

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/81416>

Please be advised that this information was generated on 2018-10-23 and may be subject to change.

CI: confidence interval; IL: interleukin; OR: odds ratio; RA: rheumatoid arthritis; SNP: single nucleotide polymorphism.
Vol 11 No 2
Teixeira
et al.

Arthritis Research & Therapy
R

Open Access

Research article

Testing for the association of the *KIAA1109/Tenr/IL2/IL21* gene region with rheumatoid arthritis in a European family-based study

Vitor Hugo Teixeira^{1,2}, Celine Pierlot¹, Paola Migliorini³, Alejandro Balsa⁴, René Westhovens⁵, Pilar Barrera⁶, Helena Alves⁷, Carlos Vaz⁷, Manuela Fernandes⁷, Dora Pascual-Salcedo⁴, Stefano Bombardieri³, Jan Dequeker⁵, Timothy R Radstake⁶, Piet Van Riel⁶, Leo van de Putte⁶, Antonio Lopes-Vaz⁷, Thomas Bardin⁸, Bernard Prum⁹, François Cornélis^{1,8,10,11}, Elisabeth Petit-Teixeira¹ for the European Consortium on Rheumatoid Arthritis Families

¹GenHotel-EA3886, Evry University – Paris 7 University Medical School, AutoCure European Consortium member, 2 rue Gaston Crémieux, 91057 Evry-Genopole, France²Faculty of Medicine, University of Coimbra, Rua Larga, 3004-504 Coimbra, Portugal³Pisa University, Lungarno Pacinotti 43, 56126 Pisa, Italy⁴La Paz Hospital, Paseo Castellana 261, 28046 Madrid, Spain⁵KUL Leuven University, Naamsestraat 63, BE-3000 Leuven, Belgium⁶Nijmegen University, Geert Grooteplein 10, 6500HB Nijmegen, The Netherlands⁷Porto San Joao Hospital, Estrada Interior da Circunvalação, 4200 Porto, Portugal⁸Rheumatology Federation in Pôle Appareil Locomoteur, Lariboisière Hospital, Assistance Publique-Hôpitaux de Paris, 2 rue Ambroise Paré, 75010 Paris, France⁹Laboratoire Statistique et Génome, Genopole, Tour Evry 2, 91000 Evry, France¹⁰Clinical Genetics Unit in Pôle Laboratoire-Imagerie-Pharmacie Lariboisière Hospital, Assistance Publique-Hôpitaux de Paris, 2 rue Ambroise Paré, 75010 Paris, France¹¹Adult Genetics Unit, Centre Hospitalier Sud Francilien, Evry-Corbeil, 59 bd H Dunant, 91106 Corbeil-Essonnes, France

Corresponding author: Elisabeth Petit-Teixeira, epetit@polyarthrite.netR45

Received: 22 Jan 2009 Revisions requested:

17 Feb 2009

Revisions received:

6 Mar 2009

Accepted: 20 Mar 2009 Published: 20 Mar 2009

Arthritis Research & Therapy 2009, 11:R45 (doi:10.1186/ar2654)

This article is online at: <http://arthritis-research.com/content/11/2/R45>

© 2009 Teixeira *et al.*; licensee BioMed Central Ltd.

This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Introduction A candidate gene approach, in a large case–control association study in the Dutch population, has shown that a 480 kb block on chromosome 4q27 encompassing *KIAA1109/Tenr/IL2/IL21* genes is associated with rheumatoid arthritis. Compared with case–control association studies, family-based studies have the added advantage of controlling potential differences in population structure. Therefore, our aim was to test this association in populations of European origin by using a family-based approach.

Methods A total of 1,302 West European white individuals from 434 trio families were genotyped for the rs4505848, rs11732095, rs6822844, rs4492018 and rs1398553 polymorphisms using the TaqMan Allelic discrimination assay (Applied Biosystems). The genetic association analyses for each SNP and haplotype were performed using the Transmission Disequilibrium Test and the genotype relative risk.

Results We observed evidence for association of the heterozygous rs4505848-AG genotype with rheumatoid arthritis ($P = 0.04$); however, no significance was found after Bonferroni correction. In concordance with previous findings in the Dutch population, we observed a trend of undertransmission for the rs6822844-T allele and rs6822844-GT genotype to rheumatoid arthritis patients. We further investigated the five SNP haplotypes of the *KIAA1109/Tenr/IL2/IL21* gene region. We observed, as described in the Dutch population, a nonsignificant undertransmission of the AATGG haplotype to rheumatoid arthritis patients.

Conclusions Using a family-based study, we have provided a trend for the association of the *KIAA1109/Tenr/IL2/IL21* gene region with rheumatoid arthritis in populations of European descent. Nevertheless, we failed to replicate a significant association of this region in our rheumatoid arthritis family sample. Further investigation of this region, including detection and testing of all variants, is required to confirm rheumatoid arthritis association.

Introduction

Rheumatoid arthritis (RA) is a common autoimmune inflammatory disease of unknown cause, in which both genetic and environmental risk factors have been implicated [1]. Since the genetic contribution to RA has been estimated to be between 50% and 60%, identification of genes contributing to disease is important for the understanding of underlying biological mechanisms [2].

