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Variations of three single nucleotide polymorphisms in *ABCG2* modify Jr^a expression

Yoshiko Ogiyama, Shoichi Ito, Michiyo Irino, Tomoko Hishinuma, Tomomi Asano, Masanori Ooyama, Hiroshi Shimizu, Kunitoshi Nakagawa, Masayoshi Minegishi, Takahiro Osabe, Kenichi Ogasawara, Ikuo Wada, Hitoshi Ohto

ABSTRACT

Aim: The present study reports a single nucleotide polymorphism (SNP) of *ATP-binding cassette, membrane G2 (ABCG2)* gene may influence the expression of Jr^a antigen on red blood cells (RBCs). Methods: Genomic DNAs from 474 random donors were examined for SNPs, c.-262C/T, c.376C/T, and c.421C/A by polymerase chain reaction. The amount of Jr^a antigen of RBCs was assayed by flow cytometry using human monoclonal anti-Jr^a (HIRO-133). We classified them in three groups: Group I (wild type) with no variance of three loci (c.-262C, c.376C, and c.421C), Group II with SNP of c.376T and/or c.421A but with c.-262C, and Group III with SNP loci c.-262T. Results: Compared to Group I (n = 185), Group II (n = 263) showed decreased Jr^a expression on RBCs by 20–75%, whereas SNP c.-262T (Group III) increased Jr^a expression with more than 50%. The predicted open structure of 5' untranslated region formed by c.-262T may recruit the initiation complex for translation or increase the rate of ribosomal scanning, thereby enhancing the frequency of protein synthesis. Conclusion: Among a healthy Japanese population, the SNPs c.376C>T and/or c.421C>A downregulated expression of Jr^a, whereas SNP c.-262C>T augmented Jr^a expression.

Keywords: *ABCG2*, Antigen expression, Genetic variation, Jr^a antigen

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Yoshiko Ogiyama[1], Shoichi Ito[1], Michiyo Irino[1], Tomoko Hishinuma[1], Tomomi Asano[1], Masanori Ooyama[1], Hiroshi Shimizu[1], Kunitoshi Nakagawa[1], Masayoshi Minegishi[2], Takahiro Osabe[3], Kenichi Ogasawara[3], Ikuo Wada[4], Hitoshi Ohto[5]

Affiliations: [1]Japanese Red Cross Tohoku Block Blood Center, Sendai, Miyagi, Japan; [2]Japanese Red Cross Miyagi Blood Center, Sendai, Miyagi, Japan; [3]Japanese Red Cross Central Blood Institute, Tokyo, Japan; [4]Department of Cell Science, Institute of Biomedical Sciences, Fukushima Medical University, Fukushima, Japan; [5]Department of Advanced Cancer Immunotherapy, Fukushima Medical University, Fukushima, Japan.

Corresponding Author: Hitoshi Ohto, MD, PhD, Fukushima Medical University School of Medicine, Department of Advanced Cancer Immunotherapy, Fukushima City, Fukushima 960-1295, Japan; Email: hit-ohto@fmu.ac.jp

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INTRODUCTION

Jr^a, a high-prevalence blood group antigen, was first reported in 1970 by Stroup and Macllroy [1]. Anti-Jr^a usually does not cause severe transfusion reactions or hemolytic disease of the fetus and neonate, however, the antibody may rarely develop fatal hemolysis and intrauterine fetal death [2, 3]. Revealed to be a truncated

product of *ATP-binding cassette, membrane G2 (ABCG2)* gene [4, 5] in 2012, the Jr[a] antigen was assigned to the 32nd blood group system, JR (ISBT 032), by the International Society of Blood Transfusion in 2012 [6]. Among Japanese blood donors, the frequency of Jr(a–) of around 0.06–0.07% is more frequent compared to other ethnic populations [7, 8]. The Jr(a–) phenotype is caused by any null alleles of the *ABCG2* gene, 20 or more of which have been reported [9]. As for the Japanese population, the Jr(a–) phenotype is brought by mainly (>70%) homozygotes of *ABCG2*01N.01* (c.376C>T, p.Gln126X), while the remaining <30% is brought by heterozygotes *ABCG2*01N.01* and other *ABCG2* null allele [10–12].

