Review Article

Recent advances on the genetics of rheumatoid arthritis: current topics and the future

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Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes severe joint pain and eventually joint deformity. Recent large cohort studies and the rapid progression of genotyping platforms have enabled identification of more than 30 susceptibility genes for RA. *HLA* is the major genetic determinant for RA for which a shared epitope hypothesis (70th-74th amino acids of HLA-DR β chain determine susceptibility) has been accepted. However, recent detailed single nucleotide polymorphism (SNP) typing of the *HLA* region and imputation method revealed that the most important amino acid positions of the HLA-DR β chain are the 11th in addition to the 71st and the 74th. HLA-B (at position 9) and HLA-DPB1 (at position 9) are also important determinants. This revised shared epitope hypothesis will form a new theory for *HLA* association. Another topic is that anti-citrullinated protein antibody (ACPA)-negative RA has been shown to be genetically different from ACPA-positive RA. Many susceptibility genes including *HLA* were not associated with ACPA-negative RA; however, we have shown that some *HLA* alleles are associated with ACPA-negative RA. In this review, we present some new findings regarding *HLA* as well as some recently discovered susceptibility genes for RA.

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Introduction

Since 2003, when sequencing of the human genome was completed, there has been a burst of identification of new susceptibility genes for RA. In the last several years in particular, more than 30 genes or loci have been identified as RA-related genes[1]. This activity was supported by the development of SNP genotyping platform, which enables us to type hundreds of millions of SNPs in a few weeks, even in a relatively small lab. In addition, a growing number of large cohorts were formed to tackle the elucidation of RA pathogenesis, which provided substantial power to detect genes of significance[2].

ACPA is a specific autoantibody of RA, and its target antigens are citrullinated vimentin, filaggrin, α-enolase, and others[3]. It is a useful marker not only for diagnosis of RA, but also for predicting disease course[4]. ACPA-positive RA

is clinically severer than ACPA-negative RA. Moreover, it has been suggested that ACPA-positive RA is genetically distinct from ACPA-negative RA[5, 6].

Here we present the recent advances in RA genetics and also discuss the genetic differences between ACPA-positive and ACPA-negative RA.

Human leukocyte antigen (HLA)

Genetic predisposition to RA has been investigated intensively. *HLA* is a major determinant of RA susceptibility and *HLA-DRB1**01:01, *01:02, *04:01, *04:04, *04:05, *04:08, *04:10, *04:13, *04:16, *10:01, *14:02 and *14:06 were reported to be associated with RA development. Among these *HLA-DRB1* alleles, there are common amino acid sequences at the 70th-74th residues of the HLA-DRβ chain (QKRAA, QRRAA or RRRAA), which is called a 'shared epitope' (SE)[7]. The association of *HLA-DRB1* SE with RA has been replicated in many ethnic groups[8]. However, recently the important role of Leucine at 67th position (Leu67)[9-10] and Valine at 11th position (Val11)[10] for RA development and resistant effect on RA development by Aspatic acid at 70th position (Asp70)[11] were also reported. In addition, Raychaudhuri et al. used existing genome-wide SNP data of >5,000 ACPA-positive RA cases and ~15,000 controls and imputed (expected SNP genotypes in silico from adjacent SNP gentotypes and linkage disequilibrium information) the gap SNP genotypes of HLA locus and reported the following findings. They showed that three amino acid positions (11, 71 and 74) of HLA-DRβ chain as well as single-amino acid positions in HLA-B (at position 9) and HLA-DPβ chain (at position 9) explain most of the MHC association with RA[12]. All these positions are located in peptide-binding grooves, as shown in Fig.1. Among these positions, position 11 of HLA-DRβ chain showed the strongest association with RA development ($p<10^{-581}$ for position 11). As shown in Table 1, Val11 and Leu11 are the key amino acids for susceptibility and

