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Review

Genetic basis of rheumatoid arthritis: A current review

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ABSTRACT

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases. As with other complex traits, genome-wide association studies (GWASs) have tremendously enhanced our understanding of the complex etiology of RA. In this review, we describe the genetic architecture of RA as determined through GWASs and meta-analyses. In addition, we discuss the pathologic mechanism of the disease by examining the combined findings of genetic and functional studies of individual RA-associated genes, including *HLA-DRB1*, *PADI4*, *PTPN22*, *TNFAIP3*, *STAT4*, and *CCR6*. Moreover, we briefly examine the potential use of genetic data in clinical practice in RA treatment, which represents a challenge in medical genetics in the post-GWAS era.

Contents

1. Genetic aspects of rheumatoid arthritis

Rheumatoid arthritis (RA) is one of the most common forms of autoimmune arthritis, affecting approximately 0.5–1.0% of the world's population. The serum of most RA patients contains autoantibodies, such as rheumatoid factor (RF) or anti-citrullinated protein antibodies (ACPAs), the presence of which constitutes one of the new classification criteria for RA revised in 2010 [1]. Although RF is also present in other autoimmune diseases and immunological conditions, such as chronic infection and inflammation, ACPAs have a higher specificity, suggesting a central role for citrulline as an antigenic determinant in this disease [2] (Fig. 1). This suggests that autoimmunity to citrullinated proteins may be the hallmark of RA pathogenesis. However, the rest of RA patients lack these autoantibodies, suggesting a heterogeneity in the disease etiology. In clinical practice, the appearance of biologics that target inflammatory cytokines has dramatically improved the outcome of RA, although some patients still suffer destructive arthritis that leads to disability. The limitations of current RA therapies underscore the need for further investigation of disease pathogenesis and identification of new therapeutic targets.

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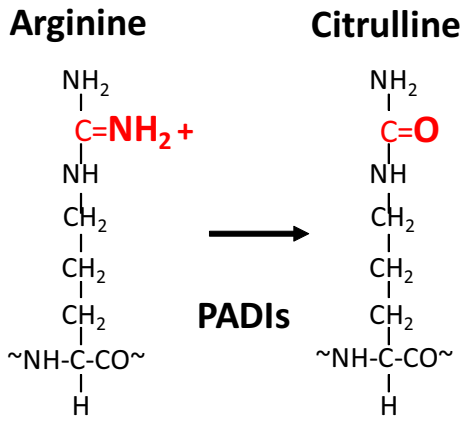

Fig. 1. Citrullination of arginine residues by PADI enzymes.

As with other complex traits, evidence from familial studies suggests that RA is caused by a combination of genetic and environmental factors. For instance, a recent population-based epidemiologic study in Sweden demonstrated that the familial odds ratio for RA is approximately 3 in first-degree relatives of RA patients and 2 in second-degree relatives [3]. In addition, higher concordance rates in monozygotic twins over dizygotic twins suggest the involvement of genetic factors [4–6]. The heritability of a disease, which is defined as the contribution of genetic variation to variation in the liability of that disease, has been estimated at around 60% in RA by the above-mentioned studies.

The establishment of a comprehensive catalog of common genetic variants in human populations by the HapMap project [7], as well as significant recent advances in genotyping technology, now enable searching of the entire genome at once for risk loci for complex diseases. This methodologic approach, now broadly known as the "genome-wide association study (GWAS)," has greatly advanced understanding of the genetic background of complex traits such as RA. In contrast, with a few exceptions, such as cigarette smoking, infections, and diet, little is known about the role of environmental factors in the development of RA. Although environmental aspects of RA are beyond the topic of this review, readers are referred to other excellent articles [8,9].

2. *HLA-DRB1* gene

Since the first evidence suggesting the involvement of human leukocyte antigens (HLAs) in RA was reported in 1969 [10], polymorphisms in the *HLA* region have been at the center of genetic studies of RA. That study demonstrated reduced lymphocyte responses in autologous mixed cultures of cells from RA patients, suggesting that polymorphisms in *HLA* genes (which encode the major histocompatibility complex [MHC] molecules that present antigens to T cells) are shared among patients [10]. Subsequently, serologic studies showed that the frequency of the HLA-DR4 serotype is higher in RA patients compared with control subjects [11,12]. Other serotypes, such as DR1, are also associated with increased risk for RA, although the increase in risk is moderate compared with that of DR4 [13]. Sequencing of *HLA-DRB1*, which encodes the polymorphic β-chain of the DR molecule, revealed that the prominent subtypes of DR4 differ between populations. For example, Europeans harbor the *04:01 and *04:04 *DRB1* alleles and East Asians harbor the *04:05 allele. In addition, several subtypes of the *DR4* allele, such as *04:02 and *04:03, were shown to protect against the disease. These observations led to the hypothesis that a conserved epitope (i.e., QKRAA/QRRAA/RRRAA) spanning amino acid residues 70–74 in the third hypervariable region of the β chain (which is now referred to as a "shared epitope [SE]") is associated with RA susceptibility [14]. Although this SE hypothesis is generally accepted, there have been several attempts to reclassify or refine it. Recently, two studies demonstrated that the amino acids at residues 11 and 13 are also independently associated with the disease, which may explain the higher risk associated with *DR4* (*04:01/*04:04/*04:05) compared with *DR1* (*01:01) [15,16].

As the importance of ACPAs in RA has become apparent over the last decade, the strong association between SE alleles and the appearance of ACPAs in RA patients has been demonstrated in multiple populations [17–20], suggesting that DR molecules encoded by SE alleles are involved in the presentation of citrullinated peptides to T cells (Fig. 2). This hypothesis is supported by the observation in human *DR4*-transgenic mice that the conversion of arginine (positively charged) to citrulline (neutral) leads to a substantial increase in HLA-peptide affinity and subsequent activation of CD4 T cells [21]. The molecular basis of these observations was determined in a recent crystal structure analysis showing that citrulline residues of peptides are accommodated within the electropositive P4 pocket of *DRB1*04:01/04, whereas the electronegative P4 pocket of the non-risk allele product *04:02 are not accommodated [22]. As the amino acid residues at positions 13 and 71 comprise the P4 pocket and directly contact the citrulline residue, the nature of these residues may be crucial in the presentation of citrullinated peptides and may explain the genetic association between *HLA-DRB1* and RA.

The primary citrullinated autoantigens that directly cause RA are poorly defined because clinical laboratory testing of serum samples from RA patients typically involves an artificial cyclic-citrullinated peptide that reacts with multiple citrullinated self proteins. However, fibrinogen, α-enolase, vimentin, immunoglobulin binding protein (BiP), and type II collagen, all of which are expressed in the synovial joint tissues, are potential candidates [23]. The primary autoantigen may differ between individuals, as a study examining patient serum samples showed that epitope spreading with an increase in the recognition of citrullinated antigens occurs before the onset of RA [24]. Differences in antibody profile between patients could depend on other genetic and environmental factors. Cigarette smoking is an environmental factor that substantially increases the risk of ACPA appearance. Intriguingly, gene-environment interactions (defined as a departure from a multiplicative model) between the *HLA-DRB1* SE allele and smoking have been reported [25–27]. Another study demonstrated that the combination of smoking and genetic factors, including *HLA-DRB1*, may determine the specificity of ACPAs in RA patients [28,29].

The association between *HLA-DRB1* and ACPA-negative RA has been relatively understudied due to the higher prevalence of APCA-positive RA. In Europeans, *HLA-DR3* (*DRB1*03:01) is associated with ACPA-negative RA [30,31]. A study of Japanese populations (in which the *DRB1*03:01 allele is rare) indicated that both ACPA- and RF-negative RA are associated with *DR14* and the *HLA-DR8* homozygote [32]. These observations suggest that the contribution of *HLA-DRB1* alleles is distinctly different in ACPA-negative RA. However, the lack of a specific serologic test for ACPA-negative RA could result in heterogeneity in studies of different cohorts. To overcome this problem, a recent study of ACPA-negative patients that statistically adjusted for the clinical heterogeneity of ACPA-negative RA identified two independent association signals in the *HLA-DRB1* and *HLA-B* gene products: serine 11 (encoded by *DRB1*03*) and aspartate 9 (encoded by *HLA-B*08*), respectively, providing additional evidence that ACPA-positive and -negative RA are genetically distinct [33].

