Relationship between allergic sensitization–associated single-nucleotide polymorphisms and allergic transfusion reactions and febrile nonhemolytic transfusion reactions in pediatric cases

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Conflict Of Interest

The authors have no potential conflicts of interest to declare.

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Abstract

BACKGROUND: Allergic transfusion reactions (ATRs) and febrile nonhemolytic transfusion reactions (FNHTRs) are common transfusion-related adverse reactions; however, their pathogenesis remains unclear and it is difficult to predict their occurrence. Single-nucleotide polymorphisms (SNPs) are related to the onset of various diseases and therapy-related adverse events; therefore, identification

of SNPs related to transfusion-related adverse reactions may help elucidate the underlying mechanism and predict onset.

STUDY DESIGN AND METHODS: We retrospectively analyzed the association between the onset of ATRs or FNHTRs and 22 allergic sensitization–related SNPs in 219 children (aged ≤ 20 years) who had hematological and oncological disease and who had received transfusions of platelets and/or red blood cell concentrate products.

RESULTS: From the 219 children, 105 had developed ATRs and/or FNHTRs at least once. The patients who developed ATRs frequently had a risk allele in rs6473223, while the patients who developed FNHTRs frequently had a risk allele in rs10893845. Furthermore, patients who developed ATRs accompanied by febrile symptoms also frequently had a risk allele in rs10893845, similar to patients who developed FNHTRs.

CONCLUSION: The results suggested that allergic sensitization is associated with the onset of ATRs and/or FNHTRs in some patients. Although further prospective evaluation is necessary, analysis of these SNPs might help provide safer transfusion therapy by predicting patients at higher risk for transfusion-related adverse reactions and further clarifying the pathogenic mechanism underlying such reactions.

Introduction

Transfusion therapy is essential supportive care in pediatric practice; however, allergic transfusion reactions (ATRs) and febrile nonhemolytic transfusion reactions (FNHTRs) occur frequently.[1,2] ATRs and FNHTRs are more common in pediatric than adult patients[3,4]; furthermore, ATRs are especially frequent in patients who have received platelet (PLT) transfusions.[1,3] Although the risk of severe ATRs is elevated in patients with the selective protein deficiencies and passive transfer of immunoglobulin E (IgE) or allergens, the mechanism underlying the common type of mild ATR remains unclear.[5,6] Even in patients who repeatedly experience ATRs because of PLT transfusions, such reactions can be prevented by using washed PLT products.[7-9] Therefore some of the factors present in plasma are thought to be strongly associated with the onset of ATRs. The pathogenic mechanism underlying FNHTRs was thought to involve leukocyte antibodies present in plasma[10] or increase in leukocyte- and PLT-derived biological response modifiers in blood components during storage.[11] The decrease in FNHTRs noted on prestorage leukocyte reduction was also thought to support these studies.[1,12-14]

Recent research indicates that the occurrence of transfusion-related adverse reactions involves multiple factors, that is, a combination of patient-derived factors in addition to donor- and product-derived factors.[6,15] While there are cases wherein no transfusion-related adverse reactions occur even when multiple blood transfusions are performed, there are also cases wherein the same patient may repeatedly develop ATRs or FNHTRs.[2,16] However, it is currently difficult to predict which patients

are likely to develop these adverse reactions. Our previous pediatric study showed that ATRs due to PLT transfusions occur more frequently in pediatric patients who are older and in patients with hematologic and malignant diseases.[1] Similarly, another study involving an adult population indicated that a background of hematological disease and younger age of the adults were risk factors.[17] A study involving an elderly population who had developed FNHTRs showed that the frequency of these adverse reactions was higher in patients who had undergone a greater number of transfusions and in patients with lymphoma or leukemia.[18] Therefore, the patient background is important at the time of transfusion. Furthermore, it remains unclear whether ATRs and FNHTRs have common risk factors.

