TISSUE ANTIGENS
IMMUNE RESPONSE GENETICS

Tissue Antigens ISSN 0001-2815

BRIEF COMMUNICATION

# *FCGR3B* gene frequencies among ethnic Thai blood donors from a regional hospital in Eastern Thailand

C. Kongmaroeng[1] & K. Kumkaen[2]

1 Division of Blood Bank, Faculty of Medical Technology, Huachiew Chalermprakiet University, Bangplee, Thailand
2 Blood Bank Unit, Division of Clinical Pathology, Rayong Hospital, Muang, Thailand

**Key words**
FCGR3B; gene frequency; human neutrophil antigen; Thais

**Correspondence**
Chalunda Kongmaroeng, PhD
Division of Blood Bank
Faculty of Medical Technology
Huachiew Chalermprakiet University
18/18 Bangna-Trad Road
Bangplee
Samut Prakan
Thailand 10540
Tel: 662 312 6414
Fax: 662 312 6414
e-mail: chalunda.hcu@gmail.com

Received 7 November 2013; revised 10 November 2014; accepted 14 December 2014

doi: 10.1111/tan.12511

## Abstract

The FCGR3B gene encodes three human neutrophil antigens which consist of HNA-1a, HNA-1b, and HNA-1c. These antigens are encoded by three alleles in the FCGR3B locus: *FCGR3B*01*, *FCGR3B*02*, and *FCGR3B*03* alleles, respectively. The frequencies of FCGR3B alleles have been reported in different ethnic populations. This study compared the FCGR3B gene frequencies among 230 unrelated healthy Eastern Thai blood donors in Rayong hospital with the previously published studies. The polymerase chain reaction-sequence-specific primers method was performed to determine FCGR3B genotypes. The results showed that the allele frequencies of *FCGR3B*01*, *FCGR3B*02*, and *FCGR3B*03* were 0.722, 0.274, and 0.009, respectively. The *FCGR3B*01* and *FCGR3B*02* frequencies found in the Eastern Thais were similar to the previous reports investigating in Northern Thais, Chinese Han, Taiwanese, and Japanese populations. Interestingly, our data showed statistically significant difference ($P < 0.05$) to Central Thais, Korean, Indian, Turkish, Australian, Tunisian, American, German, and Italian populations. In addition, one $FCGR3B_{null}$, which represents a gene deletion, was also found in this study. This information is important not only for the assessment of neutrophil antibody-mediated clinical conditions and for disease association studies but also for anthropological studies.

The current human neutrophil antigen (HNA) nomenclature is defined as five systems (HNA-1 to HNA-5) (1, 2). The HNA-1 system, which consists of three antigens (HNA-1a, HNA-1b, and HNA-1c), is the most frequent target of neutrophil alloantibodies. These antigens are polymorphic structures of the Fc gamma receptor IIIb (FcγRIIIB) localized on the surface of the human granulocytes. Antibodies against these polymorphisms is associated with allo- and autoimmune neutropenia, such as neonatal immune neutropenia (NAN), transfusion-related acute lung injury (TRALI), refractoriness to granulocyte transfusions, and febrile transfusion reactions (3–6). According to the knowledge of molecular genetics, these antigens are encoded by *FCGR3B*01*, *FCGR3B*02*, and *FCGR3B*03* alleles, respectively. The *FCGR3B*01* differs from *FCGR3B*02* by five nucleotide substitutions within exon 3 at position 141, 147, 227, 277, and 347, whereas the *FCGR3B*03* is caused by a single nucleotide substitution in the allele coding *FCGR3B*02* (7). The frequencies of these alleles have previously been characterized in different populations (8–16). The data represented the significant differences between populations which is important for risk prediction in allo- and autoimmunization to FcγRIIIB antigens. Thailand is located in a Central part of Southeast Asia. It can be divided into the following six regions: Northern, Northeastern, Western, Central, Eastern, and Southern regions (Figure 1). The Eastern region is composed of seven provinces including Chachoengsao, Chanthaburi, Chon Buri, Prachin Buri, Rayong, Sa Kaeo, and Trat provinces. Rayong is a Central of the Eastern of Thailand. Although there have been some studies mentioning on the FCGR3B gene frequencies in the ethnic Thai populations, the data on the Eastern Thais have not been reported. The purposes of this study were to describe the frequencies of FCGR3B gene in the Eastern Thai population using the polymerase chain reaction-sequence-specific primers (PCR-SSP) method and compare these frequencies with existing data on different populations.