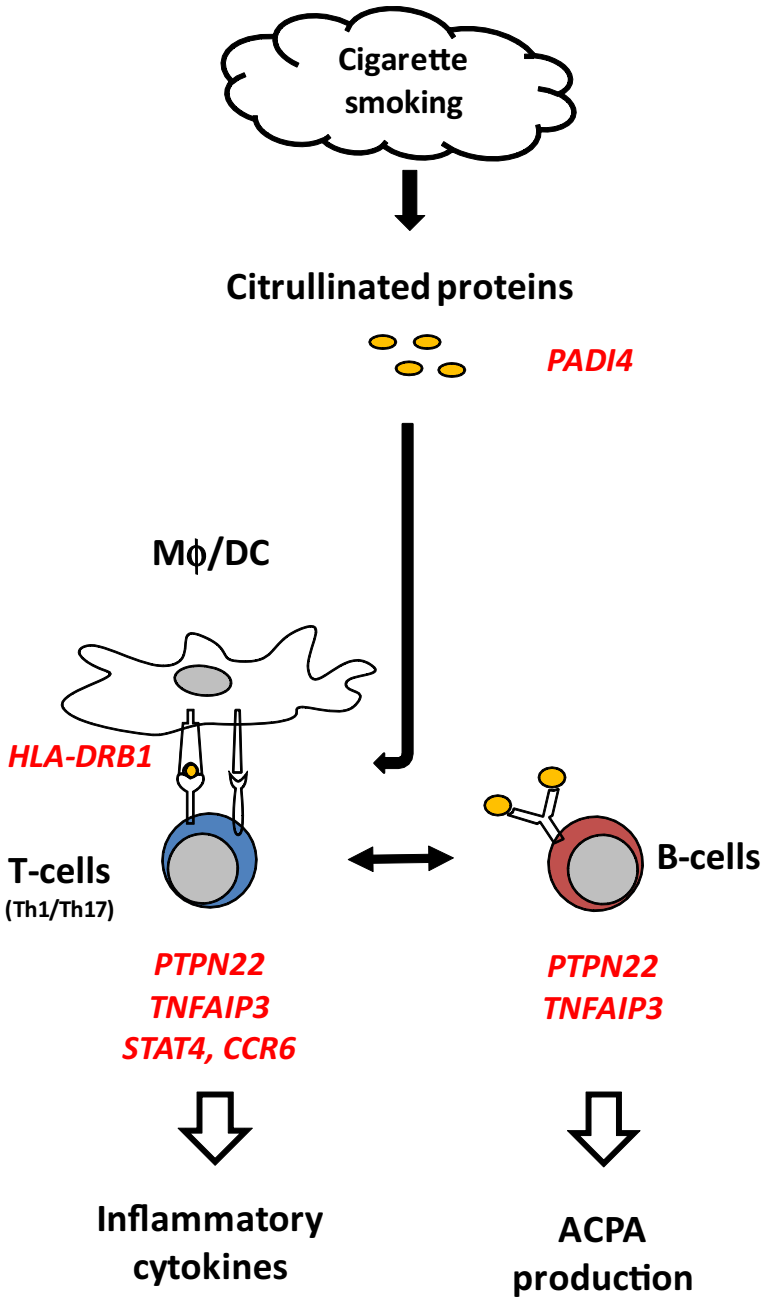

Fig. 2. Genetic factors involving autoimmunity to citrullinated proteins.

3. Insights from GWASs

In a GWAS, ~1 million single-nucleotide polymorphisms (SNPs) are simultaneously genotyped for affected patients (cases) and non-affected individuals (controls). The null hypothesis of a GWAS is that there is no association between a given SNP and disease susceptibility and is tested by comparing the allele frequency or genotype frequency between cases and controls. If the null hypothesis is rejected with a genome-wide significance level, which is usually set at $\alpha = 5 \times 10^{-8}$, the genetic marker indicates the presence of a causal variant(s) in the locus. Following the first GWAS, which was performed in a Japanese population and covered only the gene regions and not the intergenic regions [34], multiple GWASs have been performed in worldwide populations. These individual GWASs identified a number of RA-susceptibility loci, including *PADI4* [34], *PTPN22* [35], *TNFAIP3* [36], *TRAF1/C5* [37], *REL* [38], and *CCR6* [39]. However, each of these studies lacked sufficient statistical power to detect loci that have a moderate effect size. To overcome this limitation, meta-analyses of GWASs of both European and Asian populations were conducted, which increased the number of risk loci [40,41]. More recently, a multi-ethnic meta-analysis of GWASs was performed that involved collaboration between 25 study groups worldwide and a total of over 100,000 subjects (29,880 RA cases and 73,758 controls) of European or Asian ancestry [42]. This was the largest meta-analysis of autoimmune disease GWASs ever performed and identified 101 RA risk loci.

Although a GWAS can identify disease risk loci, it can directly identify neither the responsible genes nor disease-causing variants in the loci. The disease-causing variants can affect the function of the responsible genes by (1) introducing stop codons or frame-shift mutations, (2) changing the amino acid sequence, (3) affecting

alternative splicing, or (4) regulating the level of transcript expression. Among the 100 risk-associated SNPs in non-HLA RA risk loci, only 16% are in linkage disequilibrium with missense SNPs, indicating that the majority of causal variants in the risk loci affect splicing or the level of gene expression. In fact, RA-risk SNPs were found in 44 *cis*-acting expression quantitative trait loci (*cis*-eQTL) identified in peripheral blood mononuclear cells [43], indicating that disease-causing variants in the risk loci affect the expression level of genes in *cis*. Similar observations have been reported for other autoimmune diseases, indicating that the accumulation of quantitative differences in risk genes leads to disease onset [44,45].

As the regulation of gene expression in cells of the immune system, including T cells, B cells, and macrophages, is quite sophisticated, the *cis*-eQTL effects may also be cell specific. Data from recent human genome studies, such as the Encyclopedia of DNA Elements (*ENCODE*) project, provide clues that may help elucidate the underlying mechanism of cell-specific eQTL effects for many loci identified in GWASs. Using omics data (e.g., genomic, epigenomic, transcriptomic) obtained via next-generation sequencing technologies, the ENCODE project developed a comprehensive "parts list" of functional elements in the human genome that included descriptions of regulatory elements that control cells and the circumstances under which a given gene is active [46]. Analyses of non-HLA RA risk loci for enrichment in epigenetic chromatin marks revealed significant trimethylation of histone H3 at lysine 4 (H3K4me3), which is a promoter- and enhancer-specific modification associated with active transcription [47]. Among 34 cell types investigated, H3K4me3 peaks were particularly enriched in primary $CD4^+$ regulatory T cells (Treg cells) [42]. This observation suggests that a substantial proportion of RA risk variants are involved in transcriptional regulation of genes in Treg cells and that modulating the activity of Treg cells by targeting these GWAS-identified genes could be used to treat RA.

4. RA risk genes and pathogenesis

As mentioned above, GWASs have identified more than 100 RA risk loci. Although the effect of each individual locus is moderate (e.g., the odds ratio for most individual alleles ranges between 1.1 and 1.3), detailed analyses of individual loci to identify disease-causing variants and to determine the effect of the identified variants on responsible genes (e.g., gain-of-function or loss-of-function) would enhance our understanding of the disease. Examples of RA risk genes and their role in the pathogenesis of RA are discussed below.

4.1. PADI4

PADI4 was the first RA susceptibility gene identified in a GWAS of an Asian population [34]. *PADI4* is a member of the peptidyl arginine deaminase gene family and encodes an enzyme that converts arginine into citrulline in a posttranslational modification (Fig. 1). Although the physiological role of citrullination of proteins is not well understood, the specific presence of autoantibodies to citrullinated proteins (i.e., ACPAs) in RA supports the hypothesis that citrullination of autoantigens leads to autoimmunity in RA. Through in vitro assays, we have shown that transcripts of the risk haplotype of *PADI4* are more stable than transcripts of the non-risk haplotype, suggesting that increased expression and function of PADI4 could increase the risk of developing RA. Interestingly, the effect size of *PADI4* variants on the risk of developing disease differs between European and Asian populations, with greater effects observed in Asian populations [48]. One possible explanation for this genetic heterogeneity is the impact of environmental factors. For example, we demonstrated that *PADI4* variants exert an epistatic effect in conjunction with cigarette smoking, especially in males [49]. Because 40–60% of East Asian males smoke, compared with 10–30% of European males, the higher effect size of *PADI4* in Asian populations may be partially explained by the difference in smoking rates.

4.2. PTPN22

Among the RA-associated common variants outside the *HLA* region that have been identified by GWASs, the missense variant of the protein tyrosine phosphatase nonreceptor 22 (*PTPN22*) gene has the strongest effect [50]. To date, this missense variant (*PTPN22* R620W) has been associated with over 20 different autoimmune diseases in European populations, including systemic lupus erythematosus (SLE), type 1 diabetes, and Graves disease and is considered a common autoimmune gene [51]. Interestingly, this variant is very rare or is not polymorphic in Asian and African populations [52,53] and provides another example of genetic heterogeneity among populations.

PTPN22 encodes lymphoid tyrosine phosphatase (LYP), which dephosphorylates the phosphotyrosine residues of target proteins in lymphocytes. The disease-associated variant exchanges the arginine residue at position 620 in the proline-rich 1 motif to tryptophan. In vitro assays demonstrated that expression of the R620W risk allele leads to interference with the physical association between LYP and c-Src kinase (CSK), resulting in increased LYP activity. Parallel to this observation, both T-cell receptor (TCR) and B-cell receptor signaling were found to be reduced in the lymphocytes of risk allele carriers [54,55]. These observations suggest that the R620W LYP variant is a gain-of-function mutant. However, when this mutation was introduced at residue 619 of the murine ortholog of human LYP, Pep (Pep_619W), the phenotype of the knock-in mice was similar to that of Pep-deficient mice, characterized by splenomegaly and spontaneous germinal-center reactions [56]. This observation suggests that in contrast to human LYP_620W, murine Pep_619W is a loss-of-function variant. This is also supported by evidence demonstrating that TCR signaling is enhanced in both Pep-deficient and Pep_619W knock-in mice. Interestingly, an autoimmune response was observed in B6×129 Pep_619W knock-in mice, suggesting that Pep_619W triggers autoimmunity in mice in combination with other genetic factors [57]. The conflicting observations in human and mouse studies demonstrate the difficulty of translating findings from mouse models to human diseases (for a full review, see [58]).

