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Identification of 46 novel SNPs in the 130-kb region containing a myocardial infarction susceptibility gene on chromosomal band 6p21

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Abstract We identified a total of 187 single-nucleotide polymorphisms (SNPs) at 11 gene loci in the 130-kb region on chromosome 6p21 containing a gene strongly associated with myocardial infarction (MI). By comparing our data with SNPs deposited in the dbSNP database at the National Center for Biotechnology Information, 46 of these SNPs (24.6%) were considered to be novel: four were identified in the *P5-I* locus, 14 in the *MICB* locus, nine in the *BAT1* locus, one in the *ATP6V1G2* locus, six in the *NFKB1L1* locus, one in the *LTA* locus, one in the *TNF* locus, five in the *LST1* locus, four in the *LY117a* locus, and one in the *AIF-1* locus. The SNP map presented here should provide as useful resource not only for examining the relationships between genotypes and susceptibility to the MI phenotype, but also for scanning of complex diseases mapped to this local segment on chromosome 6.

Keywords Single-nucleotide polymorphism (SNP) · Fine-scale SNP map · Myocardial infarction critical region · Myocardial infarction susceptibility gene

Introduction

Despite the changes in life style and development of new pharmacologic approaches, coronary artery dis-

eases including myocardial infarction (MI) remain the principal causes of death in the world, indicating the importance of identifying genetic and environmental factors in their pathogenesis (for reviews, see Braunwald 1997; Breslow 1997). By means of a genome-wide gene-based single-nucleotide polymorphism (SNP) analysis and subsequent functional assay, we previously identified the *LTA* gene as an MI susceptibility gene on 6p21, and two genetic variants in the gene that may influence lymphotoxin- α (*LTA*) function both quantitatively and qualitatively (Ozaki et al. 2002). During the search for the MI susceptibility gene in this critical region, we attempted to construct a high-density SNP map for linkage disequilibrium (LD) mapping by direct sequencing analysis and identified one extended block of intense LD with LD coefficients drop-off near *P5-I* and *AIF-1* (Ozaki et al. 2002).

In this paper, we provide a high-resolution SNP map of this MI critical region on 6p21, in which we have detected a total of 46 novel SNPs among 64 chromosomes from 16 individuals with MI and 16 control individuals.

Subjects and methods

Blood samples were obtained with written informed consent from 16 individuals with MI and 16 control individuals for this study, which was approved by the ethical committee of the RIKEN SNP Research Center. We generated a reference sequence of 130-kb by assembling sequences Y14768, AP000506, and AC004184 from the GenBank database and then designed primer sets to amplify the 11 gene loci (*AIF-1*, *LY117a*, *LST1*, *LTB*, *TNF*, *LTA*, *NFKB1L1*, *ATP6V1G2*, *BAT1*, *MICB*, and *P5-I*), excluding over most of the regions that corresponded to repetitive sequences predicted by the Repeat Masker program (<http://repeatmasker.genome.washington.edu/cgi-bin/RepeatMasker>).

Each polymerase chain reaction (PCR) was performed with 20 ng mixed genomic DNA derived from two individuals. Amplification of genomic DNA fragments by PCR, and DNA sequencing of the amplified fragments were performed according to methods described previously (Iida et al. 2003).

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Table 1 Summary of 46 novel SNPs in the MI susceptibility gene region on chromosome 6p21

