朴 京 淑 敎授指導

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Allelic polymorphism and haplotypic associations of KIR2DL4 and 3DL1 genes in 77 Korean families

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誠信女子大學校 大學院

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심사위원 _____인

심사위원 _____인

심사위원 _____인

誠信女子大學校 大學院

論 文 槪 要

NK 세포 수용체의 하나인 *KIR*는 NK 세포막에 존재하는 다양한 구조의 당단백으로 19번 염색체의 장완에 위치한 *KIR* 유전자 복합체에 의해 지배된다. *KIR*는 HLA class I 분자를 인식하여 면역반응을 일으키는데, 종양세포나 감염된 세포에서 HLA class I 의 표현이 감소되는 것을 감지하여 NK세포를 활성화시킨다. *KIR*는 각 일배체형에 포함되는 유전자의 수와 종류뿐만 아니라 각 대립유전자의 다형성에 따라 많은 다양성을 보인다.

동일한 *KIR* 유전자 조합에서 대립유전자의 다형성에 따라 일배체형의 다양화가 어떻게 나타나는지 알아보기 위해 본 실험에서는 한국인 77가족의 가계조사를 통하여 *KIR2DL4* 와 *KIR3DL1*의 고해상도 분석법인 대립유전자 형별검사를 하여 일배체형과 연쇄불평형 (linkage disequilibrium, LD) 분석을 실시하였다. 본 연구의 결과, 한국인에서 총 6가지의 *KIR2DL4* 대립유전자 (*00102, *00102J, *00202J, *005, *005J, 006)가 확인되었고, 총 5가지의 *KIR3DL1* 대립유전자 (*001, *005, *007, *1502, *JB)가 확인되었다. *KIR2DL4*의 대립유전자 빈도는 *00102(55.8%), *005(20.8%), 005J(10.7%), *006(7.5%), *00202J(4.2%), 00102J(1.0%)로 나타났고, *KIR3DL1*의 대립유전자 빈도는 *1502(55.5%), *005(10.7%), *007(7.5%),

*001(4.2%), *JB(1.0%)를 보였고, blank는 21.1%로 나타났다. 7가지의 *KIR2DL4-KIR3DL1* 일배체형이 확인되었는데, 그 중 6가지 (*2DL4**00102-*3DL1**1502, *005-blank, *005J-*005, *006-*007, *00202J-*001, *00102J-*JB) 는 0.99-1.00의 강한 양성 연쇄불평형을 보였다.

2가지의 A 일배체형은 대립유전자 형별검사에 의해 6가지로 다양화되었고 A1-1 일배체형이 가장 흔한 빈도를 보였다(53.6%). 대립유전자 형별검사에 의한 유전자형을 살펴보면, A 일배체형의 동형접합체가 가장 흔한 빈도로 나타났으며, 이 중 A1-1 일배체형의 동형접합체는 29.87%의 빈도로 나타났다. 두번째로 흔한 유전자형은 A 일배체형과 B 일배체형의 이형접합체에 해당하였고, B 일배체형의 동형접합체는 가장 낮은 빈도로 나타났다. *KIR2DL4*와 *KIR3DL1* 대립유전자 다형성에 의해 A 일배체형은 다양화 되었으나, B 일배체형은 다양화되지 않았다.

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Introduction

Natural killer (NK) cells are fundamental components of the innate immune response. NK cells, a subset of bone marrow derived granular lymphocytes, express multiple receptors, some of which have major histocompatibility complex (MHC) class I molecules as their ligands (Raulet et al., 2001; Middleton et al., 2002). Recently, research attention has turned to a group of highly polymorphic NK cell receptors, known as the killer cell immunoglobulin-like receptor (*KIR*) family (Halfpenny et al., 2004).

The *KIR* family, encoded on chromosome 19q13.4, is a member of the immunoglobulin superfamily and functions as either inhibitory or activating receptors. Classification of *KIR* genes is determined according to the number of extracellular domains, two domains (2D) or three domains (3D), and possession of either long (L) or short (S) cytoplasmic tails. Those with long cytoplasmic tails contain immunoreceptor tyrosine-based inhibitory motifs (ITIM) and have an inhibitory function, whereas those with short cytoplasmic tails have a potentially activating function (Halfpenny et al., 2004).

To date, 16 *KIR* genes (*KIR2DL1-5, KIR3DL1-3, KIR2DS1-5, KIR3DS1*) and pseudogenes (*KIR2DP1, KIR3DP1*) have been identified and they exhibit allelic as well as haplotypic variability in terms of the number and types of genes present on a haplotype (Vilches and Parham., 2002; Shilling et al., 2002; Uhrberg et al., 1997). Group A and group B

haplotypes are characterized by a dominance of genes encoding inhibitory and activating receptors, respectively (Uhrberg et al., 1997). Group A haplotypes contain seven *KIR* genes and have *KIR2DS4* as the only activating receptor. Group B haplotypes are more diverse in the *KIR* genes they contain, have more activating receptors, and are characterized by the *2DL2, 2DS1, 2DS2, 2DS3*, or *2DS5* genes (Figure 2) (Uhrberg et al., 1997). These results have subsequently been confirmed in studies of other populations (Crum et al., 2000; Witt et al., 1999 ; Norman et al., 2001; Toneva et al., 2001). Recently, the WHO Nomenclature Committee has defined group B haplotype as having one or more of the following *KIR* genes, *2DL5, 2DS1, 2DS2, 2DS3, 2DS5* and *3DS1*, and group A as having none of these genes (Marsh et al., 2003).

