*DI**A and *DI**B Allele Frequencies Among Southern Thai Blood Donors

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Abstract Diego (DI) blood group genotyping is clinically important in Asian populations. Data of Diego blood type among southern Thais is still unknown. This study aimed to report *DI**A and *DI**B allele frequencies in southern Thai blood donors and to estimate potential risk of Di^a incompatibility and alloimmunization in Thai populations. DNA samples obtained from 427 southern Thai blood donors were genotyped for *DI**A and *DI**B alleles by polymerase chain reaction with sequence-specific primer. *DI**A and *DI**B allele frequencies among southern Thais were 0.0047 and 0.9953. Their frequencies were similar to those among American Native, Italian, Filipino, Alaska Native/Aleut and Hawaiian/Pacific Islander populations; while, the frequencies significantly differed from central and northern Thai, Southeast Asian, Brazilian, Southern Brazilian, Brazilian Japanese descendants, Japanese, Han Chinese, Chinese, and Korean populations ($P < 0.05$). The Di^a incompatibility among southern Thais (0.93%) was lower than among central Thais (3.49%), corresponding to a significantly lower probability of Di^a alloimmunization ($P < 0.05$). This is the first report of *DI**A and *DI**B allele frequencies among southern

Thais, which is beneficial for not only creating information for estimating risk of alloimmunization, but also providing antigen-negative red cell donors to prevent both alloimmunization and adverse transfusion reactions.

Keywords Diego blood group · Genotyping · Southern Thais · Allele frequencies

Introduction

The Diego (DI) blood group system is clinically significant in transfusion practice. Anti-Di^a and anti-Di^b are implicated in mild and severe cases of hemolytic disease of the fetus and newborn (HDFN) and hemolytic transfusion reactions (HTRs) [1]. The Di^a and Di^b antigens are the products of *DI**A and *DI**B alleles caused by a single nucleotide polymorphism, c.2561C > T in exon 19 (*DI**A encodes Leu854 and *DI**B encodes Pro854), of the human erythrocyte membrane anion-transporter gene (*SLC4A1*) [1, 2].

The most common Diego phenotypes found in different populations are Di(a–b+), 99.9% followed by Di(a+b+) and Di(a+b–), respectively. Notably, the Di^a antigen is commonly found among native southern Americans from 2 to 54% and its frequency varies between 2 and 12% in Eastern Asian populations [3]. Related studies of *DI**A and *DI**B allele frequencies in two Thai populations revealed that these allele frequencies among northern Thais were similar to central Thais [4, 5].

Southern Thai populations have different ethnic origins compared with other regions of Thailand. Most Thai-Muslims are located in three provinces; Pattani, Yala and Narathiwat located in the southern part of Thailand near the border with Malaysia. Regarding the prevalence of Di^a antigen, its frequency varies among Malaysian blood

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donors in three ethnic groups including Malay (1.25%), Chinese (4.01%) and Indian (0.88%) [6]. The differences of blood group antigen frequencies in populations are involved in alloimmunization and production of antibody specificities in transfusion-dependent patients. Until now, the data of Diego blood type among southern Thai blood donors is still unknown. This study aimed to report *DI* A* and *DI* B* allele frequencies among southern Thai blood donors and to estimate the potential risk of Di^a incompatibility and alloimmunization in Thai populations.

Materials and Methods

EDTA-anticoagulated blood from 1588 unrelated healthy Thai blood donors was included. In all, 427 and 1161 samples obtained from the Regional Blood Centre 12th Songkhla, Thai Red Cross Society, Songkhla, and the National Blood Centre, Thai Red Cross Society (NBC–TRC), Bangkok, Thailand. All 427 samples were from Thai-Muslim donors living in the three southern border provinces of Pattani, Yala and Narathiwat. Genomic DNA was extracted from peripheral blood samples using the Genomic DNA extraction kit (REAL Genomics, RCBioscience, Taipei, Taiwan), then stored at -20°C until used for genotyping. A PCR-SSP technique for genotyping of *DI* A* and *DI* B* alleles was performed as previously described [4].

Gene frequencies were calculated by gene counting method. The Chi-square (χ^2) test was used to evaluate whether the observed genotype frequencies agree with the expected ones under the Hardy–Wienberg equilibrium. The differences in allele frequencies between southern Thai and other populations were compared using a Chi-square test of homogeneity. The frequencies of Di^a incompatibility were estimated, based on the *DI* A* gene frequencies of three Thai populations. The percentage of Di^a incompatibilities was calculated by $[DI^*A/DI^*A(DI^*B/DI^*B + DI^*A/DI^*B) + DI^*B/DI^*B(DI^*A/DI^*A + DI^*A/DI^*B)]$; *DI* A/DI* A*, *DI* A/DI* B* and *DI* B/DI* B* were the percentage of genotype frequencies in each Thai population. In addition, the estimation risk of Di^a alloimmunization was obtained by multiplying the probability of having a predicted $\text{Di}(a-)$ phenotype frequency by the probability of having a predicted $\text{Di}(a+)$ phenotype frequency. All statistical analyses were conducted using the SPSS, version 16.0 (SPSS Inc., Chicago, IL, USA). A *P* value <0.05 was considered statistically significant.

