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Identification of 45 novel SNPs in the 83-kb region containing peptidylarginine deiminase types 1 and 3 loci on chromosomal band 1p36.13

Received: 23 March 2004 / Accepted: 25 March 2004 / Published online: 19 May 2004
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Abstract Peptidylarginine deiminases (PADIs) are post-translational modification enzymes that catalyze the conversion of protein-bound arginine residues into citrulline residues in the presence of calcium ions. Among PADIs, *PADI4* was identified as a rheumatoid arthritis-susceptibility gene (Suzuki et al. in Nat Genet 34:395, 2003). We identified a total of 87 single nucleotide polymorphisms (SNPs) in *PADI1* and *PADI3* gene loci. Following a comparison of our data with SNPs in the dbSNP database in the National Center for Biotechnology Information, 45 SNPs are considered to be novel: 33 were identified in the *PADI1* gene locus and 12 in the *PADI3* gene locus. We also identified two insertion–deletion polymorphisms in introns of the *PADI1*. The high-resolution map that we constructed in this study will serve as a useful resource for analyzing gene scans of complex diseases mapped to this local segment on chromosomal band 1p36.13.

Keywords Single nucleotide polymorphism (SNP) · Japanese population · High-density SNP map · Rheumatoid arthritis · Peptidylarginine deiminase 1 gene · Peptidylarginine deiminase 3 gene

Introduction

Peptidylarginine deiminases (PADIs) are post-translational modification enzymes that catalyze the conversion of protein-bound arginine residues into citrulline residues in the presence of calcium ions. To date, four human *PADI* genes, type 1 to type 4, have been isolated and characterized by cDNA sequences, expression patterns, and biochemical analyses (Nakashima et al. 1999; Kanno et al. 2000; Ishigami et al. 2002; Guerrin et al. 2003). Three of four genes, *PADI1*, *PADI3*, and *PADI4*, lie within an approximately 160-kb region on chromosome 1p36.13. *PADI2* is located at 86-kb distal to the *PADI1,3,4* gene cluster.

We previously reported one of four *PADI* genes, *PADI4*, to be a rheumatoid arthritis-susceptibility gene by a case-control association study using single nucleotide polymorphisms (SNPs) and subsequent functional assays (Suzuki et al. 2003). In the course of this association study, we constructed fine-scale SNP maps of the *PADI* gene cluster region by a direct sequencing method and the SNP information derived from the JSNP database (Haga et al. 2002). In this report, we describe more detailed information of genetic variations in the 83-kb region corresponding to the *PADI1/PADI3* loci, including 45 novel SNPs and two insertion–deletion polymorphisms in the Japanese population.

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Subjects and methods

Samples of peripheral blood were obtained with written informed consent from 48 healthy Japanese volunteers for this study. Polymerase chain reaction (PCR) experiments and DNA sequencing were performed according to the methods described previously (Iida et al. 2003). In brief, on the basis of the genomic sequence from the Genbank database (accession number AL590644.13), we designed primer sites to amplify each gene locus in its entirety, excluding most of the regions that corre-

Fig. 1 A fine-scale single nucleotide polymorphism (SNP) map in the 83-kb region including the *PADI1* and *PADI3*. Exons and introns are represented by *rectangles* and *horizontal lines*, respectively. SNPs are indicated above the *lines* (designations correspond to the left-most column of Table 1). Two insertion–deletion polymorphisms are indicated below the *map*

sponded to human repetitive sequences predicted by the RepeatMasker program (<http://www.repeatmasker.genome.washington.edu/cgi-bin/RepeatMasker>). 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