For the population study, 230 buffy coats collected from healthy unrelated ethnic Thai blood donors from the blood bank unit in Rayong Hospital, who lived in the Eastern of Thailand, consisting of Rayong, Chon Buri, Chanthaburi, and Trat provinces, were selected. All participants were interviewed to confirm the ethnic background for at least two generations. They were also asked for their informed consent, in accordance with the Human Research Ethics committee in Huachiew Chalermprakiet University. The buffy coat was


**Figure 1** The National Research Council divides Thailand into six geographical regions, based on natural features including landforms and drainage, as well as human cultural patterns. They are namely the North, Northeast, Central, East, West, and South Region of Thailand. Each of the six geographical regions differs from the others in population, basic resources, natural features, and level of social and economic development. The diversity of the regions is in fact the most pronounced attribute of Thailand's physical setting.

lysed by red blood cell lysis buffer, and then the genomic DNA was extracted by using guanidine method (17). The genotyping system was validated by using DNA from *FCGR3B*01+, *02−, *03−*, *FCGR3B*01−, *02+, *03−* and *FCGR3B*01+, *02+, *03−* individuals, which had previously been determined by DNA sequencing method. Furthermore, a reference DNA from *FCGR3B*01+, *02+, *03+* kindly provided by Dr Sentot Santoso from the Institute for Clinical Immunology and Transfusion Medicine, Justus Liebig University, Giessen, Germany, was used to confirm the result of *FCGR3B*03* positive. The ambiguity genotype was confirmed by using DNA sequencing method. Reaction was analyzed in a capillary sequencer (ABI Prism 3730XL DNA analyzer, PE Applied Biosystems, Foster City, CA) by the Pacific Science Co., Ltd. Bangkok, Thailand. Six SSP of FCGR3B alleles, published by Bux *et al.* and Steffensen *et al*, were used to determine FCGR3B genotypes (18, 19). A pair of primers for part of the human growth hormone (HGH) gene was used as an internal control (IC). The polymerase chain reaction (PCR) master mix consisted of 1 µl of 100 ng/µl DNA sample, 0.3 µl of 5 U/µl Taq polymerase (Vivantis, Kuala Lumpur, Malaysia), 6.7 µl reaction buffer [10x PCR buffer, 0.6 mM dNTPs (Vivantis), and 2.0 mM $MgCl_2$], and 5 µl of 0.4 µM working primers. A total volume of 13 µl PCR reaction was amplified in a thermal cycler (Mastercycler vapo.protect, Eppendorf, Hamburg, Germany). The PCR conditions started with a pre-PCR heat-activation step (5 min at 95°C), followed by 30 cycles of denaturation (95°C, 1 min), annealing (60°C, 1 min), and extension (72°C, 1 min), and were finally terminated after a 5-min extension at 72°C. The PCR product was visualized in 2% agarose gel using a UV transilluminator and photographed by gel documentation system. The genotype and allele frequencies were calculated by the direct counting method using Microsoft Excel version 2007. Genotype distributions were tested for deviation from Hardy–Weinberg equilibrium using Excel spreadsheets from Michael H. Court's (2005–2008) online calculator (http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls). The difference in allele frequencies between the Eastern Thais and other populations was compared using chi-squared ($\chi^2$) test. *P*-values less than 0.05 were considered statistically significant for all tests. Phylogenetic tree was constructed using the neighbor-joining method in phylip 3.68 (20). Figure 2 shows the PCR-SSP genotyping gel with homozygous and heterozygous FCGR3B control DNAs. Visible PCR products at 141, 156, and 191 bp indicate the presence of SSP for HNA-1a, -1b, and -1c, respectively. The internal positive control product (IC) was seen at 439 bp. The result of FCGR3B genotype and allele frequencies among 230 Eastern Thai blood donors are shown in Table 1. One hundred twenty-seven individuals were typed as **01/*01* including 125 (54.3%) from *FCGR3B*01+, *02−, *03−* genotype and 2 (0.9%) from *FCGR3B*01+, *02−, *03+* genotype. Seventy-eight (33.9%) individuals were typed as **01/*02* from *FCGR3B*01+, *02+, *03−*, and 24 (10.4%) were typed as **02/*02* from *FCGR3B*01−, *02+, *03−*. In addition, one (0.4%) individual presented the genotype of *FCGR3B*01, *02,* and **03* as $FCGR3B_{null}$. This sample was confirmed by PCR amplification of FCGR3B and HGH genes using specific primers published by Terzian *et al.* (21), to be a deletion of FCGR3B gene. The observed frequencies are consistent with the expected frequencies under the Hardy–Weinberg equilibrium. The result of the chi-squared test was not statistically significant ($P > 0.05$). The allele frequencies among the Eastern Thai population revealed

