Jpn. J. Infect. Dis., 55, 2002

Laboratory and Epidemiology Communications

Polymorphisms and Haplotypes of the *CD209L* Gene and Their Association with the Clinical Courses of HIV-Positive Japanese Patients

Noriko Kobayashi[1], Hitomi Taguchi-Nakamura[1], Mieko Goto[1], Tetsuya Nakamura[2], Koichiro Nakamura[3], Wataru Sugiura[4], Aikichi Iwamoto[1,2] and Yoshihiro Kitamura[1]*

[1]*Division of Infectious Diseases, Advanced Clinical Research Center and*
[2]*Division of Infectious Diseases and Applied Immunology,*
Institute of Medical Science, University of Tokyo,
Shirokanedai 4-6-1, Minato-ku, Tokyo 108-8639,
[3]*Department Dermatology, Faculty of Medicine, University of Tokyo,*
Hongo, Bunkyo-ku, Tokyo 113-0033 and
[4]*AIDS Research Center, National Institute of Infectious Diseases,*
Musashimurayama, Tokyo 208-0011

Communicated by Hiroshi Yoshikura

(Accepted September 19, 2002)

The human liver-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin protein (CD209L, L-SIGN, DC-SIGNR [1,2]) is a type II membrane protein homologous to the dendritic cell (DC)-specific counterpart (CD209, DC-SIGN [1-3]) and is expressed in liver and lymph nodes but not in DCs (1). Reportedly, CD209 is required for activation of resting T cells through its binding to ICAM-3, which stabilizes the DC/T-cell contact zone (4). CD209L may have a similar function.

CD209L as well as CD209 were shown to capture human immunodeficiency virus type I (HIV) virions by binding HIV gp120 with carbohydrate recognition domains (CDRs) and to transmit them to T lymphocytes (2-3). Therefore, the genetic polymorphisms of *CD209L* and *CD209* may affect HIV pathogenesis. Studies to test such a hypothesis have not been conducted, so far, at least on *CD209L*. The human *CD209L* gene consists of 8 exons and spans about 6.5 kb on chromosome 19 (1,2). Exon 4 encodes the neck domain and exons 5-7 CRDs. IMSUT/JST (http://snp.ims.u-tokyo.ac.jp) has discovered 5 single-nucleotide polymorphisms (SNPs; one in intron 4, one in exon 5, and three in intron 5) in the *CD209L* gene in a Japanese population (IMS-JST025120 to IMS-JST025124). Soilleux et al. (1) described a variable-number-of-tandem-repeats (VNTR) of a 69 bp-sequence in exon 4; this VNTR results in 23 aa repeats in the neck domain of CD209L. In the present study, we typed the 5 SNPs in DNA samples from 115 Japanese people (59 HIV-infected patients, 56 uninfected individuals). We also typed the VNTR polymorphism (2) in the same samples.

The frequencies of SNP genotypes ranged from 2.6% (T/T genotype at IMS-JST 025123 in intron 5) to 71.3% (G/G genotype at IMS-JST 025121 in exon 5) (upper part of Fig. 1). We identified 5 alleles of the VNTR polymorphism with various copies of the repeat unit ranging from 5 to 9 in 115 Japanese individuals (lower part of Fig. 1), whereas Bashirova et al. (2) found 7 alleles in 350 Caucasian individuals with repeats ranging from 3 to 9 copies. In both studies, the most common and the second most common alleles were those containing 7 and 5 copies of a repeat unit, respectively. In contrast, the third most common copy number of the repeat unit was 9 in our study while it was 6 in Bashirova et al.'s (2). The observed allelic frequencies at each SNP and the VNTR polymorphism in our population were generally in Hardy-Weinberg equilibrium.

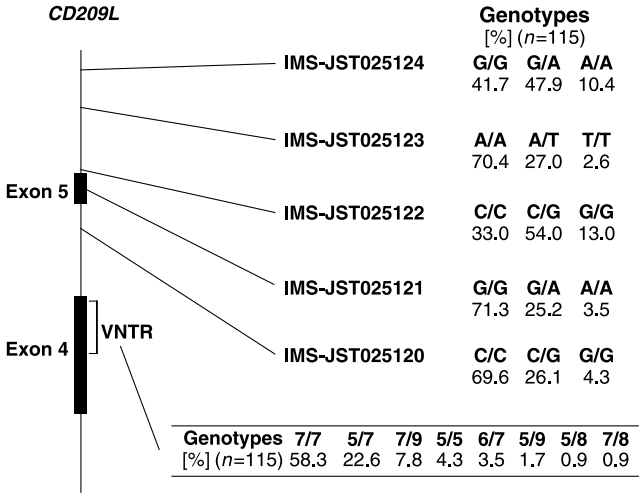

Fig. 1. Summary of genotype frequencies in the *CD209L* gene. At left, a region spanning from Intron 3 to Intron 5 of the human *CD209L* gene is drawn proportionally. At right, the frequencies (%) of genotypes at 5 SNP sites (ID: IMS-JST025120-025124) as well as the frequency (%) of the VNTR genotype are shown. The VNTR genotypes are shown with their copy numbers of the repeat units. We amplified appropriate regions by means of polymerase chain reaction: information on reaction conditions and primers are listed at http://snp.ims.u-tokyo.ac.jp. We typed the 5 SNPs using the ABI PRISM SNaPshot Multiplex Kit (PE Biosystems, Foster City, Calif., USA) in DNA samples from 115 Japanese people (56 HIV-uninfected and 59 HIV-positive individuals). We also typed the VNTR polymorphism by means of polymerase chain reaction as described (2) in the same samples.