Table 1 Effect estimates of the 3 amino acids associated with risk of RA

HLA-DRβ1 amino acid at position			multivariate OR	95%CI	*HLA-DRB1* alleles
11	71	74			
Val	Lys	Ala	4.44	4.02-4.91	*04:01
Val	Arg	Ala	4.22	3.75-4.75	*04:08, *04:05, *04:04, *10:01
Leu	Arg	Ala	2.17	1.94-2.42	*01:02, *01:01
Pro	Arg	Ala	2.04	1.59-2.62	*16:01
Val	Arg	Glu	1.65	1.24-2.19	*04:03, *04:07
Asp	Arg	Glu	1.65	1.29-2.10	*09:01
Val	Glu	Ala	1.43	1.04-1.96	*04:02
Pro	Ala	Ala	1.00	Reference	*15:01, *15:02
Ser	Arg	Ala	0.88	0.77-1.00	*11:01, *11:04, *12:01
Ser	Arg	Leu	0.71	0.57-0.89	*08:01, *08:04
Ser	Lys	Arg	0.63	0.54-0.73	*03:01
Ser	Glu	Ala	0.59	0.51-0.68	*11:02, *11:03, *13:01, *13:02

Estimate effects for haplotypes of *HLA-DRB1*. For each haplotype, the multivariate effect is given as an odds ratio (OR), taking the most frequent haplotype (Pro-Ala-Ala) in the control samples as the reference (that is, given that the haplotype has an OR of 1). Classical shared epitope alleles are shown in bold. This table is modified from a previous report[12].

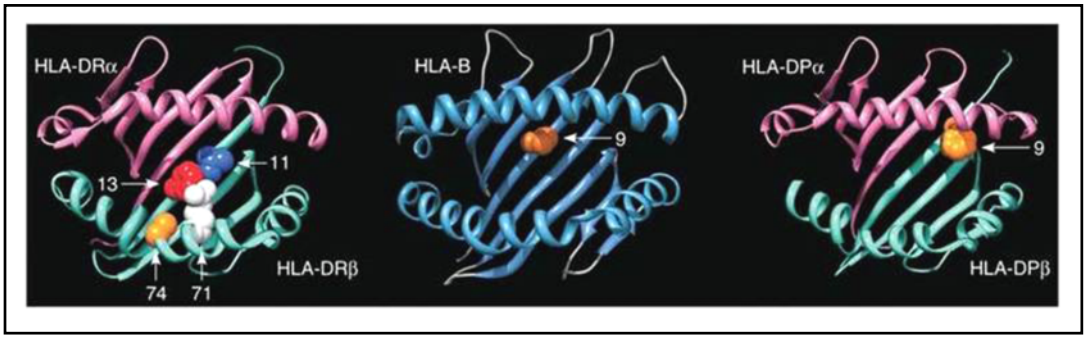

Fig.1
Three-dimensional ribbon models for the HLA-DR, HLA-B and HLA-DP proteins. Key amino acid positions identified by the association analysis are highlighted. This figure is taken from a previous report[12].

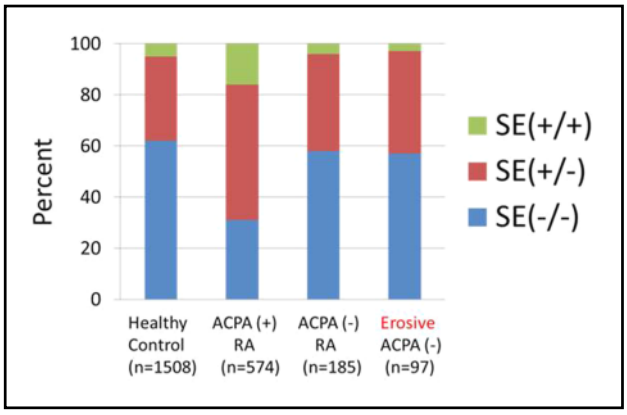

Fig.2
Prevalence of individuals carrying double SE, single SE or no SE is shown in healthy control, ACPA-positive RA, ACPA-negative RA and ACPA-negative RA with typical bone erosion as determined by X-ray. This clearly shows that ACPA-negative RA is distinct from ACPA-positive RA. This figure is illustrated based on our previous report[13].