Comparison of gene organization for two A haplotypes and one B haplotype showed that human *KIR* haplotypes are organized around three conserved framework genes: *KIR3DL2* and *KIR3DL3* at either end and *KIR2DL4* in the middle. Between the framework genes are two regions of variable gene content. In these regions, gene duplication, deletions, and hybridization by asymmetrical recombination are likely facilitated by the close proximity of the genes and the sequence similarity of the intergenic sequence (Martin et al., 2000; Wilson et al., 2000).

According to the data recently reported by Hsu et al (2002), the *KIR* haplotypes are composed of two separate halves: the centromeric half bordered upstream by *KIR3DL3* and comprising those *KIR* genes upstream of anchor gene *KIR2DL4*, and the telomeric half bordered

downstream by *3DL2* and comprising those *KIR* genes downstream of *2DL4*. Pairing variants of the centromeric half with variants from the telomeric half encompasses nearly all identified haplotype permutations. There are rare *KIR* haplotypes, which do not fit into this model. These haplotypes can, however, readily be explained by recombination, gene duplication and inversion (Hsu et al., 2002).

Previous studies of *KIR* diversity have focused on either low resolution genotyping that determines the presence or absence of *KIR* genes (Uhrberg et al., 1997; Crum et al., 2000; Witt et al., 1999; Norman et al., 2001; Toneva et al., 2001) or cDNA sequencing to assess their allelic polymorphism (Colonna and Samaridis., 1995). But the *KIR* receptors are very polymorphic both in the number of genes expressed in an individual and the alleles present for a gene (Middleton et al., 2002; Wagtmann et al., 1995; Rajalingam et al., 2001; Gardiner et al., 2001; Viches et al., 2000). Allelic polymorphism further differentiates *KIR* haplotypes having the same gene content by combining analysis of families with *KIR* typing at level of both genes and alleles. Together these two mechanisms of diversification individualize the human *KIR* genotype.

In the present study, high resolution typing was targeted to the 2 *KIR* loci for which most variants had been reported: *KIR2DL4* (OMIM 604945) and *KIR3DL1* (OMIM 604946). This study carried out identification of *KIR2DL4* and *3DL1* at allele level in 77 unrelated Korean families and elucidated the *KIR* haplotypes of the parents by segregation

analysis.

The goal of this study is to determine how genetic diversity of *2DL4* and *3DL1* genes are related and, more specifically, to examine the extent of allelic polymorphism underlying *KIR* haplotypes sharing similar or identical gene content.

Materials and Methods

As most *KIR*-specific amplicons are longer than 1000 bp, it is important to use undegraded DNA to avoid false-negative results in the SSP typing of *KIR* genes (Gómez-Lozano et al., 2002). To avoid this problem, newly extracted DNA from frozen peripheral blood mononuclear cells was used in this study. Family samples of 77 unrelated Korean families comprising 352 members (154 parents and 198 children) were available for the study. For these families, haplotype analysis of *KIR* genes, based mainly on the gene content of 16 *KIR* genes, has been reported (Whang et al., 2005). In this study, allelic polymorphisms of *KIR2DL4* and *3DL1* genes were investigated.

Molecular typing

Genomic DNA was extracted from frozen mononuclear cells using the LaboPass^{TM} Blood Mini kit (COSMOGENTECH, Seoul, Korea). Allelic typing of *KIR2DL4* and *KIR3DL1* genes was performed using 17 PCR-SSP reactions, and the primers were designed to discriminate allele-specific polymorphims (Yawata et al., personal communication). Allele typing was conducted using primers and PCR amplifications shown in Table 1. PCR amplification conditions are listed as NT, M7, H, M2, and M5 reflecting the annealing temperature used (Figure 1). Condition N,

initial denaturation was 95℃ for 5min; five cycles of 97℃ for 20s, 62℃ for 45s, and 72℃ for 90s were followed by 26-30 cycles of 95℃ for 20s, 60℃ for 45s, and 72℃ for 90s; finally, a 7-min extension at 72℃ was performed. For condition M7, annealing temperature were 65℃ during initial five cycles and 61℃ for the remaining cycles; for condition H, annealing temperature were 68℃ and 64℃; for condition M2, annealing temperature were 68℃ and 64℃; for condition M5, annealing temperature were 65℃ and 62℃. Amplification of genomic DNA was performed in 10 ㎕ reactions using 100 ng DNA, 0.4U Taq DNA polymerase, 1 ㎕ 10X buffer (67mM Tris-HCL, pH8.8, 16 mM$(NH_4)_2SO_4$, 2 mM $MgCl_2$, 100 μM dNTPs). *KIR* primers were used at 1 μM. As an internal control, primers specific for a 560-bp fragment of the HLA-DRA gene (5'-ACCTGTCACCACAGGAGTGTC-3' and 5'-CAGACCCACAGTCAGGCCC-3') were included at a 0.5 μM final concentration in all of the above SSP typing reactions. Amplification products were electrophoresed on 1.5% agarose gels and visualized with ethidium bromide.

Haplotype analysis

KIR haplotypes were determined by segregation analysis in families. In assigning genes to specific haplotypes, the following assumptions were made (Hsu et al., 2002): 1) all haplotypes contained *KIR3DL3, 2DL4,* and *3DL2*; 2) haplotypes contained either *2DL2* or *2DL3,* but not both; 3) haplotypes contained either *3DP1* or *3DP1* variant (*3DP1v*), but not both.

