Evidence for Natural Selection on Leukocyte Immunoglobulin-like Receptors for HLA Class I in Northeast Asians

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Human leukocyte antigen (HLA) plays a critical role in innate and adaptive immunity and is a well-known example of genes under natural selection. However, the genetic aspect of receptors recognizing HLA molecules has not yet been fully elucidated. Leukocyte immunoglobulin (Ig)-like receptors (LILRs) are a family of HLA class I-recognizing receptors comprising activating and inhibitory forms. We previously reported that the allele frequency of the 6.7 kb *LILRA3* deletion is extremely high (71%) in the Japanese population, and we identified premature termination codon (PTC)-containing alleles. In this study, we observed a wide distribution of the high deletion frequency in Northeast Asians (84% in Korean Chinese, 79% in Man Chinese, 56% in Mongolian, and 76% in Buryat populations). Genotyping of the four HapMap populations revealed that *LILRA3* alleles were in strong linkage disequilibrium with *LILRB2* alleles in Northeast Asians. In addition, PTC-containing *LILRA3* alleles were detected in Northeast Asians but not in non-Northeast Asians. Furthermore, flow-cytometric analysis revealed that the *LILRB2* allele frequent in Northeast Asians was significantly associated with low levels of expression. F_{ST} and extended-haplotype-homozygosity analysis for the HapMap populations provided evidence of positive selection acting on the *LILRA3* and *LILRB2* loci. Taken together, our results suggest that both the nonfunctional *LILRA3* alleles and the low-expressing *LILRB2* alleles identified in our study have increased in Northeast Asians because of natural selection. Our findings, therefore, lead us to speculate that not only HLA class I ligands but also their receptors might be sensitive to the local environment.

Introduction

Human leukocyte antigen (*HLA*) genes are well-known examples of genes under natural selection.[1] HLA molecules play a critical role in innate and adaptive immunity by acting as self-markers recognized by HLA class I receptors—such as leukocyte immunoglobulin (Ig)-like receptor (LILR), killer cell Ig-like receptor (KIR), and NKG2—or by presenting self and nonself peptides to T cell receptors. Because of evolutionary and functional significance of the *HLA* genes, numerous studies have demonstrated strong associations between *HLA* alleles and autoimmune or infectious diseases. However, to date, the genetic aspects of receptors recognizing HLA molecules are not fully understood.

Leukocyte immunoglobulin (Ig)-like receptors (LILRs) are a multigene family of HLA class I-recognizing receptors.[2] The *LILR* family comprises 11 expressed genes and two pseudogenes. LILRs consist of activating (LILRA1-6) and inhibitory (LILRB1-5) receptors expressed mainly on the myeloid cells, although some LILRs are expressed on lymphoid cells. Both LILRB1 and LILRB2 bind to a broad range of classical and nonclassical HLA class I molecules and inhibit the stimulatory signals triggered via activating receptors. This suggests that not only NK cells and T cells, but also other cells expressing LILR, are able to monitor the cell-surface expression of HLA class I molecules.[3,4] Numerous studies have shown that the *LILR* genes are highly polymorphic and that they have accumulated disease-associated polymorphisms.[5–14] On the other hand, the murine homolog of LILR is thought to be the paired Ig-like receptor (PIR), which consists of activating (PIR-A) and inhibitory (PIR-B) forms. PIR can also bind to various mouse major histocompatibility complex (MHC) class I molecules. In addition, PIR-B-deficient mice exhibit aberrant B cell activation, impaired dendritic cell maturation, increased T helper cell 2 (Th2) responses, and exacerbated graft-versus-host disease.[15,16]

LILRA3 [MIM 604818] is unique among *LILRs* in that the gene exhibits a presence or absence variation due to a 6.7-kb deletion[6,7,9,17] and encodes a secreted protein.[18–20] We have previously described the allele frequency of the 6.7-kb *LILRA3* deletion as extremely high (71%) in the Japanese population as compared with the Thai, African, and European-descended populations.[13] Furthermore, we identified a splice-acceptor mutation in intron 1 resulting in three alternatively spliced isoforms with premature-termination codons (PTCs) in exon 3, thus suggesting that natural selection has acted on the *LILRA3* gene. However, it cannot be ruled out that these observations suggest a hitchhiking effect of variations in genes closely linked to *LILRA3*. Accordingly, the aims of this study are to examine

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the geographical distribution of variations in the *LILRA3* region for the Northeast Asian and HapMap populations and to investigate the evolutionary and functional significance of the *LILRA3* and adjacent genes.

Material and Methods

Genomic DNA Samples

Genomic DNA samples from four healthy, unrelated Northeast Asian volunteers, including 49 Korean (in China), 47 Man (in China), 47 Mongolian, and 48 Buryat individuals, were previously collected for the study of *HLA* polymorphisms.[21–23] The genomic DNA samples used by the International HapMap Project were obtained from the Coriell Cell Repository: 44 individuals from the Japanese population in Tokyo (JPT), 45 individuals from the Han Chinese population in Beijing (CHB), 59 individuals (parents) from the Centre d'Etude du Polymorphisme Humain (CEPH) collection (CEU), and 58 individuals (parents) from the Yoruba population in Ibadan, Nigeria (YRI). This study was reviewed and approved by the research-ethics committees of the Tokyo Metropolitan Red Cross Blood Center and the Faculty of Medicine of the University of Tokyo.