Single-nucleotide polymorphisms (SNPs) have been reported to be associated with the onset of various diseases and with adverse events due to medical treatment.[19,20] Because several kinds of SNPs related to allergic diseases have also been reported[21,22]; if SNPs involved in ATRs are identified, then this would enable much safer transfusion therapy. However, currently, not much research has been performed on transfusion-related adverse reactions in relation to SNPs. Therefore, we focused on SNPs associated with allergic disease and examined the relationship between ATR and/or FNHTR occurrence and allergy-related SNPs in pediatric patients with hematological and oncological diseases, the population most at risk for developing transfusion-related adverse reactions.

Material and Methods

Patient selection

We retrospectively analyzed data for pediatric patients (aged ≤ 20 years) who had hematological and oncological disease and who had been transfused with red blood cell (RBC) concentrate and/or PLT concentrate (PC) at Nagano Children's Hospital from April 2003 to March 2020. All occurrences of ATRs and/or FNHTRs due to these transfusions during this study period were also analyzed. The current study includes cases reported in some previous studies.[1,2] This study was approved by the institutional ethical review board (approval number: 30-23).

Transfused blood products

All transfused blood products were obtained from the Japanese Red Cross Blood Society. The PC products used in this study were obtained from blood type–matched single-donor apheresis.[2] RBC concentrates were prepared from 200 ml or 400 ml samples of whole blood obtained from single donors on the basis of blood type.[2] Prestorage leukocyte reduction and diversion of the first aliquot of blood were performed for each blood product according to the passage of time, as previously described.[1] All the blood products had been obtained after 20-minipool nucleic acid testing at the Japanese Red Cross Blood Society; subsequently, they had been exposed to irradiation before transfusion.[8,9]

Definitions of ATR and FNHTR

ATR and FNHTR were defined as per previous studies: ATR was diagnosed when at least one symptom, such as rash, pruritus, urticaria, flushing, and respiratory distress, had occurred during transfusion or within 4 h of its completion.[2] FNHTR was defined by pyrexia (≥38°C or ≥1°C above baseline) that could be accompanied by chills, rigor, hypertension, tachycardia, and dyspnea without other clinical symptoms.[2,15] These symptoms occurred within 4 h after the completion of transfusion, and no other causes, such as hemolysis or bacterial infection, were indicated. We also classified the severity of ATR and FNHTR cases according to the criteria previously described.[2]

Genotyping of SNPs associated with allergic sensitization

DNA was extracted from the peripheral blood samples or conserved bone marrow cells by using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). We genotyped 22 SNPs previously reported to be associated with allergic sensitization.[21,22] The risk allele for allergic sensitization for each SNP had also been reported in a previous study.[22] SNP genotyping was performed with Cycleave polymerase chain reaction (PCR), allele-specific PCR analyses and restriction fragment length polymorphism (RFLP)-PCR analysis. The primers and probes used are listed in Table I. For Cycleave PCR, the primers and probes were designed using the Cycleave PCR Assay Designer (SNPs) (Takara Bio Inc., Shiga, Japan), and PCR was

performed on a LightCycler 96 system (Roche, Basel, Switzerland) at 95 °C for 30 s, followed by 45 cycles at 95 °C for 5 s, 55 °C for 10 s, and 60 °C for 15 s.

Allele-specific PCR and RFLP-PCR were utilized for analyzing SNPs for which the primers and probes could not be designed using Cycleave PCR Assay Designer (SNPs). They were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Waltham, Massachusetts, USA). The SNPs of rs9860547, rs10893845, and rs9303280 were analyzed using allele-specific PCR, while those of rs10189629 was analyzed using RFLP-PCR. In the SNPs of rs9860547 and rs10893845, amplification was performed using GoTaq Colorless Master Mix (Promega, Wisconsin, USA), with a final concentration of 0.2 μM for each primer, at 95 °C for 2 min, followed by 30 cycles at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s. In the SNP of rs9303280, amplification was performed using Paq5000 (Agilent Technologies, Santa Clara, California, USA), with a final concentration of 0.2 μM for each primer, at 95 °C for 2 min, followed by 30 cycles at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. In that of rs10189629, amplification was performed using the HotstarTaq Master Mix (QIAGEN, Hilden, Germany), with a final concentration of 0.5 μM for each primer, at 95 °C for 15 min, followed by 35 cycles at 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. After amplification, the PCR products were digested using *Hind*III (Takara Bio Inc., Shiga, Japan) at 37 °C for 60 min. Electrophoresis of the RFLP

