Original Article

ความถี่ของยีน *KIR2DS3* ในประชากรชาวไทย

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บทคัดย่อ

บทนำ: *KIR2DS3* เป็นยีนที่ทำหน้าที่สร้างโปรตีน KIR2DS3 (หรือที่รู้จักในนาม NKAT7) ซึ่งอยู่บนผิวของ natural killer (NK) cell และมีบทบาทเกี่ยวกับการตอบสนองทางภูมิคุ้มกัน ยีนนี้มีภาวะพหุสัณฐานสูง และพบความถี่ของยีนได้แตกต่างกันมากในแต่ละกลุ่มประชากร นอกจากนี้พบว่ายีน *KIR2DS3* มีความสัมพันธ์กับโรคติดเชื้อหลายชนิด อาทิ การติดเชื้อไวรัสตับอักเสบซี การติดเชื้ออีโบลา มาลาเรีย และวัณโรค วัตถุประสงค์ของงานวิจัยนี้คือ เพื่อศึกษาความถี่ของยีน *KIR2DS3* ในประชากรชาวไทยและการกระจายความถี่ของยีนนี้ในแต่ละภูมิภาคของประเทศไทย **วิธีการดำเนินการวิจัย:** ทำการสุ่มอาสาสมัครสุขภาพดีที่ไม่มีความสัมพันธ์กันทางเครือญาติจำนวน 100 ราย จากสี่ภูมิภาคเท่า ๆ กัน (ภูมิภาคละ 25 ราย) ตามภูมิลำเนาซึ่งแบ่งโดยกรมการปกครอง กระทรวงมหาดไทย จากนั้นทำการเจาะเลือดอาสาสมัครเพื่อสกัดดีเอ็นเอด้วยวิธี Chelex method และทำการเพิ่มปริมาณดีเอ็นเอและตรวจวิเคราะห์หายีน *KIR2DS3* ด้วยเทคนิคปฏิกิริยาลูกโซ่โพลีเมอเรสโดยอาศัยไพรเมอร์จับจำเพาะ **ผลการวิจัย:** พบยีน *KIR2DS3* ในอาสาสมัครจำนวน 42 ราย จากอาสาสมัครทั้งหมด 100 ราย คิดเป็น 42% โดยพบการกระจายความถี่ของยีนในอาสาสมัครแต่ละภูมิภาคดังนี้ ภาคเหนือพบ 44% (11 ราย จาก 25 ราย) ภาคกลางพบ 72% (18 ราย จาก 25 ราย) ภาคตะวันออกเฉียงเหนือพบ 20% (5 ราย จาก 25 ราย) และภาคใต้พบ 32% (8 ราย จาก 25 ราย) **สรุปผลการวิจัย:** ในประชากรชาวไทยสามารถพบภาวะพหุสัณฐานของยีน *KIR2DS3* ได้ และความถี่ที่พบของยีนนี้จะแตกต่างกันตามภูมิภาคของประเทศ โดยประชากรในภาคตะวันออกเฉียงเหนือจะพบยีนนี้ได้น้อยที่สุดเมื่อเทียบกับภูมิภาคอื่นของประเทศ ดังนั้นข้อมูลเหล่านี้จะเป็นประโยชน์ในอนาคตต่อการศึกษาความสัมพันธ์ของยีน *KIR2DS3* กับการเกิดโรคต่าง ๆ ในประชากรชาวไทย

คำสำคัญ: Killer cell immunoglobulin-like receptors (KIRs), *KIR2DS3*, ความถี่ของยีน, ประชากรไทย
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The Frequency of *KIR2DS3* in Thai Population

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Abstract

Introduction: KIR2DS3, also known as NKAT7, is a cell surface receptor of natural killer (NK) cell encoded by *KIR2DS3* and plays a role in immune response. *KIR2DS3* is a highly polymorphic gene, and its frequency varies greatly between populations. Furthermore, *KIR2DS3* has been found to be associated with many infection diseases, such as hepatitis C infection, Ebola infection, malaria and tuberculosis. This study aims to investigate the frequency of *KIR2DS3* in Thai population and the distribution of this gene frequency in each region of Thailand. **Methods:** A hundred of Thai healthy volunteers unrelated individuals were randomly and proportionally (25 samples per region) selected from four regions based on their residential location according to the Department of Provincial Administration, Ministry of Interior. Their DNA samples were extracted from peripheral blood using Chelex method. Then, the DNAs were amplified and identified for *KIR2DS3* gene by Polymerase Chain Reaction with Sequence-Specific Primers (PCR-SSP). **Results:** *KIR2DS3* was found in 42 out of 100 participants (42%). In each region, *KIR2DS3* was presented in 44% of the Northern (11 out of 25 samples), 72% of the Central (18 out of 25 samples), 20% of the Northeastern (5 out of 25 samples), and 32% of the Southern (8 out of 25 samples). **Conclusions:** The polymorphism of *KIR2DS3* is found in Thai population, and its frequency varies among regional parts of Thailand; people who are in the Northeastern region have less *KIR2DS3* frequency compared with the individuals of other regions. As results, these data might be of benefits for further studies in the association of *KIR2DS3* and diseases in Thai population.

