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Algorithms using genome-wide association studies for prediction of effectiveness of biologics in rheumatoid arthritis

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Abstract:

Purpose: Two anti-TNF biologics, infliximab and etanercept, are extremely useful for inflammatory diseases such as rheumatoid arthritis (RA). However, approximately 20 to 30% of RA have been described as non-responders. Here, we analyzed the statistical relationships of whole genome single nucleotide polymorphisms (SNPs) with remission or low disease activity (LDA) among RA and developed algorithms using SNPs to predict effectiveness of these biologics.

Methods: The total study subjects (first, second and validation population for each infliximab and etanercept) were 260 RA patients for infliximab and another 251 RA patients for etanercept. Effectiveness of infliximab and etanercept was assessed using DAS28-CRP. In first, second and the combined population, relationships of 277,339 SNPs with remission and LDA were analyzed using case-control analyses by Fisher's exact tests for each infliximab and etanercept. We picked up SNPs ($P < 0.05$) from each first, second and combined population. Then, 10 SNPs with lower P value in the combined population were selected from common SNPs among the first, second and combined populations. We developed algorithms using the 10 SNPs to predict effectiveness of infliximab and etanercept. In the validation population, availability of the algorithms was evaluated.

Results: Using combined infliximab-remission and LDA algorithms in the validation population, the accuracy was 90.4%. Using combined etanercept-remission and LDA algorithms in validation population, the accuracy was 76.8%.

Conclusions: The combined use of our two algorithms using SNPs may be very useful in the prediction of remission or LDA before treatment with infliximab or etanercept.

Keywords:

infliximab, etanercept, effectiveness, single nucleotide polymorphism, GWAS

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1. Introduction

Rheumatoid arthritis (RA) is a progressive autoimmune

disease well defined by widely accepted symptoms such as chronic joint inflammation and structural damage [1]. In RA, the first purpose of treatment is to control symptoms

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and avoid joint damage. Predicting the prognosis of RA is very important in order to manage the disease. However, its clinical features and the molecular pathways are heterogeneous [2,3]. Therefore, the responses to treatments vary among RA patients, which make it difficult to predict RA patient's responsiveness to drugs.

In treatment for RA at present, using biological agents including tumor necrosis factor (TNF) blockades is extremely useful because these agents specifically inhibit immune responses and inflammation. Achievement of remission or low disease activity (LDA) in biological treatments for RA is currently one of the most important therapeutic targets. The importance of remission or LDA as outcomes has been highlighted by European League Against Rheumatism (EULAR) recommendations [4]. However, several studies have reported that approximately 20 to 30% of RA patients treated with these agents experience only transient clinical improvement or have no benefit because of individual differences such as lifestyles, past history and genetic factors among the patients [5,6]. Therefore, predicting the effectiveness of biologics is not easy before treatment. So far, several studies have reported that many single nucleotide polymorphisms (SNPs) affect biological efficacy [7–9]. These studies, however, only focused on SNPs located on genes associated with the TNF cascade, potentially missing other SNPs affecting efficacy. It has also been reported that some genes expression profiles in blood could predict the response to a biological agent [10]. However, using SNPs has not thus far been reported.

In this study, in order to develop algorithms using SNPs to predict effectiveness of infliximab (IFX) and etanercept (ETN) before their first administration, we analyzed the statistical relationships of whole genome SNPs with remission or LDA among IFX- or ETN-treated RA patients. Then, we examined whether the algorithms could predict the effectiveness of these biologics.

2. Material and methods

2.1. Study subjects

We recruited 511 RA patients receiving treatment of IFX or ETN from 6 hospitals (A to F hospital). The patients were divided into three populations by IFX or ETN treatment. The first population for IFX treatment included 94 RA patients recruited from hospital A. The second population for IFX treatment included 92 RA patients recruited from hospitals B, C, D, E or F. The validation population for IFX treatment included 72 RA patients recruited from all hospitals. The first population for ETN treatment included 88 RA patients recruited from hospital A. The second population for ETN treatment included 81 RA patients recruited from hospitals B, C, D, E or F. The validation population for ETN treatment included 73 RA patients recruited from all hospitals. Written informed consent to participate in this study was obtained from each patient. This study was ap-

proved by the ethical committee for analytical research on the human genome of the Matsubara Mayflower Hospital.

