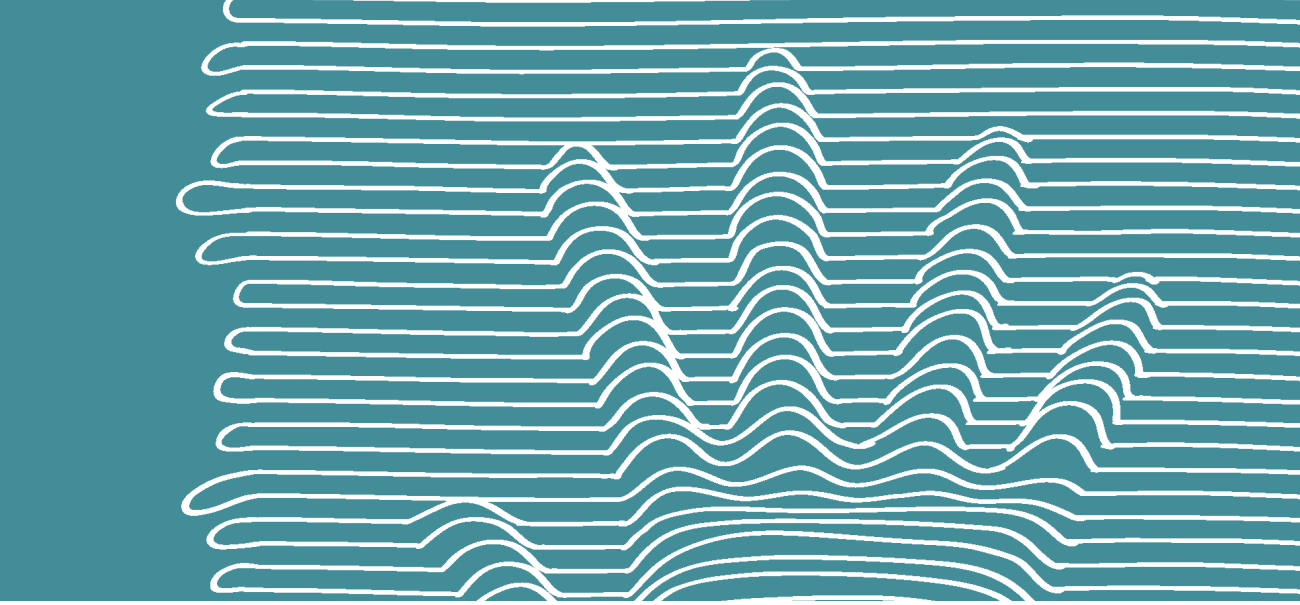

CHAPTER 5

Novel genetic association of the VTCN1 region with rheumatoid arthritis

N.A. Daha, B.A. Lie, L.A. Trouw, G. Stoeken, J.J.M. Schonkeren, B. Ding, T.K. Kvien, M.W. Schilham, L. Padyukov, T.W.J. Huizinga, R.E.M. Toes

Ann Rheum Dis. 2012 Apr;71(4):567-71

ABSTRACT

Objective

Based upon findings in juvenile idiopathic arthritis, the genetic contribution of the VTCN1 region to rheumatoid arthritis (RA) susceptibility and anticitrullinated protein antibody (ACPA) status was investigated. VTCN1 is known to play a pivotal role in regulation of the immune system and, in soluble form, has previously been associated with higher disease activity.

Methods

Ten VTCN1 polymorphisms were genotyped in 1237 Dutch patients with RA and 1055 healthy controls. Significant findings were replicated in two independent RA populations of northern European descent consisting of 2826 patients and 2122 healthy controls. Allele distribution was analysed using a χ^2 test and combined analysis of all studies was performed using the Mantel-Haenszel fixed effects method.

Results

A significant association with two polymorphisms was observed in the Dutch RA population. Replication of these findings showed an overall significant association with rs4376721 and rs10923217 (OR 1.13, 95% CI 1.03 to 1.24, p = 0.013 and OR 0.78, 95% CI 0.67 to 0.91, p = 0.0011, respectively). Stratification for ACPA status revealed an association in the ACPA-negative subset for rs4376721 (OR 1.19, 95% CI 1.05 to 1.35, p = 0.0071), while no overall significance could be observed in the ACPA-positive population. rs10923217 was associated with both subsets of the disease.

