

## SHORT COMMUNICATION

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**High-resolution SNP map in the 55-kb region containing the selectin gene family on chromosome 1q24–q25**

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**Abstract** Immunoglobulin A nephropathy (IgAN) is the most common form of glomerulonephritis characterized by predominant IgA deposits in glomerular mesangium. By means of a genome-wide case-control association study, we previously demonstrated that eight single-nucleotide polymorphisms (SNPs) within the selectin gene cluster are significantly associated with IgAN. Here we provide more detailed information of variations corresponding to selectin loci, consisting of 88 SNPs and two insertion–deletion polymorphisms in the Japanese population: 27 in 5′ flanking regions, 1 in 5′ untranslated regions, 6 within coding regions, 46 in introns, 4 within 3′ untranslated regions, and 4 in 3′ flanking regions. The SNP map presented here will be a useful resource not only for examining the relationships between selectin genotypes and susceptibility to the IgAN phenotype, but also for analyzing gene scans of complex diseases mapped to this local segment on chromosome 1.

**Key words** Immunoglobulin A nephropathy · E-selectin · L-selectin · Single-nucleotide polymorphism (SNP) · Fine-scale SNP map

**Introduction**

Immunoglobulin A nephropathy (IgAN; MIM161950), the most common type of glomerulonephritis worldwide, is characterized by deposits of IgA-containing immunocomplexes with proliferation of the glomerular mesangium; approximately 20%–30% of IgAN patients develop pro-

gressive renal failure 20 years after onset (see review by Monteiro et al. 2002). The pathogenesis of IgAN remains largely unknown, although several genes encoding angiotensin-converting enzyme, angiotensinogen, platelet-activating factor acetylhydrolase, and T-cell receptor have been suggested as candidates that increase susceptibility (see reviews by Hsu et al. 2000; Tanaka et al. 2000). In addition, Gharavi et al. (2000) also mapped the familial IgAN gene to 6q22–23 by linkage analysis using 30 IgAN families. More recently, we reported that single-nucleotide polymorphisms (SNPs) in the HLA-DRA gene are significantly associated with an increased risk of IgAN in Japanese patients [ $P = 0.000001$ ; odds ratio = 1.91 (95% confidence interval 1.46–2.49); Akiyama et al. 2002].

The selectins represent a family of three vascular cell adhesion molecules that appear to modulate the migration of leukocytes from blood into extravascular tissue, and share 60%–70% identity between the amino acid sequences of their lectin domains (Bevilacqua et al. 1991; Bevilacqua and Nelson 1993). Several lines of evidence suggest that increased expression of selectins has been observed in some patients with IgAN (Lai et al. 1994; Roy-Chaudhury et al. 1996; Kennel-De March et al. 1999). Recently, we identified the association IgAN and some specific SNPs within the E-selectin (*SELE*)/L-selectin (*SELL*) gene cluster on chromosome 1q24–25, by means of a case-control association study that was based on linkage disequilibrium (Takei et al. 2002). Simultaneously, we also demonstrated the accumulation of *SELE* and *SELL* gene products in interstitial infiltrates of renal tissues in a patient with IgAN. In this report, we describe a more detailed SNP map in the 55-kb region corresponding to *SELE/SELL* gene loci, containing 88 SNPs and two insertion–deletion polymorphisms in the Japanese population.

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**Subjects and methods**

Blood samples were obtained with written informed consent from 48 healthy Japanese volunteers for this study,

