Original article

Relationship between allergic sensitisation-associated single-nucleotide polymorphisms and allergic transfusion reactions and febrile non-haemolytic transfusion reactions in paediatric cases

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Background - Allergic transfusion reactions (ATR) and febrile non-haemolytic transfusion reactions (FNHTR) are common transfusion-related adverse reactions; however, their pathogenesis remains unclear and it is difficult to predict their occurrence. Single-nucleotide polymorphisms (SNP) are related to the onset of various diseases and therapy-related adverse events; therefore, identification of SNP related to transfusion-related adverse reactions may help to elucidate the underlying mechanism and predict the onset of these reactions.
Materials and methods - We retrospectively analysed the association between the onset of ATR or FNHTR and 22 allergic sensitisation-related SNP in 219 children (aged ≤20 years) who had haematological and oncological diseases and who had received transfusions of platelets and/or red blood cell concentrates.
Results - Among the 219 children, 105 had developed an ATR and/or FNHTR at least once. The patients who developed ATR frequently had a risk allele in rs6473223, while the patients who developed FNHTR frequently had a risk allele in rs10893845. Furthermore, patients who developed ATR accompanied by febrile symptoms also frequently had a risk allele in rs10893845, similar to patients who developed FNHTR.
Discussion - The results suggested that allergic sensitisation is associated with the onset of ATR and/or FNHTR in some patients. Although further prospective evaluation is necessary, analysis of these SNP might help to provide safer transfusion therapy by predicting patients at higher risk of transfusion-related adverse reactions and further clarifying the pathogenic mechanism underlying such reactions.

Keywords: *ATR, children, FNHTR, polymorphism, SNP.*

INTRODUCTION

Transfusion therapy is essential supportive care in paediatric practice; however, allergic transfusion reactions (ATR) and febrile non-haemolytic transfusion reactions (FNHTR) occur frequently[1,2]. ATR and FNHTR are more common in paediatric than adult patients[3,4]; furthermore, ATR are especially frequent in patients who have received platelet transfusions[1,3]. Although the risk of severe ATR is elevated in patients with 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

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who repeatedly experience ATR because of platelet transfusions, such reactions can be prevented by using washed platelet products[7-9]. Therefore, some of the factors present in plasma are thought to be strongly associated with the onset of ATR. The pathogenic mechanism underlying FNHTR was thought to involve leucocyte antibodies present in plasma[10] or an increase in leucocyte- and platelet-derived biological response modifiers in blood components during storage[11]. The decrease in FNHTR noted following pre-storage leucocyte 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 some patients do not have transfusion-related adverse reactions even when multiple blood transfusions are performed, there are also cases of the same patient repeatedly developing ATR or FNHTR[2,16]. However, it is currently difficult to predict which patients are likely to develop these adverse reactions. Our previous paediatric study showed that ATR due to platelet transfusions occur more frequently in paediatric patients who are older and in patients with haematological and malignant diseases[1]. Similarly, another study involving an adult population indicated that a background of haematological disease and younger age of the adults were risk factors[17]. A study involving an elderly population who had developed FNHTR 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]. The patient's background is, therefore, important at the time of transfusion. Furthermore, it remains unclear whether ATR and FNHTR have common risk factors.

Single-nucleotide polymorphisms (SNP) 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 SNP related to allergic diseases have also been reported[21,22], if SNP involved in ATR are identified, this would enable much safer transfusion therapy. However, currently, not much research has been performed on transfusion-related adverse reactions in relation to SNP. We, therefore, focused on SNP associated with allergic disease and examined the relationship between the occurrence of ATR and/or FNHTR and allergy-related SNP in paediatric patients with haematological and oncological diseases, the population most at risk of developing transfusion-related adverse reactions.

MATERIALS AND METHODS

Patients

We retrospectively analysed data from paediatric patients (aged ≤20 years) who had haematological and oncological disease and who had been transfused with a red blood cell concentrate and/or platelet concentrate at Nagano Children's Hospital between April 2003 and March 2020. All occurrences of ATR and/or FNHTR due to these transfusions during this study period were also analysed. 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 platelet concentrates used in this study were obtained from blood type-matched single-donor apheresis[2]. Red blood cell 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 leucocyte 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. The products were subsequently irradiated prior to transfusion[8,9].