Conclusion

These results indicate a novel genetic association with the VTCN1 region in RA susceptibility.

INTRODUCTION

Although the aetiology of rheumatoid arthritis (RA) is largely unknown, it is thought that both environmental and genetic factors have a role in the development of the disease[1]. To date, over 30 genetic regions have been identified to associate with RA susceptibility. Most of these loci have been identified by genome-wide association studies (GWAS) performed in autoantibody-positive patient populations[2]. It is known, however, that RA is a heterogeneous disease and data indicate that distinct risk factors play a role in the anticitrullinated protein antibody (ACPA) subsets[3]. Further investigation in these subsets is needed to elucidate the genetic backgrounds.

Although many genes for complex diseases such as RA have been identified, it is thought that only part of the genetic susceptibility to these diseases can be explained by these loci. Part of this 'missing heritability' is speculated to be due to rare genetic variations which can be identified by resequencing genomes of patients[4]. Additionally, it is well recognised that GWAS are probably underpowered to detect all common disease variants and further investigation of these variants might lead to novel genetic associations[5].

As more autoimmune susceptibility loci are being identified, it has become clear that part of these genes overlap between autoimmune diseases. Recently, a GWAS in juvenile idiopathic arthritis (JIA) identified the VTCN1 region to be associated with JIA and observed this region as the strongest signal next to human leucocyte antigen[6].

VTCN1 encodes B7-H4 which has been reported as a negative regulator of T cell responses in vitro by inhibiting proliferation and cytokine production[7,8]. However, in B7-H4 deficient mice, no strong phenotype of hyperactivation of T and B cells was observed[9]. It has been shown that the protein is expressed in tumour-associated suppressive macrophages[8]. Recently, it was found that high levels of the soluble form of B7-H4 are seen more frequently in patients with RA than in healthy controls and are associated with higher disease activity as measured by the 28-joint disease activity score[10]. Likewise, in B7-H4 knockout mice the progression of inflammation in a collagen-induced arthritis model was accelerated compared with wild-type mice, which suggests a relevant role for B7-H4 in arthritis[10].

The aim of the present study was to identify the genetic contribution of the VTCN1 region to RA susceptibility and the clinically well-defined subsets, characterised by ACPA status.

METHODS

Patients

A total of 1257 patients with RA, all of whom met the American College of Rheumatology (ACR) 1987 criteria, were recruited from hospitals in the western part of The Netherlands. ACPA status was obtained for 940 patients. The characteristics of the patients have been described previously[11]. The control group consisted of 1060 healthy subjects randomly selected by the Immunogenetics and Transplantation Immunology section of the Leiden

University Medical Center.

For validation purposes, two independent western European cohorts were included. The first cohort consisted of 953 Norwegian patients with RA and 1121 healthy Norwegian controls. All patients fulfilled the ACR criteria and originated from two cohorts: the Oslo RA register (ORAR) and the Norwegian arm of the European Research on Incapacitating Disease and Social support. Follow-up has been described previously[12,13]. ACPA status was available for 893 patients. The second cohort consisted of 1908 Swedish patients with RA and 1061 healthy Swedish controls recruited from different parts of Sweden between May 1996 and December 2005. All patients originated from the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study and met the ACR criteria for RA. ACPA status was obtained for all patients. A more detailed description of the EIRA study has been presented previously[14]. The study was approved by the ethics committee at the Karolinska Institutet and by the Regional Stockholm ethics committee.

The power to detect an association within the combined population was >80%, calculated by Quanto, assuming an OR of 1.20.