4.3. TNFAIP3

Several GWASs almost simultaneously reported an association between the tumor necrosis factor-α-induced protein 3 (*TNFAIP3*) locus and RA [36] and SLE [59,60]. *TNFAIP3* encodes the ubiquitin-editing enzyme A20, which is a key player in the negative feedback regulation of NF-κB signaling. Two functional variants that may cause the diseases have been identified to date, one of which is a missense variant involving substitution of phenylalanine with cysteine at amino acid position 127, which in vitro assays have shown results in impaired A20 function [60]. The other variant is a TT>A polymorphic dinucleotide located 42 kb downstream of the *TNFAIP3* promoter that reduces the avidity of NF-κB subunit binding, resulting in reduced *TNFAIP3* expression [61]. Both of these variants impair the function of A20 and consequently augment NF-κB signaling. As these two variants are in linkage disequilibrium, both may simultaneously contribute to disease development. In mice, specific ablation of *Tnfaip3* in myeloid cells results in spontaneous development of a severe polyarthritis resembling RA [62]. These mice have high serum levels of inflammatory cytokines, including TNF-α, which is consistent with sus-

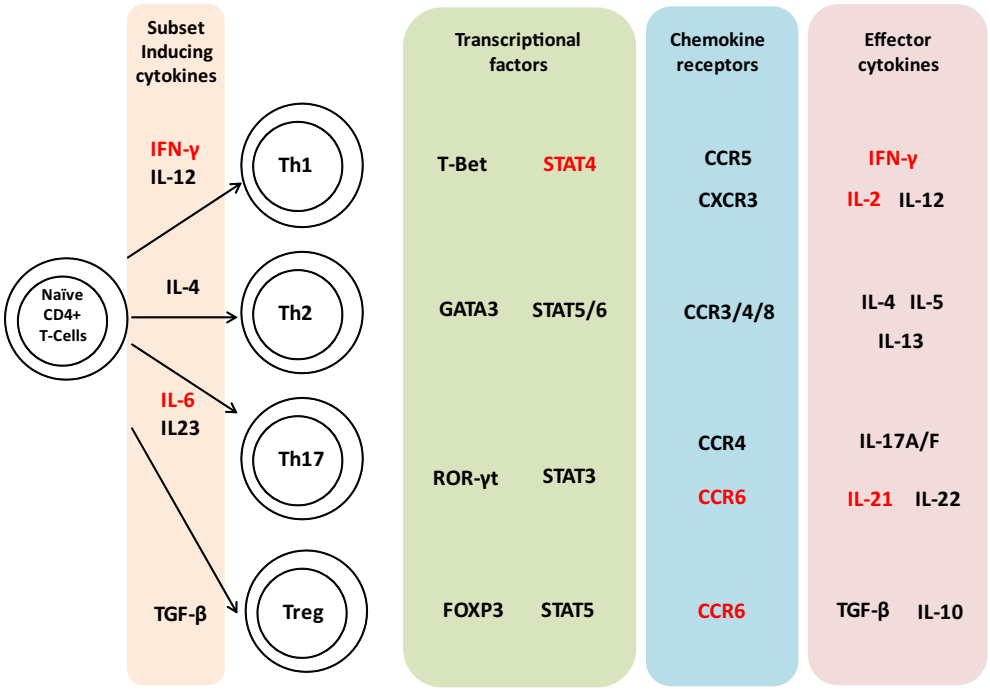

Fig. 3. $CD4^+$ helper T-cell subsets and their regulating factors.

tained NF-κB activation in macrophages. Interestingly, A20 is frequently inactivated by somatic mutations and/or deletions in B-lineage lymphomas [63]. Similar observations have been reported with somatic mutations in genes associated with lymphomas, such as *REL*, *FCRL3*, and *DDX6*, in which common variants cause RA [42], revealing contrasts in the etiologies of these diseases.

4.4. STAT4

An association between the signal transducer and activator of transcription 4 (*STAT4*) gene locus and the autoimmune diseases RA and SLE was first reported in a European population [64], and this association has been repeatedly confirmed in GWASs in multiple populations. An association between this locus and other autoimmune diseases, including systemic sclerosis and Sjögren syndrome, has also been reported [65]. Allelic expression analyses revealed that *STAT4* is overexpressed in carriers of the risk allele, suggesting that an increase in the function of the *STAT4* gene product results in these autoimmune diseases [66]. Members of the STAT family of proteins act as transcription factors; they are activated upstream by JAK proteins and mediate signaling associated with many inflammatory cytokines. STAT4 plays a critical role in type I interferon signaling. In fact, expression of the risk variant of *STAT4* is associated with greater IFN-α-induced gene expression in peripheral blood cells of SLE patients [67]. STAT family members also play essential and non-redundant roles in the differentiation of Th1, Th2, and Th17 helper proinflammatory T cells. Both Th1 and Th17 cells are thought to be involved in autoimmune disorders, as *STAT4* is involved in the differentiation of Th1 cells (Fig. 3). Interestingly, GWASs have revealed that *STAT3*, which plays a role in the differentiation of Th17 cells, is associated with the development of Crohn's disease [68], psoriasis [69], and multiple sclerosis [70]. Because Th17 cells are thought to play a significant role in the pathology of these autoimmune diseases, the *STAT3* variant may drive the activity of Th17 cells in these diseases. These observations suggest that different helper T-cell subsets may play the central role in different diseases in which the genetic factors that drive the activity of each subset also differ.

4.5. CCR6

In a GWAS in a Japanese population, we identified an association between the C—C chemokine receptor type 6 (*CCR6*) locus and RA [39]. We then examined the *CCR6* region for causal variants and identified a dinucleotide polymorphism (*CCR6DNP*) in the 5′-flanking region that influences the binding of nuclear proteins and enhances the transcriptional activity of *CCR6*. The risk allele exhibits greater enhancing activity and the level of *CCR6* transcription is higher in cells with the risk genotype. *CCR6DNP* is also associated with the positive status of IL-17A in the serum of RA patients, suggesting that *CCR6DNP* influences the activity of Th17 cells. In the SKG mouse model of arthritis, which involves a mutation in *Zap70*, Ccr6+Th17 cells are recruited into inflamed joints by the Ccr6 ligand Ccl20. Administration of anti-Ccr6 blocking antibodies substantially relieves the inflammation, suggesting that Ccr6+Th17 cells play a role in the pathogenesis of arthritis [71]. Although these data strongly support the hypothesis that Th17 cells are involved in RA, an association between the *CCR6* locus and disease development has only been confirmed for Crohn's and Basedow's diseases and not for other Th17-related diseases, such as psoriasis and multiple sclerosis, in which the *STAT3* variant increases the risk, as mentioned above.

Because the gene is also expressed in other T-cell subsets, *CCR6* can influence the activity of Treg and γδ T cells. Recently, another *CCR6*-expressing T-cell type, designated exFoxp3 Th17, was identified in a murine arthritis model [72]. In this model, CD25 lo Foxp3+ $CD4^+$ T cells cease to express Foxp3 and undergo transdifferentiation into Th17 cells. These exFoxp3 Th17 cells are more potent osteoclastogenic T cells than are naïve $CD4^+$ T cell-derived Th17 cells, although the roles of counterpart cells in humans is not clear. Taken together, these observations suggest that the differential effects of *CCR6* variants in different autoimmune diseases may be linked to the cells that drive the diseases.

5. Missing heritability

The GWAS approach has proven to be a powerful means of identifying risk loci that control complex traits under the common disease–common variant hypothesis, which assumes that common variants of modest effect are responsible for common diseases [73]. However, it is becoming apparent that common variants can explain only a small proportion of the heritability of these diseases. In RA, the 100 risk loci identified outside the *HLA* region explain only 5.5% and 4.7% of the total risk of developing the disease (which involves both genetic and environmental components) in Europeans and Asians, respectively [42]. An analysis of GWAS data using a Bayesian inference approach estimated that hundreds to thousands of associated loci harboring common causal variants, including *HLA-DRB1* and GWAS-identified loci, could explain only ~30% of the disease risk (about a half of heritability) [74]. The remaining heritability could be explained by the effects of rare variants, which are usually defined as minor allele frequencies <1%. The signals from rare variants are difficult to detect in a conventional GWAS because the majority of genetic markers used in this type of study are common variants. However, the emergence of next-generation sequencing technologies within the last 5 years now enable resequencing of the entire genome. Among the rare variants in the coding region, missense variants predicted to be damaging are more prevalent than variants predicted to be benign, whereas most common variants are predicted to be benign, consistent with studies demonstrating that rare variants in coding regions are under purifying selection [75]. This evidence suggests that the contribution of each individual variant to disease development should be higher for a rare missense variant than a common missense variant, warranting the sequencing of protein-coding regions based on priority. In a recent study attempting to determine the roles of rare variants in RA, deep exon sequencing of 25 biological candidate genes from GWAS-identified loci was performed and resulted in the identification of an accumulation of missense variants in the *IL2RA* and *IL2RB* genes [76]. A more comprehensive approach involves whole-exome sequencing, which decodes all protein-coding genes. However, to date no study has succeeded in identifying RA-associated rare variants using whole-exome sequencing, primarily due to insufficient statistical power. For example, a causal rare variant with a frequency of 0.2% and relative risk of 10 using a sample set of 200 cases and 200 controls has only 0.2% power to be detected at the conventional GWAS significance threshold ($\alpha = 5 \times 10^{-8}$) [75], indicating that lower-cost sequencing technologies that provide greater statistical power are needed to analyze rare variants.

6. Clinical use of genetic data in RA

In the final section of this review, we discuss the use of genetic data in clinical practice as it pertains to treating RA. The use of genetic data represents a challenge in the post-GWAS era because RA is a very heterogeneous disease with an outcome that is difficult to predict. The heterogeneity of RA can be partially explained by genetic factors; that is, the specific combination of genetic factors in an individual can determine the outcome of the disease. In this context, GWAS data can be used to predict an individual's disease phenotype. Phenotype prediction has been intensely investigated in RA with respect to two outcomes: disease severity and drug response.