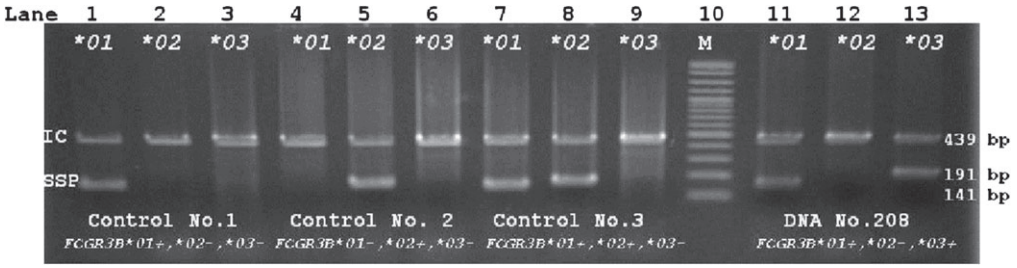


**Figure 2** Validation of FCGR3B genotyping by polymerase chain reaction-sequence-specific primers (PCR-SSP) method. Lane no. 10 shows a 100 bp-DNA ladder marker (VC 100 bp DNA Ladder, Vivantis, Kuala Lumpur, Malaysia). The amplification products (439 bp) of the internal control (IC) are present in each lane. Lane no. 1 to no. 3 are positive controls for *FCGR3B*01+, *02–, *03–* DNA, which only present positive PCR product (141 bp) in lane no. 1, consisting of specific *FCGR3B*01* primer set (SSP). On the other hand, the *FCGR3B*01–, *02+, *03–* DNA only presents a positive PCR product (156 bp) in lane no. 5, which consists of specific *FCGR3B*02* primer set. For *FCGR3B*01+, *02+, *03–* DNA, the PCR products (141 and 156 bp) are present both in the reaction for *FCGR3B*01* and *FCGR3B*02* primer sets (lane no. 7 and 8). In addition, the *FCGR3B*03* positive individual DNA shown in the lane no. 13, consists of specific *FCGR3B*03* primer set.