Definitions of allergic transfusion reaction and febrile non-haemolytic transfusion reaction

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

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Genotyping of single nucleotide polymorphisms associated with allergic sensitisation

DNA was extracted from peripheral blood samples or conserved bone marrow cells by using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). We genotyped 22 SNP previously reported to be associated with allergic sensitisation[21,22]. The risk allele for allergic sensitisation for each SNP had 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 (SNP) (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 utilised for analysing SNP for which the primers and probes could not be designed using the Cycleave PCR Assay Designer (SNP). They were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Waltham, MA, USA). The SNP of rs9860547, rs10893845, and rs9303280 were analysed using allele-specific PCR, while those of rs10189629 was analysed using RFLP-PCR. In the SNP of rs9860547 and rs10893845, amplification was performed using GoTaq Colorless Master Mix (Promega, Fitchburg, WI, 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, CA, 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 HotstarTaq Master Mix (Qiagen), 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.) 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 occurrence of ATR or FNHTR were analysed with Fisher's exact test. The odds ratio and 95% confidence intervals (95% 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 Centre, 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 II provides summaries of the 219 cases analysed in this study. Of these 219 patients, 105 had developed an ATR and/or FNHTR at least once; there were 71 cases of ATR only, 19 cases of FNHTR only, and 15 cases of both ATR and FNHTR. Among the 86 ATR, 39 (45.3%) were grade I mild ATR with transient symptoms, and 36 (41.9%) were grade II ATR that required transfusion discontinuation. In addition, there were 11 cases (12.8%) of grade III ATR showing anaphylactic symptoms. On the other hand, 31 (91.2%) of the 34 FNHTR were classified as grade I with fever <38.0 °C, while the remaining (8.8%) were grade II with fever ≥38.0°C and <39.0 °C.

Our analysis included only cases from one institution and only paediatric cases of haematological 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 III**).

Next, the relationship between 22 SNP and the onset of ATR or FNHTR was analysed in the study population. Patients who had developed an ATR had a 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 IV-A**). Patients who had developed a FNHTR had a 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 IV-B**).

We also analysed patients who developed febrile symptoms along with ATR. Our findings showed that these patients had

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Table I - *Primers and probes used in this study*

(A) Cycleave polymerase chain reaction				
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		

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Table I - *Primers and probes used in this study (continued from previous page)*

(B) Allele-specific polymerase chain reaction		
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) Restriction fragment length polymorphism polymerase chain reaction		
SNP ID	**Alleles**	**Primers (5'-3')**
rs10189629	C/A	F: GTGAGACTGCATTGGGAGCT
		R: ATTGAACATCTGCTCGGCGA

Uppercase letters in the probe sequence indicate SNP sites, and the parentheses indicate RNA. SNP: single-nucleotide polymorphism; ID: identity.

Table II - *Characteristics of the patients analysed*

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

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Table III - *Comparison of the minor allele frequency for each single nucleotide polymorphism between cases included in this study and the controls*

Gene	SNP ID	Position[a]	Risk allele[b]	Minor allele frequency		p-value
				This study	1000 genome project	
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

[a]The positions of single nucleotide polymorphisms and genes are according to NCBI build 37.3. [b]The risk allele in the minor allele is indicated with the symbol*.

a 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 IV-C**), similar to the patients with FNHTR.