Keywords: Killer cell immunoglobulin-like receptors (KIRs), *KIR2DS3*, gene frequency, Thai population
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Introduction

Killer cell immunoglobulin-like receptors (KIRs) are transmembrane glycoproteins expressed by natural killer (NK) cells and some T cells. Some of KIRs are recognized by human leukocyte antigen (HLA) class I molecules, found on most nucleated cells, and activate signaling to regulate the immune responses (Vilches and Parham, 2002). KIRs are encoded by 17 genes in the leukocyte receptor complex located at chromosome 19q13.4 (Marsh et al., 2003). The KIRs can be divided into two forms, activating KIRs and inhibitory KIRs, depending on the signal transduction after recognition with their ligands. The nomenclature of KIRs is based on the number of extracellular immunoglobulin-like domains (2D for two domains, 3D for three domains), which is responsible for ligand recognition, and the length of cytoplasmic tails (L for long and S for short) which is responsible for signaling transduction either activating or inhibitory function (Campbell and Purdy, 2011).

KIR2DS3 (killer cell immunoglobuli-like receptor, two domains, short cytoplasmic tail, 3), also known as NKAT7, is encoded by *KIR2DS3*. It is one of the activating KIRs expressed by natural killer cells (Carrington and Norman, 2003). Like other KIR genes, *KIR2DS3* is a polymorphic gene. The possession of *KIR2DS3* can be found presence or absence in a given individual. Moreover, the frequency of *KIR2DS3* is also found highly variable across populations, *i.e.* the gene is missing in the Tarahumarus (native) people of Mexico, while the highest *KIR2DS3* frequency (81%) is found in the Australian Aborigines (The Allele Frequency Net Database, 2007: http://www.allelefrequencies.net.).

Currently, the ligand of *KIR2DS3* and its mechanism to interact with natural killer cells are not yet understood (VandenBussche et al., 2009). However, a number of studies demonstrated that *KIR2DS3* is associated with many infection diseases, such as hepatitis C virus infection (Dring et al., 2011; Keane et al., 2013), Ebola virus infection (Wauquier et al., 2010), malaria (Olaniyan et al., 2014) and pulmonary tuberculosis (Lu et al., 2012). Therefore, the objectives of this study are to investigate the frequency of *KIR2DS3* in Thai population and explore the distribution of its frequency in each region of Thailand to provide basic information for further study such as the disease association of *KIR2DS3* in Thai population.

Methods

Study populations

A hundred of Thai healthy volunteers unrelated individuals were randomly and proportionally (25 samples per region) selected from four regions based on their residential location in Thailand, including the Northern, the Central, the Northeastern and the Southern, according to the geographical guideline suggested by the Department of Provincial Administration, Ministry of Interior, Thailand. The selection criteria for volunteers were: age over 18 years old, both Thai nationality and ethnicity, living in the four regions mentioned above for at least 3 generations (by interview). All participants gave written consent, and the study was approved by the Ethics Committee of Ubon Ratchathani University, Thailand (Reference number: 7/2555).

DNA sampling and DNA extraction

Genomic DNA was extracted from 30 µL of EDTA-anti-coagulated blood using Chelex method described below. Then, DNA was checked and calibrated to 10 ng/µL by using Nanodrop 2000c Spectrophotometer (Thermo Scientific, USA).

Chelex method: One mL of sterile distilled water was added into 30 µL of EDTA-anti-coagulated blood and incubated at room temperature for 5 minutes, followed by 1 minute centrifugation at 8,000 rpm. Then, the supernatant was removed and repeated again by mixing with 1 mL of sterile distilled water. 100 µL of 5% Chelex-100 resin (Bio-Rad®, USA) was added and vortexed for 10 seconds. Then, the mixture was incubated at 100°C for 12 minutes. After cooling for a while at room temperature, the mixture was vortexed for 10 seconds and centrifuged at 14,000 rpm for 20 minutes. The supernatant was transferred carefully to a new 1.5 mL microcentrifuge tube.