Genotyping of the *LILRA3* and *LILRB2* Genes

An *LILRA3*-deletion polymorphism was genotyped by the PCR-SSP typing method, and the splice-acceptor mutation at nucleotide position 161 of intron 1, which causes PTC in exon 3, was detected by the sequencing-based typing method in Northeast Asian and HapMap populations, performed as described previously.[13] Direct sequencing of the *LILRB2* gene in the HapMap populations focused on the functional regions including the 568-bp promoter region, the 5′-untranslated region (UTR), the signal sequence region, four Ig-like domains, the cytoplasmic domain, and the 3′-UTR. Nomenclature of sequence variations was based on the recommendations of the Human Genome Variation Society (HGVS). Primer information is shown in Table 1. First, four amplicons for sequencing were produced by PCR with each set of primers, then sequenced by each sequencing primer. Direct sequencing was carried out with an ABI PRISM 3100 Genetic Analyzer (Foster City, CA, USA). Derived alleles were determined by sequence alignment for *LILRB2* and chimpanzee *LIRb* (*LILRB2* homolog) with the use of CLUSTAL W on MEGA3 software.[24] *LILRB2* (accession no. NC_000019.8 ranging from 59470132 to 59476762 of reverse complemented strand) and *LIRb* (accession no. NC_006486.2 ranging from 59981107 to 59987522 of reverse complemented strand) sequences were obtained from NCBI.

Flow-Cytometric Analysis

Whole blood samples derived from twenty-one healthy Japanese individuals were stained with PE-conjugated monoclonal human CD85d (ILT4/LILRB2) antibody (Beckman Coulter), and this was followed by the lysing of red blood cells with OptiLyse C Lysis Solution (Beckman Coulter). Cell preparations were analyzed on a FC500 flow cytometer (Beckman Coulter). Mann-Whitney U test was used for the assessment of differences in cell-surface expression levels of LILRB2 between genotypes.

Data Analysis

The HapMap Phase II genotype data and our genotyping data of *LILRA3* and *LILRB2* in the HapMap populations were used in the following analyses. Linkage disequilibrium (LD) parameters (D′ and r^2) and haplotype frequencies were estimated with the Haploview program.

We used the F_{ST} statistic for evaluation of the population differentiation at the *LILRA3* and *LILRB2* loci. For generation of the empirical distributions of F_{ST} between Northeast Asian (JPT and CHB, denoted hereafter as "JPT+CHB") and European (CEU) populations and between Northeast Asian (JPT+CHB) and African (YRI) populations, the F_{ST} statistic was applied to 40,072 SNPs on chromosome 19, which included polymorphic SNPs in the four HapMap populations, extracted by removal of monomorphic SNPs in all of the HapMap populations from the HapMap Phase II data. Values beyond the 95th percentile were regarded as significant.

Extended haplotype homozygosity (EHH)[25] was calculated for the detection of evidence of positive selection acting on the *LILRA3* and *LILRB2* genes. EHH at a distance of "x" from the core region is defined as the probability that two randomly chosen chromosomes from the samples carrying a tested core haplotype are homozygous at all SNPs for the entire interval from the core region to x. Relative EHH (REHH) is the ratio of the EHH on the tested core haplotype to the EHH of the grouped set of core haplotypes at the region not including the core haplotype tested. First, we inserted our genotype data into the HapMap Phase II SNP data on chromosome 19, and the haplotype phase was then estimated for the entire chromosome with the fastPHASE v.1.2.3 program.[26] The phased data was used for EHH analysis, implemented in the SWEEP v1.1 program, on our tested core haplotypes. REHH was calculated at a 0.25 cM distance from the core on both sides. For assessment of statistical significance, the empirical data were divided equally into 20 bins on the basis of haplotype frequencies, and the REHH values for the tested core haplotypes were ranked within the bin. Values beyond the 95th percentile were regarded as significant.

Table 1. Primer Information for Direct Sequencing of the *LILRB2* Gene

Method	Primer Name	Sequence (5′ → 3′)
PCR (Amplicon 1)	Promoter-f-A	TGCTACACATACGTTTCTCCATTAC
	Intron1-r	CAAATAGAACTGGTGCTTCTTGT
Sequencing (Amplicon 1)	Promoter-f-A	TGCTACACATACGTTTCTCCATTAC
	Promoter-r	CCGGGAAGAAACCTCTGAGT
	Promoter-f-B	GGTGCAGAGGGACGGCTAAGGAC
	Intron1-r	CAAATAGAACTGGTGCTTCTTGT
PCR (Amplicon 2)	Intron1-f	CCAGCCTCTGAGTGTCCACACA
	Intron5-r	GAGACTCAGGGAACTCCAGA
Sequencing (Amplicon 2)	Intron1-f	CCAGCCTCTGAGTGTCCACACA
	Intron3-r	TCCCCATCCCCAGCTGCACGGA
	Intron3-f	CAGCTGGGGATGGGGATTAGA
	Intron4-r	GGAGACACCCCTGAGAGCCT
	Intron4-f	GCTCTGCCCTCAGGAAGGA
	Intron5-r	GAGACTCAGGGAACTCCAGA
PCR (Amplicon 3)	Intron5-f	AAGATCAGCAGTGGTGAGGCA
	Intron7-r-A	TGCTGGCGATGCCGCTGAGTGTGC
Sequencing (Amplicon 3)	Intron5-f	AAGATCAGCAGTGGTGAGGCA
	Intron6-r	GACCACCCCCCGCCTCATCCT
	Intron6-f	AGAAAACAGAGACAGAGGCTCCTA
	Intron7-r-B	CCCACTGGCTGAGCCCCG
PCR (Amplicon 4)	Intron13-f-A	ACAAAATGCAAATAAATGCGCCA
	UTR-r	TGATTTTAGTTTTCTCGGTTAAC
Sequencing (Amplicon 4)	Intron13-f-B	AGGACAGACAGATGGACACTGAGA
	UTR-r	TGATTTTAGTTTTCTCGGTTAAC