Moreover, the presence of *ABCG2*01W.01* (c.421C>A, p.Gln141Lys) diminishes Jr[a] antigen expression on red cells of individuals with the Jr(a+) phenotype [13]. Thus, Jr[a] expression on RBCs should be suppressed in individuals with heterozygotes of *ABCG2*01N.01* and *ABCG2*01W.01*. On the other hand, we have suspected that another genetic variance c.-262C>T increases the amount of Jr[a] of RBCs. This implies that expression of Jr[a] is also controlled by unknown mechanisms and may influence pathogenesis associated with anti-Jr[a].

The association between the above alleles and Jr[a] expression on RBCs throughout the normal population has rarely been reported. Having a higher frequency of Jr(a–) individuals in the Japanese population than in other ethnic groups, this is important when looking for compatible RBCs for transfusion purposes, since only 0.06% are Jr(a–) among blood donors in Japan [8], but transfusion cells must be Jr(a–) and not Jr(a+[w]). Hence, we studied the associations between each of three Jr[a]-associated alleles, c.-262C/T, c.376C/T, and c.421C/A, and the expression levels of Jr[a] on RBCs.

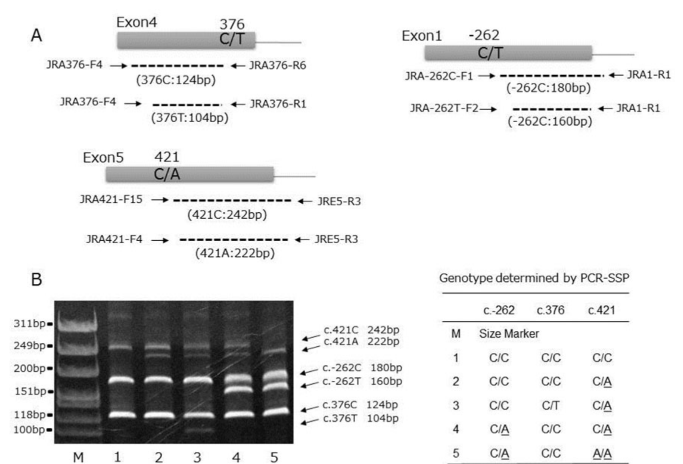

Figure 1: (A) Primer design. (B) Electrophoresis pattern.

MATERIALS AND METHODS

Samples

The blood samples used were from 474 random blood donors who gave written informed consent for research use at the time of blood donation. The study design has been approved by the institutional ethics committee of the Japanese Red Cross Society (#2016-030).

DNA sequence analysis

Genomic DNA was isolated from whole blood with a kit (DNA blood mini kit, QIAamp, Qiagen, Tokyo, Japan). Polymerase-chain reaction (PCR)-sequence-specific primers (SSP) were performed to determine the three SNPs, c.-262C/T, c.376C/T, and c.421C/A. Detailed information of primers and conditions of PCR-SSP are shown in Figure 1 and Table 1.

RNA secondary structure prediction

RNA secondary structure was estimated by the RNAstructure Web server (http://rna.urmc.rochester.edu/RNAstructureWeb/Servers/Predict1/Predict1.html) using the MaxExpect algorithm, which calculates partition functions for base-pair and single-strand probabilities to find the most probable structure [14].