In addition, *2DS4*-containing haplotypes were assumed to contain either *2DS4F* (full-length *2DS4*) or *2DS4D* (a variant form of *2DS4* with a 22 bp deletion), but not both.

In the assessment of the *KIR* haplotypes, group B haplotypes were defined by the presence of one or more of the following genes: *KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5*, and *KIR3DS1* (Marsh et al., 2003). Conversely, group A haplotypes were defined by the absence of all these genes.

Statistical analysis

Phenotype frequencies (PF) and gene frequencies (GF) of *KIR2DL4* and *KIR3DL1* alleles were determined by direct counting of alleles by segregation analysis. Linkage disequilibrium (LD) values for two locus associations were calculated according to Mattiuz and coworkers (Mattiuz et al., 1971). Relative LD (r) values for two locus associations were calculated by dividing the LD value by the maximum LD value possible for the haplotype, which was determined as outlined previously (Bauer et al., 1980). P values of two-locus associations were calculated using Chi-square test or Fisher's exact test.

Results

Locus frequencies and genotype profiles in the low resolution KIR typing

Initial low resolution PCR-SSP typing was performed in a previous study, to determine the presence or absence of 16 *KIR* genes and pseudogenes (Whang et al., 2005). The results showed that all parents typed positive (100%) for the 3 framework genes (*2DL4, 3DL2*, and *3DL3*) and the 2 pseudogenes (*2DP1* and *3DP1*). In addition, 2DL1 (99.4%), *2DL3* (99.4%), *3DL1* (94.2%), and *2DS4* (94.2%) were present at high frequencies of >90%.

A total of 29 different *KIR* genotypes were identified in 154 parents of 77 unrelated Korean families (Figure 3). Group A haplotype was divided into two subtypes, A1 and A2 containing *KIR2DS4F* (full length *KIR2DS4*) and *KIR2DS4D* (variant form with a 22-bp deletion *KIR2DS4*), respectively. Genotype #1 corresponds to haplotype A1A1 homozygous state containing *KIR2DSF* and genotype #2 corresponds to haplotype A1A2 heterozygous state containing *KIR2DS4F* and *KIR2DS4D*. These two genotypes accounted for more than 50% of the panel. The distribution of genotypes, in terms of combination of haplotypes was: AA 85 (55.2%), AB 56 (36.4%), and BB 13 (8.4%).

Allele frequencies of *KIR2DL4* and *KIR3DL1*

Six of the eleven *KIR2DL4* and five of the ten *3DL1* alleles were detected in this study: *KIR2DL4**00102, *00102J, *00202J, *005J *005, and *006; *KIR3DL1**001, *005, *007, *1502, and *JB (Table 2). The

detected *KIR2DL4* and *KIR3DL1* alleles segregated as expected in most of the family segregation analyses.

The most common alleles were *KIR2DL4**00102 and *KIR3DL1**1502. These two alleles showed allele frequencies of >50%. Less frequent alleles with frequencies of >5% were: *2DL4**005 (20.8%), *005J (10.7%), *006 (7.5%); *3DL1**005 (10.7%), *007 (7.5%). Other alleles showed frequencies of <5% (Table 2).

In Tables 3 and 4, the estimated phenotype and gene frequencies of the *KIR2DL4* and *KIR3DL1* alleles in Korean and those of other populations are illustrated. *KIR2DL4* allele frequencies in Korea were similar to those of Hong Kong. *KIR2DL4**00102 and *005 is predominant in most populations. Distribution of *KIR3DL1* alleles is markedly different among different ethnic groups. *KIR3DL1**001 is predominant in Northern Irish and North Indian, and *KIR3DL1**1502 is predominant in Korean.

Analysis of two-locus *KIR* haplotypes

Two-locus *KIR* haplotypes were analyzed and shown in Table 5. This yielded 7 distinct two-locus haplotypes. Analysis of the *KIR2DL4*-*KIR3DL1* haplotypes in the present study of Koreans showed an exclusive association between *KIR2DL4* and *KIR3DL1* alleles: *KIR2DL4**00102-*KIR3DL1**1502 (55.5%), *KIR2DL4**005-*KIR3DL1* blank (20.8%), *KIR2DL4**005J-*KIR3DL1**005 (10.7%), *KIR2DL4**006-*KIR3DL1**007 (7.5%). Comparison of the haplotypes revealed striking patterns of LD between alleles of *KIR2DL4* and *KIR3DL1* (Table 6).

The *2DL4**00102 and *3DL1**1502 alleles were associated exclusively

with each other, as were *2DL4**005 and *3DL1* blank. In addition, the *2DL4**005J and *3DL1**005 alleles were also associated exclusively with each other, as were *2DL4**006 and *3DL1**007. There was a corresponding significant positive LD in haplotypes 1-6 while negative LD was obserbed between pairs of *2DL4**00102 and *3DL1* blank (haplotype 7).

In this study, parents with *3DL1**1502 carried the full length form of *2DS4* without exception (Figure 4).

Diversification of *KIR* haplotypes by allelic polymorphism of *KIR2DL4* and *3DL1*.

Within each family, the segregation of *KIR* alleles was determined and used to define *KIR* haplotypes. In the previous study, a total of 29 different *KIR* genotypes, and 19 (2 group A and 18 group B) haplotypes were identified in 154 parents. Group A haplotype was divided into two subtypes, A1 and A2, containing *KIR2DS4F* and *KIR2DS4D*, respectively (Whang et al., 2005).