that *FCGR3B*01* is more frequent than *FCGR3B*02*. The frequencies of *FCGR3B*01*, **02,* and **03* were 0.722, 0.274, and 0.009, respectively. Because phenotyping requires a large number of freshly isolated granulocytes and antisera are very rare for the serologic assays, genotyping by PCR with SSPs is widely used in human leukocyte antigen, human platelet antigen, and HNA genotyping. This genotyping method is rapid, accurate, and gives a reliable result. Although real-time PCR-SSP is quicker than classical PCR-SSP, it requires dedicated and expensive equipment. Therefore, the classical PCR-SSP genotyping method is widely used in developing countries and small-scale laboratories. Alloantibodies to FCGR3B have been implicated as a cause of alloimmune neutropenia and TRALI in Asian and Caucasian populations. The knowledge of HNA frequencies in different populations is important for genotyping in these patients and their donors. In this study, the result shows that the frequency of FCGR3B in the Eastern Thais is similar to the previous published data regarding Northern Thais and other Asian populations (Chinese Han, Taiwanese, and Japanese), in whom *FCGR3B*01* was found more frequently than *FCGR3B*02*. Comparison of the allele frequencies between ethnic Thai populations shows a similarity in allele frequencies between Eastern and Northern Thais, whereas these frequencies are significantly different from Central Thais, where *FCGR3B*02* is the high-frequency antigen (16). These results suggest the genetic difference of the Central Thais, resulting in migration and marriage between Thais and different ethnic populations, particularly around the area of Bangkok. When comparing to non-Thai populations, the frequencies of FCGR3B in Eastern Thais are quite similar to those found in case of Asian populations ($P > 0.05$), whereas these frequencies are significantly different from those reported in Korean, Asian Indian, Turkish, and Caucasian (Australian, Tunisian, American, German, and Italian) populations ($P < 0.05$). This result confirms the significantly different genotype frequencies in different ethnical groups relative to the basis of their geographic location. The phylogenetic tree (Figure 3) shows the relationship among 14 populations based on FCGR3B frequencies. It illustrates that the Eastern Thai population is more closely related to Northern Thais and other Asian populations than the Central Thais. Our data showed a frequency of *FCGR3B*03* as 1% in the Eastern Thai blood donors, while 2.5%–3% was observed in Caucasian population (22). In contrast, the *FCGR3B*03+* was not found in *FCGR3B*01* homozygous individuals in the Argentinean population (10), while both in the Eastern Thais and in a Danish population (19) individuals were found to express both *FCGR3B*01* and *FCGR3B*03.* Population diversity may explain the difference to this result, however, studies of Polish families published by Gittinger FS and Bux J indicated that the *FCGR3B*03* is not always closely linked to *FCGR3B*01*, but there is also a linkage with *FCGR3B*02* (23). Previous reports identified individuals expressing *FCGR3B*01*, **02,* and **03* antigens while having three FCGR3B genes (19, 24). Interestingly, we also found one $FCGR3B_{null}$ among 230 Eastern Thais. The individual with $FCGR3B_{null}$ phenotype can develop FCGR3B-specific alloantibodies; especially pregnant women with FCGR3B deficiency may develop FCGR3B-specific alloantibodies that may cause neonatal alloimmune neutropenia (ANN). However, FCGR3B deficiency does not seem to induce severe clinical complication as FCGR3B function may be compensated by the other FCGRs. In this study, we did not perform the FCGR3B phenotype analysis of the male blood donor identified as $FCGR3B_{null}$, because we are now trying to set-up the serological method in our laboratory. A detailed family study of the individual and his relatives will be performed as soon as the technique is available. In conclusion, a database of FCGR3B genotype donors has been established as a result of this study. It will be used for donor selection in case of HNA-matched granulocyte transfusion. The similarity in genotype frequencies among the Eastern Thais, Northern Thais, and Asian populations would lead one to believe that the incidence of the immune neutropenia and TRALI, caused by FCGR3B antibodies, in these groups would also be similar.

**Table 1** FCGR3B genotype and allele frequencies in ethnic Thai blood donors and other populations

| Population | Number | Genotype frequencies | | | Allele frequencies | | | References |
|---|---|---|---|---|---|---|---|---|
| | | *01/*01 | *01/*02 | *02/*02 | *01 | *02 | *03 | |
| Eastern Thais | 230 | 0.552 | 0.339 | 0.104 | 0.722 | 0.274 | 0.009 | This study |
| Central Thais | 500 | 0.224 | 0.648 | 0.128 | 0.548 | 0.452 | 0.004 | 16 |
| Northern Thais | 300 | 0.467 | 0.420 | 0.113 | 0.680 | 0.320 | 0.000 | 16 |
| Chinese Han | 493 | 0.426 | 0.483 | 0.091 | 0.667 | 0.333 | 0.000 | 15 |
| Taiwanese | 138 | 0.457 | 0.449 | 0.094 | 0.681 | 0.319 | 0.000 | 13 |
| Korean | 200 | 0.285 | 0.590 | 0.125 | 0.580 | 0.420 | NT | 25 |
| Japanese | 523 | 0.369 | 0.509 | 0.122 | 0.623 | 0.377 | 0.000 | 26 |
| Indian | 92 | 0.160 | 0.550 | 0.280 | 0.339 | 0.661 | 0.000 | 8 |
| Turkish | 118 | 0.161 | 0.534 | 0.305 | 0.420 | 0.564 | 0.030 | 12 |
| Danish | 200 | 0.150 | 0.430 | 0.420 | 0.365 | 0.635 | 0.030 | 27 |
| Tunisian | 98 | 0.122 | 0.489 | 0.377 | 0.311 | 0.668 | 0.000 | 8 |
| American | 90 | 0.110 | 0.380 | 0.510 | 0.367 | 0.633 | 0.000 | 8 |
| German | 260 | 0.127 | 0.492 | 0.381 | 0.373 | 0.627 | 0.050 | 11 |
| Italian | 200 | | | | 0.282 | 0.692 | NT | 15 |

NT, not test.