The nature of progressive joint damage in RA varies considerably between individuals. Patients who would experience more rapid progression need more extensive therapy, such as the early use of biologics. Disease severity can be quantified by assessing and scoring the degree of joint damage using radiographic imaging. Regression analyses can then be performed to test associations between changes in radiologic scores and variant genotypes. The most extensively investigated gene to date is *HLA-DRB1*, which has also been shown to have the strongest effect on the severity of disease [77–79]. In addition, several studies have reported potential associations between various candidate genes and disease severity [80–84]. GWAS-identified loci have also been investigated, and a recent analysis involving a Japanese cohort demonstrated that polymorphisms in *PADI4* are associated with radiographic progression of RA [79]. More recently, a GWAS on the radiological progression rate in autoantibody-positive RA patients identified an association in an SNP at *SPAG16* gene, which is shown to be expressed in the synovial tissues of RA patients [85]. Although these lines of evidence indicate that genetic variants can influence on the severity of disease, individual alleles of these genes could not predict disease severity sufficiently for clinical practice use due to their moderate effect.

Another important clinical phenotype that ideally should be predictable based on genetic data is an individual's response to a drug. The advent of biologic therapies such as treatment with anti-TNF antibodies has revolutionized the treatment of RA, but a substantial proportion of patients (20–40%) will not respond to these therapies. In addition, some patients who do not respond to one biologic therapy (e.g., anti-TNF antibodies) may respond to another (e.g., anti-IL-6R antibodies). Therefore, if the response to a biologic agent can be predicted, unnecessary costs and potential side effects can be avoided. Several GWASs examining the response to anti-TNF antibody therapy provided evidence suggesting an association between drug response and genes involved in signaling, including the *CD84* locus [86–88]. However, as with the prediction of disease severity, individual loci cannot sufficiently predict an individual's drug response. The observations resulting from attempts to predict both disease severity and drug response clearly indicate that single genetic factors are insufficient for predicting clinical phenotype and that we need to establish a polygenic approach in combination with analyses of environmental factors using appropriate statistical models.

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References

[1] D. Aletaha, T. Neogi, A.J. Silman, J. Funovits, D.T. Felson, C.O. Bingham 3rd, N.S. Birnbaum, G.R. Burmester, V.P. Bykerk, M.D. Cohen, B. Combe, K.H. Costenbader, M. Dougados, P. Emery, G. Ferraccioli, J.M. Hazes, K. Hobbs, T.W. Huizinga, A. Kavanaugh, J. Kay, T.K. Kvien, T. Laing, P. Mease, H.A. Menard, L.W. Moreland, R.L. Naden, T. Pincus, J.S. Smolen, E. Stanislawska-Biernat, D. Symmons, P.P. Tak, K.S. Upchurch, J. Vencovsky, F. Wolfe, G. Hawker, Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative, Arthritis Rheum. 62 (2010) (2010) 2569–2581.

[2] G.A. Schellekens, B.A. de Jong, F.H. van den Hoogen, L.B. van de Putte, W.J. van Venrooij, Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies, J. Clin. Invest. 101 (1998) 273–281.

[3] T. Frisell, M. Holmqvist, H. Kallberg, L. Klareskog, L. Alfredsson, J. Askling, Familial risks and heritability of rheumatoid arthritis: role of rheumatoid factor/anti-citrullinated protein antibody status, number and type of affected relatives, sex, and age, Arthritis Rheum. 65 (2013) 2773–2782.

[4] A.J. Silman, A.J. MacGregor, W. Thomson, S. Holligan, D. Carthy, A. Farhan, W.E. Ollier, Twin concordance rates for rheumatoid arthritis: results from a nationwide study, Br. J. Rheumatol. 32 (1993) 903–907.

[5] A.J. MacGregor, H. Snieder, A.S. Rigby, M. Koskenvuo, J. Kaprio, K. Aho, A.J. Silman, Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins, Arthritis Rheum. 43 (2000) 30–37.

[6] D. van der Woude, J.J. Houwing-Duistermaat, R.E. Toes, T.W. Huizinga, W. Thomson, J. Worthington, A.H. van der Helm-van Mil, R.R. de Vries, Quantitative heritability of anti-citrullinated protein antibody-positive and