In this study, allelic polymorphism of *KIR2DL4* and *3DL1* genes diversified haplotypes with similar gene content, mainly for the group A haplotypes (Figure 4). Six distinct A haplotypes (A1-1~A2-3), composed of same gene content, and 18 B haplotypes, composed of variable gene content, were defined.

Therefore, allelic polymorphism diversified A haplotypes with same gene content. B haplotypes having more complicated combinations of *KIR*

genes than the A haplotypes were not further diversified by allelic polymorphism of *KIR2DL4* and *3DL1* genes. In the previous study, 18 distinct B haplotypes were unambiguously defined in the 154 parents (Whang et al., 2005), and particular B haplotypes were associated with particular *KIR2DL4* and *3DL1* haplotypes.

Analysis of *KIR2DL4* and *3DL1* alleles in the family panel reveled segregation of 24 (6 group A and 18 group B) different haplotypes (Table 7). A1 haplotype was divided into three types, A1-1 (52.3%), A1-2 (1%), A1-3 (0.3%), and A2 haplotype was divided into three haplotypes also, A2-1 (3.6%), A2-2 (9.4%), A2-3 (6.8%). B haplotypes were not further diversified.

KIR haplotype combinations at the allele level were analyzed and shown in Table 8. Group A-1 haplotype in a homozygous state is occurring at the highest frequency in this panel (29.87%).

A total of 44 different haplotype combinations were identified, 10 group A haplotypes in homozygous state, 24 group A and B haplotypes in heterozygous state and 10 group B haplotypes in homozygous state. On the other hand, in the previous study, a total of 31 (A1B4=B1B3, A1B7=A2B3) different haplotype combinations were identified, 3 group A haplotypes in homozygous state and 18 group A and B haplotypes in heterozygous state, and 10 group B haplotypes in homozygous state (Whang et al., 2005).

Discussion

Systematic sequence analysis of *KIR* cDNA indicated that *KIR* haplotypes vary in gene content and *KIR* genes exhibit allelic polymorphism (Valiante et al., 1997). In the present study, two highly polymorphic *KIR* genes, *KIR2DL4* and *3DL1*, were analyzed in 352 members of 77 unrelated Korean families. *KIR3DL1* and *2DL4* contain 50 and 19 polymorphic sites, respectively (Yawata et al., 2002). The genetic organization of *KIR* haplotypes can best be studied by analysis of continuous genomic sequence on a haplotype (Wende et al., 1999; Rajalingan et al., 2001) or by segregation analysis based on a family study.

The *KIR* are members of the immunoglobulin superfamily, and some of them directly recognize polymorphic *HLA* determinants. *KIR2DL4* has been associated with *HLA-G* specificity and *KIR3DL1* with *HLA-B* specificity (Yawata et al., 2002).

Group A haplotype diversity is mostly due to the allelic variations, whereas group B haplotype diversity is mostly due to differences in gene content (Yawata et al., 2002). It thus appears that *KIR* diversity involves major contributions from both gene content and allelic polymorphism. In this study, allelic polymorphism did not diversified group B haplotypes, and particular group B haplotypes were associated with particular *KIR2DL4* and *3DL1* haplotypes at allelic level.

Allelic polymorphism diversifies *KIR* haplotypes having the same set of

genes. But this effect was only apparent for the A haplotypes, which all have the same arrangement of *KIR* genes. In this study, subgroups of A and B haplotypes were analyzed using allele level typing of *KIR2DL4* and *3DL1* genes, and two group A haplotypes were diversified to 6 different haplotypes but B haplotypes were not further diversified. Assessment of A and B haplotype diversity in the present study is likely to be an underestimate because allele level typing was not done for all *KIR* genes.

Analysis of the high resolution typing obtained for the *KIR2DL4* and *3DL1* genes revealed striking patterns of LD. The high LD between *KIR2DL4* and *3DL1* is considered to be caused by close proximity of these loci.

In conclusion, this study demonstrates that group A haplotypes are diversified by combinations of *KIR2DL4* and *3DL1* alleles in the telomeric half of the haplotype. In contrast, diversification of group B haplotype is mostly due to difference in gene content.

This is the first study on high resolution genotyping of *KIR2DL4* and *3DL1* in Koreans based on a family study. The result of this study would be useful as basic data on *KIR* polymorphisms in Koreans for studies of *KIR* gene polymorphisms associated with susceptibilities to infections, tumors and autoimmune disease, as well as the clinical outcome of organ transplantations.