Genotyping methods

Ten single nucleotide polymorphisms (SNPs) were selected on the basis of their previous significant association with JIA.6 rs6673837, rs2358817, rs10923217, rs6669320, rs10923223 and rs12046117 were genotyped in 985 patients and 865 controls using MassArray matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (Sequenom, San Diego, California, USA) with a success rate of >97%. rs2051047, rs4376721, rs12038533 and rs7415876 were genotyped in 1257 patients and 1060 controls using Taqman allelic discrimination assays (AppliedBiosystems, Bleiswijk, The Netherlands) with a success rate of >95%.

In the Norwegian dataset, rs4376721 and rs10923217 were genotyped using MassArray matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (Sequenom). The genotyping success rate was >95%.

Data from the Swedish EIRA study were retrieved from GWAS published previously[15]. After removing outliers, a total of 1147 ACPA-positive patients and 1079 controls were used for analysis.

Statistical analysis

Analysis of allele distribution for association in the Dutch population and carriage of the minor allele in the replication study was performed using a χ^2 test with one degree of freedom. ORs and 95% CI were calculated using the Statcalc module of EpiInfo Software (Centers for Disease Control and Prevention, Atlanta, Georgia, USA). P values <0.05 were considered significant and genotype frequencies in controls did not deviate from Hardy-Weinberg equilibrium ($p<0.05$). Combined analysis of the genotypes of all studies was performed using the Mantel-Haenszel fixed effects method. Heterogeneity across studies was analysed using the Breslow-Day test.

RESULTS

Ten polymorphisms which have previously been shown to be associated with JIA were genotyped in a Dutch RA population. Both rs4376721 and rs10923217 showed significant associations with RA (OR 1.21, 95% CI 1.07 to 1.38, p = 0.003 and OR 0.87, 95% CI 0.76 to 0.99, p = 0.031, respectively; table 1).

In this analysis we did not correct for multiple testing as we wished to replicate all the associations observed in a second stage. To validate the observed associations, the two significant polymorphisms rs4376721 and rs10923217 were replicated in two independent RA populations of western European descent. Since the genotype frequencies indicated a dominant inheritance, carriage of the minor allele was also analysed. A p value <0.025 was considered significant.

Table 1. *Analysis of 10 SNPs in the VTCN1 region in a Dutch rheumatoid arthritis population*

	Allele		Cases					Controls					1 vs 2		
SNP	Major	Minor	N	11	12	22	MAF	N	11	12	22	MAF	OR (95% CI)	p Value	HW controls
rs6673837	G	A	980	619	317	44	0.21	853	542	265	46	0.21	1.02 (0.86 to 1.20)	0.845	0.080
				63.1	32.35	4.5			63.54	31.07	5.4				
rs2358817	C	T	978	831	1 41	6	0.08	835	699	1 30	6	0.09	1.10 (0.86 to 1.40)	0.455	1.000
				85.0	14.42	0.6			83.71	15.57	0.7				
rs2051047	G	A	1 239	1 040	1 87	12	0.09	1057	885	164	8	0.09	1 .00 (0.81 to 1.24)	0.999	0.843
				83.94	1 5.09	1.0			83.73	15.52	0.8				
rs4376721	T	G	1 237	621	521	95	0.29	1055	481	455	119	0.33	1.21 (1.07 to 1.38)	0.003	0.490
				50.2	42.12	7.7			45.59	43.13	11.28				
rs10923217	G	C	968	230	495	243	0.51	836	245	395	196	0.47	0.87 (0.76 to 0.99)	0.031	0.145
				23.76	51.14	25.1			29.31	47.25	23.44				
rs6669320	G	A	976	706	250	20	0.15	845	615	203	27	0.15	1 .03 (0.85 to 1.24)	0.768	0.060
				72.34	25.6	2.0			72.7	24.0	3.195				
rs12038533	C	A	1 237	984	246	7	0.11	1057	839	203	15	0.11	1 .05 (0.87 to 1.28)	0.576	0.529
				79.55	1 9.89	0.6			79.3	19.2	1.4				
rs7415876	G	C	1 241	871	335	35	0.16	1057	742	279	36	0.17	1 .02 (0.87 to 1.20)	0.794	0.148
				70.19	26.99	2.8			70.2	26.4	3.4				
rs10923223	T	C	981	682	273	26	0.17	852	612	226	14	0.15	0.88 (0.73 to 1.06)	0.170	0.224
				69.52	27.83	2.7			71.83	26.53	1.6				
rs12046117	C	T	958	71 5	227	16	0.14	844	631	200	13	0.13	0.99 (0.81 to 1.20)	0.910	0.656
				74.63	23.7	1.7			74.76	23.7	1.5				

1, major (common) allele; 2, minor (rare) allele. MAF, minor allele frequency; SNP, single nucleotide polymorphism; HW, Hardy-Weinberg equilibrium.