Table 2. Allele Frequencies of the *LILRA3* Gene in Various Populations

No.	Population	N	Deletion	PTC	Functional
1	Korean[a]	49	0.84	0.13	0.03
2	Man[a]	47	0.79	0.05	0.16
3	Mongolian[a]	47	0.56	0.14	0.30
4	Buryat[a]	48	0.76	0.02	0.22
5	Japanese[13]	119	0.71	0.19	0.10
6	JPT[a]	44	0.78	0.11	0.11
7	CHB[a]	45	0.70	0.11	0.19
8	CEU[a]	59	0.17	0	0.83
9	YRI[a]	58	0.07	0	0.93
10	Palestinian[17]	100	0.10	0	0.90
11	Caucasoid (UK)[17]	172	0.26	0	0.74
12	Thai[17]	119	0.21	0	0.79
13	Pakistani[17]	92	0.10	0	0.90

[a] this study.

Results

Wide Distribution of Nonfunctional LILRA3 Alleles in Northeast Asians

A 6.7 kb *LILRA3* deletion and splice-acceptor mutation of intron 1 were genotyped in four Northeast Asian population samples and in four HapMap population samples. Table 2 shows the allele frequencies of *LILRA3* in various populations, including the eight populations examined in this study. The allele frequencies of the 6.7 kb *LILRA3* deletion were found to be remarkably high in all of the Northeast Asian samples, with the highest allele frequency seen in the Korean population samples. On the other hand, the allele frequencies of the 6.7-kb *LILRA3* deletion in the CEU and YRI population samples were low, which is consistent with the frequencies in non-Northeast Asians that were reported in a previous study.[17] It should be noted that PTC-containing alleles were widely present in Northeast Asian samples but were not detected in CEU or YRI samples. As shown in Figure 1, nonfunctional *LILRA3* alleles, including the 6.7-kb deletion and PTC-containing alleles, were predominant in Northeast Asians.

Variations of the *LILRB2* Gene and Structure of LD

For examination of LD between *LILRA3* and adjacent genes, the HapMap database was used. Although no strong LD was observed between *LILRA3* and adjacent genes, the extent of LD between *LILRA3* and *LILRB2* (MIM 604815) could not be determined because there was no informative SNP data on *LILRB2* in the HapMap database. Thus, we searched for variations of the *LILRB2* gene with direct sequencing of the genomic region corresponding to functional domains such as the 568-bp promoter, 5′-UTR, signal peptide, four Ig-like domain, cytoplasmic domain, and 3′-UTR in all of the HapMap samples used in this study. Of the identified SNPs, four were markedly differentiated between Northeast Asians (JPT+CHB) and non-Northeast Asians (CEU, YRI) (Figure 2 and Table 3). Two SNPs were located in the 5′-UTR (c.−169G→A rs448092 and c.−153G→A rs448083), and the other two were nonsynonymous SNPs located in the signal sequence (c.59A→G, p.R20H rs383369) and the third Ig-like domain (c.703G→A, p.M235V rs386056). Haplotype frequencies are shown in Table 4. Strong LD was observed between the *LILRA3*-deletion polymorphism and the four *LILRB2* SNPs, whereas *LILRA3* and *LILRB2* were not in strong LD with the other adjacent genes (Figure 3).