Table 1. SSP primer mix information for *KIR2DL4* and *KIR3DL1* allele typing

KIR receptor specificity	Forward primer (5'→3')	Reverse primer (5'→3')	Amplicon length (Kb)
KIR2DL4			
1	TGACTCTTCGGTGTCACTG	GGCCGGGCTGTAAGG	1.0
2	AGGACAAGCCCTTCTGC	GAACCGTGGGGCCCA	1.0
3	AGGACAAGCCCTTCTGC	GGTCACGTTCTCTCCTGT	1.0
4	CAAGAGCCTGCGGGAC	TGCTCATGGGCAGGAGAT	0.4
5	CAAGAGCCTGCGGGAC	TCCAGCTGCTGGTACATGG	0.5
6	AGGACAAGCCCTTCTGC	AGTTCATGGGCTTCCCCT	1.0
7	CAGTGGCCATCATCCTCTTT	CTCCCTGTTCACTGTTCTGTGT	0.5
8	CAGTGGCCATCATCCTCTTC	CTCCCTGTTCACTGTTCTGTGT	0.5
KIR3DL1			
1	TCCCATCTTCCATGGCAGAT	TAGGTCCCTGCAAGGGCAA	1.7
2	CCATCGGTCCCATGATGCT	ATAGGAGCTCCGGGAGCTG	1.6
3	CCATCGGTCCCATGATGCT	ACGTTCATGGGCTCCCCG	1.6
4	ATCTCTAAGGACCCCTCAA	AGAGAGAAGGTTTCTCATATG	1.7
5	AACCCCAGACACCTGCACG	GTACAAGATGGTATCTGTAG	0.7
6	TACAAAGAAGACAGAATCCACA	GGAGCTGACAACTGATAGGG	1.6
7	GGTTCTGTTACTCACACCT	AGAGTGACGGAAAGAGCCA	1.8
8	TCTTCGGTGTCACTATCG	GAGCTGACAACTGATAGGA	1.6
9	CAGACACCTGCATGTTCTC	GTACAAGATGGTATCTGTAG	0.8

Table 2. *KIR2DL4* and *3DL1* allele frequencies in 154 parents of 77 unrelated Korean families

KIR receptor specifity	Phenotype frequency[a]		Allele frequency	
	n = 154	%	n = 308	%
KIR2DL4				
*00102	122	79.2	172	55.8
*00102J	3	1.9	3	1.0
*00202J	13	8.4	13	4.2
*005J	30	19.5	33	10.7
*005	55	35.7	64	20.8
*006	23	14.9	23	7.5
*00101	0	0.0	0	0.0
*00201	0	0.0	0	0.0
*00202	0	0.0	0	0.0
*003	0	0.0	0	0.0
*007	0	0.0	0	0.0
KIR3DL1				
*001	13	8.4	13	4.2
*002	0	0.0	0	0.0
*003	0	0.0	0	0.0
*004	0	0.0	0	0.0
*005	30	19.4	33	10.7
*006	0	0.0	0	0.0
*007	23	14.9	23	7.5
*008	0	0.0	0	0.0
*1502	121	78.6	171	55.5
*JB	3	1.9	3	1.0
blank			65	21.1

[a]: phenotype frequency = n / total number of individuals tested.

Table 3. Frequency of *KIR2DL4* alleles in various populations

KIR2DL4	Korea (n=154)		Hong Kong (n=40)		Oman (n=40)		Northern Ireland (n=162)		Xhosa (n=50)		Cuban mulatto (n=42)	
	PF(%)[a]	GF[b]	PF(%)	GF	PF(%)	GF	PF(%)	GF	PF(%)	GF	PF(%)	GF
*00102	79.2	0.558	82.5	0.563	60.0	0.388	48.8	0.275	74.0	0.520	64.3	0.393
*00201	0	0	0	0	20.0	0.100	37.0	0.204	20.0	0.120	35.7	0.179
*00202	0	0	7.5	0.038	42.5	0.250	30.3	0.157	4.0	0.020	19.1	0.119
*005	35.7	0.357	52.5	0.313	35.0	0.213	55.6	0.327	50.0	0.340	45.2	0.262
*006	14.9	0.149	17.5	0.088	10.0	0.050	6.8	0.037	0	0	9.5	0.048
Reference	This study						Williams et al., 2004					

[a] : phenotype frequency (%) , [b] : gene frequency

Table 4. Frequency of *KIR3DL1* alleles in various populations

KIR 3DL1	Korean (n=154)		Northern Irish (n=100)		North Indian (n=58)	
	PF(%)[a]	GF[b]	PF(%)	GF	PF(%)	GF
*001	8.4	0.042	41	0.232	38.5	0.216
*002	0	0	24	0.128		
*003	0	0	14	0.073		
*004	0	0	31	0.169	8.4	0.043
*005	19.4	0.107	26	0.140	24.1	0.129
*006	0	0	0	0		
*007	14.9	0.075	11	0.057		
*008	0	0	6	0.030		
*009	0	0	4	0.020		
*1502	78.6	0.555				
*JB	1.9	0.010				
blank		0.211		0.151		0.612
Reference	This study		Halfpenny et al., 2004		Halfpenny et al., 2004	

[a] : phenotype frequency (%) , [b] : gene frequency

Table 5. Haplotype frequencies of *KIR2DL4* and *3DL1* in 154 parents of 77 unrelated Korean families

Haplotype	*KIR2DL4*	*KIR3DL1*	n=308	frequency
1	*00102	*1502	171	0.555
2	*005	blank	64	0.208
3	*005J	*005	33	0.107
4	*006	*007	23	0.075
5	*00202J	*001	13	0.042
6	*00102J	*JB	3	0.010
7	*00102	blank	1	0.003

Table 6. Linkage disequilibrium (LD) analysis for *KIR2DL4* and *KIR3DL1* alleles in 154 parents of 77 unrelated Korean families

		*2DL4**00102	*2DL4**00102J	*2DL4**00202J	*2DL4**005	*2DL4**005J	*2DL4**006
*3DL1**001	Δ			0.040			
	r			1			
	h			0.042			
	p			3.8×10^{-19}			
*3DL1**005	Δ					0.096	
	r					1	
	h					0.107	
	p					2.3×10^{-35}	
*3DL1**007	Δ						0.069
	r						1
	h						0.075
	p						7.1×10^{-28}
*3DL1**1502	Δ	0.244[a]					
	r	0.99[b]					
	h	0.555[c]					
	p	4.5×10^{-34} [d]					
*3DL1**JB	Δ		0.010				
	r		1				
	h		0.010				
	p		1.7×10^{-6}				
*3DL1*blank	Δ	-0.115			0.164		
	r	-0.97			1		
	h	0.003			0.208		
	p	2.7×10^{-2}			0.6×10^{-4}		

[a] LD (Δ) values were calculated according to Mattiuz et al.(1971); [b] relative LD (1=threshold);
[c] haplotype frequencies of *KIR2DL4* and *KIR3DL1*; [d] p value by Chi-squre test or Fisher's exact test.