products was performed on a 2% agarose gel.

Statistical analysis

The minor allele frequency for each SNP in this study group was compared with that of a Japanese healthy control group for which data were obtained from the 1000 Genomes Project.[23,24] The differences in the minor allele frequency for each SNP between the study and control groups and the relationship between the risk allele and ATR/FNHTR occurrence were analyzed with Fisher's exact test. The odds ratio and 95% confidence intervals (CI) for ATR/FNHTR were estimated with the homozygous low-risk allele as the reference and are shown according to the presence of the risk allele in each SNP. The odds ratio and 95% CI were undetectable when no ATR/FNHTR cases were included in the group of homozygous pattern of low-risk allele. Statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan)[25] and BellCurve for Excel (Social Survey Research Information Co., Ltd., Tokyo, Japan). Statistical significance was defined as $P < 0.05$.

Results

Table 2 provides summaries of the 219 cases analyzed in this study. Of these 219 patients, 105 had developed ATRs and/or FNHTRs at least once; there were 71 cases of ATRs only, 19 cases of FNHTRs only, and 15 cases of both ATRs and FNHTRs. Among 86 ATRs, 39 (45.3%) were grade I mild ATRs

with transient symptoms, and 36 (41.9%) were grade II ATRs that required transfusion discontinuation. In addition, there were 11 cases (12.8%) of grade III ATRs showing anaphylactic symptoms. On the other hand, 31 (91.4%) of the 34 FNHTRs were classified as grade 1 with fever <38.0 °C, the remaining of which (8.8%) corresponded to grade 2 with fever ≥ 38.0 °C and <39.0 °C. Our analysis included only cases from an institution and only pediatric cases of hematological and oncological diseases. To determine whether this caused bias, we first compared the minor allele frequencies with those from a database containing data from the Japanese population; however, no significant difference was observed between the frequencies (Table 3).

Next, the relationship between 22 SNPs and the onset of ATRs or FNHTRs was analyzed in the study population. Patients who had developed ATRs showed significant frequency of a risk allele (T) in the SNP rs6473223 [odds ratio, 2.281; 95% CI, 1.018−5.111; p = 0.044; Table 4(A)]. Patients who had developed FNHTRs showed significant frequency of a risk allele (G) in the SNP rs10893845 [odds ratio, 3.767; 95% CI, 1.701−8.339; p = 0.001; Table 4(B)].

We also analyzed patients who developed febrile symptoms along with ATRs. Our findings showed that these patients had higher frequency of the risk allele (G) in the SNP rs10893845 [odds ratio, 5.500; 95% CI, 1.140−26.533; p = 0.023); Table 4(C)], similar to the patients with FNHTRs.