Table 2. *Replication of rs4376721 and rs10923217 and stratification for anticitrullinated protein antibody status in populations of northern European descent*

			Cases				Controls					1 vs 2		11 vs 12+22	
SNP	Population		N	11	12	22	MAF	N	11	12	22	MAF	OR 95% CI)	p Value	OR (95% CI)	p Value
rs4376721	Dutch		1237	621	521	95	0.29	1055	481	455	119	0.33	1.21 (1.07 to 1.38)	0.0026	1.20 (1.02 to 1.42)	0.0277
				50.2	42.1	7.7			45.6	43.1	11.3					
		ACPA positive	531	261	224	46	0.30						1.15 (0.98 to 1.36)	0.0780	1.15 (0.93 to 1.43)	0.1799
				49.2	42.2	8.7										
		ACPA negative	404	207	164	33	0.28						1.23 (1.02 to 1.47)	0.0229	1.25 (0.99 to 1.59)	0.0533
				51.2	40.6	8.2										
	Norway		918	466	374	78	0.29	1061	491	471	99	0.32	1.13 (0.99 to 1.30)	0.0693	1.20 (1.00 to 1.43)	0.0465
				50.8	40.7	8.5			46.3	44.4	9.3					
		ACPA positive	533	270	219	44	0.29						1.14 (0.97 to 1.34)	0.1149	1.19 (0.96 to 1.48)	0.0986
				50.7	41.1	8.3										
		ACPA negative	327	172	123	32	0.29						1.15 (0.94 to 1.40)	0.1555	1.29 (1.00 to 1.66)	0.0454
				52.6	37.6	9.8										
	Sweden		1 908	952	767	189	0.30	1061	524	449	88	0.29	0.97 (0.87 to 1.10)	0.6559	1.02 (0.88 to 1.19)	0.7909
				49.9	40.2	9.9			49.4	42.3	8.3					
		ACPA positive	1143	557	470	116	0.31						0.94 (0.83 to 1.07)	0.3640	0.97 (0.82 to 1.16)	0.7582
				48.7	41.1	10.1										
		ACPA negative	765	395	297	73	0.29						1.02 (0.88 to 1.19)	0.7346	1.09 (0.90 to 1.32)	0.3435
				51.6	38.8	9.5										
	Combined		4063	2039	1662	362		3177	1496	1375	306		1.09 (1.02 to 1.17)	0.0158	1.13 (1.03 to 1.24	0.0131

Table 2. *Continued.*

		ACPA positive	2207	1088	913	206							1 .05 (0.97 to 1.15)	0.2365	1.07 (0.97 to 1.20)	0.1669
		ACPA negative	1496	774	584	138							1.11 (1.01 to 1.23)	0.0285	1.19 (1.05 to 1.35)	0.0071
rs1092321	Dutch		968	230	495	243	0.507	836	245	395	196	0.471	0.87 (0.76 to 0.99)	0.0309	0.75 (0.61 to 0.93)	0.0077
				23.8	51.1	25.1			29.3	47.2	23.4					
		ACPA positive	381	1 00	188	93	0.509						0.92 (0.77 to 1.10)	0.3567	0.86 (0.65 to 1.14)	0.2721
				26.2	49.3	24.4										
		ACPA negative	257	61	127	69	0.484						0.84 (0.68 to 1.02)	0.0750	0.75 (0.54 to 1.05)	0.0819
				23.7	49.4	26.8										
	Norway		910	193	491	226	0.518	1033	258	534	241	0.492	0.90 (0.79 to 1.02)	0.1010	0.81 (0.65 to 1.00)	0.0497
				21.2	54.0	24.8	25.0	51.7	23.3							
		ACPA positive	525	1 05	286	134	0.472						0.87 (0.74 to 1.01)	0.0585	0.75 (0.58 to 0.98)	0.0281
				20.0	54.5	25.5										
		ACPA negative	327	71	177	79	0.488						0.92 (0.77 to 1.10)	0.3617	0.83 (0.61 to 1.13)	0.2298
				21.7	54.0	24.2										
	Combined		1878	423	986	469		1869	503	929	437		0.91 (0.84 to 1.00)	0.0533	0.78 (0.67 to 0.91)	0.0011
		ACPA positive	906	205	474	227							0.92 (0.82 to 1.03)	0.1351	0.80 (0.66 to 0.96)	0.0185
		ACPA negative	584	132	304	148							0.91 (0.80 to 1.04)	0.1642	0.79 (0.64 to 0.99)	0.0393