Table 1: Primers used for PCR-SSP*

SNPs	Primer		Sequence (5'→3')	mM**
c.421C/A	JRA421-F4	Sense	CTGACAGTGAGAGAAAACTTAA	0.13
	JRA421-F15	Sense	ACTACAACACTACCCGTGAGTGACGGTGAGCGAAAACTTAC	0.08
	JRE5-R3	Antisense	TACAGGAAACTTCTGAATCAG	0.21
C.-262C/T	JRA-262C-F1	Sense	CAAGGCCCGCGCGTCCTCCCGAAGAGGCAGTGCCCGCCAC	0.04
	JRA-262T-F2	Sense	GAAGAGGCAGTGCCCCGCTAT	0.17
	JRA1-R1	Antisense	GTCACCCGGACCTTCCAAAC	0.21
c.376C/T	JRA376-F4	Sense	CCAAGTGGATTATCTGGAGATG	0.08
	JRA376-R6	Antisense	TTGTCTCCTTTGTCTTTTACCAAACCCACTAATACTTTCTTG	0.04
	JRA376-R1	Antisense	CAAACCCACTAATACTTACTTA	0.04

*The PCR conditions included initial denaturation at 94°C for 3 minutes, 35 cycles of denaturation (94°C, 15 seconds) and annealing/extension (59°C, 1 minute), followed by final extension at 72°C for 5 minutes.

**Final concentration in the PCR mixture.

Jr^a antigen intensity analysis by flow cytometry

The antigen expression intensity was evaluated by flow cytometry with FACSCalibur™ (Becton Dickinson, Tokyo, Japan) using in-house monoclonal anti-Jr^a (HIRO-133) and fluorescence-conjugated (ab′)2 to human IgG (H+L) (Abliance, Germany) as a second antibody. The amount of Jr^a antigen was expressed as mean fluorescence intensity (MFI).

Statistics

The estimated amount of Jr^a expressed on RBCs by flow cytometry was compared among healthy donors with different genetic variations at three loci of *ABCG2* genes with Microsoft Excel software (Microsoft Excel 2016, Microsoft, USA) using Student's *t*-test. Values of $p < 0.01$ were considered statistically significant.

RESULTS

Of the 474 blood donors, none were found to be c.376T homozygote corresponding to Jr(a–). Instead, the wild type group (Group I) with no variance at three loci (c.-262C, c.376C, and c.421C), a variant group (Group II) at least one locus at either or both c.421C>A and/or c.376C>T, and another variant group (Group III) with mutation at c.-262C>T, regardless of loci c.421 and c.376, were found in 185 (39.0%), 263 (55.5%), and 26 (5.5%) subjects, respectively, as shown in Table 2.

Comparing the Jr^a expression on RBCs of the two variants groups with Group I, the Group II showed significantly ($p < 0.01$) lower expression by 20–75%. Especially, when c.376C was altered to T, which generates premature termination of the gene, Jr^a was more seriously diminished than one locus SNP at c.421C>A.

Expression of Jr^a of Group III with c.-262C>T had significantly upregulated compared to that Group I or Group II nearly 1.5-fold as shown in Table 2 and Figure 2.

To understand why c.-262C>T increases expression of Jr^a, we estimated the most probable RNA structure of the entire 5′ untranslated region of 546 bases (UTR) and evaluated the effect of the c.-262C>T mutation. As shown in Figure 3, a 238-bp segment from c.-455 to c.-218 likely formed a major branch (red box) in the 5′ UTR. A single base substitution at c.-262 was predicted to cause a large structure transition by losing a -262C: -244G pair at a stem (indicated by an arrow in Panel A), which may increase the number of unpaired nucleotides (indicated by an arrow in Panel B).

DISCUSSION

In this study, we found that nearly 40% of blood donors that we have studied had a 20–25% downregulated expression of Jr^a due to a SNP at locus c.421C>A, that may not be a serious obstacle in correctly typing Jr^a

Table 2: Variations of three *ABCG2* gene loci and their influence on Jr^a antigen expression among 474 Jr(a+) Japanese blood donors