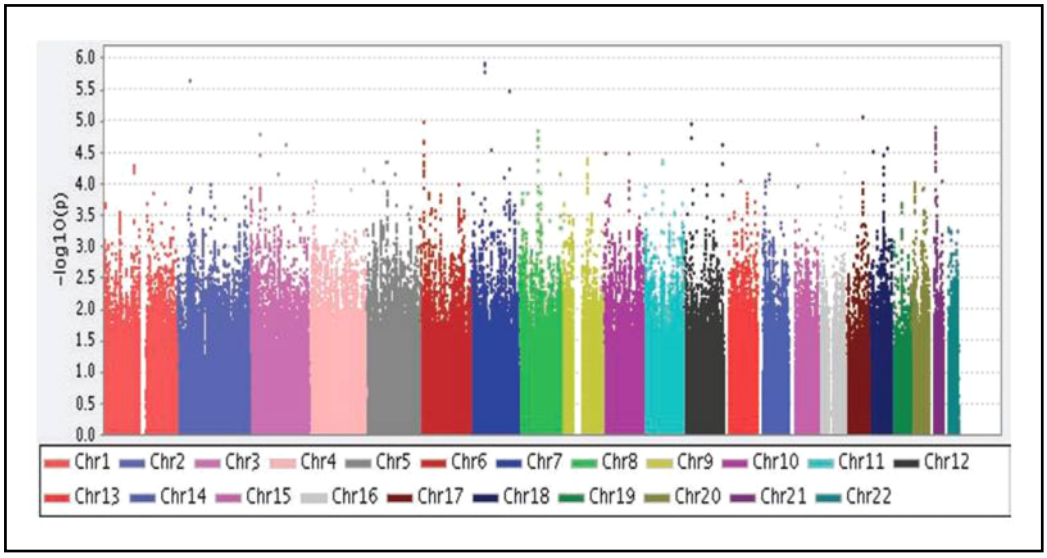

Fig 3
Probability plot for association with ACPA-negative RA (n=774) versus healthy controls (n=1079). This figure is taken from a previous report[6].

Ser11 is protective, for example, even though positions 71 and 74 are the SE types, Ser11 offsets such effects. Since most of the SE alleles have Valine or Leucine at position 11, Leucine at position 67, and do not have Serine at position 11 nor Aspatic acid at position 70, the results of previous studies using SE would not have been affected by the recent findings. Thus, key amino acid positions of HLA-DRβ chain for RA development seem to be 11th, 70th, 71st, and 74th positions and there still are some debates which positions have the primary effect. Anyway, these positions seem to be important for citrullinated peptide presentation.

HLA association with ACPA-negative RA

In 2005, a Dutch group reported that the association of SE was only exhibited with ACPA-positive RA and no association was seen with the ACPA-negative RA patients[10]. We have replicated the results in the Japanese population, and also showed that similar results were obtained even when we selected only bone-erosive ACPA-negative RA[13], which strongly suggests that this observation is not due to the contamination of non-RA arthritic diseases in ACPA-negative RA subset (Fig.2).

First of all, is there a genetic predisposition for ACPA-negative RA? From a twin study, heritability of ACPA-negative RA has been estimated and is thought to be as high as that of ACPA-positive RA[14].

Next, is *HLA* associated with ACPA-negative RA? A genome-wide association study (GWAS) meta-analysis of ACPA-negative RA showed that *HLA-DR* locus in chromosome 6 had no peak of association (see Fig.3)[6], suggest-

ing that the impact of *HLA* for development of ACPA-negative RA is not as large as that of ACPA-positive RA. In the study, the p-value of the *HLA* locus for ACPA-positive RA reached the order of 10^{-60}; in contrast, that for ACPA-negative RA reached the order of 10^{-4}. However, this does not mean that *HLA* is not associated with ACPA-negative RA, but probably means that ACPA-positive RA is a rather homogeneous subset in terms of *HLA* usage compared with ACPA-negative RA. ACPA-negative RA might have more variations of autoantigen (probably not citrullinated). In ACPA-positive RA, *HLA* usage is rather homogeneous, probably because citrullinated proteins or peptides are the common autoantigens among such patients that have SE-carrying *HLA*.

What *HLA* alleles are associated with ACPA-negative RA? In Caucasians, *HLA-DR3* and *DR13* have been reported to be associated with ACPA-negative RA[15-17]. As *HLA-DR3* association was seen in 3 independent European cohorts, it is probably true in Caucasians. In Japanese, we found that multiple *HLA-DRB1* alleles, including *12:01, *14:03 and *04:05, were associated with ACPA-negative RA susceptibility in the Japanese population[18]. *HLA-DR3* alleles were not shown because they are very rare in Japanese. We also found that *HLA-DRB1**15:02 and *13:02 were protective against ACPA-negative RA development. It is noteworthy that one of the SE alleles, *HLA-DRB1**04:05, was associated with ACPA-negative RA. Other SE alleles were not associated with ACPA-negative RA. This implies that the association of *04:05 with ACPA-negative RA is not due to the common amino acid sequence of SE because SE-carrying alleles other than *04:05 are not associated. Therefore, other mechanisms are suggested.