**Table 1.** Characterization of 90 variations in the *SELE/SELL* gene cluster on chromosome 1q24-25

I.D.	Region	Exon	Position <sup>a</sup>	5' Flanking sequence <sup>b</sup>	Variation <sup>c</sup>	3' Flanking sequence <sup>b</sup>	Substitution	Identity to dbSNPs	Previous report
LE1	5' Flanking region		-6137	cattcagaaagctctctaataaccag	C/T	ccG/Tatccctactgtttctccgcttggg			
LE2	5' Flanking region		-6134	tcaggaaagttcttaataaacgC/Tcc	G/T	atccactgtttcttccgcttgggaat			
LE3	5' Flanking region		-6095	ttgttctccgttggaaatcaccaga	G/A	ttgtctatttgaagaaatcccaatg			
LE4	5' Flanking region		-5723	ttacaagtgagggtctggcttccagctcc	G/A	aagaagtgctacaaatgatagtaaat			
LE5	5' Flanking region		-5098	tctactaagactctcaaaagtaactag	A/G	tgtagcagtagagcttftttatggag			
LE6	5' Flanking region		-4923	gtcacttaaccaataataactcaactct	C/T	gatagcttfttggcagctgtgtgtgg			
LE7	5' Flanking region		-4555	tttgaccgttcttctctctttttc	C/T	cateT/Catgtagcggatgcttccccatg			
LE8	5' Flanking region		-4550	accgttcttctctttcttctcC/Tc	T/C	atgtagcggatgcttccccatgagaaa			
LE9	5' Flanking region		-4473	tgtagggcgggcttgaaaaccggcaatcc	G/A	gtaacgctagctactcttcagagcttgg			
LE10	5' Flanking region		-4433	agctacatctcagactggggatcag	T/A	ggagcctgaaagccttaaacagcctgt			
LE11	5' Flanking region		-4397	ctgaaagcctctactcagctgttctt	G/C	gggtatatttggatatttgaagaggg			
LE12	5' Flanking region		-4064	tccatgacctgatttgcagtaaacctc	G/A	ggggcagggcacacagaaaatactgtgg			
LE13	5' Flanking region		-2904	agccccatgtctcagaaccagacacag	T/C	T/Catctcagtgagctgtgtatggca			
LE14	5' Flanking region		-2903	ggcccatgtctcagaaccagacacag/c	A/G	gaagagtacaggaacttgggtagacaag			
LE15	5' Flanking region		-2851	ggfcaagatgtctcagaagaagagggca	A/G	atctcagtgagagctgtatggcaagga			
LE16	5' Flanking region		-2362	taggagttaacgacttaattctatg	A/C	ataataatgtagcttattgatag			
LE17	5' Flanking region		-2230	tattcacttataaaataaataat	A/G	cA/TgataatgtataaaccA/Gcttgac			
LE18	5' Flanking region		-2228	ttcacttataaaataaataatA/Gc	A/T	gataatgtataaaccA/Gcttgaccttg			
LE19	5' Flanking region		-2210	taaaactatA/GcA/Tgataatgataa	A/G	ctgacattctggcctaaataataaata			
LE20	5' Flanking region		-2177	tgacattcgcctaaataatcaataa	C/T	tatatagtgattactctctgaattt			
LE21	5' Flanking region		-1948	ttttggaattttctctttctt	T/A	tttttggcttgacatttatagaac			
LE22	5' Flanking region		-1897	ttatgaacacagaaagctgtaacaa	G/A	taagagaaaacagggcttftaccactac			
LE23	5' Flanking region		-1755	ggcctgaaacacactacagaataggaat	T/G	aaaatgatccccattactgttaccat			