DISCUSSION

We were able to find candidate SNP that may be related to the development of ATR and FNHTR in paediatric patients with haematological and oncological diseases. In this study, rs6473223 was identified as a SNP that may be related to the occurrence of ATR. 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 SNP 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 ATR[6,15]. Savage *et al.* indicated that hay fever and food allergy are more frequent in patients with ATR to platelet transfusions[36]. Furthermore, total and aeroallergen-specific IgE concentrations in patients with ATR were found to be higher than those in patients without ATR[36,37]. However, the previous studies only utilised 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 ATR in the current study, we believe that this is an important finding. Our previous studies did not completely clarify the association

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Table IV - *Association of risk allele frequency for each single nucleotide polymorphism with the development of allergic transfusion reactions, febrile non-haematolytic transfusion reactions and allergic transfusion reactions accompanied by febrile symptoms*

Gene	SNP ID	Risk allele[a]	(A) ATR			(B) FNHTR			(C) ATR 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

[a]The risk allele in the minor allele is indicated with the symbol *.
ATR: allergic transfusion reaction; CI: confidence interval; FNHTR: febrile non-haemolytic transfusion reaction; OR: odds ratio; SNP: single-nucleotide polymorphism.

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between patients' allergy history and the occurrence of ATR[1,17], possibly because not all the patients' information could be accurately collected during a retrospective analysis. Furthermore, patients who do not have symptoms of allergies may still have elevated levels of aeroallergen-specific IgE. Therefore, it is considered difficult to predict the occurrence of ATR on the basis of medical interviews conducted to determine a patient's allergic history. More studies are required to further clarify the pathogenesis of ATR. It is hoped that future research on different kinds of SNP analyses and/or measurement of specific IgE antibodies may help to predict the risk of ATR, thereby enabling much safer transfusion therapy.

A study pertaining to the relationship between FNHTR and cytokine-related SNP indicated a relationship between these transfusion reactions and the polymorphism *IL1RN * 2· 2*, which may be related to serum interleukin-1β levels[38]. The SNP analysed in the current study had been previously reported to be involved in allergic sensitisation; we found that even FNHTR were associated with one of these SNP. rs10893845 is located near the *ETS1* gene at 11q23.421. *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 FNHTR and allergic constitution remains unclear. When patients develop pyrexia after blood transfusion, acute haemolytic transfusion reaction, transfusion-related acute lung injury, and microbial contamination should be considered as possible causative factors because FNHTR is a diagnosis of exclusion[15,41]. ATR are rarely suspected on the basis of febrile symptoms[41]. However, pyrexia associated with the development of ATR is not rare in children, and the same patient may often experience both ATR and FNHTR[2]. It is, therefore, speculated that the onset of ATR and FNHTR could overlap. It is possible that allergic predisposition is associated with both ATR and FNHTR. In our current study, patients who developed pyrexia along with ATR also frequently had a risk allele in rs10893845, similar to patients with FNHTR. This finding indicates that, in some paediatric patients diagnosed with FNHTR, both the conventional pathogenic mechanism and the allergic constitution of the patient may be involved in some manner. Elucidation of the pathogenic mechanism underlying ATR and FNHTR in future studies would help improve their management.

The current study identified potential SNP that could be related to ATR or FNHTR but has some limitations. First, a limited number of cases from a single institution were included. Second, the subjects were limited to paediatric patients with haematological and malignant diseases, which is a population expected to have a high incidence of ATR/FNHTR. It is, therefore, unclear whether our results can be extrapolated to the adult population and to paediatric patients with other 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 forms of ATR, our findings cannot be generalised to all cases of ATR of varying severity. To resolve these issues and to confirm our results, a large-scale multi-institutional prospective study will need to be performed 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 a restrictive transfusion approach, in high-risk patients should be further investigated.

CONCLUSIONS

In this study, we found one SNP each related to allergic sensitisation could be associated with ATR and FNHTR. Taken together with the findings of a previous study involving allergen-specific IgE, our results suggest that a recipient's allergic constitution could be a risk factor for developing ATR. Our results also indicate an association between FNHTR and SNP related to allergic sensitisation. It was, therefore, speculated that the allergic constitution of the patients is involved in the pathogenic mechanism underlying FNHTR in some paediatric cases. Analysing these SNP could help to predict which patients are likely to develop these transfusion-related reactions and may help to clarify the pathogenic mechanism underlying ATR and FNHTR.

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AUTHORSHIP CONTRIBUTIONS

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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The Authors declare no conflicts of interest.

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