Jr^a antigen expression	Loci at						
	c.-262	c.376	c.421	n (%)	[Subtotal %]	MFI* (%)**	[Range MFI]
Group I (Regular or wild type)	C/C	C/C	C/C	185 (39.0)		32.9 (100, reference)	[24.8–40.3]
Group II	C/C	C/C	C/A	179 (37.8)	[263, 55.5%]	26.0 (79)	[19.2–32.7]
(Downregulated)	C/C	C/C	A/A	55 (11.6)		18.8 (57)	[12.7–24.3]
	C/C	C/T	C/C	20 (4.2)		14.5 (44)	[10.0–19.5]
	C/C	C/T	C/A	9 (1.9)		8.1 (25)	[6.0–10.8]
Group III	C/T	C/C	C/C	15 (3.2)	[26, 5.5%]	54.2 (165)	[44.5–62.9]
(Upregulated)	C/T	C/C	C/A	10 (2.1)		46.0 (140)	[41.4–52.1]
	C/T	C/C	A/A	1 (0.2)		29.3 (89)	
	C/T	C/T	C/C	0			
	C/T	C/T	C/A	0			

*MFI: mean fluorescence intensity.
**Percentile compared to Group I (regular or wild type).

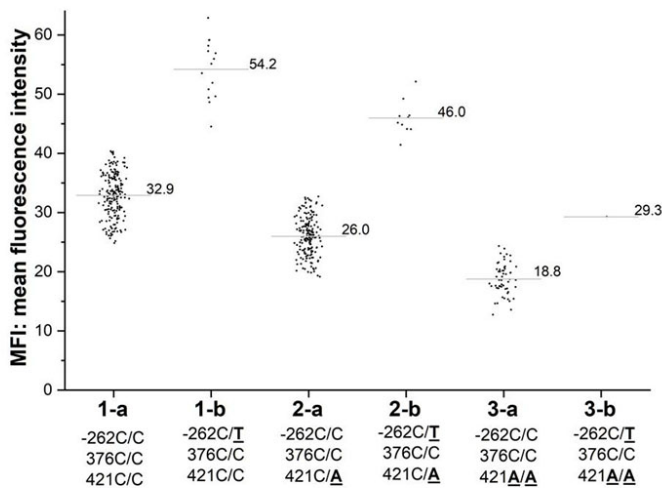

Figure 2: Variation of Jr^a antigen in red cells harboring SNP at c.421 and/or c.-262 among individuals with c.376C/C genotype. Each point represents the amount of Jra antigens as evaluated by flow cytometry.

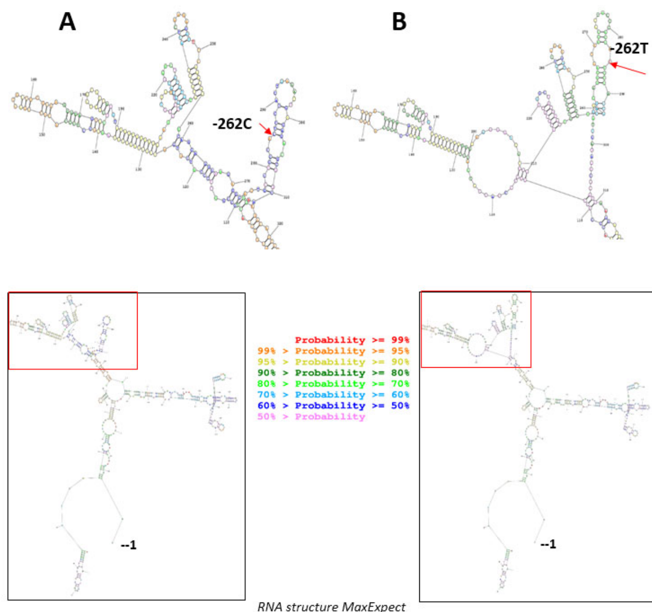

Figure 3: RNA secondary structure prediction of ABCG2 5′ UTR. The most probable RNA structure of the entire 5′ UTR (from –1 to –546) was predicted using the MaxExpect algorithm. A region (red box) of the large fork, including c.-262C (A) or c.-262/T (B), is enlarged from the whole structure of the 5′ UTR (black box). The color indicates the probability of local structures.

using human-derived anti-Jr^a reagents. On the other hand, a variance of c.376C>T alone (4% of donors), or a combination of c.376C>T with c.421C>A (2% of donors) diminished Jr^a antigen expression seriously; the latter expressed Jr^a only 25% of that expressed by the wild type. In this case, it is likely that Jr(a+^{w}) may be mistyped as Jr(a–) due to its weak signal when weak anti-Jr^a reagents are used. Similarly, patients' weak anti-Jr^a cannot be detected correctly but falsely judged negative when weaker Jr(a+) cells are used for antibody screening.