LE24	5' Flanking region		-290	cttttaagaacatagacttatttatt	T/G	aattaaaaatcagcttatttggctat			
LE25	5' Flanking region		-44	tctataaaggcctcagccgagtagtg	T/C	tcagctgttctggctgacttcaacaaa			
LE26	Intron 1		213	ataaagatttgcactgagctgtgctggc	C/T	aaaacttaggatgctgcttgaacat			Ohnishi et al. (2000)
LE27	5' Untranslated region	2	-19	cctcagctcacttgggtaagcag	G/T	aaagaaacttgaagcactgattcttca		rs1805193	
LE28	Intron 2		12	aagtgagatcaaatgacctattccccag	C/T	caatlagaccaagatttcttctcgcact		rs932307	
LE29	Intron 2		224	acagtgctcttggctacactaagctc	A/C	tgcatgtgagattcaatagcatggttca			
LE30	Intron 2		300	tactataatgttggagtgagtggtataa	A/G	ataaagcttagttattcttgatttct		rs727909	
LE31	Intron 2		376	aaataaagtcgctgttctctacgtac	G/A	gtaccT/Cagtggtggcagctcaaaaaga			
LE32	Intron 2		382	aaataaagtcgctgttctctacgtac	T/C	tttacttttgaagaatgagaaaagtctg			
LE33	Intron 2		439	aaataaagtcgctgttctctacgtac	T/C	cagtggttctctgtaaaagtgagat			Ohnishi et al. (2000)
LE34	Intron 4		1157	aaataaagtcgctgttctctacgtac	C/T	atctacccttgaagcagcttctcactc		rs2076059	
LE35	Intron 5		652	atgcccctaaagattgacataggaacttgg	G/A	aaatccctcagtggtgagtgatgctg		rs1534904	
LE36	Intron 5		748	ctctctgtaaaatcaatgctctt	A/C	ccagcaatggggtcgtggaatgttccaa		rs5363	
LE37	Coding region	6	741	ctcagtggtgagtgatgctgagcaaa	T/C	tgtaaacctcctacaccctgctctg			
LE38	Intron 7		325	ggaaagcaataatgagaaatgatttga	A/G	ttaatgtctttaaattagtgccatgac		rs1076638	
LE39	Intron 7		736	gaagaacagctcaggaactaatgctc	A/G	ccaagctactctatgacctgcaaat		rs1076637	
LE40	Intron 7		883	gcttgaatgactgggttctcctcaacaac	G/A	aaaagaactatgaaagacttgggaactg			
LE41	Intron 8		33	acagcttgttttttttttttttaagat	A/G	tgctggagatgcaaaaactctaaagtg		rs5367	
LE42	Intron 8		82	cttgggaacttgggttacttgggaaacgta	T/A	gittccagctgtagagctgagctgctccac			
LE43	Intron 8		123	gcaacaacttctaaagctctctctg	T/C	tcccctattggagaatctaccctcaagctc		His445His	
LE44	Coding region	9	1335	gccccgaagggttggfaggtgctc	T/C	atggatcaactcaacttgaagcactc		His468Tyr	
LE45	Coding region	9	1402	gcttccagctgtagggaggttgaatta	C/T	ttacttcaatgtgactttaaagcaagt			Ohnishi et al. (2000)
LE46	Intron 9		126	tacatctgaactaagtagcgtctacac	T/A	ttcctcagaccttcttctcttact			
LE47	Intron 10		506	ggacaggcaggagacttcaacatcaat	C/T	tcctctggcttggaaatgcttaccgaaag		rs5355	
LE48	Coding region	11	1723	ggacttccctcctcagacttaccacattt	C/T	tgatgataaacataatcttcttattat		rs5359	
LE49	3' Untranslated region	14	2977	tttgcatctctacaagatgttctgacagat	A/G				

