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SHORT COMMUNICATION

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Identification of 20 novel SNPs in the guanine nucleotide binding protein alpha 12 gene locus

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Abstract Heterotrimeric guanine nucleotide binding proteins (G proteins) regulate various signals from transmembrane receptors to intracellular effectors thereby mediating cell growth, differentiation, and apoptosis. We have been publishing a series of genetic variations detected in the genomic regions corresponding to the potential drug target genes. As an addition to genetic information reported earlier, we provide here 20 novel single nucleotide polymorphisms (SNPs) in the region corresponding to a gene encoding α subunits of G_{12} protein, *GNA12*, in the Japanese population: 16 in introns, two in the coding region, and two in the 3′ flanking region. We also identified 12 genetic variations of other types from this locus. The collection of genetic variations reported here will serve as a useful resource for analyzing potential associations between genotypes and susceptibility to common diseases as well as efficacy and/or adverse reactions to drugs.

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Introduction

Heterotrimeric guanine nucleotide binding proteins (G proteins), which are composed of α, β, γ subunits, act as a molecular switch that mediate a wide variety of extracellular signals from G-protein coupled receptors to effector molecules within cells (see a review by Dhanasekaran et al. 1998). On the basis of sequence similarities of the α subunits of G protein, they are divided into four groups: G_s, G_i, G_q, and G_{12} (Hurowitz et al. 2000). It is also reported that members of the G_{12} subfamily, consisting of $G_{\alpha 12}$ and $G_{\alpha 13}$, are ubiquitously expressed and share more than 67% amino-acid sequence identity (Strathmann and Simon 1991). The gene encoding human $G_{\alpha 12}$, *GNA12*, was originally identified as a transforming gene by means of an expression cloning method to search the putative oncogene for soft-tissue sarcoma (Chan et al. 1993). Overexpression of wild-type $G_{\alpha 12}$ leads to the oncogenic transformation of NIH3T3 cells in a serum-dependent manner. Subsequent functional analyses revealed that $G_{\alpha 12}$ as well as $G_{\alpha 13}$ are involved in the regulation of various signaling pathways, such as cell growth, differentiation, cytoskeletal changes, and apoptosis (see reviews by Radhika and Dhanasekaran 2001; Kurose 2003).

Single nucleotide polymorphisms (SNPs) at some gene loci are indicated to be useful as DNA markers of individual risk for adverse drug reactions or susceptibility to complex diseases. To establish the bases of SNP information for genetic studies of complex diseases and responsiveness to drug therapy, we have been focusing on isolating SNPs in gene loci encoding proteins involved in the metabolism, transport, and signaling of drugs. So far, high-density SNP maps containing

Guanine nucleotide binding protein (G protein) alpha 12 *(GNA12)*,123-kb

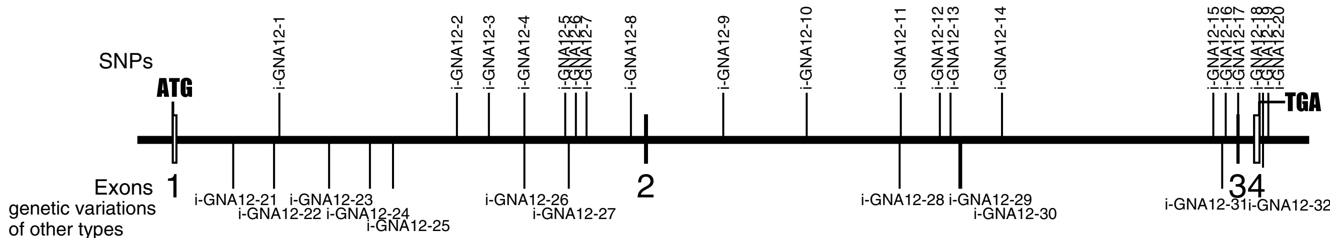

Fig. 1 Genomic organization and locations of 32 genetic variations in *GNA12* locus. Exons and introns are represented by *rectangles* and *horizontal lines*, respectively. Single nucleotide polymorphisms (SNPs) are indicated above the lines (designations correspond to the left-most column of Table 1). Genetic variations of other types, where present, are indicated below the maps. However, the complete 5′ untranslated sequences and 3′ untranslated sequences of *GNA12* was as yet unidentified in the database we used