ID	Region	Exon	Position ^a	5' Flanking sequence ^b	SNP ^c	3' Flanking sequence ^b	Substitution	Repetitive sequence
MHC class I region ORF								
i-P5-1-1	5' Flanking region		-19	ttataagtcctcgcggcc	T/C	accgggactcagattctccc		
i-P5-1-2	Coding region	2	198	ggggatccctgggtccaca	T/C	gaaactctctaccctcatt	His66His	
i-P5-1-3	Coding region	2	312	tctacccgtgtgtctctgg	G/A	cacatactgtccaatcccc	Gly104Gly	
i-P5-1-4	3' Untranslated region	2	466	gcaaaatccacagacagtg	G/A	gtfgaaggaaatgggataatgg		
MHC class I polypeptide-related sequence B								
i-MICB-1	5' Flanking region		-327	tcgggatgcaggaggcgga	T/C	agggtccagggtcgtgctcat		
i-MICB-2	Intron 1		6941	ccctcatctcacctccact	A/G	tgcaccagaccacactggga		
i-MICB-3	Coding region	2	86	ccagagccccacagtcttc	A/G	ttacaacctatggtgctgt		
i-MICB-4	Coding region	4	699	ggctccaggtctatcccg	A/G	aatacacactgacctggcg	Arg29His	
i-MICB-5	Intron 5		187	ataaggtcttcC/Tcaagccag	A/G	gcctggggcaaggcttcA/Gaa	Arg233Arg	
i-MICB-6	Intron 5		205	agA/Ggtggggcaaggcttc	A/G	aataccagctgtggcctcc		
i-MICB-7	Intron 5		255	aagtgaagggtggcagcag	A/G	gaagggtgtggcaccctgctc		
i-MICB-8	Intron 5		347	tgggggtgagttgggaaacac	A/G	tgcgtatgtctgaaggccctg		
i-MICB-9	Intron 5		989	gtcggccccatgacctgag	A/C	agatgggaaagggttctgctccc		
i-MICB-10	Intron 5		1760	ccccccaggtcttcgatgg	T/G	gtcccaagttaaggagctgccc		
i-MICB-11	Intron 5		2223	cacaacatgcaccaggtgg	G/A	gcatttaccctgttccctct		
i-MICB-12	3' Flanking region		22	gacatttctgtctgtgtt	C/T	atttcattgcgtctgggagcgg		
i-MICB-13	3' Flanking region		53	tcctgggagcggataaaggt	A/G	agggttcaaggaggaaaggagag		
i-MICB-14	3' Flanking region		670	tttggcataaaataaaacag	C/G	taccataaaaggaaaaactgt		
HLA-B associated transcript 1								
i-BAT1-1	5' Flanking region		-1949	actgtagaagaaaaactggag	T/C	gtaggagcaaggtctctttg		
i-BAT1-2	5' Flanking region		-1382	tcactgaagacaagcttact	T/G	caccaagaatttgaaccaat		
i-BAT1-3	Intron 1		684	gcttgcaacgccccctccc	C/A	ccatgctaattacaagaaggc		
i-BAT1-4	Intron 2		30	ctcttggggcgtgagtgctc	A/C	ttgggtctctttaaagggtaca		
i-BAT1-5	Intron 2		1037	taattattctctcccccc	C/G	caacttttagtccagcatga		
i-BAT1-6	Intron 7		62	ccagctgtagcagaaccctg	G/A	atattaaagtacacttttatt		
i-BAT1-7	Intron 7		1155	gcaatttgaattctgaact	C/T	tgtgtacctcagaggccag		
i-BAT1-8	Intron 10		172	ttctgaccttattgtgggt	T/G	tcgtT/Gcttctcatat		
i-BAT1-9	Intron 10		177	acctttatgggggtT/Gtctg	T/G	cttctctcatatattacat		
ATPase, H + transporting, lysosomal 13-kDa, V1 subunit G isoform 2								
i-ATP6V1G2-1	3' Untranslated region	3	510	ttctgtgacacttataaat	A/G	tcctgtcacatctgaatct		
Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1								
i-NFKBIL1-1	3' Untranslated region	4	1304	caagtgggggtgggggtgc	A/G	ggccgccaccactgctcctt		
i-NFKBIL1-2	3' Flanking region		118	ccgtcaacacacacgcacac	A/G	ctaaacagggtctctgagctg		
i-NFKBIL1-3	3' Flanking region		248	ctgtccacctgcgaactgt	C/T	ggctggagaataatagatgga		
i-NFKBIL1-4	3' Flanking region		322	ctccccctctgctcccca	A/G	gtgaaccacatctctctcc		
i-NFKBIL1-5	3' Flanking region		695	caaactcatctcgccatt	G/C	agctgctgccccatctctaaa		
i-NFKBIL1-6	3' Flanking region		859	gggggtcccttggaactaag	G/A	taaggtagaccccaactacat		
Lymphotoxin alpha								
i-LTA-1	Intron 1		204	ctctcgggggtgggggggtg	G/C	tctctccaggggcgggagggt		
Tumor necrosis factor								
i-TNF-1	5' Flanking region		-390	atgA/Gggaggggttcagcct	T/C	cagggtctctacacacaaatc		
Leukocyte-specific transcript 1								
i-LST1-1	5' Flanking region		-511	tttacttagttctgtttca	A/G	tgccagaatgtctgctctcta		
i-LST1-2	Intron 1a		290	ggctctgggggtgaaagaac	A/G	ggcccccttccacccctcaaa		
i-LST1-3	Intron 1b		23	gfgtcatatcacctctccct	T/A	cttcccccaagtgcccacac		

