1* activity is likely to be reduced in the carrier's cells. However, since this mutation was found in a healthy volunteer, we have no information regarding its potential effect on the carrier's susceptibility to any disease or drug response.

Altogether we have collected a total of 173 SNPs among 13 *GST*s and related genes in the Japanese population. As

Microsomal glutathione S-transferase 1 (*MGST1*)

Fig. 1. SNP map spanning the 21-kb genomic region containing the *MGST1* gene. Exons are represented by vertical boxes or a line. The 46 SNPs are identified above the gene (designations correspond to the identification (ID) numbers in the left-most column on Table 1); locations of the 13 insertion-deletion polymorphisms listed in Table 2 are

indicated below the gene. Detailed information of variations isolated in this study is shown in Table 1 and Table 2. The region upstream of the dominant transcription initiation site at exon 1b is defined as the promoter region on the basis of a report described by Kelner et al (2000)

Table 1. Characterization of SNPs from the *MGST1* gene locus

ID	location		SNP (5' to 3')	repetitive seq.	dbSNP ID number
i-MGST1-1	promoter region	-1879	ttaataaaatgtttattcaat		
i-MGST1-2	promoter region	-508	tctggaccctgaacaggagg		
i-MGST1-3	promoter region	-314	cctggagattttaactttct		
i-MGST1-4	promoter region	-131	atcagcaggcgatggttact		
i-MGST1-5	intron1b	36	ggagaaggggaccgatgca		
i-MGST1-6	intron1c	456	ccccttgggacggttctcac		
i-MGST1-7	intron1c	719	gcccgcaagcattgtgtat		
i-MGST1-8	intron1c	985	cgagtaaaatttttctaccg		
i-MGST1-9	intron1c	1428	gtaaaagggaaggcggtcc		
i-MGST1-10	intron1c	2914	ctcatcaggtgtgtgcaga		
i-MGST1-11	intron1c	4274	attgtaaatagatttaacaaag		
i-MGST1-12	intron1c	4276	tgtaaatagatttaacaaagg		
i-MGST1-13	intron1c	4406	gatggctatatgaccaataa		rs1913262
i-MGST1-14	intron1c	4464	agaaagattgcagctgatag		rs1913263
i-MGST1-15	intron1c	4683	aatggcagaggactggaaat		rs1913264
i-MGST1-16	intron1c	4767	gccttctctctcagcacatt		
i-MGST1-17	intron2	2379	ttctcaaatttcattataca		
i-MGST1-18	intron2	2767	tttaactatagatgccttct		
i-MGST1-19	intron2	2974	tcactgcagcctcaacacct		
i-MGST1-20	intron2	3083	aaaaaattttagatatggg		
i-MGST1-21	intron2	3106	tccctatgttgcccaggctg		
i-MGST1-22	intron3	1495	gtcagacaatggccttcagc		
i-MGST1-23	intron3	1703	ttctcttctaagaagaagtc		
i-MGST1-24	intron3	2528	ttttggagacacttttcaga		
i-MGST1-25	intron3	2557	tccagcatcttccctttcca		
i-MGST1-26	intron3	2731	atacacatatgggaacaatta		
i-MGST1-27	intron3	3032	agagacatttagaataatatt		
i-MGST1-28	intron3	3045	atatattcccttttaaggta		
i-MGST1-29	intron3	3289	ggtttatagtggtccccccc		
i-MGST1-30	intron3	3885	gaagctgcggtccagggaag		
i-MGST1-31	intron3	3976	ggaaagctggggaactgttt		
i-MGST1-32	intron3	4288	ccattctatttgcacactgc		
i-MGST1-33	intron3	4298	tgtaactgcgttaacacagg		
i-MGST1-34	intron3	4378	aaatgtctgtccttttggca		
i-MGST1-35	intron3	4429	attggagggtgacgatctct		
i-MGST1-36	intron3	4519	tttaatagaataatgggtatt		
i-MGST1-37	intron3	4817	attgctatagaagagagtaa		
i-MGST1-38	intron3	6077	tttgaaattagtgctcttaa		
i-MGST1-39	3' untranslated region	487	aaagaaaatcatacaactca		rs11875
i-MGST1-40	3' untranslated region	525	aagaattctgtactctcgaat		rs1042669
i-MGST1-41	3' untranslated region	603	gggtaaacccattttgaata		
i-MGST1-42	3' flanking region	147	tatttgctttctctctctct		
i-MGST1-43	3' flanking region	237	cagcacgtttttctctatgaa		
i-MGST1-44	3' flanking region	1318	tggtctgtgtgcatgaaca		rs1024834
i-MGST1-45	3' flanking region	1322	tctgtgtgcatgaacatgca		rs1024835
i-MGST1-46	3' flanking region	1331	atgaacatgcacgcgtgcac		rs1024837

Nucleotide numbering is according to mutation nomenclature (Dunnen and Antonarakis, 2000)

MGST1, microsomal glutathione S-transferase 1

Table 2. Characterization of insertion–deletion polymorphisms from *MGST1* gene locus

ID	location		Variation (5' to 3')	repetitive seq.
i-MGST1-47	intron1C	904–923	tgcgattatctttggttaatt	(A) _{16–19} ggcaaatcagtcctcaatttg
i-MGST1-48	intron1C	3433–3434	ccccctcaactagaaacaa	gcagacacattaaatgttac
i-MGST1-49	intron1C	5146	actatttcaattttttttt	ggagggggagacagagtctc
i-MGST1-50	intron2	552–563	cccagcattataagaatgac	aagtgcagatgtggggaggg
i-MGST1-51	exon3	172–173	tagcatttggcaaggagaa	tgccaagaagatcttcgaa
i-MGST1-52	intron3	152–158	agaaaactggatgtctgaaa	TTGACA/delGTCCAATAT/ins
i-MGST1-53	intron3	2198–2200	ggatttttagattcctcccta	CTA/del
i-MGST1-54	intron3	2567–2568	ccctttccatttttaagtta	(T) _{10–12} A/ins
i-MGST1-55	intron3	2571–2580	tttccatttttaagtttagac	(T) _{9–11} C/ins
i-MGST1-56	intron3	3288–3289	ggtttatagtggtccccc	GAGATGTTGTGGCTCACAT/ins
i-MGST1-57	intron3	4682–4683	tcctcttcatgtctctatgt	CC/del
i-MGST1-58	3' flanking region	1359–1360	acacacacacacacacacac	ACT/del
i-MGST1-59	3' flanking region	1889–1891	ttagaatagtttctaactat	

Nucleotide numbering is according to mutation nomenclature (Dunnen and Antonarakis 2000)

ins, insertion polymorphism; del, deletion polymorphism

an adjunct to this work, we have been able to establish a high-throughput system for genotyping up to 100 million SNPs per year, using a multiplex PCR method with the Invader assay (Ohnishi et al. 2001). In the near future, this powerful system will provide data that should assist in efforts to reach the goal of personalized medicine, and should be useful also for identifying genes involved in drug efficacy and/or side effects.

All data collected so far concerning SNPs in genes encoding drug-metabolizing enzymes and the sequences of primers used for PCR and PCR conditions are available on our website (<http://snp.ims.u-tokyo.ac.jp/>).

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