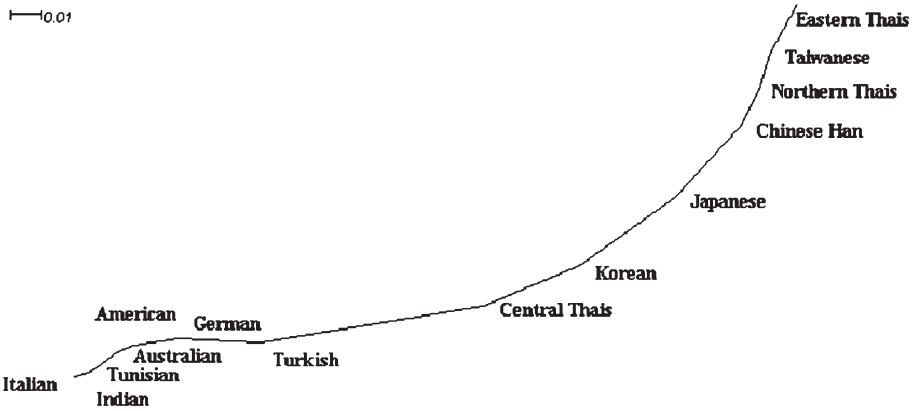


**Figure 3** Phylogenetic tree constructed by the neighbor-joining method showing the relationship between eastern Thais in Rayong province with other ethnic Thais (central and northern Thais) and other populations based on the frequencies of FCGR3B gene.

This information will be important not only for the assessment of neutrophil antibody-mediated clinical conditions and for disease association studies but also for anthropological studies.

## Acknowledgments

We sincerely thank all donors who participated in this study. We would like to thank Dr Sentot Santoso from the Institute for Clinical Immunology and Transfusion Medicine, Justus Liebig University, Giessen, Germany, for providing DNA control. We also want to express our gratitude to the staff of the Blood Bank Unit at Rayong Hospital and the Faculty of Medical Technology, Huachiew Chalermprakiet University, for their fruitful collaboration. The authors thank Dr Methee Sriprapun, Ms Sucha Joonsumlee, and Mrs Pornsuree Pongsuchat for assistance with the English-language presentation of the manuscript. This study was supported by the Research Fund, Huachiew Chalermprakiet University, Thailand, in 2010.

## Conflict of interest

The authors have declared no conflicting interests.

## References

1. Bux J. Human neutrophil alloantigens. *Vox Sang* 2008: **94**: 277–85.
2. Moritz E, Norcia AM, Cardone JD et al. Human neutrophil alloantigens systems. *An Acad Bras Cienc* 2009: **81**: 559–69.
3. Bruin M, Dassen A, Pajkrt D, Buddelmeyer L, Kuijpers T, de Haas M. Primary autoimmune neutropenia in children: a study of neutrophil antibodies and clinical course. *Vox Sang* 2005: **88**: 52–9.
4. Bux J, Jung KD, Kauth T, Mueller-Eckhardt C. Serological and clinical aspects of granulocyte antibodies leading to alloimmune neonatal neutropenia. *Transfus Med* 1992: **2**: 143–9.
5. Han TH, Chey MJ, Han KS. Granulocyte antibodies in Korean neonates with neutropenia. *J Korean Med Sci* 2006: **21**: 627–32.
6. Lucas G, Rogers S, Evans R, Hambley H, Win N. Transfusion-related acute lung injury associated with interdonor incompatibility for the neutrophil-specific antigen HNA-1a. *Vox Sang* 2000: **79**: 112–5.
7. Bux J. Molecular genetics of granulocyte polymorphisms. *Vox Sang* 2000: **78** (Suppl 2): 125–30.