anti-citrullinated protein antibody-negative rheumatoid arthritis, Arthritis Rheum. 60 (2009) 916–923.
[7] International HapMap Consortium, The International HapMap Project, Nature 426 (2003) 789–796.
[8] K.P. Liao, L. Alfredsson, E.W. Karlson, Environmental influences on risk for rheumatoid arthritis, Curr. Opin. Rheumatol. 21 (2009) 279–283.
[9] R.A. Hoovestol, T.R. Mikuls, Environmental exposures and rheumatoid arthritis risk, Curr. Rheumatol. Rep. 13 (2011) 431–439.
[10] G.P. Astorga, R.C. Williams Jr., Altered reactivity in mixed lymphocyte culture of lymphocytes from patients with rheumatoid arthritis, Arthritis Rheum. 12 (1969) 547–554.
[11] A.J. McMichael, T. Sasazuki, H.O. McDevitt, R.O. Payne, Increased frequency of HLA-Cw3 and HLA-Dw4 in rheumatoid arthritis, Arthritis Rheum. 20 (1977) 1037–1042.
[12] P. Stastny, Association of the B-cell alloantigen DRw4 with rheumatoid arthritis, N. Engl. J. Med. 298 (1978) 869–871.
[13] L. Legrand, G.M. Lathrop, A. Marcelli-Barge, A. Dryll, T. Bardin, N. Debeyre, J.C. Poirier, M. Schmid, A. Ryckewaert, J. Dausset, HLA-DR genotype risks in seropositive rheumatoid arthritis, Am. J. Hum. Genet. 36 (1984) 690–699.
[14] P.K. Gregersen, J. Silver, R.J. Winchester, The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis, Arthritis Rheum. 30 (1987) 1205–1213.
[15] B.M. Freed, R.P. Schuyler, M.T. Aubrey, Association of the HLA-DRB1 epitope LA(67, 74) with rheumatoid arthritis and citrullinated vimentin binding, Arthritis Rheum. 63 (2011) 3733–3739.
[16] S. Raychaudhuri, C. Sandor, E.A. Stahl, J. Freudenberg, H.S. Lee, X. Jia, L. Alfredsson, L. Padyukov, L. Klareskog, J. Worthington, K.A. Siminovitch, S.C. Bae, R.M. Plenge, P.K. Gregersen, P.I. de Bakker, Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis, Nat. Genet. 44 (2012) 291–296.
[17] I. Auger, M. Sebbag, C. Vincent, N. Balandraud, S. Guis, L. Nogueira, B. Svensson, A. Cantagrel, G. Serre, J. Roudier, Influence of HLA-DR genes on the production of rheumatoid arthritis-specific autoantibodies to citrullinated fibrinogen, Arthritis Rheum. 52 (2005) 3424–3432.
[18] F.A. van Gaalen, J. van Aken, T.W. Huizinga, G.M. Schreuder, F.C. Breedveld, E. Zanelli, W.J. van Venrooij, C.L. Verweij, R.E. Toes, R.R. de Vries, Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis, Arthritis Rheum. 50 (2004) 2113–2121.
[19] S. Bas, T.V. Perneger, E. Mikhnevitch, M. Seitz, J.M. Tiercy, P. Roux-Lombard, P.A. Guerne, Association of rheumatoid factors and anti-filaggrin antibodies with severity of erosions in rheumatoid arthritis, Rheumatology (Oxford) 39 (2000) 1082–1088.
[20] K. Shimane, Y. Kochi, A. Suzuki, Y. Okada, T. Ishii, T. Horita, K. Saito, A. Okamoto, N. Nishimoto, K. Myouzen, M. Kubo, M. Hirakata, T. Sumida, Y. Takasaki, R. Yamada, Y. Nakamura, N. Kamatani, K. Yamamoto, An association analysis of HLA-DRB1 with systemic lupus erythematosus and rheumatoid arthritis in a Japanese population: effects of *09:01 allele on disease phenotypes, Rheumatology (Oxford) 52 (2013) 1172–1182.
[21] J.A. Hill, S. Southwood, A. Sette, A.M. Jevnikar, D.A. Bell, E. Cairns, Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule, J. Immunol. 171 (2003) 538–541.
[22] S.W. Scally, J. Petersen, S.C. Law, N.L. Dudek, H.J. Nel, K.L. Loh, L.C. Wijeyewickrema, S.B. Eckle, J. van Heemst, R.N. Pike, J. McCluskey, R.E. Toes, N.L. La Gruta, A.W. Purcell, H.H. Reid, R. Thomas, J. Rossjohn, A molecular basis for the association of the HLA-DRB1 locus, citrullination, and rheumatoid arthritis, J. Exp. Med. 210 (2013) 2569–2582.
[23] L. Klareskog, J. Ronnelid, K. Lundberg, L. Padyukov, L. Alfredsson, Immunity to citrullinated proteins in rheumatoid arthritis, Annu. Rev. Immunol. 26 (2008) 651–675.
[24] D. van der Woude, S. Rantapaa-Dahlqvist, A. Ioan-Facsinay, C. Onnekink, C.M. Schwarte, K.N. Verpoort, J.W. Drijfhout, T.W. Huizinga, R.E. Toes, G.J. Pruijn, Epitope spreading of the anti-citrullinated protein antibody response occurs before disease onset and is associated with the disease course of early arthritis, Ann. Rheum. Dis. 69 (2010) 1554–1561.
[25] L. Klareskog, P. Stolt, K. Lundberg, H. Kallberg, C. Bengtsson, J. Grunewald, J. Ronnelid, H.E. Harris, A.K. Ulfgren, S. Rantapaa-Dahlqvist, A. Eklund, L. Padyukov, L. Alfredsson, A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination, Arthritis Rheum. 54 (2006) 38–46.
[26] S.P. Linn-Rasker, A.H. van der Helm-van Mil, F.A. van Gaalen, M. Kloppenburg, R.R. de Vries, S. le Cessie, F.C. Breedveld, R.E. Toes, T.W. Huizinga, Smoking is a risk factor for anti-CCP antibodies only in rheumatoid arthritis patients who carry HLA-DRB1 shared epitope alleles, Ann. Rheum. Dis. 65 (2006) 366–371.
[27] H. Kallberg, L. Padyukov, R.M. Plenge, J. Ronnelid, P.K. Gregersen, A.H. van der Helm-van Mil, R.E. Toes, T.W. Huizinga, L. Klareskog, L. Alfredsson, Gene-gene and gene-environment interactions involving HLA-DRB1, PTPN22, and smoking in two subsets of rheumatoid arthritis, Am. J. Hum. Genet. 80 (2007) 867–875.
[28] K. Lundberg, C. Bengtsson, N. Kharlamova, E. Reed, X. Jiang, H. Kallberg, I. Pollak-Dorocic, L. Israelsson, C. Kessel, L. Padyukov, R. Holmdahl, L. Alfredsson, L. Klareskog, Genetic and environmental determinants for disease risk in subsets of rheumatoid arthritis defined by the anticitrullinated protein/peptide antibody fine specificity profile, Ann. Rheum. Dis. 72 (2013) 652–658.
[29] H. Mahdi, B.A. Fisher, H. Kallberg, D. Plant, V. Malmstrom, J. Ronnelid, P. Charles, B. Ding, L. Alfredsson, L. Padyukov, D.P. Symmons, P.J. Venables, L. Klareskog, K. Lundberg, Specific interaction between genotype, smoking and autoimmunity to citrullinated alpha-enolase in the etiology of rheumatoid arthritis, Nat. Genet. 41 (2009) 1319–1324.
[30] K.N. Verpoort, F.A. van Gaalen, A.H. van der Helm-van Mil, G.M. Schreuder, F.C. Breedveld, T.W. Huizinga, R.R. de Vries, R.E. Toes, Association of HLA-DR3 with anti-cyclic citrullinated peptide antibody-negative rheumatoid arthritis, Arthritis Rheum. 52 (2005) 3058–3062.
[31] P. Irigoyen, A.T. Lee, M.H. Wener, W. Li, M. Kern, F. Batliwalla, R.F. Lum, E. Massarotti, M. Weisman, C. Bombardier, E.F. Remmers, D.L. Kastner, M.F. Seldin, L.A. Criswell, P.K. Gregersen, Regulation of anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis: contrasting effects of HLA-DR3 and the shared epitope alleles, Arthritis Rheum. 52 (2005) 3813–3818.
[32] C. Terao, K. Ohmura, K. Ikari, Y. Kochi, E. Maruya, M. Katayama, K. Yurugi, K. Shimada, A. Murasawa, S. Honjo, K. Takasugi, K. Matsuo, K. Tajima, A. Suzuki, K. Yamamoto, S. Momohara, H. Yamanaka, R. Yamada, H. Saji, F. Matsuda, T. Mimori, ACPA-negative RA consists of two genetically distinct subsets based on RF positivity in Japanese, PLoS One 7 (2012) e40067.
[33] B. Han, D. Diogo, S. Eyre, H. Kallberg, A. Zhernakova, J. Bowes, L. Padyukov, Y. Okada, M.A. Gonzalez-Gay, S. Rantapaa-Dahlqvist, J. Martin, T.W. Huizinga, R.M. Plenge, J. Worthington, P.K. Gregersen, L. Klareskog, P.I. de Bakker, S. Raychaudhuri, Fine mapping seronegative and seropositive rheumatoid arthritis to shared and distinct HLA alleles by adjusting for the effects of heterogeneity, Am. J. Hum. Genet. 94 (2014) 522–532.
[34] A. Suzuki, R. Yamada, X. Chang, S. Tokuhiro, T. Sawada, M. Suzuki, M. Nagasaki, M. Nakayama-Hamada, R. Kawaida, M. Ono, M. Ohtsuki, H. Furukawa, S. Yoshino, M. Yukioka, S. Tohma, T. Matsubara, S. Wakitani, R. Teshima, Y. Nishioka, A. Sekine, A. Iida, A. Takahashi, T. Tsunoda, Y. Nakamura, K. Yamamoto, Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis, Nat. Genet. 34 (2003) 395–402.
[35] Wellcome Trust Case Control Consortium, Genome-wide association study of 14,000 cases of seven common diseases and 3000 shared controls, Nature 447 (2007) 661–678.
[36] W. Thomson, A. Barton, X. Ke, S. Eyre, A. Hinks, J. Bowes, R. Donn, D. Symmons, S. Hider, I.N. Bruce, A.G. Wilson, I. Marinou, A. Morgan, P. Emery, A. Carter, S. Steer, L. Hocking, D.M. Reid, P. Wordsworth, P. Harrison, D. Strachan, J. Worthington, Rheumatoid arthritis association at 6q23, Nat. Genet. 39 (2007) 1431–1433.
[37] R.M. Plenge, M. Seielstad, L. Padyukov, A.T. Lee, E.F. Remmers, B. Ding, A. Liew, H. Khalili, A. Chandrasekaran, L.R. Davies, W. Li, A.K. Tan, C. Bonnard, R.T. Ong, A. Thalamuthu, S. Pettersson, C. Liu, C. Tian, W.V. Chen, J.P. Carulli, E.M. Beckman, D. Altshuler, L. Alfredsson, L.A. Criswell, C.I. Amos, M.F. Seldin, D.L. Kastner, L. Klareskog, P.K. Gregersen, TRAF1-C5 as a risk locus for rheumatoid arthritis – a genomewide study, N. Engl. J. Med. 357 (2007) 1199–1209.
[38] P.K. Gregersen, C.I. Amos, A.T. Lee, Y. Lu, E.F. Remmers, D.L. Kastner, M.F. Seldin, L.A. Criswell, R.M. Plenge, V.M. Holers, T.R. Mikuls, T. Sokka, L.W. Moreland, S.L. Bridges Jr., G. Xie, A.B. Begovich, K.A. Siminovitch, REL, encoding a member of the NF-kappaB family of transcription factors, is a newly defined risk locus for rheumatoid arthritis, Nat. Genet. 41 (2009) 820–823.
[39] Y. Kochi, Y. Okada, A. Suzuki, K. Ikari, C. Terao, A. Takahashi, K. Yamazaki, N. Hosono, K. Myouzen, T. Tsunoda, N. Kamatani, T. Furuichi, S. Ikegawa, K. Ohmura, T. Mimori, F. Matsuda, T. Iwamoto, S. Momohara, H. Yamanaka, R. Yamada, M. Kubo, Y. Nakamura, K. Yamamoto, A regulatory variant in CCR6 is associated with rheumatoid arthritis susceptibility, Nat. Genet. 42 (2010) 515–519.
[40] E.A. Stahl, S. Raychaudhuri, E.F. Remmers, G. Xie, S. Eyre, B.P. Thomson, Y. Li, F.A. Kurreeman, A. Zhernakova, A. Hinks, C. Guiducci, R. Chen, L. Alfredsson, C.I. Amos, K.G. Ardlie, A. Barton, J. Bowes, E. Brouwer, N.P. Burtt, J.J. Catanese, J. Coblyn, M.J. Coenen, K.H. Costenbader, L.A. Criswell, J.B. Crusius, J. Cui, P.I. de Bakker, P.L. De Jager, B. Ding, P. Emery, E. Flynn, P. Harrison, L.J. Hocking, T.W. Huizinga, D.L. Kastner, X. Ke, A.T. Lee, X. Liu, P. Martin, A.W. Morgan, L. Padyukov, M.D. Posthumus, T.R. Radstake, D.M. Reid, M. Seielstad, M.F. Seldin, N.A. Shadick, S. Steer, P.P. Tak, W. Thomson, A.H. van der Helm-van Mil, I.E. van der Horst-Bruinsma, C.E. van der Schoot, P.L. van Riel, M.E. Weinblatt, A.G. Wilson, G.J. Wolbink, B.P. Wordsworth, C. Wijmenga, E.W. Karlson, R.E. Toes, N. de Vries, A.B. Begovich, J. Worthington, K.A. Siminovitch, P.K. Gregersen, L. Klareskog, R.M. Plenge, Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci, Nat. Genet. 42 (2010) 508–514.
[41] Y. Okada, C. Terao, K. Ikari, Y. Kochi, K. Ohmura, A. Suzuki, T. Kawaguchi, E.A. Stahl, F.A. Kurreeman, N. Nishida, H. Ohmiya, K. Myouzen, M. Takahashi, T. Sawada, Y. Nishioka, M. Yukioka, T. Matsubara, S. Wakitani, R. Teshima, S. Tohma, K. Takasugi, K. Shimada, A. Murasawa, S. Honjo, K. Matsuo, H. Tanaka, K. Tajima, T. Suzuki, T. Iwamoto, Y. Kawamura, H. Tanii, Y. Okazaki, T. Sasaki, P.K. Gregersen, L. Padyukov, J. Worthington, K.A. Siminovitch, M. Lathrop, A. Taniguchi, A. Takahashi, K. Tokunaga, M. Kubo, Y. Nakamura, N. Kamatani, T. Mimori, R.M. Plenge, H. Yamanaka, S. Momohara, R. Yamada, F. Matsuda, K. Yamamoto, Meta-analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population, Nat. Genet. 44 (2012) 511–516.
[42] Y. Okada, D. Wu, G. Trynka, T. Raj, C. Terao, K. Ikari, Y. Kochi, K. Ohmura, A. Suzuki, S. Yoshida, R.R. Graham, A. Manoharan, W. Ortmann, T. Bhangale, J.C. Denny, R.J. Carroll, A.E. Eyler, J.D. Greenberg, J.M. Kremer, D.A. Pappas, L. Jiang, J. Yin, L. Ye, D.F. Su, J. Yang, G. Xie, E. Keystone, H.J. Westra, T. Esko, A. Metspalu, X. Zhou, N. Gupta, D. Mirel, E.A. Stahl, D. Diogo, J. Cui, K. Liao, M.H.