2.2. Assessment of disease activity

The Disease Activity Score with 28 joints using C-reactive protein (DAS28-CRP) was used in order to assess efficacy of IFX or ETN for each patient. Remission and LDA criteria were determined by DAS28-CRP score within 24 to 30 weeks after the first administration of IFX or ETN to each patient. Patients were categorized by their DAS28-CRP score into two groups: remission (DAS28-CRP score < 2.3) and non-remission (DAS28-CRP score $\geq$ 2.3). Patients were also categorized by their DAS28-CRP score into two groups: LDA including remission (DAS28-CRP score < 2.7) and non-LDA (DAS28-CRP score $\geq$ 2.7).

2.3. Genome-wide SNP genotyping

The patients' whole blood samples were used for DNA extraction at Mitsubishi BCL Inc. Genome wide SNP genotyping were performed at deCode genetics Inc. (Reykjavik, Iceland) using Illumina HumanHap300K chip technology (Illumina Corp., San Diego, CA, USA). After genotyping, 277,339 of 317,503 SNPs excluded SNPs with call rates < 90% and minor allele frequency < 1% were used in the case-control analysis described below.

2.4. Statistical analysis

We used the case-control analyses to analyze the relationships of 277,339 SNPs with remission/non-remission and LDA/non-LDA by Fisher's exact tests using Plink program (version 1.07). The case-control analyses were carried out in each first population, second population and the population combined first and second population.

2.5. Developing algorithms

After case-control analyses, we picked up SNPs ($P < 0.05$) that tended to be associated with remission/non-remission from each first, second and combined populations for IFX. The 10 SNPs with lower P value in combined the population were selected from common SNPs ($P < 0.05$) among first, second and combined populations. We scored each of the 10 SNPs in order to develop algorithms for prediction of IFX remission or non-remission. We gave a score of +1 to major allele homo in remission, score of 0 to hetero and score of -1 to major allele homo in non-remission. The total of 10 SNPs score was used in order to predict remission or non-remission. The optimum cut-off value was determined based on receive operating characteristic (ROC) analysis. Then, in order to evaluate functionality of this algorithm, we calculated accuracy, specificity and sensitivity of this algorithm in the validation population. The accuracy, specificity and sensitivity were calculated using formulas: (true positive + true negative)/total, true negative/(false positive + true negative) and true positive/(true positive + false negative), respectively. Similarly, algorithms for prediction of IFX LDA/non-LDA, ETN remission/non-remission and

Table 1. Clinical characteristics of patients

	infliximab (n = 260)			etanercept (n = 251)		
Population	First	Second	Validation	First	Second	Validation
Number of patients	95	92	73	88	81	82
Age (years)	58.5 ± 12.7	53.1 ± 13.1	56.0 ± 11.4	61.6 ± 11.7	53.7 ± 13.1	55.1 ± 15.1
Female (%)	73.7	89.1	82.2	75.0	93.8	80.5
Disease duration (years)	9.8 ± 8.8	10.5 ± 9.1	10.2 ± 10.1	11.2 ± 10.2	11.3 ± 8.7	8.0 ± 8.5
DAS28 (CRP)	5.0 ± 1.3	4.8 ± 1.2	4.9 ± 1.2	5.0 ± 1.2	4.7 ± 1.1	4.8 ± 1.4
high (> 5.1)	49	38	30	48	27	34
moderate + low (≤ 5.1)	46	54	43	40	54	48
Steinbrocker's radiographic stage (disease stage)						
I (Early) + II (Moderate)	20	34	32	16	23	13
III (Severe) + IV (Terminal)	72	58	25	70	57	38
unknown	3	-	-	2	1	31
Steinbrocker's functional class (degree of dysfunction)						
I + II	75	72	53	73	64	45
III + IV	17	20	4	13	16	8
unknown	3	-	-	2	1	29
Remission or non-remission						
remission	22	17	14	30	24	37
non-remission	72	75	59	58	57	45
Low disease activity or non-low disease activity						
low disease activity	29	30	22	44	38	47
non-low disease activity	66	62	51	44	43	35