approximately 6,800 genetic variations have been constructed (Iida et al. 2001a,b,c,d,e; 2002a,b,c,d; 2003; 2004; Saito et al. 2001a,b; 2002a,b,c,d; 2003a,b; Sekine et al. 2001). Furthermore, we reported several distinct mutations in the genes encoding drug metabolizing enzymes and potential drug receptors among Japanese healthy donors (a review by Iida and Nakamura 2003; Iida et al. 2004). As an addition to SNP information reported earlier, we provide here 20 novel SNPs and 12 genetic variations of other types in the *GNA12* locus.

Subjects and methods

Samples of peripheral blood were obtained with written informed consent form 48 healthy Japanese volunteers

Table 1 Characterization of 32 genetic variations in the *GNA12* locus. *ins* insertion polymorphism, *del* deletion polymorphism. An accession number of the genomic sequence obtained from Genbank is AC006028.3

I.D.	Location	Exon	Position[a]	5′ Flanking sequence[b]	Variation[c]	3′ Flanking sequence[b]	Substitution
i-GNA12-1	Intron 1		10818	tgtgatgggttagtctttct	C/G	tctgtgaggataaatgctca	
i-GNA12-2	Intron 1		29241	aggaaaaggaaataag(G/A)aat	T/C	ttttggtgggagttgcggct	
i-GNA12-3	Intron 1		32463	gccaaggctgggaaactaga	G/C	ttctggcagctttgttgctc	
i-GNA12-4	Intron 1		36276	tttttttttttctctctta	T/C	accttattttaatgctcatt	
i-GNA12-5	Intron 1		40521	ccttttccaaagccctcgat	C/A	gtcccctttctcacacagac	
i-GNA12-6	Intron 1		41460	accccaccccccacccccccc	A/C	aaaaaaatctacatccccag	
i-GNA12-7	Intron 1		42654	atttctgtatttgagttgga	C/T	gagcagggccttcccggata	
i-GNA12-8	Intron 1		47226	aacatgatcccctggctccc	G/A	ttttggtgggcgggctactt	
i-GNA12-9	Intron 2		7986	cagtggcatctggtgtcttc	C/T	ttgccgggggcttggctctc	
i-GNA12-10	Intron 2		16662	aggttttgtgagaattttgc	G/A	tttaagccaaatgaaatgct	
i-GNA12-11	Intron 2		26464	tttcatttcacgcagtcctc	G/A	aatgcagttagtgtttttct	
i-GNA12-12	Intron 2		30404	tgtgaagtaaacgctgagcc	C/G	gaccacaaccactgtgaata	
i-GNA12-13	Intron 2		31563	ggaactcggccttctccgcc	C/G	gatgaagcaaacaaactgtg	
i-GNA12-14	Intron 2		36858	gctgctgactcatcctgttg	G/A	ttttgagttagggagtgact	
i-GNA12-15	Intron 2		58844	aacctggcccttttaatgag	C/T	tgctgctgtaagacttgagg	
i-GNA12-16	Intron 2		60096	ctctagagagccggtggtca	C/T	gaggtgcacgtgctcgcccc	
i-GNA12-17	Coding region	3	534	ccttcctgacagggggagtc	G/A	gtgaagtacttcctggacaa	Ser178Ser
i-GNA12-18	Coding region	4	1062	caccacttcaccaccgccat	C/T	gacaccgagaacgtccgctt	Ile354Ile
i-GNA12-19	3′ Flanking region		341	t(aatt/del)gaggaccgtgttgtgtgt	G/C	tatgtgtgtacacacgctct	
i-GNA12-20	3′ Flanking region		1504	tatcccagggccctcgtccc	G/A	aggccgtgctgccccgagcc	
i-GNA12-21	Intron 1		6012	aaaattgtcccttttttttt	T/del	attacctattctgatggtct	
i-GNA12-22	Intron 1		10112∼10113	gcttctggggtctggaagca	CA/del	gtttggtttttatggccttg	
i-GNA12-23	Intron 1		15929∼15930	ctttcattaattaaaaaaaa	A/ins	ttttaaataaagtatcgggg	
i-GNA12-24	Intron 1		20154	ttaatttttaattttttttt	T/del	agcttgcctagccaactaga	
i-GNA12-25	Intron 1		22589∼22590	cctgtgttgaacaggcggag	AG/del	tctcccctcaggatacagca	
i-GNA12-26	Intron 1		36255∼36267	gctgtgttatcctggctagg	(T)12∼15	ctctctta(T/C)accttatttta	
i-GNA12-27	Intron 1		40754∼40755	tttaccgccttttgggtttt	T/ins	ccccattcgttacccaccac	
i-GNA12-28	Intron 2		26399∼26400	cctttgttttcctgagtgtt	AAA/ins	acatccatgattttaagggc	
i-GNA12-29	Intron 2		32564∼32565	gggaaccgccataccgtgtc	C/ins	tggattcggtgggatcgtgt	
i-GNA12-30	Intron 2		32721∼32723	acgaagcccttacaacttct	CCT/del	agaaacgaagcctgggttga	
i-GNA12-31	Intron 2		59812∼59813	gaacttgtcgtaaatcaggg	G/ins	agtgagtgcacccaacggct	
i-GNA12-32	3′ Flanking region		319∼322	ctctttttctgacgcagttt	AATT/del	gaggaccgtgttgtgtgt(G/C)t	