Guo, K. Myouzen, T. Kawaguchi, M.J. Coenen, P.L. van Riel, M.A. van de Laar, H.J. Guchelaar, T.W. Huizinga, P. Dieude, X. Mariette, S.L. Bridges Jr., A. Zhernakova, R.E. Toes, P.P. Tak, C. Miceli-Richard, S.Y. Bang, H.S. Lee, J. Martin, M.A. Gonzalez-Gay, L. Rodriguez-Rodriguez, S. Rantapaa-Dahlqvist, L. Arlestig, H.K. Choi, Y. Kamatani, P. Galan, M. Lathrop, R. consortium, G. consortium, S. Eyre, J. Bowes, N. de Vries, L.W. Moreland, L.A. Criswell, E.W. Karlson, A. Taniguchi, R. Yamada, M. Kubo, J.S. Liu, S.C. Bae, J. Worthington, L. Padyukov, L. Klareskog, P.K. Gregersen, S. Raychaudhuri, B.E. Stranger, P.L. De Jager, L. Franke, P.M. Visscher, M.A. Brown, H. Yamanaka, T. Mimori, A. Takahashi, H. Xu, T.W. Behrens, K.A. Siminovitch, S. Momohara, F. Matsuda, K. Yamamoto, R.M. Plenge, Genetics of rheumatoid arthritis contributes to biology and drug discovery, Nature 506 (2014) 376–381.

[43] H.J. Westra, M.J. Peters, T. Esko, H. Yaghootkar, C. Schurmann, J. Kettunen, M.W. Christiansen, B.P. Fairfax, K. Schramm, J.E. Powell, A. Zhernakova, D.V. Zhernakova, J.H. Veldink, L.H. Van den Berg, J. Karjalainen, S. Withoff, A.G. Uitterlinden, A. Hofman, F. Rivadeneira, P.A. t Hoen, E. Reinmaa, K. Fischer, M. Nelis, L. Milani, D. Melzer, L. Ferrucci, A.B. Singleton, D.G. Hernandez, M.A. Nalls, G. Homuth, M. Nauck, D. Radke, U. Volker, M. Perola, V. Salomaa, J. Brody, A. Suchy-Dicey, S.A. Gharib, D.A. Enquobahrie, T. Lumley, G.W. Montgomery, S. Makino, H. Prokisch, C. Herder, M. Roden, H. Grallert, T. Meitinger, K. Strauch, Y. Li, R.C. Jansen, P.M. Visscher, J.C. Knight, B.M. Psaty, S. Ripatti, A. Teumer, T.M. Frayling, A. Metspalu, J.B. van Meurs, L. Franke, Systematic identification of trans eQTLs as putative drivers of known disease associations, Nat. Genet. 45 (2013) 1238–1243.

[44] P.C. Dubois, G. Trynka, L. Franke, K.A. Hunt, J. Romanos, A. Curtotti, A. Zhernakova, G.A. Heap, R. Adany, A. Aromaa, M.T. Bardella, L.H. van den Berg, N.A. Bockett, E.G. de la Concha, B. Dema, R.S. Fehrmann, M. Fernandez-Arquero, S. Fiatal, E. Grandone, P.M. Green, H.J. Groen, R. Gwilliam, R.H. Houwen, S.E. Hunt, K. Kaukinen, D. Kelleher, I. Korponay-Szabo, K. Kurppa, P. MacMathuna, M. Maki, M.C. Mazzilli, O.T. McCann, M.L. Mearin, C.A. Mein, M.M. Mirza, V. Mistry, B. Mora, K.I. Morley, C.J. Mulder, J.A. Murray, C. Nunez, E. Oosterom, R.A. Ophoff, I. Polanco, L. Peltonen, M. Platteel, A. Rybak, V. Salomaa, J.J. Schweizer, M.P. Sperandeo, G.J. Tack, G. Turner, J.H. Veldink, W.H. Verbeek, R.K. Weersma, V.M. Wolters, E. Urcelay, B. Cukrowska, L. Greco, S.L. Neuhausen, R. McManus, D. Barisani, P. Deloukas, J.C. Barrett, P. Saavalainen, C. Wijmenga, D.A. van Heel, Multiple common variants for celiac disease influencing immune gene expression, Nat. Genet. 42 (2010) 295–302.

[45] Y. Okada, K. Shimane, Y. Kochi, T. Tahira, A. Suzuki, K. Higasa, A. Takahashi, T. Horita, T. Atsumi, T. Ishii, A. Okamoto, K. Fujio, M. Hirakata, H. Amano, Y. Kondo, S. Ito, K. Takada, A. Mimori, K. Saito, M. Kamachi, Y. Kawaguchi, K. Ikari, O.W. Mohammed, K. Matsuda, C. Terao, K. Ohmura, K. Myouzen, N. Hosono, T. Tsunoda, N. Nishimoto, T. Mimori, F. Matsuda, Y. Tanaka, T. Sumida, H. Yamanaka, Y. Takasaki, T. Koike, T. Horiuchi, K. Hayashi, M. Kubo, N. Kamatani, R. Yamada, Y. Nakamura, K. Yamamoto, A genome-wide association study identified AFF1 as a susceptibility locus for systemic lupus erythematosus in Japanese, PLoS Genet. 8 (2012) e1002455.

[46] E.P. Consortium, A. Kundaje, S.F. Aldred, P.J. Collins, C.A. Davis, F. Doyle, C.B. Epstein, S. Frietze, J. Harrow, R. Kaul, J. Khatun, B.R. Lajoie, S.G. Landt, B.K. Lee, F. Pauli, K.R. Rosenbloom, P. Sabo, A. Safi, A. Sanyal, N. Shoresh, J.M. Simon, L. Song, N.D. Trinklein, R.C. Altshuler, E. Birney, J.B. Brown, C. Cheng, S. Djebali, X. Dong, J. Ernst, T.S. Furey, M. Gerstein, B. Giardine, M. Greven, R.C. Hardison, R.S. Harris, J. Herrero, M.M. Hoffman, S. Iyer, M. Kelllis, P. Kheradpour, T. Lassmann, Q. Li, X. Lin, G.K. Marinov, A. Merkel, A. Mortazavi, S.C. Parker, T.E. Reddy, J. Rozowsky, F. Schlesinger, R.E. Thurman, J. Wang, L.D. Ward, T.W. Whitfield, S.P. Wilder, W. Wu, H.S. Xi, K.Y. Yip, J. Zhuang, B.E. Bernstein, E.D. Green, C. Gunter, M. Snyder, M.J. Pazin, R.F. Lowdon, L.A. Dillon, L.B. Adams, C.J. Kelly, J. Zhang, J.R. Wexler, P.J. Good, E.A. Feingold, G.E. Crawford, J. Dekker, L. Elinitski, P.J. Farnham, M.C. Giddings, T.R. Gingeras, R. Guigo, T.J. Hubbard, M. Kellis, W.J. Kent, J.D. Lieb, E.H. Margulies, R.M. Myers, J.A. Starnatoyannopoulos, S.A. Tennebaum, Z. Weng, K.P. White, B. Wold, Y. Yu, J. Wrobel, B.A. Risk, H.P. Gunawardena, H.C. Kuiper, C.W. Maier, L. Xie, X. Chen, et al., An integrated encyclopedia of DNA elements in the human genome, Nature 489 (2012) 57–74.

[47] G. Trynka, C. Sandor, B. Han, H. Xu, B.E. Stranger, X.S. Liu, S. Raychaudhuri, Chromatin marks identify critical cell types for fine mapping complex trait variants, Nat. Genet. 45 (2013) 124–130.

[48] Y. Kochi, A. Suzuki, R. Yamada, K. Yamamoto, Ethnogenetic heterogeneity of rheumatoid arthritis-implications for pathogenesis, Nat. Rev. Rheumatol. 6 (2010) 290–295.

[49] Y. Kochi, M.M. Thabet, A. Suzuki, Y. Okada, N.A. Daha, R.E. Toes, T.W. Huizinga, K. Myouzen, M. Kubo, R. Yamada, Y. Nakamura, K. Yamamoto, PADI4 polymorphism predisposes male smokers to rheumatoid arthritis, Ann. Rheum. Dis. 70 (2011) 512–515.

[50] A.B. Begovich, V.E. Carlton, L.A. Honigberg, S.J. Schrodi, A.P. Chokkalingam, H.C. Alexander, K.G. Ardlie, Q. Huang, A.M. Smith, J.M. Spoerke, M.T. Conn, M. Chang, S.Y. Chang, R.K. Saiki, J.J. Catanese, D.U. Leong, V.E. Garcia, L.B. McAllister, D.A. Jeffery, A.T. Lee, F. Batliwalla, E. Remmers, L.A. Criswell, M.F. Seldin, D.L. Kastner, C.I. Amos, J.J. Sninsky, P.K. Gregersen, A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis, Am. J. Hum. Genet. 75 (2004) 330–337.

[51] J. Zheng, S. Ibrahim, F. Petersen, X. Yu, Meta-analysis reveals an association of PTPN22 C1858T with autoimmune diseases, which depends on the localization of the affected tissue, Genes Immun. 13 (2012) 641–652.

[52] M. Mori, R. Yamada, K. Kobayashi, R. Kawaida, K. Yamamoto, Ethnic differences in allele frequency of autoimmune-disease-associated SNPs, J. Hum. Genet. 50 (2005) 264–266.

[53] T.C. Lins, R.G. Vieira, D. Grattapaglia, R.W. Pereira, Allele and haplotype frequency distribution in PTPN22 gene across variable ethnic groups: Implications for genetic association studies for autoimmune diseases, Autoimmunity 43 (2010) 308–316.