Table 7. *KIR* haplotype frequencies in 154 parents (308 haplotypes) of 77 unrelated Korean families

Haplotype	n = 308	frequency
A haplotype (total)	226	0.734
A1	165	0.536
A1-1	161	0.523
A1-2	3	0.010
A1-3	1	0.003
A2	61	0.198
A2-1	11	0.036
A2-2	29	0.094
A2-3	21	0.068
B haplotype (total)	82	0.266
B1	31	0.101
B2	21	0.068
B3	8	0.026
B4	6	0.019
B5	2	0.006
B8	2	0.006
Others[a]	12	0.029

[a]Others : B6, B7, B9~B18 (n=1, each)

Table 8. *KIR* genotype frequencies at the allele level in 154 parents of 77 unrelated Korean families

		A1-1	A1-2	A1-3	A2-1	A2-2	A2-3	B1	B2	B8
A1-1	n %	46[a] 29.87[b]								
A1-2	n %	1 0.65								
A1-3	n %									
A2-1	n %	4 2.60								
A2-2	n %	16 10.39	1 0.65		1 0.65	2 1.30				
A2-3	n %	12 7.79			1 0.65	1 0.65				
B1	n %	16 10.39			3 1.95	1 0.65	3 1.95	1 0.65		
B2	n %	8 5.19				3 1.95	1 0.65	3 1.95	1 0.65	
B3	n %	3 1.95				1 0.65	1 0.65	2 1.30	1 0.65	
B4	n %	2 1.30	1 0.65			1 0.65	1 0.65		1 0.65	
B5	n %	2 1.30								
B6	n %								1 0.65	
B7	n %	1 0.65								
B8	n %	1 0.65								
B9	n %	1 0.65								
B10	n %	1 0.65								
B11	n %	1 0.65								
B12	n %								1 0.65	
B13	n %									1 0.65
B14	n %				1 0.65					
B15	n %							1 0.65		
B16	n %				1 0.65					
B17	n %						1 0.65			
B18	n %			1 0.65						

[a] : number of individuals , [b] : gene frequency (%)

KIR2DL4 allele	Reaction	1	2	3	4	5	6	7	8	
00102										
00102J										
00202J										
005J										
005										
006										
00101										
00201										
00202										
003										
004										
007										
	PCR condition	M7	M7	M7	M7	M7	M7	M7	M7	
	product size (kb)	1.0	1.0	1.0	0.4	0.5	1.0	0.5	0.5	
KIR3DL1 allele	Reaction	1	2	3	4	5	6	7	8	9
001										
002										
004										
005										
006										
007										
008										
1502										
JB										
	PCR condition	NT	M2	M5	NT	M5	H	NT	NT	NT
	product size (kb)	1.7	1.6	1.6	1.7	0.7	1.6	1.8	1.6	0.8

Figure 1. Subtyping for *KIR2DL4* and *3DL1* alleles. : alleles amplified by *KIR2DL4* and *3DL1* specific primer combinations (Yawata et al., personal communication).

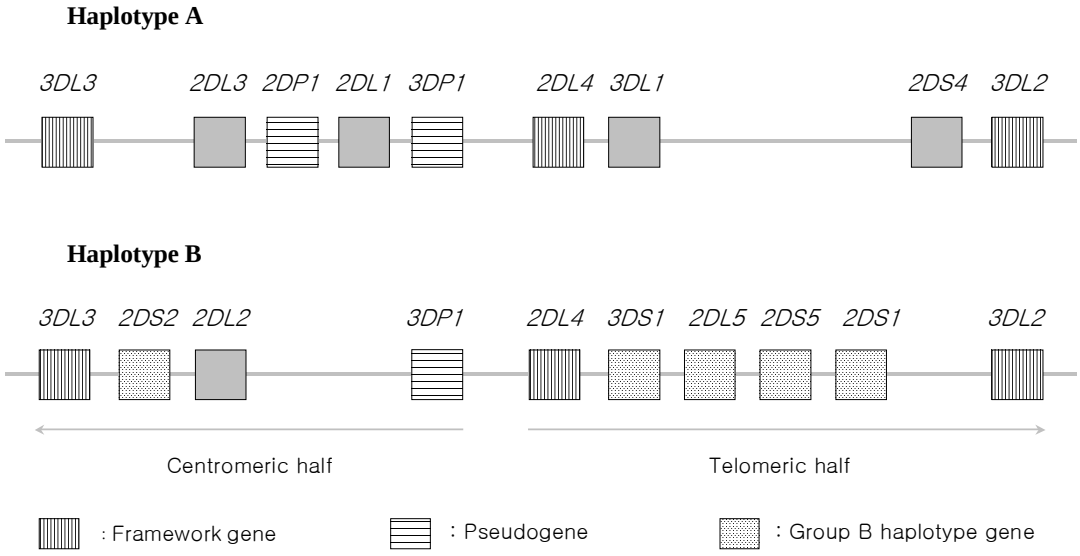

Figure 2. Organization of *KIR* genes in group A and group B *KIR* haplotypes.
Two kinds of *KIR* haplotype have been described based upon gene content, and are designated A and B. Group B haplotypes are characterized by one or more of the following genes : *KIR2DL5*, *-2DS1, -2DS2*, *-2DS3*, *-2DS5* and *–3DS1*. Group A haplotypes are characterized by the absence of all these genes. As a consequence of these differences the B haplotypes have more genes encoding activating *KIR* than A haplotypes.