Table 1. Continued

I.D.	Region	Exon	Position <sup>a</sup>	5' Flanking sequence <sup>b</sup>	Variation <sup>c</sup>	3' Flanking sequence <sup>b</sup>	Substitution	Identity to dbSNPs	Previous report
LE50	3' Untranslated region	14	3368	tcaagaagtctgctgcaac	A/G	acaagaaccaagctcaaacagagatgga		rs4786	
LE51	3' Flanking region		966	cccactgcccagttgcaatfaaat	A/T	tctatctcaaaagccagcaatatttc			
LE52	3' Flanking region		1033	cattacaagaatggctgtagaataca	T/C	tgtagggctaaaagtcataaataaata			
LE53	3' Flanking region		4213	agatcacagacagaaagggcttaccatg	C/T	caaatgtaaggaatgtagccatgggat			
LE54	3' Flanking region		4364	agtagtgcagcctggtggcagaatga	G/A	caataaatctgcgctcatcgaatgga			
LE55	Intron 5		106	ttcaaaactcagggaaatttttttt	T/ins	gttttttttaatacaattgtctctc			
LL1	5' Flanking region		-642	ctcagaagtgccactaacaggagctc	A/G	ctagggtggtagcaaggaaagcggaggg		rs2205849	
LL2	5' Flanking region		-547	tctactgaagtttgcacaactaataata	T/C	gtcgaatgcaagtttgaattgtagtatt			
LL3	Intron 1		563	ttttttttttagattgaggctgac	C/T	cattatgacaaagtagggctataacatt		rs1569457	
LL4	Intron 2		219	ctataatagccactgctagagttcaaat	C/T	ccaataaccccaaaaatcccccaatata			
LL5	Intron 2		398	attacaacagccttattaaaggacgggg	C/G	tttatactttttggctcttcccattgt			
LL6	Intron 2		1048	ggcttgtaaatgtcttttaaaatia	T/C	acagttagatataaagagttgagtggtat			
LL7	Intron 2		1274	cgtaaattgtagttatgataattacac	G/T	gtaattagttgaaatcattaacagctaca			
LL8	Intron 2		1553	taggcctgfttctctctctctctctct	A/G	gctcagcctggagtagtctagggttctttt			
LL9	Coding region	3	360	atggacgtgggtgggaaccaacaatctc	T/C	actgaaagacagagaaactggggagatgg			Ohnishi et al. (2000)
LL10	Intron 3		265	atagatctcgtgggaagacagaggaga	G/A	ctagacaatcagtgccctgataataagc	Leu120Leu	rs1051091	Ohnishi et al. (2000)
LL11	Intron 3		438	cttataaattttataaaaatgcaact	A/G	totttggtagaattttctgtaaaaatc		rs1883229	
LL12	Intron 3		558	taattgctctataaaatggactctac	C/T	gftttagcaaatggacttttaattcttt		rs1883228	
LL13	Intron 4		190	aagatttcaatgacagcaatgaaagtctga	C/T	tcacttcaacaagcttttttggatcaca		rs2205848	
LL14	Intron 4		260	cagrtgggataaaagctgcaagggttac	C/A	ataaggaattagcagtgtagattcccctc		rs2205847	
LL15	Intron 4		568	tcaaaagacagttctctaggaagcttc	C/T	tagttctgataagctccacatattctctc			
LL16	Intron 4		776	ccgaaatgaaatggacaatttaattttt	A/T	aaaaagcaatggtgtaaacataaagaat		rs2420381	
LL17	Intron 4		1134	atccagaccataaaactataaattctc	A/G	gcttttctttttagattgctctctga			
LL18	Intron 4		1500	tcttcaactgcaactctactaccatgac	C/T	gtcattatcttctctagattcttgcagt			
LL19	Intron 4		1922	tftgacagatgagaaatgaaacatgaaagt	T/G	gtagctgttactaaatggcagcagggc			
LL20	Intron 4		2038	cagaacagggaaatagatacagttctgga	G/T	aggggtgcaaatcaacaagagttaaatt			
LL21	Intron 4		2080	aatcaaaagagttaaaattctggaactgag	C/A	tagaataagaagacagaatggagtcataat			
LL22	Intron 4		2367	gaattctgatgagtcaggcttggcttctaa	G/C	gtccttgcatttgcataatgaaatcaatt			
LL23	Coding region	5	676	ccagagctgggtaccatggactgactcac	C/T	ctttgggaaactcagctcagctcacagt	Pro226Ser	rs2229569	
LL24	Intron 5		719	aaatcccttattgaaatataaaactgta	A/G	cccactactggaaatttaccggagttggac			
LL25	Intron 5		811	tgctggtagatcagaacagatgaaac	G/A	gcagagattctctgagaataatccataaga			
LL26	Intron 5		842	gcagagattctcgaataatccataaga	G/A	cccctgtagcagagccataatggcatggta			
LL27	Intron 7		1681	tatcttcaattaaattgagaatgagg	C/T	gctaggtaagtttcttaaccaatccagaaa		rs2298900	
LL28	Intron 7		1754	gaggggggtggggatggtgatgcaaga	A/G	cttcattagctatgataaccctgagagcac			
LL29	Intron 7		1845	ttagaattctgctgattgaaatcact	A/G	tcttatagtattatacagactaagaacta			
LL30	Intron 7		2093	ccccctgggataagggctattgtctaaa	T/C	ccagcattatcatttctgatttagtact			
LL31	Intron 7		3346	tcctgggctctctagacacatttggc	A/T	ccagcattatcatttctgatttagtact			
LL32	Intron 8		2598	aatgtgaaagcaggtggtgtagtggaga	C/A	tatgaaagcattttcagggccactgagc		rs2298902	
LL33	3' Untranslated region	9	1338	tcctcagtcactcgggaagattctacc	T/C	ggaccaagcttctcagcttccatttgc		rs12938	
LL34	3' Untranslated region	9	1454	gctttctgaggagaaacaataagacca	T/C	aaagggaaggattcagtggaataaaga		rs909628	
LL35	Intron 2		104	catagctctaaaggaaatatttccacag	A/G/ins	ctgttaattcttctcctctgtaaacatct			