Discussion

We were able to find candidate SNPs that may be related to the development of ATR and FNHTR in pediatric patients with hematological and oncological diseases. In this study, rs6473223 was identified as a SNP that may be related to ATR occurrence. rs6473223 has previously been found to be associated with the onset of conditions such as rhinitis, asthma, and contact dermatitis.[21,22] rs6473223 is located at 8q21.13, near the *TPD52* and *ZBTB10* genes.[21] *TPD52* is associated with B cell maturation,[26] while *ZBTB10* is a putative repressor of the Sp1 transcription factor and regulates multiple immune-related genes.[27-30] Studies on SNPs other than rs6473223 have identified loci at 8q21.13 that are thought to be associated with asthma and other allergic diseases.[31-35] The recipient's atopic predisposition could be the primary driver for the onset of ATRs.[6,15] Savage et al. indicated that hay fever and food allergy are more frequent in patients with ATRs to PLT transfusions.[36] Furthermore, total and aeroallergen-specific IgE concentrations in patients with ATRs were found to be higher than those in patients without ATRs.[36,37] However, the previous studies only utilized allergen-specific IgE to elucidate the relationship between ATR and atopic predisposition. We believe that supporting that association via other analytical methods (e.g., SNP analysis) is crucial. Thus, although only one SNP was associated with ATRs in the current study, we believe it is an important finding. Our previous studies did not completely clarify the association between the patient's allergy history and the occurrence of ATRs,[1,17] possibly because not all patient information could be accurately collected during retrospective analysis. Furthermore, patients who do not have symptoms of allergies may still have elevated aeroallergen-

specific IgE levels. Therefore, it is considered difficult to predict the occurrence of ATRs on the basis of medical interviews conducted to determine the patient's allergic history. More studies are required to further clarify the pathogenesis of ATRs. It is hoped that future research on different kinds of SNP analyses and/or measurement of specific IgE antibodies may help predict the risk of ATRs, thereby enabling much safer transfusion therapy.

A study pertaining to the relationship between FNHTRs and cytokine-related SNPs indicated a relationship between these transfusion reactions and the polymorphism *IL1RN * 2 ・2*, which may be related to the serum interleukin (IL)-1β levels.[38] The SNPs analyzed in the current study had been previously reported to be involved in allergic sensitization; we found that even FNHTRs were associated with one of these SNPs. rs10893845 is located near the *ETS1* gene at 11q23.4.[21] *ETS1* is responsible for Th2 cytokine regulation and is a negative regulator of Th17 differentiation[39,40]; it is also associated with several kinds of autoimmune diseases.[21] However, the relationship between FNHTRs and allergic constitution remains unclear. When patients develop pyrexia after blood transfusion, acute hemolytic transfusion reaction, transfusion-related acute lung injury, and microbial contamination should be considered as possible causative factors because FNHTRs constitute a diagnosis of exclusion.[15,41] ATRs are rarely suspected on the basis of febrile symptoms.[41] However, pyrexia associated with the development of ATRs is not rare in children, and the same patient may

often experience both ATRs and FNHTRs.[2] Therefore it is speculated that the onset of ATRs and FNHTRs could overlap. It is possible that allergic predisposition is associated with both ATRs and FNHTRs. In our current study, patients who developed pyrexia along with the ATRs also frequently had a risk allele in rs10893845, similar to patients with FNHTRs. This finding indicates that, in some pediatric patients diagnosed with FNHTRs, both the conventional pathogenic mechanism and the allergic constitution of the patient may be involved in some manner. Elucidation of the pathogenic mechanism underlying ATRs and FNHTRs in future studies would help improve their management.

The current study identified potential SNPs that could be related to ATRs or FNHTRs but has some limitations. First, a limited number of cases from a single institution were included. Second, the subjects were limited to pediatric patients with hematological and malignant disease, which is a population expected to have a high incidence of ATRs/FNHTRs; therefore, it is unclear whether the results could be extrapolated to the adult population and to other pediatric diseases. Third, the frequency of transfusions varies from case to case; therefore, the possibility of bias in ATR or FNHTR onset cannot be ruled out. Fourth, severe ATR can develop in patients with IgA deficiency.[42,43] Because most of our study participants only had mild cases of ATR, our findings cannot be generalized to all cases of ATR with varying disease severity. To resolve these issues and to confirm our results, it is necessary to perform a large-scale multi-institutional prospective study in the future. Furthermore,

whether genomic analysis can behave as an independent predictor to identify patients at risk and establish specific precautions, such as further manipulation of blood components or premedication or treatment options, in high-risk patients should be further investigated.