Values are mean ± SD, number of the patients

class I: Can perform all activities, class II: Moderate restriction of activities, class III: Marked restriction of activities, class IV: Incapacitated

Table 2. Selected 10 SNPs associated with infliximab remission

SNP	Chr	A1	F_R	F_N	A2	Combined <i>P</i>	OR	Gene
rs10860738	12	A	0.6154	0.3265	G	6.4×10^{-6}	3.3	-
rs2181276	1	C	0.2692	0.5276	T	6.3×10^{-5}	0.33	HIVEP3
rs751511	13	T	0.6538	0.398	C	6.5×10^{-5}	2.9	-
rs7603262	2	A	0.3077	0.5646	C	6.7×10^{-5}	0.34	AP1S3
rs763713	2	C	0.5385	0.2959	A	9.5×10^{-5}	2.8	PAIP2B
rs4629018	17	A	0.6667	0.4184	G	1.2×10^{-4}	2.8	JUP
rs7623638	3	G	0.5769	0.3367	A	1.4×10^{-4}	2.7	EPHA6
rs6598193	12	G	0.6154	0.3707	A	1.6×10^{-4}	2.7	-
rs2461224	10	G	0.2821	0.5238	A	1.9×10^{-4}	0.36	TACC2
rs12878216	14	A	0.5641	0.3265	G	2.0×10^{-4}	2.7	TRA

Chr: chromosome number, A1: allele 1, F_R: frequency of allele 1 in remission group,

F_N: frequency of allele 1 in non-remission group, A2: allele 2,

Combined *P*: *P* value in the population combined first and second population, OR: odds ratio,

Gene: Genes mapped to the SNP (NCBI genome build 106)

ETN LDA/non-LDA were developed and evaluated. We also evaluated functionality of algorithms for combined remission and LDA algorithm.

3. Results

The basic characteristics of the patients according to each population are presented in Table 1. The total patients of populations for IFX were 260 patients. The total patients of populations for ETN were 251 patients. The first population for IFX was 95 patients aged 58.5 ± 12.7 years (mean ±

SD), the second population for IFX was 92 patients aged 53.1 ± 13.1 and the validation population for IFX was 73 patients aged 56.0 ± 11.4 . The first population for ETN was 88 patients aged 61.6 ± 11.7 , the second population for ETN was 81 patients aged 53.7 ± 13.1 and the validation population for ETN was 82 patients aged 55.1 ± 15.1 .

With regard to IFX remission, 1,328 SNPs were chosen. The *P* value for 314 SNPs was less than 0.001, and for 1014 SNPs was less than 0.01. Table 2 presents the selected 10 SNPs which were strongly associated with IFX remission. Odds ratio for rs10860738 was 3.3 ($P = 6.4 \times 10^{-6}$), rs

Table 3. Selected 10 SNPs associated with infliximab low disease activity

SNP	Chr	A1	F_L	F_N	A2	Combined <i>P</i>	OR	Gene
rs10848816	12	G	0.65	0.2922	A	5.5×10^{-6}	4.5	TSPAN9
rs2288947	19	G	0.6	0.259	A	8.0×10^{-6}	4.3	TINCR
rs1933437	13	C	0.55	0.2259	T	1.0×10^{-5}	4.2	FLT3
rs7715337	5	A	0.4	0.1325	G	1.4×10^{-5}	4.4	LOC101927421
rs9486755	6	T	0.425	0.1506	C	2.0×10^{-5}	4.2	SEC63
rs7513602	1	A	0.075	0.4187	G	2.4×10^{-5}	0.11	KIF26B
rs1941238	18	G	0.45	0.1717	A	3.4×10^{-5}	3.9	-
rs7866925	9	T	0.35	0.1114	C	3.4×10^{-5}	4.3	AKNA
rs4128811	4	G	0.8	0.4548	A	3.7×10^{-5}	4.8	-
rs4078293	10	C	0.625	0.3	T	3.9×10^{-5}	3.9	-