Interestingly, we found that 5.5% of Japanese blood donors had a variance of c.-262C>T that upregulated the Jr^a expression significantly by more than 50% of that of wild type. This enhancement was also observed in individuals having c.421C>A. The variance c.-262C>T may compensate the reducing effect of c.421C>A if the *ABCG2* variant encoded by the gene with c.421C>A is functionally equivalent to that with c.421C.

RNA secondary structure was predicted that c.-262 largely influenced the 5′ UTR structure. It is tempting to speculate that the open structure formed by c.-262 may recruit the initiation complex for translation or increase the rate of ribosomal scanning, thereby enhancing the frequency of translation.

In the setting of pregnancy of women with anti-Jr^a, the fetuses/newborns born range from being asymptomatic to having life-threatening hydrops; however, the mechanism of pathogenesis and progression to a serious condition have yet to be elucidated [15]. The present study may explain the pathogenesis and offer a solution. Mother with anti-Jr^a must be theoretically *ABCG2* gene null. If her fetus is heterozygous of *ABCG2* null allele and other *ABCG2* gene then the severity of the fetus could possibly depend on the expression level of *ABCG2*. Hence, we predict that the fetus with c.-262T may be attacked severely. When the hypothesis is experimentally and clinically established, *ABCG2* genotyping should be helpful in managing fetuses and newborns that are born to mothers with anti-Jr^a.

CONCLUSION

A SNP c.-262C>T, and two others, c376C>T and/or c.421C>A in *ABCG2* gene, defined up- and downregulated expression of Jr^a, respectively, which may contribute to elucidating the mechanism of the development of severe forms of anti-Jr^a-induced hemolytic disease of the fetuses and newborns.

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Author Contributions

Yoshiko Ogiyama – Conception of the work, Design of the work, Acquisition of data, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Shoichi Ito – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Michiyo Irino – Acquisition of data, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Tomoko Hishinuma – Acquisition of data, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Tomomi Asano – Acquisition of data, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Masanori Ooyama – Acquisition of data, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Hiroshi Shimizu – Conception of the work, Design of the work, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Kunitoshi Nakagawa – Conception of the work, Design of the work, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Masayoshi Minegishi – Conception of the work, Design of the work, Drafting the work, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Takahiro Osabe – Conception of the work, Design of the work, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Kenichi Ogasawara – Conception of the work, Design of the work, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Ikuo Wada – Design of the work, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related

to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Hitoshi Ohto – Conception of the work, Design of the work, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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Written informed consent was obtained from the patient for publication of this article.

Conflict of Interest

Authors declare no conflict of interest.

Data Availability

All relevant data are within the paper and its Supporting Information files.

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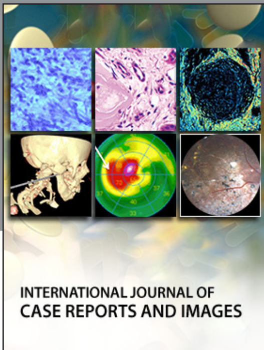
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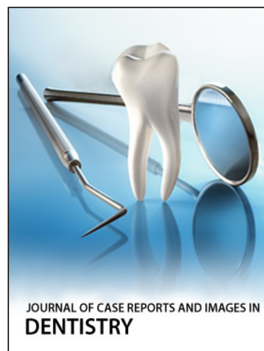
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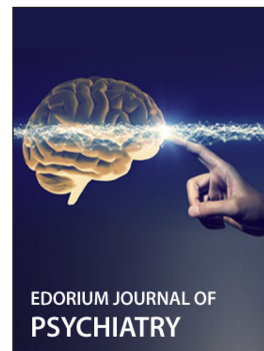
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