Conclusions

In this study, we found each one SNP related to allergic sensitization could be associated with ATRs and FNHTRs. Taken together with the findings of a previous study involving allergen-specific IgE, our results suggested that the recipient's allergic constitution would be a risk factor for developing ATRs. Our results also indicated an association between FNHTRs and SNPs related to allergic sensitization; therefore, it was speculated that the allergic constitution of the patients was involved in the pathogenic mechanism underlying FNHTRs in some pediatric cases. Analyzing these SNPs could help predict which patients are likely to develop these transfusion-related reactions and may help clarify the pathogenic mechanism underlying ATRs and FNHTRs.

Authorship contribution

Study design: RY, KM, and YA; data acquisition: YI, JK, and KK; data interpretation: YN, TT, KS, and MT; manuscript drafting: YI and RY; manuscript revision and approval: YI, RY, JK, KK, KM, YA, YN, TT, KS, and MT.

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Table 1. Primers and probes used in this study

(A) Cycleave PCR

SNP ID	Alleles	Primers (5' - 3')		Probes (5' - 3')	Probe Strand
rs2101521	G/A	F:	TCGTCCACTCAATATTTCA	Eclipse-gc(G)taaaagtt-FAM	sense
		R:	GGTACAGCTGCTTTCAGTC	Eclipse-taT(g)cgaatgt-HEX	antisense
rs1438673	T/C	F:	GCTATTGTCCAGTGGGATT	Eclipse-gtcagaa(A)aca-FAM	antisense
		R:	TTGCAGGCAGAGACATTAGT	Eclipse-gtcagaa(G)aca-HEX	antisense
rs2155219	T/G	F:	AAGCTGTGCTATATCATGTGTG	Eclipse-tctggT(g)tgt-FAM	sense
		R:	AGATGAAGATGGTCAAAGTAGG	Eclipse-gtctgg(G)gt-HEX	sense
rs690602	T/C	F:	TGTCTGCTTTCAGGGTCA	Eclipse-gagtttc(A)tca-FAM	antisense
		R:	GTTCACAGTCATTCCACTCC	Eclipse-gagtttc(G)tc-HEX	antisense
rs9266772	T/C	F:	GGACAAGGGAAACAAGGA	Eclipse-gggaaga(A)ga-FAM	antisense
		R:	GGACAAACAAGAGCCCATT	Eclipse-ggaaga(G)gag-HEX	antisense
rs10497813	T/G	F:	GTTGCAACTGAAGACAAATC	Eclipse-agtgtaT(g)atg-FAM	sense
		R:	GAAAGAGACTCCCAACCAG	Eclipse-gagtgta(G)gat-HEX	sense
rs7032572	G/A	F:	GTGACAAGAGGGCAGAATAGA	Eclipse-cc(G)tggtaaa-FAM	sense
		R:	TTTGGTTGCAGGATCAAGG	Eclipse-aT(g)gatggatg-HEX	antisense
rs6021270	T/C	F:	GCTTCAATCCCAGATTTC	Eclipse-tgaagcc(A)ct-FAM	antisense
		R:	GTCAGGTAACCTCTGTAGCA	Eclipse-gaagcc(G)ct-HEX	antisense
rs17228058	G/A	F:	AGGAAATTGTTCTGCTAGGG	Eclipse-gtctctc(G)tc-FAM	sense
		R:	ACCATAAATATACTTGGTTGCAG	Eclipse-ggtctctc(A)tc-HEX	sense
rs962993	A/G	F:	AAGTAGCATGGTATATTGT	Eclipse-cacttatg(A)ctc-FAM	antisense
		R:	AATGCTCTAGATACCTTC	Eclipse-cacttatg(G)ct-HEX	antisense
rs17388568	G/A	F:	ATCATAGAGCCAAGGATGTCA	Eclipse-ata(G)taacgcc-FAM	sense
		R:	AAGCCAGAGGTTGCAGTAAG	Eclipse-ata(A)taacgcc-HEX	sense
rs1998359	G/C	F:	TCTTGTAAAGGTGGAGTCTTG	Eclipse-tgcacata(G)tc-FAM	sense
		R:	TATTGTGGCCAATAGAGATATAA	Eclipse-gttagtaaga(G)ta-HEX	antisense
rs10174949	G/A	F:	GCATGTAAGTGTTCAGGGTTC	Eclipse-tcggg(G)ttt-FAM	sense
		R:	GCTGAGGCTACTGAGTGTTG	Eclipse-ggg(A)ttttctt-HEX	sense
rs7203459	T/C	F:	GGGTGTAATAGAGCAGGCAGA	Eclipse-cag(A)tgggc-FAM	antisense
		R:	GTAACACTGGGCACTGAGGA	Eclipse-ctcag(G)tgg-HEX	antisense
rs2107357	G/A	F:	GCCACTCTTAAGATGAAACC	Eclipse-tcaccc(G)aa-FAM	sense
		R:	GGCGTAATACAGTGAGCAA	Eclipse-ctcaccc(A)aa-HEX	sense
rs2056417	G/A	F:	TTCAAGCCACATCTACTCTA	Eclipse-aggaac(G)gg-FAM	sense
		R:	AGATGAGCCAGGTTTAATTC	Eclipse-aggaac(A)gga-HEX	sense
rs7720838-G	G/T	F:	GTGACAAAGCTCACTTCAAA	Eclipse-gacatg(G)ca-FAM	sense
		R:	GAGTGAGAGGCAGGAAATC		
rs7720838-T		F:	ATGAAACCACCCAAAGTATATG	Eclipse-ggtgatg(A)ca-FAM	antisense
		R:	TCCAGAGAATGAGGAGAGAAG		