[54] A.F. Arechiga, T. Habib, Y. He, X. Zhang, Z.Y. Zhang, A. Funk, J.H. Buckner, Cutting edge: the PTPN22 allelic variant associated with autoimmunity impairs B cell signaling, J. Immunol. 182 (2009) 3343–3347.

[55] T. Vang, M. Congia, M.D. Macis, L. Musumeci, V. Orru, P. Zavattari, K. Nika, L. Tautz, K. Tasken, F. Cucca, T. Mustelin, N. Bottini, Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant, Nat. Genet. 37 (2005) 1317–1319.

[56] J. Zhang, N. Zahir, Q. Jiang, H. Miliotis, S. Heyraud, X. Meng, B. Dong, G. Xie, F. Qiu, Z. Hao, C.A. McCulloch, E.C. Keystone, A.C. Peterson, K.A. Siminovitch, The autoimmune disease-associated PTPN22 variant promotes calpain-mediated Lyp/Pep degradation associated with lymphocyte and dendritic cell hyperresponsiveness, Nat. Genet. 43 (2011) 902–907.

[57] X. Dai, R.G. James, T. Habib, S. Singh, S. Jackson, S. Khim, R.T. Moon, D. Liggitt, A. Wolf-Yadlin, J.H. Buckner, D.J. Rawlings, A disease-associated PTPN22 variant promotes systemic autoimmunity in murine models, J. Clin. Invest. 123 (2013) 2024–2036.

[58] J. Zheng, F. Petersen, X. Yu, The role of PTPN22 in autoimmunity: learning from mice, Autoimmun. Rev. 13 (2014) 266–271.

[59] R.R. Graham, C. Cotsapas, L. Davies, R. Hackett, C.J. Lessard, J.M. Leon, N.P. Burtt, C. Guiducci, M. Parkin, C. Gates, R.M. Plenge, T.W. Behrens, J.E. Wither, J.D. Rioux, P.R. Fortin, D.C. Graham, A.K. Wong, T.J. Vyse, M.J. Daly, D. Altshuler, K.L. Moser, P.M. Gaffney, Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus, Nat. Genet. 40 (2008) 1059–1061.

[60] S.L. Musone, K.E. Taylor, T.T. Lu, J. Nititham, R.C. Ferreira, W. Ortmann, N. Shifrin, M.A. Petri, M. Ilyas Kamboh, S. Manzi, M.F. Seldin, P.K. Gregersen, T.W. Behrens, A. Ma, P.Y. Kwok, L.A. Criswell, Multiple polymorphisms in the TNFAIP3 region are independently associated with systemic lupus erythematosus, Nat. Genet. 40 (2008) 1062–1064.

[61] I. Adrianto, F. Wen, A. Templeton, G. Wiley, J.B. King, C.J. Lessard, J.S. Bates, Y. Hu, J.A. Kelly, K.M. Kaufman, J.M. Guthridge, M.E. Alarcon-Riquelme, Biolupus, G. Networks, J.M. Anaya, S.C. Bae, S.Y. Bang, S.A. Boackle, E.E. Brown, M.A. Petri, C. Gallant, R. Ramsey-Goldman, J.D. Reveille, L.M. Vila, L.A. Criswell, J.C. Edberg, B.I. Freedman, P.K. Gregersen, G.S. Gilkeson, C.O. Jacob, J.A. James, D.L. Kamen, R.P. Kimberly, J. Martin, J.T. Merrill, T.B. Niewold, S.Y. Park, B.A. Pons-Estel, R.H. Scofield, A.M. Stevens, B.P. Tsao, T.J. Vyse, C.D. Langefeld, J.B. Harley, K.L. Moser, C.F. Webb, M.B. Humphrey, C.G. Montgomery, P.M. Gaffney, Association of a functional variant downstream of TNFAIP3 with systemic lupus erythematosus, Nat. Genet. 43 (2011) 253–258.

[62] M. Matmati, P. Jacques, J. Maelfait, E. Verheugen, M. Kool, M. Sze, L. Geboes, E. Louagie, C. Mc Guire, L. Vereecke, Y. Chu, L. Boon, S. Staelens, P. Matthys, B.N. Lambrecht, M. Schmidt-Supprian, M. Pasparakis, D. Elewaut, R. Beyaert, G. van Loo, A20 (TNFAIP3) deficiency in myeloid cells triggers erosive polyarthritis resembling rheumatoid arthritis, Nat. Genet. 43 (2011) 908–912.

[63] M. Kato, M. Sanada, I. Kato, Y. Sato, J. Takita, K. Takeuchi, A. Niwa, Y. Chen, K. Nakazaki, J. Nomoto, Y. Asakura, S. Muto, A. Tamura, M. Iio, Y. Akatsuka, Y. Hayashi, H. Mori, T. Igarashi, M. Kurokawa, S. Chiba, S. Mori, Y. Ishikawa, K. Okamoto, K. Tobinai, H. Nakagama, T. Nakahata, T. Yoshino, Y. Kobayashi, S. Ogawa, Frequent inactivation of A20 in B-cell lymphomas, Nature 459 (2009) 712–716.

[64] E.F. Remmers, R.M. Plenge, A.T. Lee, R.R. Graham, G. Hom, T.W. Behrens, P.I. de Bakker, J.M. Le, H.S. Lee, F. Batliwalla, W. Li, S.L. Masters, M.G. Booty, J.P. Carulli, L. Padyukov, L. Alfredsson, L. Klareskog, W.V. Chen, C.I. Amos, L.A. Criswell, M.F. Seldin, D.L. Kastner, P.K. Gregersen, STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus, N. Engl. J. Med. 357 (2007) 977–986.

[65] J. Zheng, J. Yin, R. Huang, F. Petersen, X. Yu, Meta-analysis reveals an association of STAT4 polymorphisms with systemic autoimmune disorders and anti-dsDNA antibody, Hum. Immunol. 74 (2013) 986–992.

[66] S. Sigurdsson, G. Nordmark, S. Garnier, E. Grundberg, T. Kwan, O. Nilsson, M.L. Eloranta, I. Gunnarsson, E. Svenungsson, G. Sturfelt, A.A. Bengtsson, A. Jonsen, L. Truedsson, S. Rantapaa-Dahlqvist, C. Eriksson, G. Alm, H.H. Goring, T. Pastinen, A.C. Syvanen, L. Ronnblom, A risk haplotype of STAT4 for systemic lupus erythematosus is over-expressed, correlates with anti-dsDNA and shows additive effects with two risk alleles of IRF5, Hum. Mol. Genet. 17 (2008) 2868–2876.

[67] S.N. Kariuki, K.A. Kirou, E.J. MacDermott, L. Barillas-Arias, M.K. Crow, T.B. Niewold, Cutting edge: autoimmune disease risk variant of STAT4 confers increased sensitivity to IFN-alpha in lupus patients in vivo, J. Immunol. 182 (2009) 34–38.

[68] J.C. Barrett, S. Hansoul, D.L. Nicolae, J.H. Cho, R.H. Duerr, J.D. Rioux, S.R. Brant, M.S. Silverberg, K.D. Taylor, M.M. Barmada, A. Bitton, T. Dassopoulos, L.W. Datta, T. Green, A.M. Griffiths, E.O. Kistner, M.T. Murtha, M.D. Regueiro, J.I. Rotter, L.P. Schumm, A.H. Steinhart, S.R. Targan, R.J. Xavier, C. Libioulle, C. Sandor, M. Lathrop, J. Belaiche, O. Dewit, I. Gut, S. Heath, D. Laukens, M. Mni, P. Rutgeerts, A. Van Gossum, D. Zelenika, D. Franchimont, J.P. Hugot, M. de Vos, S. Vermeire, E. Louis, L.R. Cardon, C.A. Anderson, H. Drummond, E. Nimmo, T. Ahmad, N.J. Prescott, C.M. Onnie, S.A. Fisher, J. Marchini, J. Ghori, S. Bumpstead,

R. Gwilliam, M. Tremelling, P. Deloukas, J. Mansfield, D. Jewell, J. Satsangi, C.G. Mathew, M. Parkes, M. Georges, M.J. Daly, Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease, Nat. Genet. 40 (2008) 955–962.

[69] L.C. Tsoi, S.L. Spain, J. Knight, E. Ellinghaus, P.E. Stuart, F. Capon, J. Ding, Y. Li, T. Tejasvi, J.E. Gudjonsson, H.M. Kang, M.H. Allen, R. McManus, G. Novelli, L. Samuelsson, J. Schalkwijk, M. Stahle, A.D. Burden, C.H. Smith, M.J. Cork, X. Estivill, A.M. Bowcock, G.G. Krueger, W. Weger, J. Worthington, R. Tazi-Ahnini, F.O. Nestle, A. Hayday, P. Hoffmann, J. Winkelmann, C. Wijmenga, C. Langford, S. Edkins, R. Andrews, H. Blackburn, A. Strange, G. Band, R.D. Pearson, D. Vukcevic, C.C. Spencer, P. Deloukas, U. Mrowietz, S. Schreiber, S. Weidinger, S. Koks, K. Kingo, T. Esko, A. Metspalu, H.W. Lim, J.J. Voorhees, M. Weichenthal, H.E. Wichmann, V. Chandran, C.F. Rosen, P. Rahman, D.D. Gladman, C.E. Griffiths, A. Reis, J. Kere, Collaborative Association Study of Psoriasis, Genetic Analysis of Psoriasis Consortium, Psoriasis Association Genetics Extension, Wellcome Trust Case Control Consortium, R.P. Nair, A. Franke, J.N. Barker, G.R. Abecasis, J.T. Elder, R.C. Trembath, Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity, Nat. Genet. 44 (2012) 1341–1348.