A combined analysis of the genotypes of all studies was performed using the Mantel-Haenszel fixed effects method. 1, major (common) allele; 2, minor (rare) allele. ACPA, anticitrullinated protein antibody; SNP single nucleotide polymorphism.

A trend towards association was observed in the Norwegian cohort for both polymorphisms (rs4376721: p = 0.0456; rs10923217: p = 0.0497). This effect was in the same direction as that seen in the Dutch discovery cohort (table 2).

In the Swedish population only data on rs4376721 were available for analysis. Neither rs10923217 nor a proxy of any kind was included in the Swedish GWAS. In this study, rs4376721 did not indicate significance. Nonetheless, a combined analysis of all three independent populations did show a significant association (p=0.01). Likewise, for rs10923217, an overall significant association could be established in the combined Dutch and Norwegian cohort (p=0.001). Notably, no heterogeneity was observed between the different study populations (p>0.05) and in all studies the same allele contributed to disease (table 2). The linkage disequilibrium between the two polymorphisms was limited with a r2 of 0.30 and a D' of 0.85.

The contribution of the VTCN1 region to the ACPA subsets was also analysed. Stratification for ACPA status showed rs4376721 to be significantly associated in the ACPA-negative subset compared with healthy controls, but not in the ACPA- positive subset. The OR of the ACPA-negative subgroup was slightly higher than in the ACPA-positive subgroup, although no statistical difference was observed in the ACPA-negative subset compared with the ACPA-positive subset (OR 1.10, 95% CI 0.96 to 1.26, p=0.145). In ACPA-negative disease, all minor allele frequencies were biased in the same direction while, in ACPA-positive disease, more heterogeneity was observed (figure 1).

Stratifying for ACPA reactivity for rs10923217 demonstrated an overall association of this polymorphism for both subsets of the disease (table 2).

Expression data from the GenCord database[16] showed a significant correlation between rs6428686 (a perfect proxy for rs4376721) and VTCN1 transcript levels, with the risk allele G being associated with lower expression in primary T cells (p = 0.0069). A similar pattern was observed in lymphoblastoid cell lines (p = 0.067; see figure S1 in online supplement). These data indicate that the identified genetic variant has an impact on basal VTCN1 expression in non-progenitor immune cells.

DISCUSSION

In the present study a novel genetic risk factor for RA susceptibility was identified in a Dutch RA population and replicated in independent populations of northern European descent.

Previous studies in RA have suggested that associated loci predispose to specific subsets of the disease, characterised by ACPA status[3,17]. Also, non-genetic data indicate that ACPA-positive disease behaves differently from ACPA-negative disease[13,18]. It is therefore of relevance to analyse these potentially different forms of arthritis in a separate manner. Our results indicate that the VTCN1 region seems to associate mainly with an ACPA-negative disease subset and less with an ACPA-positive subset compared with healthy controls.