It seems there are two subsets in ACPA-negative RA based on RF positivity. Mackie et al. recently reported that *HLA-DRB1* SE is associated with ACPA(-)RF(+) RA but not with ACPA(-) RF(-) RA[19]. We have similar data for the Japanese population and showed that there are some specific *HLA-DRB1* alleles associated with ACPA(-) RF(+) RA or ACPA(-)RF(-) RA (Fig.4). For example, *04:05 and *09:01 were specifically associated with ACPA(-)RF(+) subset, and DR8/DR8 homozygote and DR14 were specifically associated with ACPA(-)RF(-) subset, whereas *12:01 was associated with both subsets. In contrast, ACPA(+)RA could not be separated by *HLA-DR* allelic usage.

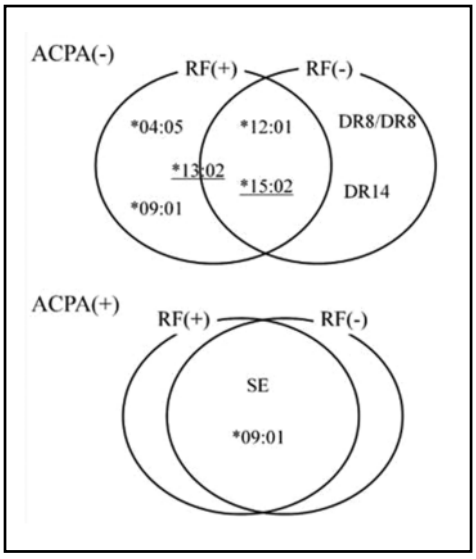

Fig.4
Scheme of HLA-DRB1 allele association with RF(+) or RF(-) subset of ACPA(-) RA or ACPA(+)RA in Japanese. Underline represents the protective allele. This figure is taken from our unpublished results.

Non-HLA genes associated with RA

A lot of genetic polymorphisms of candidate genes were tested for association with RA and reported to be associated with it, but most of them were not replicated. Perhaps the positive results are due to publication bias and relatively small sample sizes. Since 2003[20], genome-wide association studies (GWAS) have been applied to RA[21-26] and recently several meta-analyses of GWAS were performed[27-29]. Sample sizes also jumped from several hundred to tens of thousands. As a result, 30-40 genes or loci were detected to be significantly ($p<5\times10^{-8}$) associated with RA[1]. Many of these SNPs are located not in the genes (exons and introns), but near the genes, while some of the SNPs are located in exons and cause amino acid substitution (e.g. *PTPN22*). In many cases, the real causative SNPs or variants are still unknown. The list of SNPs in Table 2 shows the most strongly associated SNPs in the studies, but the real causative variants may exist somewhere else. The associated genes shown in Table 2 are classified by their main function. These genetic variants satisfied the genome-wide significance ($p<5\times10^{-8}$) or region-wide significance after Bonferroni's correction with multiple replication. Some of them are specific to Caucasians, mainly due to the absence of polymorphisms such as *PTPN22* and *RBPJ*, while some are specific to the Japanese or