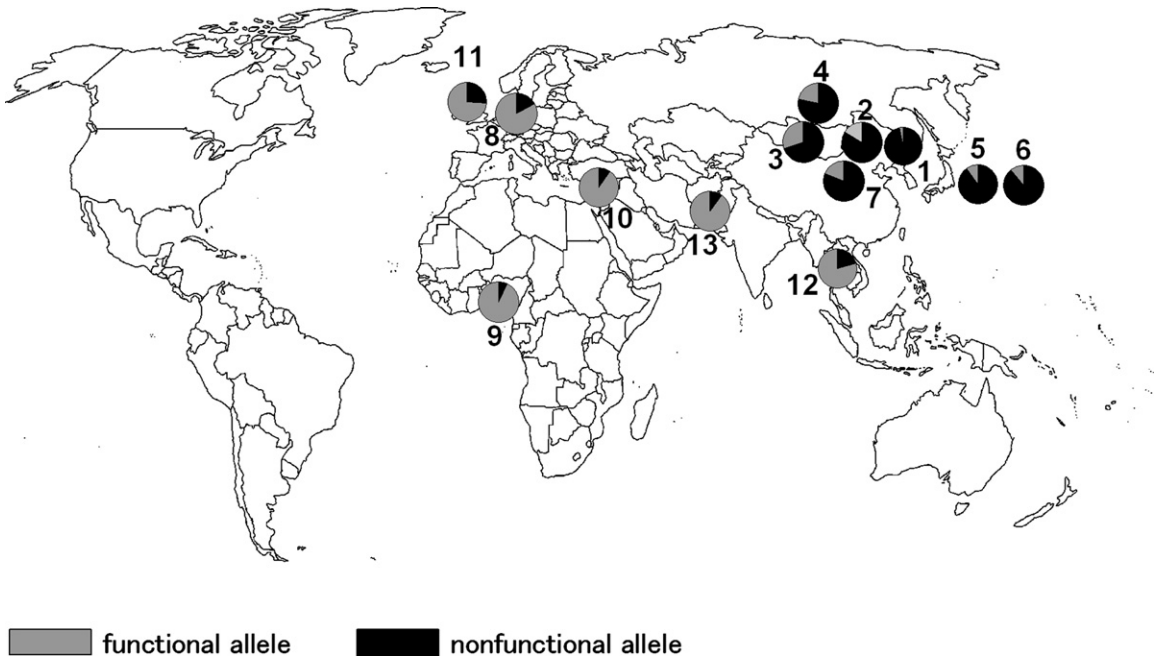

Figure 1. Geographical Distribution of *LILRA3* Allele Frequencies
The pie chart displays the allele frequencies of populations in Table 2. Each number refers to the populations shown in Table 2.

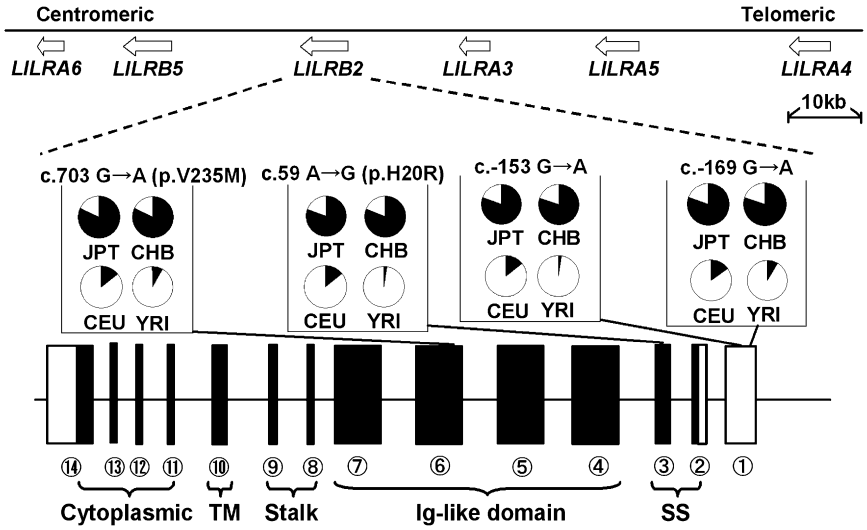

Figure 2. Schematic Diagram of the Four SNPs Identified in the *LILRB2* Gene
Each numbered block indicates an exon. White and black blocks show the UTR and the coding region, respectively. The pie chart displays the allele frequencies of the SNPs shown in Table 3. SS and TM stand for "signal sequence" and "transmembrane," respectively.

LILRB2 Genotypes and Cell-Surface Expression

The three notable *LILRB2* polymorphisms were located in the 5′-UTR and the signal sequence region, both of which potentially influence its expression. Thus, we investigated whether the *LILRB2* genotypes were associated with cell-surface expression by using flow cytometry. Given that LILRB2 is mainly expressed on monocytes, myelomonocytic cells were gated on the basis of forward- and side-scatter parameters and examined for LILRB2 cell-surface expression in 21 individuals. As shown in Figure 4, the c.59G allele, common in Northeast Asians, was significantly associated with low expression levels of LILRB2. In our samples, the c.−169A and c.−153A alleles gave the same results as the c.59G allele because of absolute LD ($r^2 = 1$). In this regard, however, it cannot be ruled out that the present association came from the LD with the primary SNPs that were not examined in this study.

Evidence for the Acting of Positive Selection on the *LILRA3* and *LILRB2* Genes in Northeast Asians

On the basis of the above data, we hypothesized that natural selection acted on *LILRA3* and *LILRB2* in Northeast Asians. It is possible to distinguish between the consequences of genetic drift and of natural selection without invoking any assumptions regarding population demography by comparing the F_{ST} of individual loci with empirical genome-wide distribution.[27] Thus, to test our hypothesis, we constructed the empirical distribution of F_{ST} for all of the SNPs on chromosome 19 in the HapMap data and then compared this with the F_{ST} values for the nonfunctional *LILRA3* polymorphism and four *LILRB2* SNPs. Figures 5A and 5B show the empirical distribution of F_{ST} between Northeast Asian and European samples and between Northeast Asian and African samples, respectively. The F_{ST} values for the nonfunctional *LILRA3* polymorphism and four *LILRB2* SNPs were significantly differentiated both between Northeast Asian and European samples and between Northeast Asian and African samples (Figure 5). Each polymorphism exhibited similar F_{ST} values because of strong LD (Table 5). Although several SNPs in genes closely linked to *LILRA3* and *LILRB2* also showed significant F_{ST} values, the *LILRA3* and *LILRB2* regions exhibited the highest F_{ST} values, as compared with the other adjacent region (Table 5).