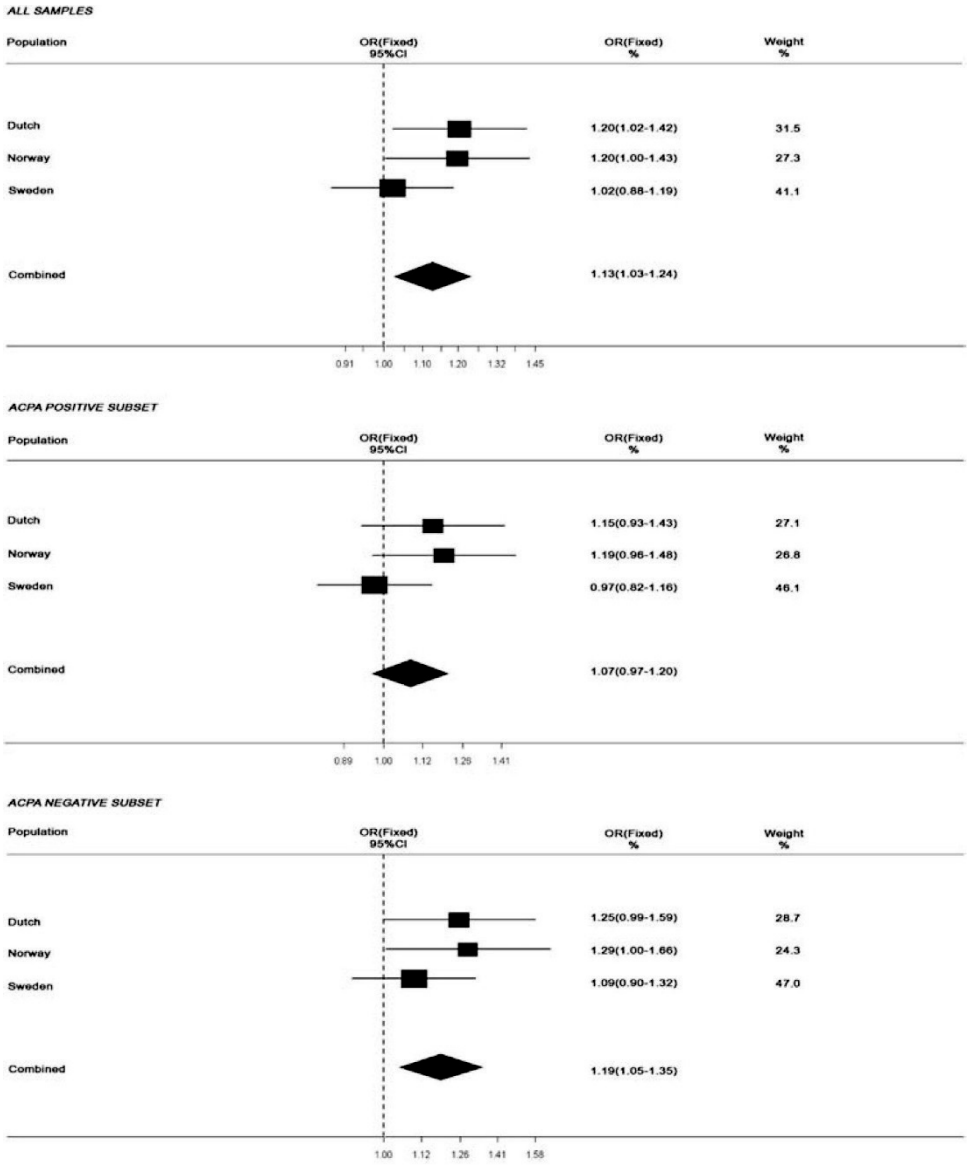

Figure 1. *Meta-analysis of association of rs4376721 for rheumatoid arthritis susceptibility and anti-citrullinated protein antibody status compared with healthy controls in three northern European rheumatoid arthritis populations. Squares represent OR values and lines represent 95% CI.*

Nonetheless, no significant difference was observed when the ACPA-negative subset was directly compared with the ACPA-positive subset. Although this lack of significance is possibly due to insufficient power (for the present sample numbers the power is ~72%), no final conclusion on this aspect can be drawn. Additional replication in independent cohorts is therefore necessary to identify a differential effect of the VTCN1 locus in the two subsets of RA.