Table 2 Candidate genes with confirmed association with rheumatoid arthritis

Gene	Best p-value	OR	Association† in		landmark SNP	SNP position	reference
			Caucasians	Japanese*			
(1)Intracellular signaling molecules and receptors							
PTPN22	9.1×10^{-74}	1.94	++	NA	rs2476601	exon	27
TRAF1-C5	4.0×10^{-14}	1.32	++	-	rs3761847	near	22
MBP	2.7×10^{-8}	1.23	-	++	rs2000811	intron	26
TNFAIP3	8.9×10^{-13}	1.22	++	++	rs6920220	near	27
BLK	5.7×10^{-9}	1.19	++	+	rs2736340	near	24
SPRED2	5.3×10^{-10}	1.13	++	+	rs934734	intron	27
TAGAP	3.8×10^{-7}	0.91	+	-	rs394581	near	44
TRAF6	3.9×10^{-6}	0.89	+	-	rs540386	intron	44
PTPRC	6.7×10^{-7}	0.88	+	-	rs10919563	intron	44
PRKCQ	4.4×10^{-6}	0.88	+	-	rs4750316	near	45
(2)Transcription factor							
REL	3.1×10^{-14}	1.25	++	-	rs13031237	intron	24
IRF5	4.2×10^{-11}	1.25	++	+	rs10488631/ rs13225818	near/near	27
STAT4	1.7×10^{-11}	1.24	++	++	rs7574865	intron	46
RBPJ	1.0×10^{-16}	1.18	++	NA	rs874040	near	27
AIRE	3.6×10^{-9}	1.18	-	++	rs2075876	intron	33
AFF3	1.0×10^{-14}	1.15	++	+	rs11676922	near	27
PRDM1	2.1×10^{-8}	1.11	++	-	rs6822844	near	44
(3)Cytokines and cytokine receptors							
CCR6	7.7×10^{-19}	1.19	++	++	rs3093024	near	25
IL2RB	4.6×10^{-8}	1.13	++	-	rs3218253	intron	47
IL2RA	1.4×10^{-11}	1.11	++	-	rs706778	intron	27
TNFRSF14	1.1×10^{-7}	0.92	+	+	rs3890745	near	45
CCL21	3.9×10^{-10}	0.87	++	-	rs951005	near	27
ANKRD55-IL6ST	9.6×10^{-12}	0.85	++	-	rs6859219	near	27
IL2-IL21	5.6×10^{-5}	0.78	+	NA	rs6822844	near	46
(4)Membrane receptors and costimulatory molecules							
HLA-DRB1	$<10^{-299}$	2.88	++	++	rs6910071	exon	27
FCRL3	8.5×10^{-7}	2.15	+	+	rs10430455	near	48
CD244	7.0×10^{-8}	1.31	-	+	rs6682654	intron	49
CD2-CD58	1.0×10^{-9}	1.13	++	-	rs11586238	near	44
CD28	1.3×10^{-9}	1.13	++	-	rs1980422	near	44
FCGR2A	1.5×10^{-5}	1.12	+	NA	rs12746613	near	44
CTLA4	6.3×10^{-9}	0.86	++	+	rs231735	near	27
CD40	2.8×10^{-9}	0.85	++	-	rs4810485	intron	27
(5)Enzymes							
PADI4	4.6×10^{-8}	1.97	+	++	rs766449	intron	20
PXK	3.1×10^{-14}	1.13	++	NA	rs13315591	near	27
DDX6	1.1×10^{-8}	0.87	++	-	rs10892279	near	28
(6)Unknown							
KIF5A-PIP4K2C	8.8×10^{-8}	0.89	+	-	rs1678542	near	45
C5orf30	4.1×10^{-8}	0.93	++	-	rs26232	intron	27

NA: not applicable due to the lack of polymorphism in Japanese

*Associations in Japanese are mainly based on our recent reports[29].

†, ++: $p<5\text{x}10^{-8}$, +: $1\text{x}10^{-4}<p<5\text{x}10^{-8}$ with confirmation in other studies, -: no association

Asians, such as *AIRE*, although the reasons for this are unknown.

It is noteworthy that the list of genes includes many T cell receptor (TCR) and costimulatory signal molecules, many NFκB signal molecules and some B-cell-activation molecules, clearly indicating the importance of T and B cells and inflammatory response, especially the NFκB signal pathway. Interestingly, many molecules such as PTPN22, TNFAIP3, CTLA4 and FCRL3 are negative regulators of receptor signaling.

Here we introduce some recently discovered RA-associated genetic polymorphisms.

1)CCR6

CCR6 encodes chemokine receptor 6, which is a surface marker of Th17, a subset of T helper cells producing IL-17. We identified that genetic variation of *CCR6* is associated with RA (p=7.7x10^{-19}, OR=1.19) in Japanese by the combination of GWAS and replication studies[25]. *CCR6* genetic polymorphism is also associated with RA in Caucasians (p=1.5x10^{-11}, OR=1.11)[27]. It is interesting that not only the identified marker SNP (rs3093024) but also the functional dinucleotide polymorphism (rs968334 and the adjacent new SNP: CA, CG and TG variants, TA was not detected) was found to be associated with CCR6 expression (CA< CG<TG) and serum IL-17 level. This is quite an important finding in that Th17 involvement in the RA pathogenesis was supported genetically because there are some arguments that Th17 is not as important in human RA as in the mouse arthritis models[30, 31]. *CCR6* variant is more strongly associated with ACPA (+) RA and is also associated with Graves' disease and Crohn's disease.