It is well known that recent positive selection could leave an imprint on human genome as extended LD.[25,28] Thus, we applied the EHH analysis proposed by Sabeti et al.[25] to our data. We selected three *LILRB2* SNPs (c.59A→G, c.−153G→A, and c.−169G→A) as the core region because the GAA haplotype was considered to be the selected candidate haplotype on the basis of the expression data above. As shown in Figures 6A and 6B, REHH values of the *LILRA3* core haplotype C-del-C (derived from rs410852, *LILRA3*-deletion polymorphism, and rs7252525) and the *LILRB2* core haplotype GAA increased upstream of the core, and the REHH values at 0.25 cM were significant in the same frequency bin (REHH = 8.47 [96.7th percentile], 5.37 [95.7th percentile]; Figures 6C and 6D, respectively). On the other hand, the REHH values of the corresponding haplotypes for CEU (*LILRA3*: rs410852-*LILRA3* deletion polymorphism-rs7252525, *LILRB2*: rs383369-rs448083-rs448092) and YRI (*LILRA3*: rs10412494-*LILRA3* deletion

Table 3. Derived Allele Frequencies of the Four SNPs Identified in the *LILRB2* Gene

SNP	Derived Allele Frequencies				Derived Allele
	JPT	CHB	CEU	YRI	
c.−169G→A	0.81	0.80	0.15	0.09	A
c.−153G→A	0.81	0.80	0.14	0.03	A
c.59A→G	0.81	0.81	0.14	0.03	G
c.703G→A	0.82	0.82	0.14	0.09	A

Table 4. Haplotype Frequencies of the *LILRB2* Gene

Haplotype[a]	JPT	CHB	CEU	YRI
AGAA	0.814	0.789	0.144	0.026
GAGG	0.186	0.166	0.847	0.914
AGGG	0	0.023	0	0
AAGA	0	0	0	0.060

[a] Haplotype names refer to the alleles of the four SNPs in order from left to right; c.703G→A, c.59A→G, c.−153G→A, and c.−169G→A.

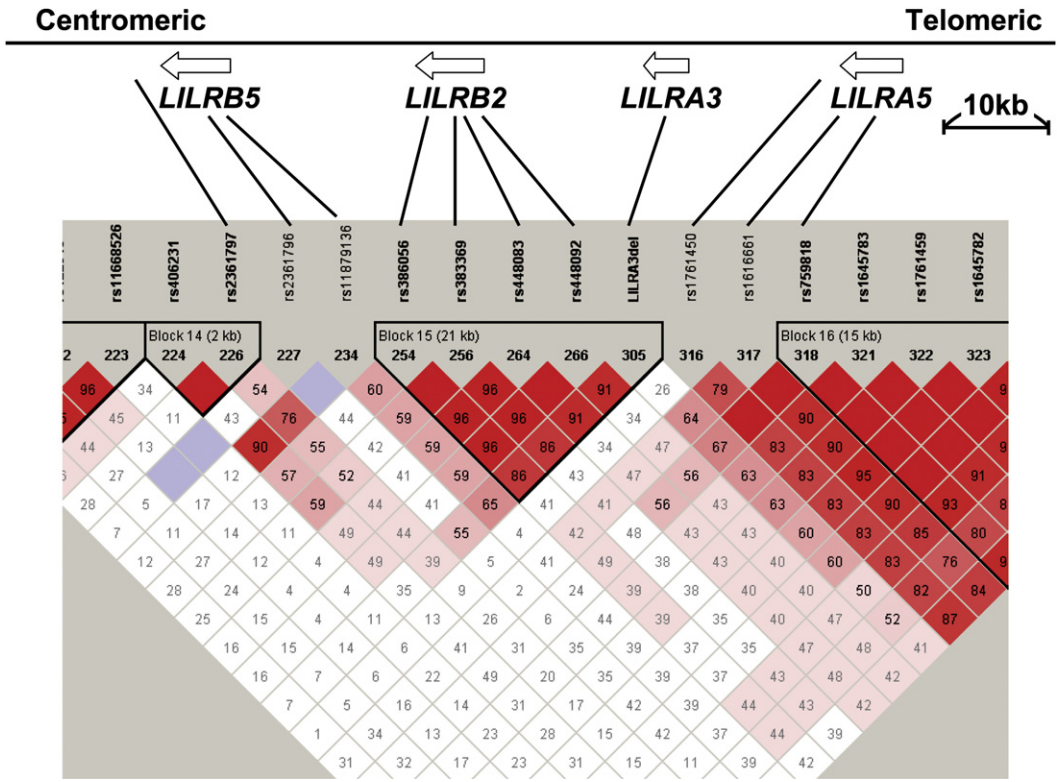

Figure 3. LD Analysis of *LILRA3*, *LILRB2*, and Adjacent Genes in JPT+CHB
The numbers within each square indicate the D′ values.

polymorphism-rs7252525, *LILRB2*: rs383369-rs448083-rs448092) were not significant (at $< 95^{th}$ percentile). To gain a better understanding of the relative significance of the *LILRA3* and *LILRB2* alleles, we performed EHH analysis on the adjacent SNPs (close to *LILRB5* and *LILRA5*) with high F_{ST} values, shown in Table 5. However, none of these SNPs showed significant REHH value (at $< 95^{th}$ percentile). These data suggest that *LILRA3* and *LILRB2* are the potential regions under natural selection in Northeast Asians.