Chr: chromosome number, A1: allele 1, F_R: frequency of allele 1 in low disease activity group, F_N: frequency of allele 1 in non-low disease activity group, A2: allele 2, Combined *P*: *P* value in the population combined first and second population, OR: odds ratio, Gene: Genes mapped to the SNP (NCBI genome build 106)

Table 4. Selected 10 SNPs associated with etanercept remission

SNP	Chr	A1	F_R	F_N	A2	Combined <i>P</i>	OR	Gene
rs10872224	6	G	0.1852	0.4304	T	9.2×10^{-6}	0.30	-
rs10484867	6	A	0.1852	0.4204	G	2.4×10^{-5}	0.31	-
rs9375195	6	A	0.2407	0.4739	G	4.6×10^{-5}	0.35	-
rs6932561	6	T	0.5943	0.3522	C	4.9×10^{-5}	2.7	-
rs2281449	6	G	0.6481	0.413	A	6.8×10^{-5}	2.6	TNFRSF21
rs2175491	15	T	0.4907	0.2652	C	7.0×10^{-5}	2.7	UNC13C
rs3212198	20	T	0.4907	0.2696	C	8.1×10^{-5}	2.6	HNF4A
rs12612022	2	C	0.2037	0.4211	T	8.5×10^{-5}	0.35	CNTNAP5
rs12216178	6	G	0.1944	0.4087	A	1.2×10^{-4}	0.35	-
rs11665898	19	G	0.4444	0.2391	A	2.2×10^{-4}	2.5	-

Chr: chromosome number, A1: allele 1, F_R: frequency of allele 1 in remission group, F_N: frequency of allele 1 in non-remission group, A2: allele 2, Combined *P*: *P* value in the population combined first and second population, OR: odds ratio, Gene: Genes mapped to the SNP (NCBI genome build 106)

2181276 was 0.33 ($P = 6.3 \times 10^{-5}$), rs751511 was 2.9 ($P = 6.5 \times 10^{-5}$), rs7603262 was 0.34 ($P = 6.7 \times 10^{-5}$), rs763713 was 2.8 ($P = 9.5 \times 10^{-5}$), rs4629018 was 2.8 ($P = 1.2 \times 10^{-4}$), rs7623638 was 2.7 ($P = 1.4 \times 10^{-4}$), rs6598193 was 2.7 ($P = 1.6 \times 10^{-4}$), rs2461224 was 0.36 ($P = 1.9 \times 10^{-4}$) and rs12878216 was 2.7 ($P = 2.0 \times 10^{-4}$) in the combined population. The cut-off value for prediction of IFX remission or non-remission was a score of 2 points (remission ≥ 2 points, non-remission ≤ 1 points). In the validation population, accuracy, specificity and sensitivity of the algorithm based on this cut-off value were 66.7%, 53.8% and 69.5%, respectively.

With regard to IFX LDA, 3,122 SNPs were chosen. The *P* value for 419 SNPs was less than 0.001, and for 2703 SNPs was less than 0.01. Table 3 presents the selected 10 SNPs which were strongly associated with IFX LDA. Odds ratio for rs10848816 was 4.5 ($P = 5.5 \times 10^{-6}$), rs2288947 was 4.3 ($P = 8.0 \times 10^{-6}$), rs1933437 was 4.2 ($P = 1.0 \times 10^{-5}$), rs7715337 was 4.4 ($P = 1.4 \times 10^{-5}$), rs9486755 was 4.2 ($P = 2.0 \times 10^{-5}$), rs7513602 was 0.11 ($P = 2.4 \times 10^{-5}$), rs

1941238 was 3.9 ($P = 3.4 \times 10^{-5}$), rs7866925 was 4.3 ($P = 3.4 \times 10^{-5}$), rs4128811 was 4.8 ($P = 3.7 \times 10^{-5}$) and rs4078293 was 3.9 ($P = 3.9 \times 10^{-5}$) in the combined population. The cut-off value for prediction of IFX LDA or non-LDA was a score of -1 points (LDA ≥ -1 points, non-LDA ≤ -2 points). In the validation population, accuracy, specificity and sensitivity of the algorithm based on this cut-off value were 82.2%, 12.5% and 90.8%, respectively.