PCR reaction and gel electrophoresis

KIR2DS3 was genotyped by polymerase chain reaction with sequence-specific primers (PCR-SSP), performed as duplex PCR, according to Vilches et al. (2007) with modifications. The 50 µL of PCR reaction consists of 100 ng of genomic DNA, 10 µL of 5x Red Load Taq Master (Jena Bioscience, Jena, Germany), 0.25 µM of HLA-DRA primers and 1 µM of KIR2DS3 primers. HLA-DRA primers were used as an internal positive control specific to non-polymorphic sequences of the *HLA-DRA* gene; the sequences and product sizes of both primers are shown in Table 1. The PCR conditions were 2 minutes of initial denaturation at 95°C, followed by 37 amplification cycles (30 seconds of denaturation at 95°C, 30 seconds of annealing at 60°C and 30 seconds for extension at 72°C). The PCR products were run on a 1.5% agarose gel electrophoresis, and stained with ethidium bromide (5 µg/mL) to photograph by gel documentation (ImageQuant™ LAS 4000, GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The identification of *KIR2DS3* from agarose gel electrophoresis is shown in Figure 1.

Table 1 PCR primers and their product sizes

Primer name	Sequence (5'-3')	Product size (bp)
KIR2DS3	F: cttgtcctgcagctcct R: gcatctgtaggttcctcct	158
HLA-DRA	F: gaggtaactgtgctcacgaacagc R: ggtccataccccagtgcttgagaag	283

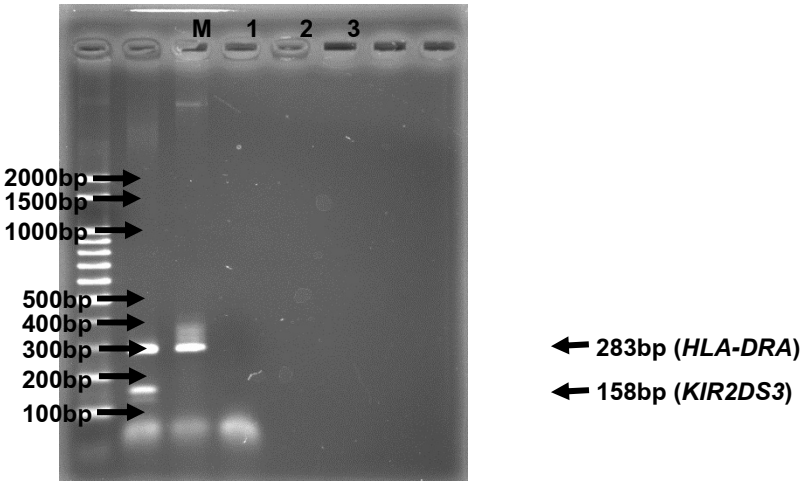

Figure 1 *KIR2DS3* genotyping by Polymerase Chain Reaction with Sequence-Specific Primers M: 100 bp ladder; Lane 1: The presence of *KIR2DS3* (158 bp) and *HLA-DRA* (283 bp) products; Lane 2: The absence of *KIR2DS3* product; Lane 3: Negative PCR

Statistical analysis

Observed frequency of *KIR2DS3* was determined by counting from the presence of *KIR2DS3* in individuals. The frequency of *KIR2DS3* was estimated by the formula: $1-\sqrt{(1-OF)}$, where OF is the observed frequency of *KIR2DS3* in a population (Single et al., 2008). The difference of *KIR2DS3* frequency among regions was tested by Pearson's Chi-square test (c^2), and *p*-value<0.05 was determined for statistically significant difference.

Results

The frequency of *KIR2DS3* in each geographical region of Thailand, examined from 100 Thai participants, is shown in Table 2. The observed frequency of *KIR2DS3* was found in 42 out of 100 individuals (42%), and the estimated *KIR2DS3* frequency was 0.24. The observed frequency of this gene in each region of Thailand was found to vary, ranges from 20% to 72%. The possession of this gene was found the highest in the individuals from the Central region, and the lowest was found in the participants from the Northeastern region. The presence of *KIR2DS3* was only found significant difference when *KIR2DS3* frequency of the Central region was compared with the other regions (p<0.05). The frequency of *KIR2DS3* in both male and female is also reported in Table 3.

Table 2 The frequency of *KIR2DS3* in each region of Thailand

Region	Number of the presence of *KIR2DS3*	Observed *KIR2DS3* frequency (in percentage)	Estimated *KIR2DS3* frequency
Northern	11 (out of 25)	44.00 %	0.25
Central*	18 (out of 25)	72.00 %	0.47
Northeastern	5 (out of 25)	20.00 %	0.11
Southern	8 (out of 25)	32.00 %	0.18
Total	**42 (out of 100)**	**42.00 %**	**0.24**

*$p < 0.05$ (statistically significant difference from the other regions)

Table 3 The frequency of *KIR2DS3* in males and females

Sex	Number of the presence of *KIR2DS3*	Observed *KIR2DS3* frequency (in percentage)	Estimated *KIR2DS3* frequency
Male	7 (out of 25)	28.00 %	0.15
Female	35 (out of 75)	46.67 %	0.27

Discussion

KIR2DS3 is a polymorphic gene which has high variation in both the individual and population levels. Among populations, the frequency of *KIR2DS3* found from 0% to 81% (http://www.allelefrequencies.net). The result of this study confirmed many previously published data on the variation of this gene, especially in Thai population which the observed frequency and estimated frequency were found to be 42.0% and 0.24, respectively. In addition, the distribution of *KIR2DS3* frequency was unequal which it was significantly increased in the individuals from the Central region of Thailand (Table 2).