Table 1 (Continued)

ID	Region	Exon	Position ^a	5' Flanking sequence ^b	SNP ^c	3' Flanking sequence ^b	Substitution	Repetitive sequence
i-LST1-4	Intron 4		437	agagagagagagagagagag	A/G	gagagagagagagagagag		
i-LST1-5	3' Untranslated region	5	597	aatttttaaaataaat	T/A	aaaaaacacatggctacc		+
LY117/Natural cytotoxicity triggering receptor 3, alternative splicing variant, LY117a								
i-LY117a-1	5' Untranslated region		-204	tcctccagcagcatctgtcc	T/C	ctctctcaggaggaggaagc		
i-LY117a-2	Coding region	2	111	gaaggatcctctgccttct	G/A	ccctgccttcaatgccag	Leu37Leu	
i-LY117a-3	Coding region	2	156	gggagactggccattggctc	C/T	gtcacgtggttcggagatga	Ser52Ser	
i-LY117a-4	Intron 3		77	gggaggaggaggacacaga	C/T	caggagaggaggagccctcgg		
Allograft inflammatory factor 1								
i-AIF-1-1	Intron 1		93	agggcagtcagctggcctag	A/G	gtagcccgggctggtgtcag		

^aNucleotide numbering is according to the mutation nomenclature (den Dunnen and Antonarakis 2000)

^bBoth 5' and 3' flanking sequences to each variation are denoted by small letters

^cSingle nucleotide polymorphism (SNP) is shown by capital letters

Fig. 1 Transcription map and locations of 46 novel single nucleotide polymorphisms (SNPs) in the 130-kb region containing a myocardial infarction susceptibility gene on chromosomal band 6p21. Each gene and its orientation are represented by an open rectangle and an arrowhead, respectively. SNPs are indicated below each gene as vertical lines

Results and discussion

By direct sequencing of DNA from 16 individuals with MI and 16 control individuals, we screened SNPs in a 130-kb genomic region corresponding to the MI critical region on 6p21, which contains 11 genes, except for most of the regions of human repetitive sequences, and identified 187 SNPs. SNPs were distributed every 275 nucleotides on average in the 51.4-kb regions screened, although their densities varied considerably in these regions. We found 68 SNPs in the *MICB* gene locus of which 8.6-kb was examined (1 SNP/127 nucleotides), whereas only two were identified in the 3.6-kb genomic region containing the *LTB* gene (1 SNP/1783 nucleotides). By a comparison of our data with SNPs deposited in the dbSNP database in National Center for Biotechnology Information (NCBI 2003), 46 of the 187 SNPs were considered to be novel (Table 1). Of the 46 SNPs, four were identified in the *P5-1* gene locus, 14 in the *MICB* gene locus, nine in the *BAT1* gene locus, one in the *ATP6V1G2* gene locus, six in the *NFKB1L1* gene locus, one in the *LTA* gene locus, one in the *TNF* gene locus, five in the *LST1* gene locus, four in the *LY117a* gene locus, and one in the *AIF-1* gene locus (Fig. 1, Table 1). The SNPs consisted of 32 transitions and 14 transversions; thus, transitions occurred 2.3 times more frequently than transversions.

The gene-based SNP map presented here should provide as useful resource not only for further examining the relationships between genotypes and susceptibility to the MI phenotype, but also for screening of genes associated with complex diseases mapped to this local segment on chromosome 6.

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