With regard to ETN remission, 1,653 SNPs were chosen. The *P* value for 521 SNPs was less than 0.001, and for 1132 SNPs was less than 0.01. Table 4 presents the selected 10 SNPs which were strongly associated with ETN remission. Odds ratio for rs10872224 was 0.30 ($P = 9.2 \times 10^{-6}$), rs10484867 was 0.31 ($P = 2.4 \times 10^{-5}$), rs9375195 was 0.35 ($P = 4.6 \times 10^{-5}$), rs6932561 was 2.7 ($P = 4.9 \times 10^{-5}$), rs2281449 was 2.6 ($P = 6.8 \times 10^{-5}$), rs2175491 was 2.7 ($P = 7.0 \times 10^{-5}$), rs3212198 was 2.6 ($P = 8.1 \times 10^{-5}$), rs12612022 was 0.35 ($P = 8.5 \times 10^{-5}$), rs12216178 was 0.35 ($P = 1.2 \times 10^{-4}$) and rs11665898 was 2.5 ($P = 2.2 \times 10^{-4}$) in combined population. The cut-off value for prediction of ETN remis-

Table 5. Selected 10 SNPs associated with etanercept low disease activity

SNP	Chr	A1	F_R	F_N	A2	Combined <i>P</i>	OR	Gene
rs676134	11	C	0.5179	0.2057	T	1.1×10^{-6}	4.1	BACE1
rs1564326	15	C	0.6786	0.3617	T	1.1×10^{-5}	3.7	-
rs711280	6	C	0.3571	0.1241	T	1.6×10^{-5}	3.9	-
rs9320236	6	G	0.4286	0.1738	T	2.3×10^{-5}	3.6	SCML4
rs3790112	16	T	0.2321	0.539	G	2.7×10^{-5}	0.26	GNAO1
rs2199724	15	A	0.7143	0.4113	C	3.3×10^{-5}	3.6	-
rs1503575	12	A	0.4821	0.2163	G	3.5×10^{-5}	3.4	-
rs12196723	6	C	0.3571	0.1312	T	3.7×10^{-5}	3.7	WDR27
rs7310134	12	A	0.4464	0.1915	G	3.8×10^{-5}	3.4	-
rs1451117	4	T	0.1429	0.4357	C	4.0×10^{-5}	0.22	-

Chr: chromosome number, A1: allele 1, F_R: frequency of allele 1 in low disease activity group,

F_N: frequency of allele 1 in non-low disease activity group, A2: allele 2,

Combined *P*: *P* value in the population combined first and second population, OR: odds ratio,

Gene: Genes mapped to the SNP (NCBI genome build 106)

sion was a score of +2 points (remission > +2 points, non-remission < +2 points). In the validation population, accuracy, specificity and sensitivity of the algorithm based on this cut-off value were 56.1%, 43.2% and 66.7%, respectively.