Comparisons of the observed frequency and estimated frequency of *KIR2DS3* in this study with other populations, including Asian, Caucasian and African, are shown in Table 4. Overall, the *KIR2DS3* frequency of Africans is nearly similar to those found in Caucasians, but the existence of this gene in both ethnics seems to be less than in Asians. However, the frequency of *KIR2DS3* in Asians is not consistent; it is low in Chinese Han, Japanese and Korean (12.5%-16.2%), moderate in Southeast Asian (25.2%-32.3%) and high in North Indian (43.1%). Regarding Thai population, the frequency of this gene is nearly identical with the Southeast Asia neighbors. Surprisingly, the result of this study showed that the frequency of *KIR2DS3* was higher than the previous published data of Thai population, and its frequency was more closely to the North Indians.

Although *KIR2DS3* frequency in Thai population has been previously reported, this study is the first study that included participants from all regions of Thailand and illustrated the distribution of this gene in each region of Thailand. A comparison of *KIR2DS3* frequency between this study and those of three studies was also performed (Table 4). By comparison, although the

frequency of *KIR2DS3* in this study was higher than those of three studies, the frequency was not different among the studies (p = 0.054). However, when it was compared with the specific population in the same area (*i.e*. the Central region vs Bangkok; Northeastern region vs Northeastern region), the results showed that the frequency of *KIR2DS3* which was found in the Central region of this study was statistically significant difference from the studies of Tammakorn et al. (2011) and Norman et al. (2001), (p < 0.01), but the frequency which was found in the Northeastern region of Thailand in this study was not different from the study of Chaisri et al. (2013) (p = 0.21). The observed difference of *KIR2DS3* frequency may have resulted from the inclusion criteria of participants. In the study of Tammakorn et al. (2011) and Norman et al. (2001), the participants may have intermarried with Chinese descents which were found most in Bangkok, especially in the metropolitan area. This combination of two gene pools may influence to reduce this gene frequency. Interestingly, although *KIR2DS3* is an autosomal gene, it was found approximately twice in Thai females comparing with Thai males (Table 3).

Table 4 Comparisons of observed *KIR2DS3* frequency and estimated *KIR2DS3* frequency in Thais with Asians, Caucasians and African**s**

Population	Number of individuals (n)	Observed *KIR2DS3* frequency (in percentage)	Estimated *KIR2DS3* frequency	Reference
Thai* (All regions)	100	42.0%	0.24	-
Thai (Northeastern)	235	32.3%	0.18	Chaisri et al. (2013)
Thai (Bangkok)	500	30.4%	0.17	Tammakorn et al. (2011)
Thai (Bangkok)	119	25.2%	0.14	Norman et al. (2001)
Cambodian	11	27.3%	0.15	Hollenbach et al. (2012)
Malaysian	120	26.7%	0.14	NurWaliyuddin et al. (2014)
Singapore Malay	80	31.3%	0.17	Lee et al. (2008)
Indonesian (Java)	45	31.0%	0.17	Velickovic et al. (2009)
Singapore Indian	80	40.0%	0.23	Lee et al. (2008)
North Indian	72	43.1%	0.25	Rajalingam et al. (2002)
Chinese Han	104	12.5%	0.06	Jiang et al. (2005)
Japanese	41	15.0%	0.08	Yawata et al. (2002)
Korean	154	16.2%	0.08	Whang et al. (2005)
UK Caucasoid	136	24.3%	0.13	Norman et al. (2001)
Germany	120	28.3%	0.15	Uhrberg et al. (2002)
African	62	19.0%	0.10	Norman et al. (2002)
Uganda	492	24.0%	0.13	Nakimuli et al. (2013)

*Present study

In conclusion, this study provides the information of *KIR2DS3* frequency in Thai population and the distribution of this gene in each region of Thailand. All this data will be useful in the future for the study of disease association of *KIR2DS3 e.g.*, tuberculosis and hepatitis C infection which are predominantly found and still present a problem in Thailand or for the other fields such as anthropology and evolutionary study.

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