8. Abid S, Zili M, Bouzid L et al. Gene frequencies of human neutrophil antigens in the Tunisian blood donors and Berbers. *Tissue Antigens* 2001: **58**: 90–2.
9. Changsri K, Tobunluepop P, Songthammawat D, Apornsuwan T, Kaset C, Nathalang O. Human neutrophil alloantigen genotype frequencies in Thai blood donors. *Blood Transfus* 2014: **12** (Suppl 1): s286–91.
10. de La Vega Elena CD, Nogues N, Fernandez Montoya A, Oyonarte S, Solis E, Muñiz-Díaz E. HNA-1a, HNA-1b and HNA-1c gene frequencies in Argentineans. *Tissue Antigens* 2008: **71**: 475–7.
11. Flesch BK, Doose S, Siebert R, Ntambi E, Neppert J. FCGR3 variants and expression of human neutrophil antigen-1a, -1b, and -1c in the populations of northern Germany and Uganda. *Transfusion* 2002: **42**: 469–75.
12. Hauck B, Philipp A, Eckstein R et al. Human neutrophil alloantigen genotype frequencies among blood donors with Turkish and German descent. *Tissue Antigens* 2011: **78**: 416–20.
13. Kissel K, Hofmann C, Gittinger FS, Daniels G, Bux J. HNA-1a, HNA-1b, and HNA-1c (NA1, NA2, SH) frequencies in African and American Blacks and in Chinese. *Tissue Antigens* 2000: **56**: 143–8.
14. Tsuno H, Matsuhashi M, Takahashi K. Granulocyte antibody detection – the role of MPHA. *ISBT Science Series* 2011: **6**: 387–90.
15. Xia W, Bayat B, Sachs U et al. The frequencies of human neutrophil alloantigens in the Chinese Han population of Guangzhou. *Transfusion* 2011: **51**: 1271–7.
16. Kaset C, Leetrakool N, Intharanut K, Nathalang O. Frequency of FCGR3B alleles in Thai blood donors. *Ann Lab Med* 2013: **33**: 426–30.
17. Kongmaroeng C, Romphruk A, Ruangwerayut R et al. HLA-B*15 subtypes in Burmese population by sequence-based typing. *Tissue Antigens* 2009: **74**: 164–7.
18. Bux J, Stein EL, Santoso S, Mueller-Eckhardt C. NA gene frequencies in the German population, determined by polymerase chain reaction with sequence-specific primers. *Transfusion* 1995: **35**: 54–7.
19. Steffensen R, Gulen T, Varming K, Jersild C. FcgammaRIIIB polymorphism: evidence that NA1/NA2 and SH are located in two closely linked loci and that the SH allele is linked to the NA1 allele in the Danish population. *Transfusion* 1999: **39**: 593–8.
20. Felsenstein J. *PHYLIP (Phylogeny Inference Package). Distributed by the author. Seattle: Department of Genome Sciences: University of Washington, 2004*.
21. Terzian CC, Chiba AK, Santos VC, Silva NP, Bordin JO. FCGR3B*03 allele inheritance pattern in Brazilian families and some new variants of gene FCGR3B. *Transfusion* 2012: **52**: 629–34.
22. Bux J. Molecular nature of granulocyte antigens. *Transfus Clin Biol* 2001: **8**: 242–7.
23. Gittinger FS, Bux J. Study of four families concerning the linkage and inheritance of the allele encoding the granulocyte-specific antigen HNA-1c (SH). *Transfusion* 2001: **41**: 847–8.
24. Koene HR, Kleijer M, Roos D, de Haas M, Von dem Borne AE. Fc gamma RIIIB gene duplication: evidence for presence and expression of three distinct Fc gamma RIIIB genes in NA(1+,2+)SH(+) individuals. *Blood* 1998: **91**: 673–9.
25. Seo DH, Park SS, Han KS. Genotype analysis of granulocyte-specific antigens in Koreans. *Korean J Clin Pathol* 1997: **17**: 1144–9.
26. Matsuhashi M, Tsuno NH, Kawabata M et al. The frequencies of human neutrophil alloantigens among the Japanese population. *Tissue Antigens* 2012: **80**: 336–40.
27. Nielsen K, Koelbaek M, Varming K, Baech J, Steffensen R. Frequencies of HNA-1, HNA-3, HNA-4, and HNA-5 in the Danish and Zambian populations determined using a novel TaqMan real time polymerase chain reaction method. *Tissue Antigens* 2012: **80**: 249–53.