rs6473223-C	C/T	F:	TTAGCAGTAGCAAGCAAATTA	Eclipse-gctg(G)cac-FAM	antisense
		R:	ACAGTCTAAGCAATCAAGGTC		
rs6473223-T		F:	TCTAGCCATTAGCAGTAGCA	Eclipse-gtgctg(A)ca-FAM	antisense
		R:	GTAACTCTGAGCTGATATGGAA		

(B) Allele-Specific PCR

SNP ID	Alleles	Primers (5' - 3')	
rs9860547	G/A	F1:	ATGCTCAACTCACTGTACG
		F2:	AATGCTCAACTCACTGTACA
		R:	CCAGTAGTGGGATTGCTGTA
rs10893845	G/T	F:	TGCATCATGTAGTGAGGTGA
		R1:	GAAAGTCCCGATAAGAAGTAC
		R2:	GAAAGTCCCGATAAGAAGTAA
rs9303280	C/T	F1:	CCCAGCCTTGTTGCTAAC
		F2:	CCCAGCCTTGTTGCTAAT
		R:	ATGAGGGGCAGAGGAGAATC

(C) RFLP-PCR

SNP ID	Alleles	Primers (5' - 3')	
rs10189629	C/A	F:	GTGAGACTGCATTGGGAGCT
		R:	ATTGAACATCTGCTCGGCGA

Abbreviations: PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single-nucleotide polymorphism.

Uppercase of the probe sequence indicate SNP sites, and parentheses indicate RNA.

Table 2. Characteristics of the patients analyzed.