Genotype # Haplotype combinations	KIR genes																				No. of KIR genes[c]	Genotypes	
	2DL							3DL			2DS						3DS	2DP	3DP			Number	%
	1	1v	2	3	4	5.1	5.2	1	2	3	1	2	3	4F[a]	4D[b]	5	1	1	1	1v			
1. A1A1	■			■	■			■	■	■				■				■	■		7	47	30.5
2. A1A2	■			■	■			■	■	■				■	■			■	■		7	33	21.4
3. A1B1	■			■	■	■		■	■	■	■			■		■	■	■	■		11	16	10.4
4. A1B2	■			■	■	■		■	■	■	■		■	■			■	■	■		11	8	5.2
5. A2B1	■			■	■	■		■	■	■	■				■	■	■	■	■		11	7	4.5
6. A2A2	■			■	■			■	■	■					■			■	■		7	5	3.2
7. A1B4 (3) B1B3 (2)	■		■	■	■	■		■	■	■	■	■		■		■	■	■	■	■	13	5	3.2
8. A2B2	■			■	■	■		■	■	■	■		■		■		■	■	■		11	4	2.6
9. A1B3	■		■	■	■			■	■	■		■		■				■	■	■	9	3	1.9
10. A1B7 (1) A2B3 (2)	■		■	■	■			■	■	■		■		■	■			■	■	■	9	3	1.9
11. B1B2	■			■	■	■			■	■	■		■			■	■	■	■		10	3	1.9
12. A1B5	■			■	■			■	■	■		■		■	■			■	■	■	8	2	1.3
13. A2B4	■		■	■	■	■		■	■	■	■	■			■	■	■	■	■	■	13	2	1.3
14. A2B17	■			■	■			■	■	■					■			■	■		7	1	0.6
15. B1B1	■			■	■	■			■	■	■					■	■	■	■		9	1	0.6
16. A2B14	■	■	■	■	■		■	■	■	■		■	■	■	■			■	■		11	1	0.6
17. A1B11	■	■		■	■		■	■	■	■		■	■	■	■			■	■		10	1	0.6
18. A1B8	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■		15	1	0.6
19. B1B15	■		■	■	■	■	■		■	■	■	■				■	■	■	■		12	1	0.6
20. A1B10	■			■	■			■	■	■	■			■			■	■	■		9	1	0.6
21. A2B16	■			■	■	■		■	■	■	■		■		■			■	■		10	1	0.6
22. A1B9	■		■	■	■			■	■	■		■		■				■	■	■	9	1	0.6
23. B2B6	■		■	■	■	■	■		■	■	■	■	■			■	■	■	■		13	1	0.6
24. B8B13		■	■		■	■	■		■	■	■	■	■			■	■	■	■		12	1	0.6
25. B2B4	■		■	■	■	■			■	■	■	■	■			■	■	■	■	■	12	1	0.6
26. B2B12	■	■		■	■	■	■	■	■	■	■	■	■		■		■	■	■		13	1	0.6
27. B2B2	■			■	■	■			■	■	■		■				■	■	■		9	1	0.6
28. B2B3	■		■	■	■	■		■	■	■	■	■	■	■			■	■	■	■	13	1	0.6
29. A1B18	■		■	■	■		■	■	■	■	■	■		■		■		■	■		12	1	0.6
Frequency(%)	99.4	3.2	14.3	99.4	100	86.4	5.2	94.2	100	100	37.7	16.9	16.2	80.5	39.6	26.6	36.4	100	100	11.7			

Figure 3. *KIR* genotypes in 154 parents of 77 unrelated Korean families.
[a] full length *2DS4* ; [b] deleted *2DS4* ; [c] number of *KIR* genes except pseudogenes (*2DL5.1* and *2DL5.2* were counted as separate genes) ; [d] PCR-SSP product showed an amplicon size different from usual 3DP1 variant. Except for 3 genotypes (#21, 27 and 28), all of the genotypes listed have been observed previously in other populations (Yawata et al., 2002).