With regard to ETN LDA, the picked up SNPs were 2,695 SNPs. The *P* value for 320 SNPs was less than 0.001, and for 2375 SNPs was less than 0.01. Table 5 presents the selected 10 SNPs which were strongly associated with ETN LDA. Odds ratio for rs676134 was 4.1 ($P = 1.1 \times 10^{-6}$), rs1564326 was 3.7 ($P = 1.1 \times 10^{-5}$), rs711280 was 3.9 ($P = 1.6 \times 10^{-5}$), rs9320236 was 3.6 ($P = 2.3 \times 10^{-5}$), rs3790112 was 0.26 ($P = 2.7 \times 10^{-5}$), rs2199724 was 3.6 ($P = 3.3 \times 10^{-5}$), rs1503575 was 3.4 ($P = 3.5 \times 10^{-5}$), rs12196723 was 3.7 ($P = 3.7 \times 10^{-5}$), rs7310134 was 3.4 ($P = 3.8 \times 10^{-5}$) and rs1451117 was 0.22 ($P = 4.0 \times 10^{-5}$) in the combined population. The cut-off value for prediction of ETN LDA was a score of -2 points (LDA > -2 points, non-LDA < -2 points). In the validation population, accuracy, specificity and sensitivity of the algorithm based on this cut-off value were 73.2%, 50.0% and 76.4%, respectively.

Using algorithm combined IFX-remission and LDA algorithms in the validation population, accuracy, specificity and sensitivity were 90.4%, 57.1% and 98.3%, respectively. Using combined ETN-remission and LDA algorithms in the validation population, accuracy, specificity and sensitivity were 76.8%, 62.2% and 88.9%, respectively.

4. Discussion

To our knowledge, our study is the first to develop algorithms to predict effectiveness of biologics such as IFX and ETN using only the results of genome-wide association studies (GWAS) among Japanese RA patients.

It has been reported that the SNPs in genes such as Human Leukocyte Antigen-DRB 1 (HLA-DRB1) [7], Interleukin-10 (IL-10) [11], Interleukin-1 receptor antagonist (IL-1RN) [12] and Transforming Growth Factor- β 1 (TGF β

1) [13] affects the effectiveness of IFX or ETN. However, these studies have shown the relationships of only a few SNPs in genes involved in the TNF cascade with remission, and LDA in IFX and ETN treatment. Therefore, it is likely that SNPs in unknown genes associated with drug responsiveness were overlooked. In this regard, GWAS is powerful tool to collectively identify SNPs associated with drug response [14]. By using GWAS, it has been clear that three genomic regions showed significant or borderline significant associations with anti-TNF response in Japanese RA patients [15]. Moreover, as a predictor of response to ETN therapy in RA, GWAS identified *CD84* [16]. Thus, conducting GWAS is valuable in identifying SNPs associated with the effectiveness of IFX or ETN. So far, it has not been established that SNPs can be used as prediction method for responsiveness of these biologics. In this study, we developed the algorithms using only SNPs based on the result of GWAS.

In our study, 10 SNPs were used for each algorithm. In case SNPs are used to predict the effectiveness of biologics, it seems that using more SNPs raises the accuracy of predicting the effectiveness. On the other hand, using more SNPs is difficult because of problems such as high cost and time required for analyzing. In addition, using more SNPs makes the algorithms for prediction complicated. However, algorithms using fewer SNPs probably show low accuracy of prediction. We, thus, selected 10 SNPs with lower *P* value and developed the algorithms.

A previous study revealed that an eight-gene blood expression profile could predict the response to IFX in RA with an 85.7% prediction accuracy [10]. Another previous study also revealed that a comprehensive transcriptome analysis of white blood cells was capable of predicting efficacy of IFX for RA patients [17]. These reports, however, were based on blood RNA expression levels. Because blood RNA expression level may vary day to day, the result of prediction for response to biologics may vary. We, thus, tested prediction methods of IFX or ETN responses using

SNPs as the most basic non-variable biomarker. Our SNP algorithm for prediction of remission or LDA in IFX treatment showed higher accuracy (90.4%) than the two previous reports. With regard to ETN, a previous study has reported that cytokine profiling by proteomic analysis may predict responsiveness of ETN in RA patients with an 84.4% accuracy [18]. Our SNP algorithm for prediction of remission or LDA in ETN treatment showed a little lower accuracy (76.8%) than this previous study. Nevertheless, our SNP algorithm can predict the responsiveness more quickly and cheaply than the cytokine profiling by proteomic analysis. Furthermore, another previous study has reported that even diagnostic sensitivity and specificity of rheumatoid factor for rheumatoid arthritis were 69% and 85%, respectively [19]. In same study, the diagnostic sensitivity and specificity of antibodies against cyclic citrullinated peptide (anti-CCP antibodies) were also 67% and 95%, respectively [19]. Our algorithms have a high usefulness compared to these sensitivity or specificity. Thus, it seems that our SNP algorithm is quite useful.