Patient parameters	Data for patients analyzed (N = 219)
Age (years), median (range)	4.2 (0.0-19.2)
Sex, male:female	133: 86
Disease	
Acute lymphoblastic leukemia	(n=43)
Acute myeloid leukemia	(n=21)
Aplastic anemia	(n=4)
Autoimmune hemolytic anemia	(n=2)
Ependymoma	(n=4)
Ewing sarcoma	(n=7)
Fibrosarcoma	(n=3)
Germ cell tumor	(n=2)
Hepatoblastoma	(n=6)
Hodgkin lymphoma	(n=4)
Immune thrombocytopenia	(n=2)
Langerhans cell histiocytosis	(n=4)
Medulloblastoma	(n=12)
Myelodysplastic syndrome	(n=5)
Neuroblastoma	(n=33)
Non-Hodgkin lymphoma	(n=21)
Pleuropulmonary blastoma	(n=2)
Primitive neuroectodermal tumor	(n=3)
Rhabdomyosarcoma	(n=16)
Wilms tumor	(n=8)
Yolk sac tumor	(n=4)
Others	(n=13)

Table 3. Comparison of the minor allele frequency for each SNP between cases included in this study and the controls.

Gene	SNP ID	Position[1)]	Risk allele[2)]	Minor allele frequency		*P*-value[4)]
				This study	1000 genome project[3)]	
PEX14	rs2056417	1: 10581658	G	0.148	0.159	0.726
ID2 -[]— RNF144A	rs10174949	2: 8442248	G	0.288	0.274	0.779
IL18R1 —[]- IL1RL2	rs10189629	2: 102879464	C	0.091	0.067	0.361
PLCL1	rs10497813	2: 198914072	G	0.272	0.308	0.351
LPP	rs9860547	3: 188128979	A*	0.393	0.433	0.346
TLR6 —[]- TLR1	rs2101521	4: 38811551	G	0.651	0.716	0.107
ADAD1	rs17388568	4: 123329362	A*	0.094	0.106	0.671
PTGER4 -[]— DAB2	rs7720838	5: 40486896	T*	0.183	0.168	0.741
CAMK4 —[]- WDR36	rs1438673	5: 110467499	C	0.484	0.500	0.736
MICA -[]— HLA-B	rs9266772	6: 31352113	C*	0.185	0.236	0.142
HLA-DQB1 -[]— HLA-DQA1	rs6906021	6: 32626311	C*	0.365	0.332	0.429
ZBTB10 -[]— TPD52	rs6473223	8: 81268155	T	0.402	0.423	0.609
IL33 -[]— RANBP6	rs7032572	9: 6172380	G*	0.005	0.000	1.000
CELF2 —[]- GATA3	rs962993	10: 9053132	C	0.187	0.240	0.073
LRRC32 —[]- C11orf30	rs2155219	11: 76299194	T*	0.564	0.365	0.104
ETS1 -[]— KIRREL3	rs10893845	11: 128186882	G*	0.247	0.298	0.181
SSTR1 —[]- MIPOL1	rs1998359	14: 38077148	G*	0.087	0.106	0.469
SMAD3	rs17228058	15: 67450305	G*	0.025	0.024	1.000
CLEC16A	rs7203459	16: 11230703	T	0.048	0.082	0.107
IL21R -[]— IL4R	rs2107357	16: 27410829	A	0.817	0.803	0.667
GSDMB	rs9303280	16:17: 38074031	C	0.711	0.688	0.580
NFATC2	rs6021270	20: 50141264	T	0.034	0.058	0.206

1) Positions of SNPs and genes are according to NCBI build 37.3.

2) Risk allele in the minor allele has been indicated with the symbol *.

3) Minor allele frequencies for the Japanese healthy controls were obtained from the data collected from the 1000 Genomes Project (ref 23, 24).

4) Differences in minor allele frequencies for the two groups was analyzed with Fisher's exact test.

Abbreviations: SNP, single-nucleotide polymorphism.

Table 4. Association of risk allele frequency for each SNP with the development of ATRs, FNHTRs, and ATRs accompanied by febrile symptoms.