dbSNP, Database of Single-Nucleotide Polymorphisms; ins, insertion polymorphism

<sup>a</sup>Nucleotide numbering is according to the mutation nomenclature (Dunnen and Antonarakis 2000)<sup>b</sup>Both 5' and 3' flanking sequences to each variation are denoted by small letters<sup>c</sup>Variation is shown by capital letters



**Fig. 1.** Fine-scale single-nucleotide polymorphism (SNP) maps in the 55-kb genomic region containing *SELE* (accession number M24736.1) and *SELL* (accession number AJ246000.1) genes. Exons and introns are represented by *rectangles* and *horizontal lines*, respectively. SNPs

are indicated *above the genes* (designations correspond to the left-most column on Table 1). Other types of variation, where present, are indicated *below the genes*. *Tel*, Telomere; *Cen*, Centromere

which was approved by the ethical committee of the RIKEN SNP Research Center. We obtained genomic sequence containing the *SELE/SELL* gene cluster (Genbank accession number AL021940.1), and then designed primer sets to amplify both gene loci, excluding most regions that corresponded to repetitive sequences predicted by the RepeatMasker program (<http://repeatmasker.genome.washington.edu/cgi-bin/RepeatMasker>). Each polymerase chain reaction (PCR) was performed with 20 ng of mixed genomic DNA derived from three individuals. All 16 mixed samples (per 96 chromosomes) 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 were used 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 healthy individuals, we screened SNPs in a 55-kb genomic region corresponding to the *SELE/SELL* gene locus, except the regions of human repetitive sequences. DNA sequencing of an approximately 32.1-kb region identified a total of 90 variations, including 88 SNPs and two insertion–deletion polymorphisms. A fine-scale SNP map and detailed information of variations in the 55-kb region are shown in Fig. 1 and Table 1. Regional distributions of the SNPs identified in the gene cluster were

as follows: 27 in 5' flanking regions, 1 in 5' untranslated regions, 6 within coding regions, 46 in introns, 4 within 3' untranslated regions, and 4 in 3' flanking regions. The overall genomic distribution of genetic variations was calculated to be 1/365 bp in the 32.1-kb regions we sequenced. By comparing our data with an earlier report (Ohnishi et al. 2000), and with the SNPs deposited in the dbSNP database at the National Center for Biotechnology Information, we were able to consider 57 of 88 SNPs (65%) to be novel (as of the beginning of October 2002).

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