Distribution of Killer Cell Immunoglobulin-Like Receptor Genes in Thai Blood Donors

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Background: *Killer cell Immunoglobulin-like Receptors (KIRs) are members of a group of molecules expressed on the surfaces of natural killer (NK) cells and some T cells. KIRs recognize MHC class I molecules on target cells. The interaction of these molecules regulates NK cell reactivity. The KIR gene cluster is highly polymorphic in individuals and different populations.*
Objective: *Determine the frequencies and diversities of KIR genes among the Thai population.*
Results: *Seventeen KIR genes and common subtypes were identified in 500 healthy Thai blood donors by PCR-SSP. The framework genes KIR2DL4, KIR3DL2, KIR3DL3, and KIR3DP1 were present in all individuals (100%). The observed frequencies of KIR genes vary in the presented population. The most frequent non-framework KIR gene was KIR2DL1 (98.4%) while the least frequent was KIR2DL5B (24.2%).*
Conclusion: *It was observed that the Thai population shows polymorphism of the KIR genes and the diversities of KIR genes in Thai differed from other populations. These data might be of benefit to future studies of the KIR gene and its association with diseases.*

Keywords: *KIR genes, PCR-SSP, Thai blood donors, Framework genes, Polymorphism*

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Killer cell Immunoglobulin-like Receptors (KIRs) are glycoprotein expressed on the surfaces of natural killer (NK) cells and some T cells. KIR can be divided according to the number of extra-cellular domains, two domains (2D) or three domains (3D), and the possession of either long (L) or short (S) cytoplasmic tails[1]. Most KIRs are able to recognize HLA class I molecules and ligands on target cells[2]. The interaction between KIRs and their ligands can lead to either an inhibitory signal or an activating signal[3]. These interactions could explain successful immune responses to viral infections and cancers[4].

KIR genes are located in leukocyte receptor complex (LRC) on chromosome 19q13.4[5]. The KIR gene family displays a high degree of polymorphism. The KIR locus is polymorphic with respect to both gene content and sequence. KIR haplotypes contain between 7 and 12 KIR genes, and up to 107 alleles for individual KIR genes have been described[5]. All known KIR haplotypes are flanked at their centromeric end by KIR3DL3 and at their telomeric end by KIR3DL2, together with the centric KIR3DP1 and KIR2DL4. These constitute the framework genes[3,5].

To date, at least 15 functional KIR genes and two pseudogenes have been identified among different populations[2]. Genetic studies of KIR in many human populations have shown the variation of the frequencies of KIR genes, genotypes, and haplotypes among different ethnic groups. Previous studies of KIR genes in a Thai population have been reported by Norman et al[6,7]. However, the number of samples in their studies was low and only 14 KIR genes were typed without pseudogene typing. Furthermore, new primers used for identifying KIR genes have recently been designed. Thus, a study of the frequencies and diversities of KIR genes in Thais, using a large number of samples and new primers, is needed. The aims of the present study were to determine the frequencies and diversities of KIR genes among the Thai population.

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Material and Method

Population samples

Stored DNA samples from 500 healthy Thai blood donors at the Blood Bank, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, were studied. The samples were previously collected from plateletpheresis donors between November 2001 and January 2007. Genomic DNA was extracted from 10 ml of EDTA blood using the modified salting-out method, the following manufacturer's instruction (Dynal® SSP Sets, Dynal AS, Oslo, Norway). The present study was approved by the Ethics Committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University.

KIR Genotyping

Samples from 500 healthy Thai blood donors were typed for 17 KIR genes, including 15 KIR genes (2DL1, 2DL2, 2DL3, 2DL4, 2DL5A, 2DL5B, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, and 3DS1) and two pseudogenes (2DP1 and 3DP1) using a KIR Genotyping SSP Kit (Dynal Biotech, Pel-Freez Clinical Systems, Brown Deer, WI, U.S.A.). The common subtypes of the KIR2DS4 allele (*001/002 and *003-007) and KIR3DP1 allele (*001/002/004 and *003) were additionally typed. The procedure of PCR-SSP amplification was performed following the manufacturer's instruction. The PCR amplifications were performed under conditions recommended by manufacturers: one cycle at 95°C for 1 minute, 30 cycles of 94°C for 20 seconds, 63°C for 20 seconds and 72°C for 90 seconds, then a hold at 4°C. Amplified products were run into each well of 2% agarose gel in 0.5 x TBE buffer, stained with ethidium bromide and visualized by UV transilluminator and photographed. The results of KIR genotyping were valid only when the internal control bands were seen in every well, except negative control well, and the four framework genes were present in each genotype.

Statistical analysis

The observed frequencies of KIR genes were calculated by direct counting. The gene frequencies were estimated by the formula:

Gene frequency (Gf) = $1-\sqrt{(1-OF)}$, when OF = observed frequencies of KIR gene in population. Pearson's Chi-square test was used to determine the difference of gene frequency between the samples of Thai population in the present study and other populations. P-value < 0.05 was set for statistically significant difference.