By direct sequencing of DNA from 48 Japanese individuals, we explored SNPs in an 83-kb genomic region including the *PADI1/PADI3* loci except most of the regions containing human repetitive sequences. The ex-

tent of each screened genomic sequence was 16.8-kb for the *PADI1* locus and 13.1-kb for the *PADI3* locus. Consequently, we identified a total of 87 SNPs from the regions (SNPs were distributed every 344 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 45 of these SNPs to be novel as of beginning of March 2004; 33 were identified in the *PADI1* locus and 12 in the *PADI3* locus (Fig. 1). Detailed information for these genetic variations is given in Table 1. Subregional distributions of novel SNPs were as follows: one in 5' flanking regions, four in coding regions, 35 in introns, two in 3' untranslated regions, and three in 3' flanking regions. Of four SNPs identified in the coding regions, three were nonsynonymous substitutions: 1289T>A (Ile430Asn) in exon 11 and 1652G>A (Arg551His) in exon 15 of the *PADI1*, and 380G>A (Gly127Glu) in exon 4 of the *PADI3*. All three could affect structures and/or biological functions of the respective gene products. In addition, the overall frequencies of nucleotide substitutions were counted as 31% for A/G, 33% for C/T, 7% for A/C, 11% for C/G, 11% for G/T, and 7% for T/A. The transitions occurred 1.8 times more frequently than transversions. We also found two insertion–deletion polymorphisms in the introns of the *PADI1*.

The SNP map that we constructed in this study will serve as a useful resource for analyzing gene scans of complex diseases mapped to this local segment on chromosomal band 1p36.13. We hope that the genetic variations can contribute to further investigations for designing personalized medical care.

Table 1 Characterization of 45 novel SNPs and two insertion–deletion polymorphisms in the *PADI1* and *PADI3* gene loci. *dbSNP* database of single nucleotide polymorphisms, *del* deletion polymorphism