Our genome-wide SNP association analysis found some SNP loci mapped on notable or near notable genes. Rs 12878216 associated with IFX remission in our study is located on the T cell receptor alpha locus. Because T cell receptor-mediated T cell activation was enhanced in the peripheral blood, this SNP might have some kind of influence on the T cell immune responses in IFX treatment [20]. Rs 2281449 associated with ETN remission in our study is located on the TNF receptor superfamily member 21 gene (*TNFRSF21*). It has been reported that *TNFRSF21*, also known as death receptor 6 (DR6), enhances B cell expansion, survival and humoral responses in mice [21]. *TNFRSF21* also has an important regulatory role in limiting T cell immune responses [22]. Furthermore, the expression of *TNFRSF21* is induced by TNF-alpha through activation of NF-kappaB [23]. These studies have suggested that this rs 2281449 SNP might affect B cell and T cell immune responses in treatment of ETN used as TNF blockade. Rs 6932561 associated with ETN remission in our study is located on the 5'-flanking region of the *CD83* gene. The *CD83* gene locus was associated with RA in the Japanese population [24]. In RA, soluble CD83 levels were higher in synovial fluid than in plasma [25]. Early RA patients also increased levels of soluble CD83 in plasma [26]. However, the level of soluble CD83 in plasma did not correlate with RA activity [26]. In addition, the release of soluble CD83 was independent of TNF-alpha [26]. Therefore, relationship of CD83 with RA should be further examined in future research. Rs11665898 associated with ETN remission in our study located on the 5'-flanking region of the *CD97* gene. In rheumatoid synovial tissues, CD97 and its ligand CD55 expression are expressed at high levels, and the CD97/CD55 pair may be of primary importance in maintaining and amplifying synovial inflammation [27]. This SNP located on the 5'-flanking region might affect the responsiveness of ETN through affecting CD97 expression and rheumatoid

synovial inflammation.

Interestingly, there were no SNPs overlapping between IFX and ETN remission or LDA. This might be caused by the structural difference between IFX and ETN. IFX is a chimeric antibody against TNF-alpha, whereas ETN is a soluble form of extracellular region of TNF receptor.

Our algorithms have a limitation. These are mainly for Japanese RA patients because this is based on the results of GWAS among Japanese RA patients. It is well known that allele frequencies of most SNPs vary in different ethnic groups. Allele frequencies of SNPs we selected for our algorithms also varied compared with the allele frequencies of other ethnic groups reported in the HapMap database (<http://www.ncbi.nlm.nih.gov/snp>). Therefore, our algorithms may not be applicable to non-Japanese RA patients. It is also well known that allele frequency of SNPs such as rs671 in aldehyde dehydrogenase 2 and rs1229984 in alcohol dehydrogenase 1B associated with alcohol sensitivity and drinking behavior vary from place to place within Japan [28,29]. Therefore, there is possibility that the SNPs we selected for our algorithms have area-specific allele frequency. However, our study RA patients were recruited from several regions in Japan. Thus, our algorithms seems to be applicable to most Japanese RA patients.

5. Conclusions

This is the first report of algorithms to predict the effectiveness of IFX or ETN using 10 SNPs selected from the results of genome wide SNP association analysis. Our SNP algorithms are very easy to use, and the algorithms combined IFX- or ETN-remission and LDA algorithms showed high accuracy (90.4% and 76.8%, respectively). Therefore, our algorithms may be very useful in the prediction of remission or LDA before treatment of IFX or ETN. Furthermore, this scoring method using SNPs may contribute to tailor-made treatments with biologics in the future.

Conflicts of interest

The authors have declared that no conflicts of interest exist.

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