Results

Seventeen KIR genes and common subtypes in Thai blood donors were identified in all of the samples. In the present study, the framework genes KIR2DL4, KIR3DL2, KIR3DL3 and KIR3DP1 were found in all individuals. Conversely, the non-framework KIR genes were found differently in each individual. The observed frequencies and estimated gene frequencies are illustrated in Table 1.The frequency of inhibitory KIR genes was more than 90%. The percentage of KIR2DL1, KIR2DL3, and KIR3DL1 were 98.4%, 96.6% and 92.6%, respectively (Table 1, Fig. 1). However, the frequencies of activating KIR genes were lower than inhibitory KIR genes. KIR2DS4 with the frequencies of 92.6% (Gf = 0.73) was the only one activating KIR with the frequency more than 50% in the presented population (Table 1, Fig. 1). The rest of activating KIR genes had the frequency less than 50%, KIR2DS1 (42.6%), KIR2DS2 (37.6%), KIR2DS3 (30.4%), KIR2DS5 (31%) and KIR3DS1 (41.6%). Furthermore, the authors also determined the frequencies of KIR2DS4 and KIR3DP1 subtypes. It was observed that the frequencies of KIR2DS4*001/002, KIR2DS4 *003-007, KIR3DP1*001/002/004 and KIR3DP1*003 alleles were 63.6% (Gf = 0.4), 57% (Gf = 0.34), 21.2% (Gf = 0.11) and 98.6% (Gf = 0.88), respectively.

Table 1. The frequencies of KIR genes in 500 individuals

	KIR gene	Frequency (%)	Gene frequency
Inhibitory KIR	2DL1	98.4	0.87
	2DL2	37.4	0.21
	2DL3	96.6	0.82
	2DL4	100	1
	2DL5A	39.6	0.22
	2DL5B	24.2	0.13
	3DL1	92.6	0.73
	3DL2	100	1
	3DL3	100	1
Activating KIR	2DS1	42.6	0.24
	2DS2	37.6	0.21
	2DS3	30.4	0.17
	2DS4*001/002	63.6	0.4
	2DS4*003-007	57	0.34
	2DS5	31	0.17
	3DS1	41.6	0.24
Pseudogene	2DP1	98.4	0.87
	3DP1*001/002/004	21.2	0.11
	3DP1*003	98.6	0.88

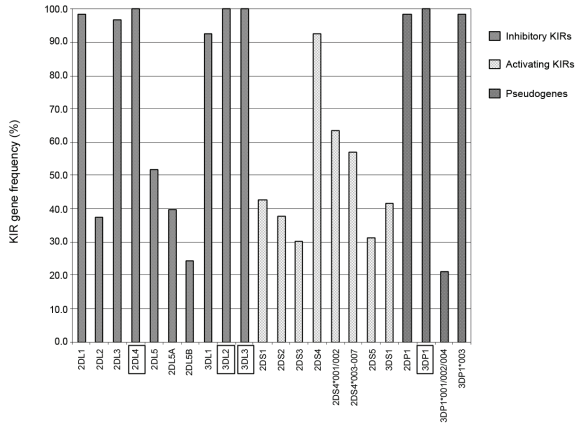

Fig. 1 Comparison of inhibitory KIRs, activating KIRs, and pseudogenes

Discussion

In the present study, all of the 17 KIR genes and their common subtypes were identified among 500 healthy Thai blood donors using PCR with sequence-specific primer (PCR-SSP). The present study showed that KIR gene frequencies varied in the presented population (range 24.2-98.4%). The percentages of inhibitory KIR genes were higher than activating KIR genes, except for KIR2DS4. The highest frequency was KIR2DL1 (98.4%), while the lowest was KIR2DL5B (24.2%). The authors also observed the frequencies of common subtypes of KIR2DS4 allele (*001/002 and *003-007) and the KIR3DP1 allele (*001/002/004 and *003), which have not been identified in the previous studies of Thai population[6,7]. The most common KIR3DP1 alleles was KIR3DP1*003 (98.8%). Comparing the frequency of KIR3DP1 alleles between the presented population and Caucasian population resulted in that the estimated gene frequency of KIR3DP1*003 alleles was higher in the presented Thai population (0.88) than that in Caucasian population (0.72), while the estimated gene frequency of KIR3DP1*001/002/004 alleles was lower (0.11 and 0.17, respectively)[8]. For subtype of KIR2DS4, it can be divided into 2 forms; the full-length form (KIR2DS4 *001/002), and the deleted form (KIR2DS4*003-007). The deleted form of KIR2DS4 is translated into non-functional activating receptor. The frequencies of the two forms of KIR2DS4, full-length (63.6%) and deleted forms (57%) in the presented population were different from that in Caucasian populations. Among Caucasian populations, the full-length form of KIR2DS4 was less frequent than the deleted form (35.5 and 78.8%, respectively)[8].