I.D.	Location	Exon Position ^a	5' Flanking sequence ^b	Variation ^c	3' Flanking sequence ^b	Substitution	Repetitive
Peptidylarginine deiminase type 1							
i-PADI1-1	5' Flanking region	–194	gcaggtgagtcctgctcgc	C/T	cggggcccccagttgaagct		
i-PADI1-2	Intron 1	117	aggatgctggcaggagatag	C/A	agagaaggtggagttggctg		
i-PADI1-3	Intron 1	432	agggacgtgtgtggtgactt	G/T	acccttggagggtcagccaga		
i-PADI1-4	Intron 1	2,838	tactgtgtgggagggggaag	C/A	ccccagaagaggaggtgttg		
i-PADI1-5	Intron 1	3,916	ttacaggattgacactgaca	C/G	taatttccagcgaccgacag		
i-PADI1-6	Intron 1	9,170	gtgactcctgtagtgtgaat	G/C	atttggcctttttatttgg		
i-PADI1-7	Intron 1	9,223	aaatctgtgacctcacctga	C/A	tgtgcatttggggacatgcc		
i-PADI1-8	Intron 1	9,244	tgtgcatttggggacatgcc	A/G	gtactgcatactctgggggtt		
i-PADI1-9	Intron 1	15,573	gccctgttggtttgcctcg	C/G	gtgccctgtagcctgagttt		
i-PADI1-10	Intron 2	215	ccagtatcacacatgtacac	G/A	tgagtactccacacaaacac		
i-PADI1-11	Intron 2	277	cagattcacagacaactca	C/T	attaatccctgtattcagcc		
i-PADI1-12	Intron 8	385	ccttgctgctatggacatg	G/A	gaaccagctctctgcaagcc		
i-PADI1-13	Intron 8	541	gaccactggaaaaagccaca	G/T	ttgagctggctcataagttaa		
i-PADI1-14	Intron 9	51	cagcctggcttggggaaagg	G/A	aagccgcacaggtgctctgat		
i-PADI1-15	Intron 9	102	tcgctagagaagccacggca	G/A	cctggccaggcctctgggg		
i-PADI1-16	Intron 9	336	aaccacccgaaaaaggtcac	T/C	gtggctttttcatctctccc		
i-PADI1-17	Intron 10	2,098	cctcgtcctgtcctcactga	C/T	ctctccttcttgggggtggg		
i-PADI1-18	Coding region	1,289	ggaataccccctgggcccga	T/A	cctcatcgggagcagcttcc	Ile430Asn	
i-PADI1-19	Intron 11	442	ttaaaagggtccttgaggta	T/C	gaagttccccaggggcaggtgg		
i-PADI1-20	Intron 11	590	aacgctcaaacaggtggcga	T/C	gtctgttaggtcctgctgag		
i-PADI1-21	Intron 11	647	ctgcaggttaaccaagaatg	A/T	(C/G)gtgtgcagt(T/C) cagggtcct		
i-PADI1-22	Intron 11	648	tgcaggttaaccaagaatg (A/T)	C/G	gtgtgcagt(T/C)cagggtcctg		
i-PADI1-23	Intron 11	658	caaagaatg(A/T) (C/G)gtgtgcagt	T/C	cagggtcctgttgggtcccc		
i-PADI1-24	Intron 11	730	aagccctggagatcaccttc	C/T	tgtttgtggggtttttgcat		
i-PADI1-25	Intron 11	797	aagccctggctgacctctg	G/A	tctccagcccagaggagta		
i-PADI1-26	Intron 11	840	cagtgtctgagaagggtcacag	C/T	ctcacctggtgaatctggca		
i-PADI1-27	Intron 11	915	cctggcaccttcccaggcac	G/A	taactgggtgcccagacagg		
i-PADI1-28	Intron 12	229	aggaatgatattgataataa	T/C	tggtaatatttattgagcac		+
i-PADI1-29	Intron 14	91	ggttgggtcagagattcagc	G/A	ctggagctctcatggctcctg		
i-PADI1-30	Intron 14	503	ggcaggaggggccttaccac	T/G	tcccggtagtcccagcgct		
i-PADI1-31	Coding region	1,652	gaaatgcattgactggaacc	G/A	taatgtgctgaagcgggagc	Arg551His	
i-PADI1-32	3' Flanking region	410	gcaggcagtgaggagggaag	G/T	cttcccgggtctctctcage		
i-PADI1-33	3' Flanking region	974	ctgagaagaagactgtgggta	G/A	gaaggagtgagcattgacag		
i-PADI1-34	Intron 9	245	cctcacttctacctctgccc	C/del	tcaccaggtcttagggaaga		
i-PADI1-35	Intron 14	815–824	cttggccagtgattatgctct	TCCTAGTCCT/ del	gggctgccagctgcctcct		
Peptidylarginine deiminase type 3							
i-PADI3-1	Intron 1	324	gctgcaggttgggctgagga	T/C	ctgtctggggcacacaagaa		
i-PADI3-2	Intron 2	254	gggataatcataaccctgt	C/T	tcctatcattgctgtggagt		+
i-PADI3-3	Coding region	380	ttgcgacctgaactgtgagg	G/A	aaggcaggacaggaactttg	Gly127Glu	
i-PADI3-4	Intron 7	275	aataaaggcagtgaggagg	A/G	ctccattcctgtaagtcta		
i-PADI3-5	Intron 9	71	gaggccttatttgggggcc	A/G	ccgagaacatccaggagga		
i-PADI3-6	Coding region	1,080	ctgggctacgttcaggcgcc	G/T	cacaagacctcccgttgtt	Pro360Pro	
i-PADI3-7	Intron 10	375	gggtcagaccaaccctgttc	C/T	gttacttacatgctgtgtga		+
i-PADI3-8	Intron 11	1,261	agtgggaatttaaaatatac	G/A	tatctggctgggtgcagtgg		
i-PADI3-9	Intron 11	1,326	tgtgaggtcaggcgggcag	A/T	tcacttgaggtcaggagttc		+
i-PADI3-10	3' UTR	2,571	cccagatgttgacctcacc	C/T	agcgtgagctgtcacatagt		
i-PADI3-11	3' UTR	3,082	cgaggtgtgtgccagctaca	C/T	gtgtgttctgtatgggtcca		
i-PADI3-12	3' Flanking region	484	cagaaacacactcaagctca	C/G	cccgggctgggtggcctagag		

^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^cVariation is shown by capital letter

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