2)AIRE

AIRE is a key regulatory molecule of self-antigen presentation in medullary thymic epithelial cells (mTEC). *AIRE* knockout mice lack expression of organ-specific peripheral antigens (e.g. insulin, salivary protein 1, type II collagen) in the mTEC of thymus, which leads to the development of organ-specific autoimmune diseases[32]. Combination of GWAS and replication studies in Japan revealed that genetic polymorphisms of the *AIRE* gene are associated with RA[33]. There were two SNPs with genome-wide significance, one of which is located in an intron and correlated with the decreased expression of *AIRE* gene. This is in concordance with *AIRE* knockout mice developing more rapid and severe collagen-induced arthritis[34]. The other SNP is located in exon 7, which introduces amino acid alteration (S278R) at the SAND domain, and these two SNPs are in strong linkage disequilibrium. Such altered AIRE molecule may have reduced AIRE function.

3)MBP

MBP encodes myelin basic protein, which is a constituent of the myelin sheath of peripheral nerves. We conducted GWAS and replication studies with 2 different cohorts and identified *MBP* as a susceptibility gene for RA[26]. We also found that ～70% of RA patients have anti-MBP antibody in the serum. This was surprising because MBP is an autoantigen for multiple sclerosis (MS) and RA patients do not show such neurological symptoms as MS patients do. However, soon we found that this is not so surprising. First, MBP has several isoforms and the long isoform of MBP is called Golli-MBP[35, 36]. Identified SNP is located in the intron of *Golli-MBP*. *Golli-MBP* is expressed in the hematopoietic cells and was shown to function as a negative regulator of TCR signaling through PKC[37]. *Golli-MBP* knockout T cells showed stronger reaction than the wild-type T cells[38]. Moreover, we found that anti-MBP antibody in the sera of RA recognized citrullinated MBP, but not non-citrullinated MBP. Since MBP is a well-known antigen that is physiologically citrullinated and a number of citrullinated proteins are the targets of RA autoantibodies[39], it is not surprising that MBP becomes one of the targets of RA autoimmunity. However, it has not been well studied how the *MBP* polymorphism is linked to the pathogenesis of RA. The *MBP* polymorphism is not associated with RA in Caucasians.

4)TNFAIP3

The *TNFAIP3* gene encodes a cytoplasmic zinc finger protein that possesses both ubiquitination and deubiquitination properties and is a major negative regulator of TNF-induced NF-κB signaling pathways. *TNFAIP3* polymorphism showed relatively high odds ratio for RA in both Caucasians and Japanese (odds ratios of 1.22 and 1.35, respectively). Several different polymorphisms have been associated with autoimmunity, including a nonsynonymous coding SNP (Phe127Cys), with some evidence of reduced negative regulatory ability for TNF-induced NF-κB signaling[40]. In addition to *TNFAIP3*, a number of genes related to NF-κB signaling (e.g. *TRAF1, CD40, Rel* and

NFKBIE) were reported to be associated with RA, clearly indicating the importance of NF-κB signaling in the pathogenesis of RA.

In the near future: rare variants

The genetic influence of each polymorphism is very modest (OR mostly ranging from 1.1 to 1.5). Therefore, there is no obvious clinical utility to predict the development of RA with such polymorphisms. This may change as the obtained knowledge becomes more complete, but currently all the known genetic variants can explain only ～15% of the genetic component[41]. This will not change very much even though we have found >100 associated genes with common variants (SNPs). Since most of the GWASs adopt common SNPs with a population prevalence of >3-5%, there may be some rare genetic variants with high genetic impacts. Sialic acid acetylesterase (*SIAE*) is an enzyme that negatively regulates B lymphocyte antigen receptor signaling and is required for the maintenance of immunological tolerance. By sequencing the *SIAE* exons, various defective variants were found in various autoimmune diseases including RA[42]. Defective variants were found in only 2 out of 648 (0.3%) healthy European subjects, whereas 24 out of 923 (2.6%) autoimmune disease patients had defective variants (OR=8.62). The odds ratio for RA was 8.31. Although this result was not successfully replicated in a larger study[43], some unknown rare variants may have strong impacts on the development of RA.

Now that the sequencing technology has developed markedly and is becoming less expensive, finding rare genetic variants associated with RA by whole-genome sequencing is realistic. As a first step, researchers started sequencing only exons of the whole genome, which is called the exome sequence, because it is much more economical than whole-genome sequencing. However, in the very near future, it is announced that the whole-genome sequence of one person can be read for $1,000 in a day. From this point onwards, it will be more realistic to understand completely the impact of genetic variants on the development of RA.

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