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Genetic profiles of killer-cell immunoglobulin-like receptors and HLA ligands in Thai blood donors

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ABSTRACT

Killer-cell immunoglobulin-like receptors (KIRs) play an important role in natural killer (NK) cell regulation. Interaction of KIRs with human leukocyte antigen (HLA) class I molecules can transmit signals to regulate the function of NK cells. In this study, the diversities of *KIR* genes and their ligands in 500 Thai blood donors were investigated. The coexistence of inhibitory *KIRs* (*iKIR*), activating *KIRs* (*aKIR*) and their ligands in the same individuals were also analyzed. Overall, 36 *KIR* genotypes were identified. The most common genotype was genotype AA1 (40.8%). All individuals carried at least one *iKIR*-HLA pair whereas 18% of the individuals lacked *aKIR*-HLA pair. The most common compound *KIR*-HLA profile was the presence of 3 *iKIR*-HLA pairs with 1 *aKIR*-HLA pair (21.4%). The most common compound gene profile of *KIR*-HLA pairs was the combined presence of *KIR2DL3-C1*, *3DL1-Bw4*, *3DL2-A3/A11* and the full length *KIR2DS4*-its ligands (8%). This study provided a comprehensive analysis of the *KIR*-HLA profiles in Thai blood donors in regards to *KIR* genotypes, HLA ligands, *KIR*-HLA ligand pairs and compound gene profiles of both *iKIRs* and *aKIRs* and their ligands. These findings will be useful as baseline information for further studies in the associations of *KIR* genes and various diseases.

1. Introduction

Natural killer (NK) cells are the effector lymphocytes of innate immunity and have an important role in the control of viral infections and tumors. NK cell effector functions are controlled by integrated signals from activating and inhibitory receptors [1]. Killer-cell immunoglobulin-like receptors (KIRs) represent one of the human NK cell receptor families that recognize class I human leukocyte antigen (HLA) molecules as their ligands. Upon engaging with its ligand, each KIR receptor transmits an inhibitory or activating signal that regulates NK cell activities.

The *KIR* gene family consists of 15 functional genes and two pseudogenes [1]. The *KIR* genes are highly polymorphic [2]. In addition, there is extensive diversity in the gene content, giving rise to haplotypic diversity and different genotypes. KIR haplotypes are broadly divided on the basis of gene content into Groups A and B. Group A haplotypes are uniform in terms of gene content. They contain only one activating *KIR* gene and at least six inhibitory *KIR* genes. Group B haplotypes are more varied and encodes more activating receptors. Studies on *KIR* gene frequencies in different populations showed remarkable variation in the frequencies of KIR genes and haplotypes between different ethnic populations, possibly due to the selection pressure conferred by pathogens [2–4]. To date, 553 KIR genotypes have been identified in worldwide populations [5].

Specific HLA class I molecules have been shown to serve as ligands for KIRs. HLA-C molecules with asparagine at position 80 (HLA-C1 group alleles) are ligands for KIR2DL2 and 2DL3. KIR2DS2 has been shown to bind weakly to HLA-C1 [6], although this has not been conclusively verified [7]. HLA-C molecules with lysine at position 80 (HLA-C2 group alleles) are ligands for KIR2DL1 and 2DS1. HLA-Bw4 motif (HLA-Bw4) serves as a ligand for KIR3DL1. The Bw4 epitope with isoleucine at position 80 (Bw4-80I) serves as a better ligand for KIR3DL1 than the Bw4 epitope with threonine at position 80 (Bw4-80T) [8]. Previous studies suggested that

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HLA-Bw4 allotypes are the putative ligands for KIR3DS1 [2,9,10]. HLA-A3 and -A11 serve as ligands for KIR3DL2 [11]. HLA-A11 and subsets of HLA-C1 (C*01:02, C*14:02, C*16:01) and -C2 (C*02:02, C*04:01, C*05:01) are ligands for the full length form of KIR2DS4 (KIR2DS4f) [12]. The deleted form of KIR2DS4 is not functional. The ligands of the other KIRs remain unknown or inconclusive.

It has become increasingly clear that the strength of HLA-KIR interactions has functional significance and can affect the outcome of infections and autoimmune diseases. Indeed, it has been shown that the *KIR2DL3*-HLA-C1 combination is associated with the resolution of hepatitis C virus (HCV) infection [13] and the *KIR3DL1/S1*-HLA-Bw4-80I genes have been implicated with the slowing down of human immunodeficiency virus (HIV) disease progression [14]. The knowledge on KIR genes combined with HLA ligands in the general population could contribute to the understanding on autoimmune diseases, infection models, reproductive failures and cancers. Although the frequencies of *KIR* genes, HLA ligands and inhibitory *KIR*-HLA combinations in Thais have previously been described [15,16], data regarding the compound gene profiles of both activating and inhibitory *KIR*s-their HLA ligands in Thai population is still lacking. Therefore, the aim of this study was to comprehensively analyze *KIR* genotypes, *HLA ligands*, matched *KIR*-HLA ligand pairs and compound gene profiles of both inhibitory and activating *KIR*s combined with their HLA ligands in Thai blood donors. Furthermore, the population in the present study was the current residents of the Bangkok metropolitan region and would be mostly Thai-chinese admixtures. Therefore, another aim of this study was to compare the frequencies of HLA ligands and matched *KIR*-HLA ligand pairs between our population and other populations, including Northeastern Thais (NE Thais).