[a]Nucleotide numbering is according to the mutation nomenclature (den Dunnen and Antonarakis 2000)
[b]Both 5′ and 3′ flanking sequences to each single nucleotide polymorphism (SNP) are denoted by small letters
[c]Variation is shown by capital letters

for this study. The SNP screening method described in an earlier report by Haga et al. (2002) was the principal technique applied in this study. Each polymerase chain reaction (PCR) was performed using 20 ng of a mixture of genomic DNAs from three individuals. All 16 mixed samples were amplified in the GeneAmp PCR system 9700 (PE Applied Biosystems, Foster City, CA, USA) under the following conditions: initial denaturation at 94°C for 2 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 2 min, and postextension at 72°C for 7 min. Products obtained from the PCR experiments served as templates for direct sequencing and detection of SNPs using the fluorescent dye-terminator cycle-sequencing method. 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

We performed direct sequencing of DNAs from 48 healthy individuals in a total of 36-kb regions (excluding the parts corresponding to human repetitive sequences) that corresponded to 29.3% of the 123-kb genomic region containing *GNA12*. We identified 86 SNPs in this region (SNPs were distributed every 419 nucleotides on average). By comparing our data with the SNPs deposited in the dbSNP database in the National Center for Biotechnology Information, USA, we considered 20 of these SNPs to be novel as of the beginning of April 2004. The exon-intron organization of *GNA12* and locations and detailed information of the 20 novel SNPs are illustrated schematically in Fig. 1 and Table 1, respectively. Subregional distributions of novel SNPs were as follows: 16 in introns, two in the coding region, and two in the 3′ flanking region. The overall frequencies of nucleotide substitutions were counted as 30% for A/G, 35% for C/T, 10% for A/C, and 25% for C/G. The transitions occurred 1.9 times more frequently than transversions. Both of the two substitutions found in the coding region were synonymous substitutions: one was 534G > A (Ser178Ser) in exon 3, and the other was 1062C > T (Ile354Ile) in exon 4. We also identified 12 genetic variations of other types in the region.

Altogether, we have collected 32 genetic variations, including 20 SNPs and 12 genetic variations of other types, in *GNA12* locus by screening 96 Japanese healthy donors. We hope that genetic variations can contribute to further investigations for designing personalized medicine.

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