Construction of haplotypes and calculation of their frequen-

*Corresponding author: Tel: +81-3-5449-5336, Fax: +81-3-5449-5427, E-mail: yochan@ims.u-tokyo.ac.jp

Table 1. Frequencies of haplotypes constructed from the SNPs and VNTR polymorphism in the human *L-SIGN* gene

Haplotype	Frequency[2)]	VNTR	SNP ID [IMS-JST]				
ID[1)]	$n = 218$[3)]	Copy number	025120	025121	025122	025123	025124
I	0.414	7	C	G	C	A	G
II	0.305	7	C	G	G	A	A
III	0.168	5	G	A	C	T	G
IV	0.041	9	C	G	G	A	G
V[4)]	0.032	7	C	G	G	A	G
VI[4)]	0.014	7	C	G	C	A	A
VII	0.014	5	G	A	C	A	G

[1)] The VNTR polymorphism with copies other than 5, 7, and 9 were excluded because of their low frequencies.
[2)] Haplotypes with frequencies of less than 0.01 are not listed. The seven haplotypes shown in this table accounted for 0.988 of the constructed haplotypes.
[3)] The total number of alleles analyzed were 218: 90 from AD patients, 110 from HIV-infected individuals, and 18 from healthy individuals.
[4)] Observed only in the HIV-infected population.

cies were carried out using the Arlequin algorithm (Genetics and Biometry Laboratory, Geneva, Switzerland; Table 1). The rarest polymorphisms (6- and 8-copy VNTRs) were excluded from this analysis. Among the 109 tested subjects (218 alleles), we identified 13 haplotypes, three of which were more frequent than 10% and, as a whole, accounted for 88.7% of the observed alleles.

Among the 13 haplotypes, the A-allele at the SNP in exon 5 (IMS-JST 025121) never coincided with the haplotypes that possessed the 7- copy VNTR allele. This suggested a possible selective disadvantage of the A-allele over the G-allele at the SNP in Exon 5 in the combination of the 7- copy VNTR. The A to G change at this SNP position resulted in Asp substitution for Asn in CRDs.

Among HIV-infected patients (5 untreated, and 54 treated), the A allele in intron 5 at IMS-JST025124 was associated with the higher number of lowest $CD4^+$ cell counts during the whole clinical course (statistical significance: A/A vs. G/G and G/A, $P = 0.0087$ by Mann-Whitney's U Test; Fig. 2A). In 44 patients, whose $CD4^+$ cells were counted on more than three occasions before the start of anti-retroviral treatment, this A allele was again associated with the higher number of the lowest CD4 cell counts during untreated periods (statistical significance: A/A vs. G/G and G/A; $P = 0.0069$ by Mann-Whitney's U Test; Fig. 2B).

The present study was approved by the ethics committee of IMSUT and all the enrolled subjects gave their written informed consent. We thank Noriaki Hosoya and Maiko Nakarai for assistance. We thank Drs. Arman Bashirova and Mary Carrington for information regarding primers for polymerase chain reaction. We thank Drs. Kunito Yoshiike, Tadahito Kanda, and Itsuro Inoue for their critical reading of the manuscript. NK is a recipient of a Research Residency from Japan Foundation for AIDS Prevention. This work was supported by grants from the Ministry of Health, Labour and Welfare of Japan; the Japan Human Sciences Foundation; a Grant-in-Aid for Scientific Research (A) from Japan Society of the Promotion of Science (JSPS); and the Japan Health Sciences Foundation.

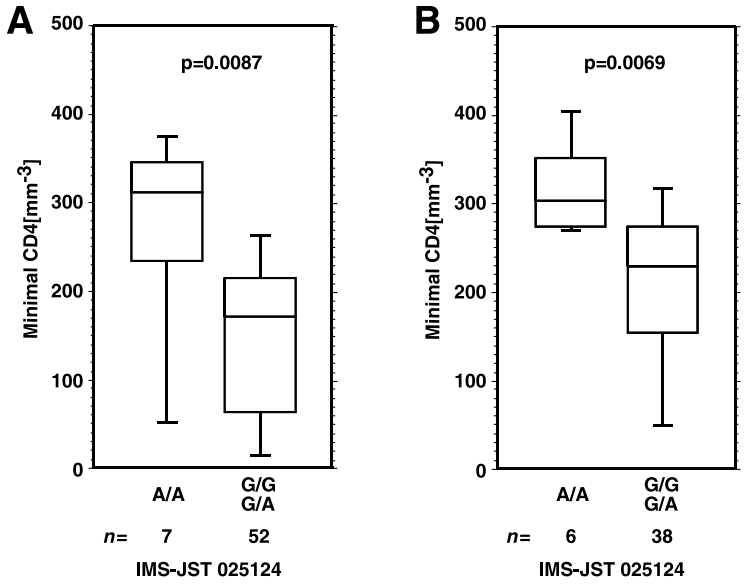

Fig. 2. Box plots of minimal CD4 cell counts.
(A) The lowest CD4 cell counts during the whole clinical course for the A/A genotype group and the G/G, G/A genotype groups at IMS-JST025124. Each box plot shows the median, 10th, 25th,75th, and 90th percentiles. *P* values between the A/A group and the other groups in Mann- Whitney's U Test are shown at the top of the panel. (B) Same as in (A) except that the lowest CD4 cell counts during untreated periods are shown.

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