Results

Among 427 southern and 1161 central Thai blood donors, the *DI* B/DI* B* genotype was observed in 423 and 1119 samples (99.06 and 96.38%), respectively; the *DI* A/DI* B* genotype

was observed in 4 and 42 samples (0.94 and 3.62%), respectively. The *DI* A/DI* A* genotype was not found in both groups. The determined *DI* A* and *DI* B* genotypes in 427 southern Thai blood donors were consistent with the Hardy–Wienberg equilibrium ($\chi^2 = 0.025$, $P = 0.8734$). The *DI* A* and *DI* B* alleles were observed with a frequency of 0.005 and 0.995 among southern Thais. The *DI* A* allele frequency among southern Thais was significantly lower than among central ($P < 0.01$) and northern Thais ($P < 0.05$) [5]. *DI* A* and *DI* B* allele frequencies between southern Thais and other populations were compared. Allele frequencies were similar to American Native, Italian (Naples), Filipino, Alaska Native/Aleut and Hawaiian/Pacific Islander populations ($P > 0.05$) [7, 8]. In contrast, these allele frequencies significantly differed from Southeast Asian, Brazilian, Southern Brazilian, Brazilian Japanese descendants, Japanese, Han Chinese (Shanghai), Chinese and Korean populations [7, 9–15], as shown in Table 1. An estimation of the Di^a incompatibilities in three Thai populations showed values of 0.93% (southern Thais), 3.49% (central Thais) and 3.54% (northern Thais). The risk of Di^a alloimmunization among central and northern Thais was significantly higher than among southern Thais ($P < 0.05$), as shown in Table 2.

Discussion

In Thailand, blood group antigen and allele frequencies have been studied among central, northeast and northern Thais [4, 5, 16–18]. However, these frequencies have not yet been studied in southern Thailand. This study is the first to investigate *DI* A* and *DI* B* allele frequencies in Thai-Muslim populations, which have maintained their own religion, culture and lifestyle behaviors.

For the Diego genotyping results, the *DI* B* allele was the most common among southern Thais similar to central and northern Thais and those observed among other populations [4, 5, 7, 9–13]. Only heterozygous *DI* A/DI* B* was found in southern and central Thais and homozygous *DI* A/DI* A* was not found. Interestingly, the predicted $\text{Di}(a+)$ phenotype among southern Thais is closely related with Malay- and Indian-Malaysians but not related with Chinese-Malaysians [6]. This study would be beneficial in supporting the hypothesis of the relationship between genetic and geographic distance in Thai populations. However, further studies of other blood group alleles are needed to confirm this evidence.

The lower probability of Di^a incompatibility among southern Thais resulting in a threefold increased risk of Di^a alloimmunization among $\text{Di}(a-b+)$ individuals from central and northern Thais, which were consistent with the compared allele frequencies in Thais. This finding may be due to the combined effects of group isolations and religious or cultural

Table 1 *DI**A** and *DI**B** allele frequencies among different populations

Populations	Number	Allele frequencies		χ^2	<i>P</i> value
		<i>DI*<i>A</i></i>	<i>DI*<i>B</i></i>		
Southern Thai	427	0.005	0.995		
Central Thai	1161	0.018	0.982	6.948	0.0084
Northern Thai [5]	300	0.018	0.982	5.163	0.0231
Southeast Asian [7]	942	0.015	0.985	4.062	0.0439
Brazilian [9]	4326	0.018	0.982	7.671	0.0056
Southern Brazilian [10]	373	0.028	0.972	12.771	< 0.001
Brazilian Japanese descendants [11]	209	0.043	0.957	22.114	< 0.001
Japanese [7]	1022	0.043	0.957	27.462	< 0.001
Han Chinese (Shanghai) [12]	403	0.045	0.955	26.512	< 0.001
Chinese (Shen-Zhen) [13]	1766	0.036	0.964	21.899	< 0.001
Chinese (Chengdu) [14]	300	0.060	0.940	39.694	< 0.001
Korean (Seoul) [15]	116	0.047	0.953	21.418	< 0.001
Korean [7]	1033	0.058	0.942	41.069	< 0.001
American Native [7]	970	0.011	0.989	1.877	0.1707
Italian (Naples) [8]	225	0.000	1.000	0.860	0.3537
Filipino [7]	1333	0.008	0.992	0.399	0.5273
Alaska Native/Aleut [7]	621	0.007	0.993	0.053	0.8186
Hawaiian/Pacific Islander [7]	522	0.006	0.994	0.000	1.000

Table 2 Estimations of *Di^a* incompatibilities and risk of *Di^a* alloimmunization in three Thai populations

Population	Number	The frequencies of predicted <i>Di^a</i> phenotype		<i>Di^a</i> incompatibilities (%)	Risk of <i>Di^a</i> alloimmunization
		Negative	Positive		
Southern Thai	427	0.992	0.009	0.93	0.009
Central Thai	1161	0.964	0.036	3.49	0.035 ^a
Northern Thai [5]	300	0.964	0.036	3.54	0.035 ^a

^a*P* < 0.05

practices such as within-group marriages. Moreover, the NBC–TRC revealed that the prevalence of anti-*Di^a* found in multitransfused Thai patients was 1.2%, similar to a report in the southern Thais [19, 20], corresponding to a calculated risk of *Di^a* alloimmunization among southern Thais. For patients with anti-*Di^a*, it would not be difficult to find antigen-negative blood because most Thai donors are negative for *Di^a* antigens. However, the probability of finding compatible blood in transfusion-dependent patients with multispecific antibodies including anti-*Di^a* may vary depending on other antibody specificities.

Conclusions

This is the first study to report *DI**A** and *DI**B** allele frequencies among southern Thai blood donors, which is beneficial for not only creating information to estimate risk

of alloimmunization, but also providing antigen-negative red cell donors to prevent both alloimmunization and adverse transfusion reactions.

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Compliance with Ethical Standards

Conflict of interest The authors who have taken part in this study declare that they do not have anything to disclose regarding the conflict of interest with respect to this manuscript.

Ethical Approval This study was approved by the Research Ethics Committee, National Blood Centre, Thai Red Cross Society and the Committee on Human Rights Related to Research Involving Human Subjects, Thammasat University, Pathumtani, Thailand.

Informed Consent Informed consent was obtained from all individual participants included in this study.

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