Discussion

In an earlier study, F_{ST} analysis of the *LILRA3* alleles was performed on populations including Thai, Palestinian, African, and European-descended;[17] however, Northeast Asians have not yet been examined. In this study, we showed that remarkably high frequencies of nonfunctional *LILRA3* and low-expressing *LILRB2* alleles identified in our study were observed in Northeast Asians and significant F_{ST} values were obtained in the empirical distributions of F_{ST}. Moreover, PTC-containing alleles were detected in Northeast Asian populations, but not in the other populations examined. Similarly, polymorphisms in another HLA class I receptor, *KIR*, also have the unique characteristics found in Northeast Asians: *KIR* shows extensive genetic variability,[29,30] and Northeast Asians have a high frequency of the group A *KIR* haplotype with less activating forms.[31–36] Given that no significant LD was observed between *LILRA3* and *KIR*,[17] the group A *KIR* haplotype and nonfunctional *LILRA3* and low-expressing *LILRB2* alleles might have been independently increased in Northeast Asians due to natural selection. Considering that both KIR and LILR are members of the HLA class I-recognizing receptor family, the HLA-receptor system might be sensitive to the local environment and could be important for pathogen-host interaction. Therefore, it will be interesting in the future to examine the *LILRA3* and *LILRB2* alleles and their functions in the different populations.

One speculative mechanism for the acting of natural selection on *LILRB2* is that LILRB2 might have been used for the entry of some type of pathogen endemic present or past in Northeast Asia. It is known that deficiency of Duffy antigen prevents Plasmodium vivax from invading erythrocytes and has a high F_{ST} value in humans, which is suggestive of natural selection.[37] Therefore, low-expressing LILRB2 might be advantageous against some type of pathogen. Alternatively, Th1/Th2 balance might be important for the clearance of pathogen. PIR-B-deficient mice showed skewed Th2 responses, increasing interleukin 4 production, and decreasing interferon γ production.[15] Furthermore, LILRA2 was upregulated in lesions of lepromatous patients and suppressed innate host defense by shifting cytokine production from interleukin-12 toward interleukin-10 and blocking antimicrobial activity triggered by Toll-like receptors.[38] Thus, LILRB2 expression might be

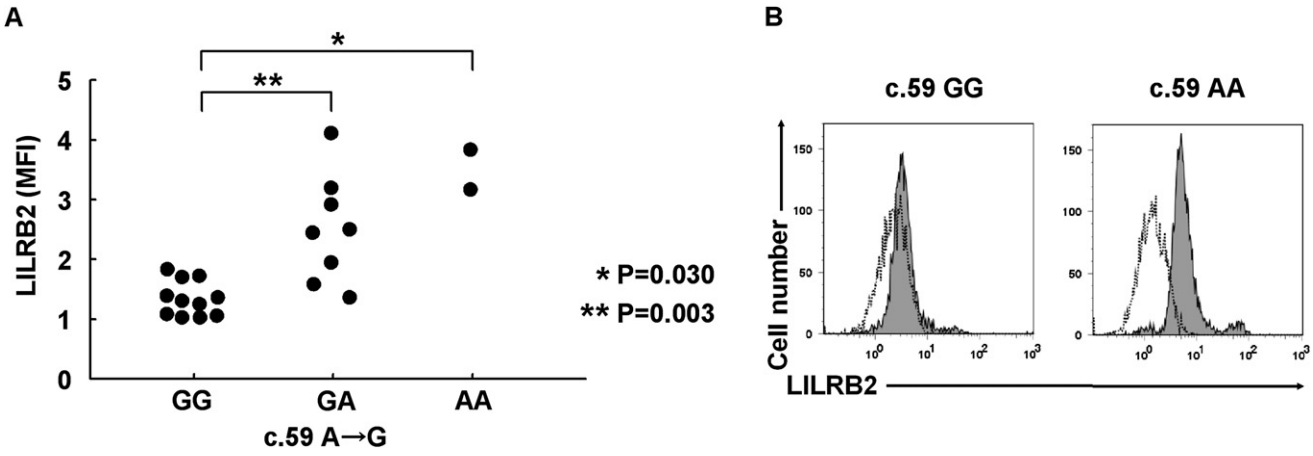

Figure 4. Association of *LILRB2* c.59GG, GA, and AA Genotypes with Cell-Surface Expression

(A) Vertical and horizontal axes indicate median fluorescence intensity (MFI) and *LILRB2* genotypes (c.59GG, GA, AA), respectively. p values were calculated with the Mann-Whitney U test.