A comparison of the frequencies of the KIR genes in the Thai population and other populations is shown in Table 2. The KIR2DL2, KIR2DS2 and KIR2DS3 gene frequencies were significantly higher ($p < 0.05$) among the presented Thai population than other Asian populations (Chinese Han, Korean and Japanese)[9-11]. For KIR2DL5, the Chinese Han and Korean groups had lower frequencies, but the Japanese had similar frequencies, compared to Thais in the present study ($p < 0.05$). The KIR gene frequencies in the Vietnamese were similar to the Thais in the present study, except for KIR2DL3, which was lower than the Thais in the

Table 2. Comparison of 12 KIR genes between Thai and other populations

Population	KIR gene frequency (%)											
	2DL1	2DL2	2DL3	2DL5	3DL1	2DS1	2DS2	2DS3	2DS4	2DS5	3DS1	2DP1
Thai (n = 500)	98.4	37.4	96.6	51.6	92.6	42.6	37.6	30.4	92.6	31.0	41.6	98.4
Chinese Han (n = 104)	99.0	17.3*	99.0	34.6*	94.2	33.7	17.3*	12.5*	94.2	23.0	32.8	99.0
Korean (n = 154)	99.4	14.3*	99.4	38.3*	94.2	37.7	16.9*	16.2*	94.2	26.6	36.4	100.0
Japanese (n = 41)	100.0	14.6*	100.0	39.0	97.6	34.1	14.6*	14.6*	97.6	24.4	29.3	100.0
Vietnamese (n = 59)	98.0	45.0	66.0*	NT	88.0	37.0	41.0	34.0	88.0	NT	41.0	NT
Labanese (n = 120)	99.2	59.2*	88.3*	58.3	95.8	40.8	59.2*	37.5	95.0	30.8	35.8	96.7
Italian Caucasian (n = 217)	95.0	53.0*	88.0*	ND	96.0	36.0	53.0*	33.0	ND	28.0	35.0	ND
Argentinean Caucasoid (n = 402)	96.0	56.0*	87.0*	56.0	95.0	46.0	55.0*	29.0	95.0	36.0	42.0	96.0
Australian Aborigines (n = 67)	71.6*	79.0*	67.0*	NT	55.0*	82.0*	85.0*	80.6*	50.7*	NT	77.6*	NT

* $p < 0.05$

NT = not tested; ND = no data

The framework genes (KIR2DL4, 3DL2, KIR3DL3 and KIR3DP1) were not tested for Chi-square test

present study ($p < 0.05$)[12]. Furthermore, the KIR2DL2, KIR2DL3 and KIR2DS2 frequencies in the Thais from the present study were significantly different from non-Asian populations (Lebanese, Italian Caucasian and Argentinean Caucasoid, $p < 0.05$)[13-15]. In addition, the frequencies of all KIR genes among the Thais in the present study were significantly different from the Australian Aborigines ($p < 0.05$)[12].

In summary, it was found from the present study that there was a great diversity in the Thai population and these diversities differed from the other populations. The results in the present study might serve as useful normal control data in the future studies of KIRs and their association with diseases.

Potential conflicts of interest

None.

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การกระจายของ KIR genes ในผู้บริจาคโลหิตที่เป็นประชากรไทย

ชุติมา ธรรมกรณ์, ทัศนีย์ มงคลสุข, ดวงตะวัน ธรรมาณิชานนท์, สามารถ ภคกษมา, พิมพรรณ กิจพ่อค้า

KIRs เป็นกลุ่มสมาชิกของโมเลกุลที่มีการแสดงออกบนผิวเซลล์ของ NK cells และ T cells บางชนิด โดย KIRs มีความสามารถในการจดจำโมเลกุลของ MHC class I ที่อยู่บนเซลล์เป้าหมาย การเกิดปฏิกิริยาของ KIRs กับ MHC class I สามารถควบคุม NK cells reactivity และพบว่ากลุ่มของ KIR gene มีความหลากหลายอย่างมาก ในแต่ละบุคคล และแต่ละกลุ่มประชากร วัตถุประสงค์ในการศึกษาคือ ศึกษาความถี่ ความหลากหลายของ KIR gene จำนวน 17 gene และ common subtypes ในกลุ่มประชากรไทย การศึกษา KIR gene ทำในกลุ่มผู้บริจาค โลหิต จำนวน 500 รายโดยวิธี PCR-SSP พบว่าทุกรายมียีนที่เป็น framework genes จำนวน 4 ยีนคือ KIR2DL4, KIR3DL2, KIR3DL3 และ KIR3DP1 พบความถี่ของ KIR genes มีความหลากหลายในกลุ่มประชากรที่ศึกษานี้ และพบว่ายีน KIR2DL1 มีความถี่สูงสุดคือ 98.4% และยีน KIR2DL5B มีความถี่ต่ำสุดคือ 24.2% ผลการศึกษาพบว่า KIR genes มีความหลากหลายในประชากรไทย และความหลากหลายของ KIR genes ในประชากรไทยมีความแตกต่างจาก กลุ่มประชากรอื่น ประโยชน์ที่ได้จากการศึกษานี้ สามารถนำไปใช้ในการศึกษาความสัมพันธ์ของ KIR gene กับโรค ต่าง ๆ ได้ในอนาคต