[70] E. Jakkula, V. Leppa, A.M. Sulonen, T. Varilo, S. Kallio, A. Kemppinen, S. Purcell, K. Koivisto, P. Tienari, M.L. Sumelahti, I. Elovaara, T. Pirttila, M. Reunanen, A. Aromaa, A.B. Oturai, H.B. Sondergaard, H.F. Harbo, I.L. Mero, S.B. Gabriel, D.B. Mirel, S.L. Hauser, L. Kappos, C. Polman, P.L. De Jager, D.A. Hafler, M.J. Daly, A. Palotie, J. Saarela, L. Peltonen, Genome-wide association study in a high-risk isolate for multiple sclerosis reveals associated variants in STAT3 gene, Am. J. Hum. Genet. 86 (2010) 285–291.

[71] K. Hirota, H. Yoshitomi, M. Hashimoto, S. Maeda, S. Teradaira, N. Sugimoto, T. Yamaguchi, T. Nomura, H. Ito, T. Nakamura, N. Sakaguchi, S. Sakaguchi, Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model, J. Exp. Med. 204 (2007) 2803–2812.

[72] N. Komatsu, K. Okamoto, S. Sawa, T. Nakashima, M. Oh-Hora, T. Kodama, S. Tanaka, J.A. Bluestone, H. Takayanagi, Pathogenic conversion of Foxp3(+) T cells into TH17 cells in autoimmune arthritis, Nat. Med. 20 (2014) 62–68.

[73] W.Y. Wang, B.J. Barratt, D.G. Clayton, J.A. Todd, Genome-wide association studies: theoretical and practical concerns, Nat. Rev. Genet. 6 (2005) 109–118.

[74] E.A. Stahl, D. Wegmann, G. Trynka, J. Gutierrez-Achury, R. Do, B.F. Voight, P. Kraft, R. Chen, H.J. Kallberg, F.A. Kurreeman, Diabetes Genetics Replication and Meta-analysis Consortium, Myocardial Infarction Genetics Consortium, S. Kathiresan, C. Wijmenga, P.K. Gregersen, L. Alfredsson, K.A. Siminovitch, J. Worthington, P.I. de Bakker, S. Raychaudhuri, R.M. Plenge, Bayesian inference analyses of the polygenic architecture of rheumatoid arthritis, Nat. Genet. 44 (2012) 483–489.

[75] A. Kiezun, K. Garimella, R. Do, N.O. Stitziel, B.M. Neale, P.J. McLaren, N. Gupta, P. Sklar, P.F. Sullivan, J.L. Moran, C.M. Hultman, P. Lichtenstein, P. Magnusson, T. Lehner, Y.Y. Shugart, A.L. Price, P.I. de Bakker, S.M. Purcell, S.R. Sunyaev, Exome sequencing and the genetic basis of complex traits, Nat. Genet. 44 (2012) 623–630.

[76] D. Diogo, F. Kurreeman, E.A. Stahl, K.P. Liao, N. Gupta, J.D. Greenberg, M.A. Rivas, B. Hickey, J. Flannick, B. Thomson, C. Guiducci, S. Ripke, I. Adzhubey, A. Barton, J.M. Kremer, L. Alfredsson, Consortium of Rheumatology Researchers of North America, Rheumatoid Arthritis Consortium International, S. Sunyaev, J. Martin, A. Zhernakova, J. Bowes, S. Eyre, K.A. Siminovitch, P.K. Gregersen, J. Worthington, L. Klareskog, L. Padyukov, S. Raychaudhuri, R.M. Plenge, Rare, low-frequency, and common variants in the protein-coding sequence of biological candidate genes from GWASs contribute to risk of rheumatoid arthritis, Am. J. Hum. Genet. 92 (2013) 15–27.

[77] A. MacGregor, W. Ollier, W. Thomson, D. Jawaheer, A. Silman, HLA-DRB1*0401/0404 genotype and rheumatoid arthritis: increased association in men, young age at onset, and disease severity, J. Rheumatol. 22 (1995) 1032–1036.

[78] C.M. Weyand, K.C. Hicok, D.L. Conn, J.J. Goronzy, The influence of HLA-DRB1 genes on disease severity in rheumatoid arthritis, Ann. Intern. Med. 117 (1992) 801–806.

[79] T. Suzuki, K. Ikari, K. Yano, E. Inoue, Y. Toyama, A. Taniguchi, H. Yamanaka, S. Momohara, PADI4 and HLA-DRB1 are genetic risks for radiographic progression in RA patients, independent of ACPA status: results from the IORRA cohort study, PLoS One 8 (2013) e61045.

[80] A. Barton, J. Bowes, S. Eyre, D. Symmons, J. Worthington, A. Silman, Investigation of polymorphisms in the PADI4 gene in determining severity of inflammatory polyarthritis, Ann. Rheum. Dis. 64 (2005) 1311–1315.

[81] B. Joven, N. Gonzalez, F. Aguilar, B. Santiago, M. Galindo, J. Alcami, J.L. Pablos, Association between stromal cell-derived factor 1 chemokine gene variant and radiographic progression of rheumatoid arthritis, Arthritis Rheum. 52 (2005) 354–356.

[82] R. Knevel, A. Krabben, A.G. Wilson, E. Brouwer, M.K. Leijsma, E. Lindqvist, D.P. de Rooy, N.A. Daha, M.P. van der Linden, S. Tsonaka, A. Zhernakova, H.J. Westra, L. Franke, J.J. Houwing-Duistermaat, R.E. Toes, T.W. Huizinga, T. Saxne, A.H. van der Helm-van Mil, A genetic variant in granzyme B is associated with progression of joint destruction in rheumatoid arthritis, Arthritis Rheum. 65 (2013) 582–589.

[83] R.J. Mathews, J.I. Robinson, M. Battellino, C. Wong, J.C. Taylor, G. Biologics in Rheumatoid Arthritis, S. Genomics Study, S. Eyre, S.M. Churchman, A.G. Wilson, J.D. Isaacs, K. Hyrich, A. Barton, D. Plant, S. Savic, G.P. Cook, P. Sarzi-Puttini, P. Emery, J.H. Barrett, A.W. Morgan, M.F. McDermott, Evidence of NLRP3-inflammasome activation in rheumatoid arthritis (RA); genetic variants within the NLRP3-inflammasome complex in relation to susceptibility to RA and response to anti-TNF treatment, Ann. Rheum. Dis. 73 (2014) 1202–1210.

[84] D.P. de Rooy, A. Zhernakova, R. Tsonaka, A. Willemze, B.A. Kurreeman, G. Trynka, L. van Toorn, R.E. Toes, T.W. Huizinga, J.J. Houwing-Duistermaat, P.K. Gregersen, A.H. van der Helm-van Mil, A genetic variant in the region of MMP-9 is associated with serum levels and progression of joint damage in rheumatoid arthritis, Ann. Rheum. Dis. 73 (2014) 1163–1169.

[85] R. Knevel, K. Klein, K. Somers, C. Ospelt, J.J. Houwing-Duistermaat, J.A. van Nies, D.P. de Rooy, L. de Bock, F.A. Kurreeman, J. Schonkeren, G. Stoeken-Rijsbergen, Q. Helmer, M.P. van der Linden, M. Kern, N. Manjarrez-Orduno, L. Rodriguez-Rodriquez, P. Stinissen, T.W. Huizinga, R.E. Toes, S. Gay, P.K. Gregersen, V. Somers, A.H. van der Helm-van Mil, Identification of a genetic variant for joint damage progression in autoantibody-positive rheumatoid arthritis, Ann. Rheum. Dis. (2013), Available online.

[86] C. Liu, F. Batliwalla, W. Li, A. Lee, R. Roubenoff, E. Beckman, H. Khalili, A. Damle, M. Kern, R. Furie, J. Dupuis, R.M. Plenge, M.J. Coenen, T.W. Behrens, J.P. Carulli, P.K. Gregersen, Genome-wide association scan identifies candidate polymorphisms associated with differential response to anti-TNF treatment in rheumatoid arthritis, Mol. Med. 14 (2008) 575–581.

[87] D. Plant, J. Bowes, C. Potter, K.L. Hyrich, A.W. Morgan, A.G. Wilson, J.D. Isaacs, Wellcome Trust Case Control Consortium, British Society for Rheumatology Biologics Register, A. Barton, Genome-wide association study of genetic predictors of anti-tumor necrosis factor treatment efficacy in rheumatoid arthritis identifies associations with polymorphisms at seven loci, Arthritis Rheum. 63 (2011) 645–653.

[88] J. Cui, E.A. Stahl, S. Saevarsdottir, C. Miceli, D. Diogo, G. Trynka, T. Raj, M.U. Mirkov, H. Canhao, K. Ikari, C. Terao, Y. Okada, S. Wedren, J. Askling, H. Yamanaka, S. Momohara, A. Taniguchi, K. Ohmura, F. Matsuda, T. Mimori, N. Gupta, M. Kuchroo, A.W. Morgan, J.D. Isaacs, A.G. Wilson, K.L. Hyrich, M. Herenius, M.E. Doorenspleet, P.P. Tak, J.B. Crusius, I.E. van der Horst-Bruinsma, G.J. Wolbink, P.L. van Riel, M. van de Laar, H.J. Guchelaar, N.A. Shadick, C.F. Allaart, T.W. Huizinga, R.E. Toes, R.P. Kimberly, S.L. Bridges Jr., L.A. Criswell, L.W. Moreland, J.E. Fonseca, N. de Vries, B.E. Stranger, P.L. De Jager, S. Raychaudhuri, M.E. Weinblatt, P.K. Gregersen, X. Mariette, A. Barton, L. Padyukov, M.J. Coenen, E.W. Karlson, R.M. Plenge, Genome-wide association study and gene expression analysis identifies CD84 as a predictor of response to etanercept therapy in rheumatoid arthritis, PLoS Genet. 9 (2013) e1003394.