(B) Representative flow-cytometry histogram of c.59GG (left) and c.59AA (right) genotypes. Vertical and horizontal axes show cell number and fluorescence intensity, respectively. "Open" and "Closed" histograms illustrate staining with an isotype-matched control and an anti-LILRB2, respectively.

involved in regulating Th1/Th2 balance, and skewed Th1/Th2 balance might be advantageous against some pathogen. In addition, LILRB2 is the major receptor for HLA-G, the expression of which is restricted to placental cytotrophoblasts.[39] Evidence of balancing selection at the HLA-G promoter region has been reported, and low-expressing HLA-G haplotypes might be favored in the presence of infection during pregnancy,[40] which suggests that the level of interaction between HLA-G and LILRB2 expressed on decidual macrophages is important in some environment. Finally, recent studies have shown that PIR-B, LILRB1, and LILRB3 could directly recognize bacteria,[41] and cytotoxic T cell lymphocyte escape mutations of HIV-1 could increase recognition of peptide-HLA class I complexes by LILRB2, leading to functional impairment of myelomonocytic cells.[42] Hence, low-expressing LILRB2 might be able to prevent immune evasion from some type of pathogen.

On the other hand, it is difficult to explain how natural selection has operated to increase nonfunctional *LILRA3* alleles in Northeast Asians, because the function of LILRA3 is still unknown. It has been speculated that LILRA3 could act as an antagonist of the other LILRs or as a soluble ligand

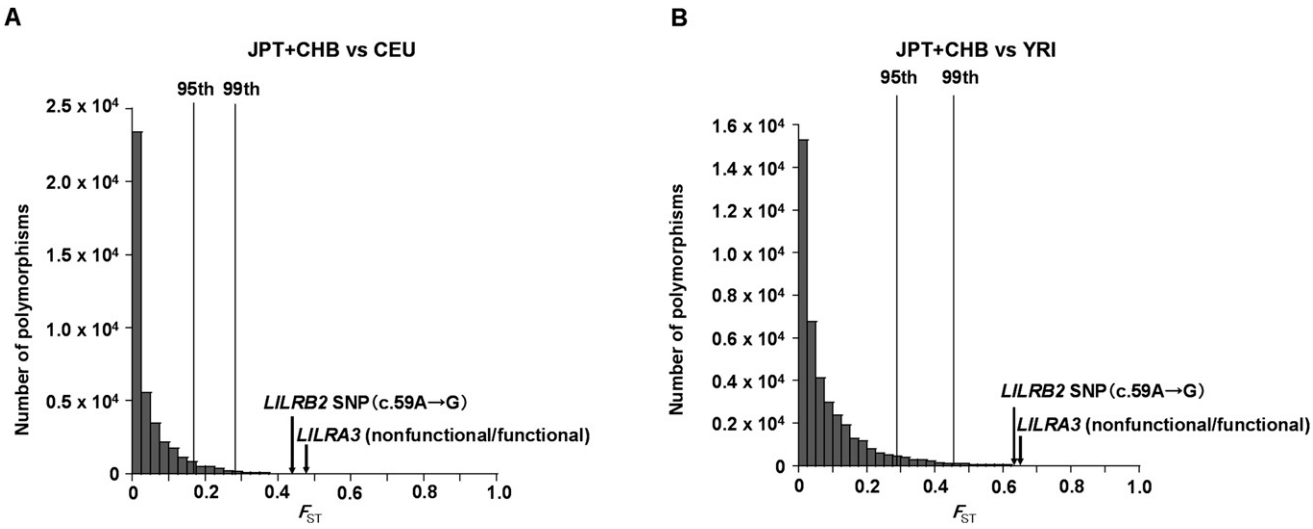

Figure 5. F_{ST} Analysis of *LILRA3* and *LILRB2*

Empirical distributions of F_{ST} between (A) JPT+CHB and CEU and (B) JPT+CHB and YRI, for all of the SNPs on chromosome 19 in the HapMap data, are shown. Vertical and horizontal axes indicate the number of polymorphisms and F_{ST} values, respectively. The 95th and 99th percentiles are shown. The *LILRB2* SNP in the signal sequence (c.59A→G) is indicated as a representative of the four SNPs shown in Table 3.

Table 5. F_{ST} Values of *LILRA3*,*LILRB2*, and the Other Adjacent Region

Polymorphism	F_{ST} Value (Percentile)	
	JPT+CHB versus CEU	JPT+CHB versus YRI
LILRA3		
(functional/nonfunctional)	0.48 (99.9th)	0.65 (99.9th)
LILRB2		
c.−169G→A (rs448092)	0.42 (99.9th)	0.52 (99.5th)
c.−153G→A (rs448083)	0.44 (99.9th)	0.62 (99.8th)
c.59A→G (rs383369)	0.44 (99.9th)	0.63 (99.8th)
c.703G→A (rs386056)	0.46 (99.9th)	0.54 (99.6th)
Downstresm of *LILRB2* (close to *LILRB5*)		
rs7409450	0.37 (99.8th)	0.42 (98.7th)
Upstream of *LILRA3* (close to *LILRA5*)		
rs651279	0.27 (98.8th)	0.62 (99.8th)
rs1761455	0.27 (98.8th)	0.62 (99.8th)

The SNPs adjacent to *LILRB5* and *LILRA5*, of which F_{ST} values exceeded the 95th percentile in both JPT+CHB versus CEU and JPT+CHB versus YRI, are shown.

to other receptors.[43] Recent preliminary experimentation has shown that recombinant LILRA3 stimulated the proliferation of T cells in a mixed-lymphocyte reaction,[44] thus suggesting that LILRA3 regulates T cell response. Alternatively, given that LILRA3 deficiency was significantly associated with multiple sclerosis (MS)[11] and significant Th1 shift was seen in MS patients,[45] LILRA3 might also regulate Th1/Th2 balance. In this regard, LILRA3 might function in concert with LILRB2, given that strong LD was observed between *LILRA3* and *LILRB2*.