The polymorphisms typed within this study were selected on the basis of their previous significant association with JIA[6]. It is notable that the association pattern of the variants seems to be different between the two diseases. First, the most significant SNP in JIA was not significantly associated within the Dutch cohort. Also, the distribution of the genotype frequencies is different between RA and JIA, which is mainly visible for rs4376721. Therefore, very subtle differences in minor allele frequency in the control or patient groups are prone to affect data and lead to different results, indicating the need for replication. One could also speculate that protein function or expression is affected differently by different genetic variants, which could explain the difference in disease outcome and disease course. Although indirect, and although it is not known whether VTCN1 is constitutively expressed, analysis of expression data from the GenCord database indeed shows differential expression for the different genotypes in lymphoid cells.

CONCLUSION

In conclusion, this study has identified the VTCN1 region as a new genetic region associated with susceptibility to RA.

REFERENCES

1. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet 2010;376:1094-108.
2. Stahl EA, Raychaudhuri S, Remmers EF et al. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nat Genet 2010;42:508-14.
3. Huizinga TW, Amos CI, van der Helm-van Mil AH, et al. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. Arthritis Rheum 2005;52:3433-8.
4. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. Nature 2009;461:747-53.
5. Elbers CC, van Eijk KR, Franke L, et al. Using genome-wide pathway analysis to unravel the etiology of complex diseases. Genet Epidemiol 2009;33:419-31.
6. Hinks A, Barton A, Shephard N, et al. Identification of a novel susceptibility locus for juvenile idiopathic arthritis by genome-wide association analysis. Arthritis Rheum 2009;60:258-63.

7. Sica GL, Choi IH, Zhu G, et al. B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. Immunity 2003;18:849-61.

8. Yi KH, Chen L. Fine tuning the immune response through B7-H3 and B7-H4. Immunol Rev 2009;229:145-51.

9. Suh WK, Wang S, Duncan GS, et al. Generation and characterization of B7-H4/B7S1/B7x-deficient mice. Mol Cell Biol 2006;26:6403-11.

10. Azuma T, Zhu G, Xu H, et al. Potential role of decoy B7-H4 in the pathogenesis of rheumatoid arthritis: a mouse model informed by clinical data. PLoS Med 2009;6:e1000166.

11. Kurreeman FA, Padyukov L, Marques RB, et al. A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis. PLoS Med 2007;4:e278.

12. Haugeberg G, 0rstavik RE, Uhlig T, et al. Bone loss in patients with rheumatoid arthritis: results from a population-based cohort of 366 patients followed up for two years. Arthritis Rheum 2002;46:1720-8.

13. Syversen SW, Gaarder PI, Goll GL, et al. High anti-cyclic citrullinated peptide levels and an algorithm of four variables predict radiographic progression in patients with rheumatoid arthritis: results from a 10-year longitudinal study. Ann Rheum Dis 2008;67:212-17.

14. Padyukov L, Silva C, Stolt P et al. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. Arthritis Rheum 2004;50:3085-92.

15. Plenge RM, Seielstad M, Padyukov L, et al. TRAF1-C5 as a risk locus for rheumatoid arthritis: a genome-wide study. N Engl J Med 2007;357:1199-209.

16. Dimas AS, Deutsch S, Stranger BE, et al. Common regulatory variation impacts gene expression in a cell type-dependent manner. Science 2009;325:1246-50.

17. Padyukov L, Seielstad M, Ong RT, et al. A genome-wide association study suggests contrasting associations in ACPA-positive versus ACPA-negative rheumatoid arthritis. Ann Rheum Dis 2011;70:259-65.

18. van der Woude D, Young A, Jayakumar K, et al. Prevalence of and predictive factors for sustained disease-modifying antirheumatic drug-free remission in rheumatoid arthritis: results from two large early arthritis cohorts. Arthritis Rheum 2009;60:2262-71.

19. Yang TP, Beazley C, Montgomery SB, et al. Genevar: a database and Java application for the analysis and visualization of SNP-gene associations in eQTL studies. Bioinformatics 2010;26:2474-6.