2. Materials and methods

2.1. Study samples and DNA isolation

The study population consisted of 500 Thai blood donors between the ages of 21 and 66 (with a mean age of 38.7 ± 9.9 years) from the Blood Bank, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. The blood samples were collected from Thai blood donors between November 2001 and January 2007. Although the subjects are currently the residents of the Bangkok Metropolitan Region, a number of subjects originated from different geographic regions of Thailand. This study was approved by the Ethics Committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University. Genomic DNA was extracted from whole blood using the modified salting-out method.

2.2. KIR Genotyping and haplotype assignment

The DNA samples of 500 Thai blood donors were typed for *KIR* genes using the commercially available KIR Genotyping SSP Kit (Dynal Biotech, Pel-Freez Clinical Systems, Brown Deer, WI, USA). The frequencies of seventeen *KIR* genes and common subtypes in the population have previously been reported [17]. In this study, the *KIR* genotypes and ID numbers were identified on the basis of the Allele Frequency Net Database [5]. The *KIR* genotypes were defined according to the presence or absence of the 16 *KIR* genes, including *KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1* and *3DP1*.

Group A and B haplotypes were predicted according to the following rules: (1) the A haplotype contains a well-defined set of seven *KIR* genes and two *KIR* pseudogenes (*2DL1, 2DL3, 2DL4, 2DS4, 3DL1, 3DL2, 3DL3, 2DP1* and *3DP1*); (2) the B haplotype includes various combinations of stimulatory receptors. The frequencies of A and B haplotypes were calculated using the following formula: haplotype A = $(2n_{AA} + n_{AB})/2n$ and haplotype B = $(2n_{BB} + n_{AB})/2n$, where n_{AA}, n_{AB} and n_{BB} are the numbers of individuals with haplotype group AA, AB and BB, respectively and n is the total number of individuals.

2.3. HLA class I typing

The HLA class I typing of 500 Thai blood donors were performed. The typing of HLA-A and HLA-B alleles were performed using INNO-LiPA HLA-A Update Plus and HLA-B Update plus (Innogenetics, Gent, Belgium) which were line probe assays based on the reverse-hybridization principle. Alleles were assigned by the reaction patterns of the probes. The typing of HLA-C alleles was performed using polymerase-chain reaction sequence-specific primers (PCR-SSP; Micro SSP™ HLA DNA typing) (One Lambda, Canoga Park, CA, USA). HLA ligand binding specificities for KIRs were considered according to Kulkarni et al. [1].

2.4. Statistical analysis

The linkage disequilibrium (LD) between pairs of *KIR* gene loci was estimated using Cramer's V statistic [18], which was computed from the contingency tables of the presence/absence counts and was referred to as Wn*. The Wn* value was not calculated when a *KIR* gene was present at 100%. The chi- square test was used to test for nonrandom association between the pairs of genes. P values <0.05 were considered statistically significant.

The observed frequencies of HLA class I ligands, of matched *KIR*-HLA pairs and of *KIR*-HLA compound profiles were determined by direct counting. Hardy-Weinberg equilibrium test was verified for the quality of HLA and KIR genotyping. The differences in frequencies of HLA ligands and of matched *KIR*-HLA pairs among populations were analyzed by the chi-square test and Fisher's exact test using Stata statistical software, version 11.0 (Stata Corp., Collage station, Tx). P values <0.05 were considered statistically significant.

The hierarchical cluster analysis was performed based on the frequencies of four *KIR*-HLA ligand pairs (*KIR2DL1-C2, 2DL2-C1, 2DL3-C1* and *3DL1-Bw4*) using the IBM SPSS 19.0 software (IBM Corporation, New York, USA). For details, see the Methods section in the Supplementary Appendix.

3. Results

3.1. KIR genotypes

The distributions of carrier frequencies and gene frequencies of the 15 *KIR* genes and 2 pseudogenes in 500 Thai blood donors have previously been published [17]. In the present study, the *KIR* genotypes and the genotype ID numbers of the study population were identified. The frequencies of AA and Bx genotypes in our study did not diverge from the Hardy-Weinberg equilibrium. A total of 36 different *KIR* genotypes were identified (Supplementary Fig. S1). The most common genotypic profile in the study population was AA1 (40.8%) (Fig. 1). In the Bx genotypic groups, genotype ID2 was the most frequent *KIR* profile with a frequency of 11.2%. The remaining 31 *KIR* profiles were all less than 5%. The group A haplotype was more frequent (65.2%) in our population than the group B haplotype (34.8%), at a ratio of 1.87:1.

3.2. Linkage disequilibrium (LD)

The associations between pairs of *KIR* loci were analyzed in the study population. The genes present in all individuals (*KIR2DL4,*