In conclusion, we found that nonfunctional *LILRA3* and low-expressing *LILRB2* alleles identified in our study were predominant in Northeast Asians and that these polymorphisms were significantly differentiated between Northeast Asians and non-Northeast Asians, as compared with the other genomic loci. Furthermore, the *LILRA3* and *LILRB2* core haplotypes showed significant long-range homozygosity. Taken together, these data provide evidence of natural selection operating on *LILRA3* and *LILRB2* in Northeast Asians. Although the causative agents for natural selection are unclear, our results will offer clues to a better understanding of the biological significance of the HLA-receptor system.

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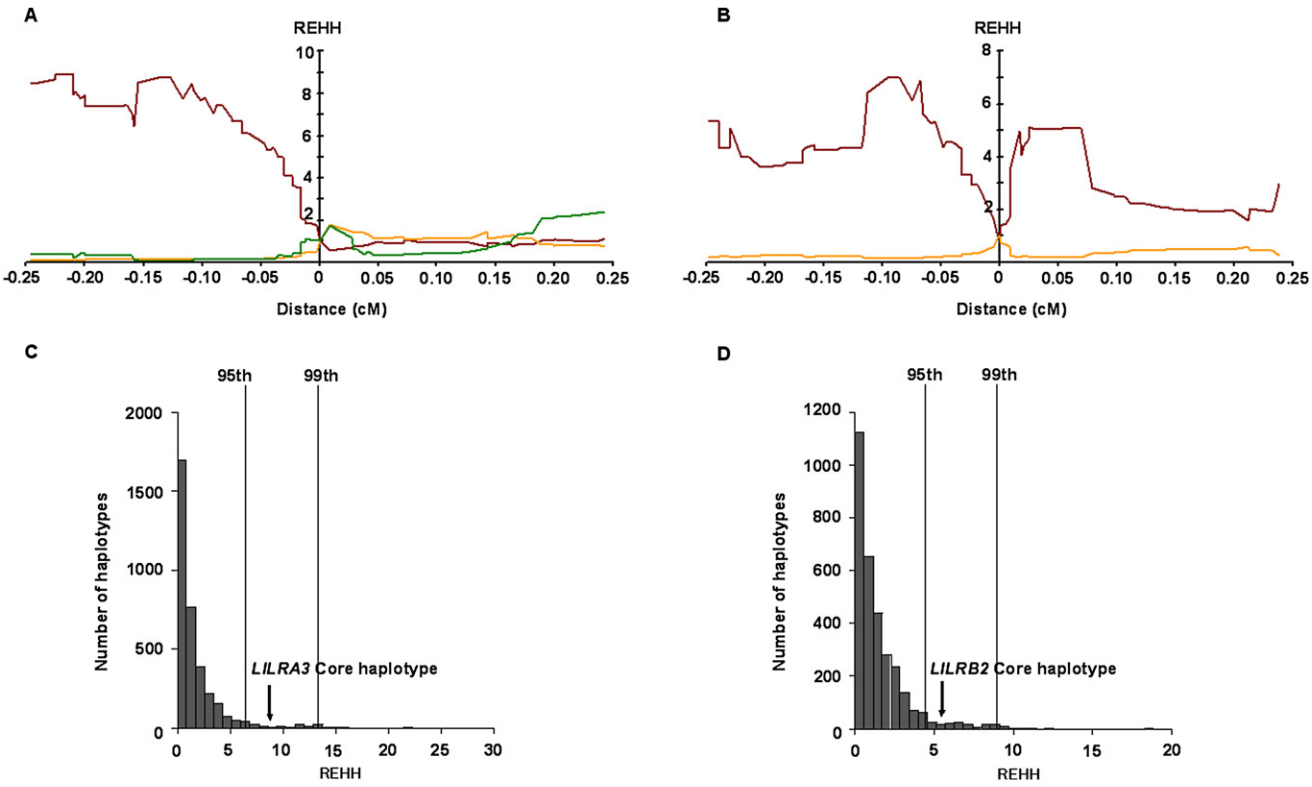

Figure 6. EHH Analysis of *LILRA3* and *LILRB2* Core Haplotypes

REHH versus distance (cM) plots of (A) *LILRA3* and (B) *LILRB2* core haplotypes are shown. Deep red line indicates the tested core haplotype. Empirical distributions of the REHH values in the same frequency bin are displayed in (C) (*LILRA3* bin; 0.7–0.75) and (D) (*LILRB2* bin; 0.80–0.85). The 95th and 99th percentiles are indicated.

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Web Resources

The URLs for data presented herein are as follows:

Haploview program, http://www.broad.mit.edu/mpg/haploview/
HapMap, http://www.hapmap.org/
HGVS, http://www.hgvs.org/
NCBI, http://www.ncbi.nlm.nih.gov/
Online Mendelian Inheritance of Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
SWEEP program, http://www.broad.mit.edu/mpg/sweep/

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