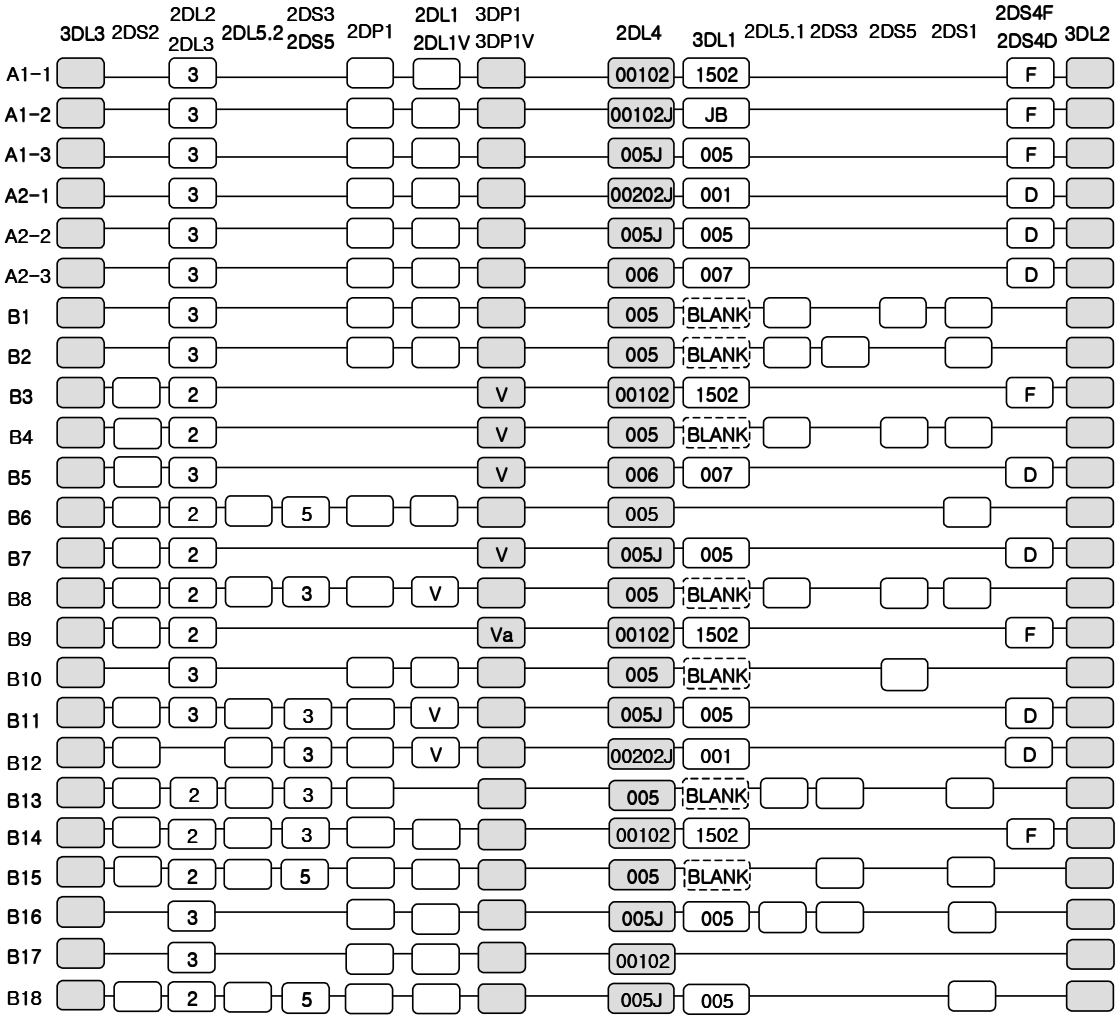

Figure 4. *KIR* haplotype with *2DL4* and *3DL1* at the allele level in 154 parents of 77 families defined by segregation analysis based on a haplotype model suggested by Hsu et al (2002). Shaded boxes denote framework *KIR* genes. Dotted boxes denote *KIR3DS1*. *3DP1Va* denotes a further variant of *3DP1V*.

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Abstract

Allelic polymorphism and haplotypic associations of KIR 2DL4 and 3DL1 genes in 77 Korean families

Yoon, Jung Ah

Department of Biology

Graduate School

SungShin Women's University

Killer cell immunoglobulin-like receptor (*KIR*) genes constitute a multigene family on human chromosome 19q13.4, and to date 16 *KIR* genes and pseudogenes have been identified. In human populations, diversity in *KIR* genotypes arises from variations in gene content and allelic polymorphism. To examine how allelic polymorphism diversifies *KIR* haplotypes with similar or identical combinations of *KIR* genes, allele typing of *KIR2DL4* and *3DL1* was performed in 77 unrelated Korean families (352 individuals including 154 parents). These family samples were previously studied for the presence of 16 *KIR* genes and pseudogenes and haplotype analysis revelaled presence of 2 group A and 18 group B haplotypes. *KIR2DL4* and *3DL1* allele typing was performed by PCR-sequence specific primer method. Six different *KIR2DL4* alleles and 5 different *KIR3DL1* alleles were detected: *KIR2DL4**00102 (allele frequency 55.8%), *005 (20.8%), *005J (10.7%), *006 (7.5%),

*00202J (4.2%), *00102J (1.0%); *KIR3DL1**1502 (55.5%), *005 (10.7%), *007 (7.5%), *001 (4.2%) and *JB (1.0%). Segregation analysis of the families defined a total of 308 independent *KIR* haplotypes in 154 parents. Seven different *KIR2DL4-3DL1* haplotypes were identified and six of the haplotypes revelaled strong positive linkage disequilibrium with relative linkage disequilibrium values of 0.99-1.00. Two different group A haplotypes were diversified into 6 different haplotypes by allelic polymorphisms of these two *KIR* genes. Group B haplotypes were not further diversified by allelic polymorphisms of these genes. The results suggest that the *KIR* locus is polygenic and polymorphic within the human population.

감사의 글

부족한 저에게 꾸준한 관심과 애정을 주시고 처음부터 끝까지 정성스레 지도해주신 박경숙 교수님과 박명희 교수님께 감사드립니다. 그 가르침을 마음에 새겨 성실하고 최선을 다해 살아가겠습니다. 그리고 바쁘신 와중에도 귀한시간을 내주시어 심사해주시고 많은 조언을 해주신 송영욱 교수님께도 감사를 드립니다.

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