Gene	SNP ID	Risk allele[1)]	(A) ATRs			(B) FNHTRs			(C) ATRs accompanied by febrile symptoms		
			OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value
PEX14	rs2056417	G	-	-	1.000	-	-	1.000	-	-	1.000
ID2 -[]— RNF144A	rs10174949	G	1.823	0.684 - 4.860	0.258	0.587	0.201 - 1.715	0.351	-	-	0.604
IL18R1 —[]- IL1RL2	rs10189629	C	-	-	1.000	-	-	1.000	-	-	1.000
PLCL1	rs10497813	G	0.900	0.276 - 2.932	1.000	-	-	0.221	-	-	1.000
LPP	rs9860547	A*	1.386	0.788 - 2.438	0.319	1.600	0.723 - 3.540	0.337	0.608	0.171 - 2.165	0.512
TLR6 —[]- TLR1	rs2101521	G	0.592	0.341 - 1.027	0.069	0.566	0.271 - 1.182	0.134	1.049	0.287 - 3.828	1.000
ADAD1	rs17388568	A*	1.119	0.561 - 2.232	0.859	0.714	0.258 - 1.972	0.636	1.090	0.223 - 5.334	1.000
PTGER4 -[]— DAB2	rs7720838	T*	0.962	0.543 - 1.705	1.000	1.423	0.673 - 3.008	0.432	3.043	0.831 - 11.140	0.095
CAMK4 —[]- WDR36	rs1438673	C	1.023	0.550 - 1.901	1.000	0.783	0.347 - 1.766	0.528	0.818	0.204 - 3.277	0.724
MICA -[]— HLA-B	rs9266772	C*	1.213	0.672 - 2.190	0.547	1.066	0.474 - 2.400	0.838	1.585	0.432 - 5.815	0.493
HLA-DQB1 -[]— HLA-DQA1	rs6906021	C*	0.920	0.530 - 1.597	0.780	0.844	0.404 - 1.767	0.706	0.672	0.189 - 2.393	0.532
ZBTB10 -[]— TPD52	**rs6473223**	**T**	**2.281**	**1.018 - 5.111**	**0.044**	1.628	0.537 - 4.936	0.465	1.873	0.230 - 15.247	1.000
IL33 -[]— RANBP6	rs7032572	G*	-	-	0.521	5.576	0.340 - 91.368	0.287	-	-	1.000
CELF2 —[]- GATA3	rs962993	C	0.313	0.056 - 1.747	0.214	-	-	0.593	0.221	0.023 - 2.090	0.247
LRRC32 —[]- C11orf30	rs2155219	T*	1.009	0.562 - 1.809	1.000	0.816	0.378 - 1.762	0.688	1.077	0.270 - 4.296	1.000
ETS1 -[]— KIRREL3	**rs10893845**	**G***	0.749	0.432 - 1.298	0.331	**3.767**	**1.701 - 8.339**	**0.001**	**5.500**	**1.140 - 26.533**	**0.023**
SSTR1 —[]- MIPOL1	rs1998359	G*	0.981	0.471 - 2.041	1.000	0.857	0.307 - 2.386	1.000	0.552	0.068 - 4.500	1.000
SMAD3	rs17228058	G*	0.565	0.146 - 2.191	0.534	1.222	0.252 - 5.921	0.682	2.211	0.255 - 19.200	0.409
CLEC16A	rs7203459	T	0.644	0.040 - 10.434	1.000	-	-	1.000	-	-	1.000
IL21R -[]— IL4R	rs2107357	A	1.202	0.676 - 2.139	0.557	0.847	0.381 - 1.883	0.842	0.221	0.027 - 1.776	0.173
GSDMB	rs9303280	C	0.910	0.371 - 2.234	0.822	1.902	0.423 - 8.553	0.541	1.022	0.123 - 8.466	1.000
NFATC2	rs6021270	T	-	-	1.000	-	-	1.000	-	-	1.000

1) Risk allele in the minor allele has been indicated with the symbol *.

Abbreviations: ATR, Allergic transfusion reaction; CI, confidence interval; FNHTR, febrile nonhemolytic transfusion reaction; OR, odds ratio; SNP, single-nucleotide polymorphism.