In addition to human leucocyte antigen (*HLA*) [3] – the first identified genetic risk factor – and four other replicated regions – including the protein tyrosine phosphatase N22 gene (*PTPN22*) [4], the TNF receptor-associated factor 1 gene (*TRAF1*)/complement component factor 5 (*C5*) locus [5-7], the 6q23 locus near the TNF α -induced protein 3 gene (*TNFAIP3*) [8,9], and the signal transducer and activator of transcription 4 (*STAT4*) gene [10,11] – a new genetic region associated with RA was described in the Dutch population [12]. This region encompassing *KIAA1109/Tenr/IL2/IL21* is contained in a large block (480 kb) of linkage disequilibrium located on chromosome 4q27 and includes the *IL2* and *IL21* genes, which are both plausible functional candidate loci for RA. Five SNPs (rs4505848, rs11732095, rs6822844, rs4492018 and rs1398553) of the block were investigated in a sample set consisting of 1,047 RA patients and 929 controls, showing a significant association of the rs6822844-T allele and the rs6822844-GT genotype ($P = 0.0002$, odds ratio (OR) = 0.72, 95% confidence interval (CI) = 0.61 to 0.86; and $P = 0.0009$, OR = 0.71, 95% CI = 0.58 to 0.87, respectively) and of a specific haplotype (AATGG) ($P = 0.00025$, OR = 0.70, 95% CI = 0.57 to 0.85) with RA [12].

Furthermore, in the North American Rheumatoid Arthritis Consortium and in the Epidemiological Investigation of Rheumatoid Arthritis populations, rs6822844 and rs1398553 SNPs were significantly associated with RA ($P = 0.01$, OR = 0.84, 95% CI = 0.74 to 0.96; and $P = 0.003$,

OR = 1.17, 95% CI = 1.05 to 1.30, respectively) [6]. In addition, the last meta-analysis performed with the North American Rheumatoid Arthritis Consortium, the Epidemiological Investigation of Rheumatoid Arthritis and the Wellcome Trust Case Control Consortium studies, with 3,393 RA patients and 12,462 controls, observed evidence of association of 4q27 with RA in an independent replication, suggesting that 4q27 is a true-positive association [13].

Given that association studies compare the frequencies in patients versus healthy individuals, unknown biases in control frequencies may lead to spurious associations. Family-based association studies therefore remain important to definitively establish association, especially when small effect sizes as well as variability in allele frequency in different populations are observed. With the aim to further substantiate the association at the *KIAA1109/Tenr/IL2/IL21* gene region, we therefore took advantage of one of the largest reported European family resources dedicated to RA family-based studies.

Materials and methods

Study design and study population

DNA was available from 434 white trio families from Western Europe through the European Consortium on Rheumatoid Arthritis Families collection followed by the selection of individuals fulfilling the American College of Rheumatology 1987 criteria for RA [14], according to the rheumatologist in charge of the patient. Each family consisted of one RA patient and both parents, with the ethnicity as determined by grandparental origin.

Characteristics of the 434 RA European Caucasian families are reported in Table 1. The 434 families included 308 families from France, 51 families from Italy, 32 families from Spain, 20 families from Belgium, nine families from the Netherlands and 14 families from Portugal. Families with an additional affected sibling and with RA patients

Table 1
Characteristics of rheumatoid arthritis (RA) index cases

Characteristic	RA families (n = 434)
Females (n (%))	375 (86%)
Mean age at disease onset (years) (\pm standard deviation)	31.1 (9.7)
Mean disease duration (years) (\pm standard deviation)	9.8(8.5)
With bone erosions (n (%))	336 (77%)
Seropositive for rheumatoid factor (n (%))	323 (76%) (out of 425 RA patients)
Seropositive for anti-cycle citrullinated peptides antibodies (n (%))	214 (76%) (out of 282 RA patients)
Carrying at least one <i>HLA-DRB1</i> shared epitope allele (n (%)) ^a	152 (77%) (out of 197 RA patients)
Carrying at least one PTPN22-1858T allele (n (%))	115 (27%)
Carrying the <i>TRAF1/CS</i> rs10818488-A variant (n (%))	277 (64%)

^aDRB1*0101, DRB1*0102, DRB1*0401, DRB1*0404, DRB1*0405, DRB1*0408, DRB1*1001.

aged <18 years were excluded. All individuals provided written informed consent and the study was approved by the Ethics Committee of Hôpital Kremlin-Bicêtre (Paris, France).

Genotyping

All samples were genotyped using the TaqMan allelic discrimination assay according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Centre d'Etude du Polymorphisme Humain DNA samples were co-genotyped with all our samples, with no inconsistencies detected. The genotyping success rate in the 434 trio families was 100% (parents and probands included).

Statistical analysis

Using the previously reported frequencies for the rs6822844-T allele in Dutch RA patients (14.1%) and in controls (18.5%) [12], the power to detect an association was calculated using the method described by Garnier and colleagues [15]. The Hardy-Weinberg equilibrium was checked in the control group (constituting the nontransmitted parental chromosomes from the trio) prior to analysis. The association analysis relied on the Transmission Disequilibrium Test, which for a given allele compares its transmission from heterozygous parents to RA patients with the transmission expected from Mendel's law (that is, 50%) [16].

Secondly, we used the genotype relative risk, which compares the affected offspring's genotype with the control genotype derived from nontransmitted parental chromosomes [17]. ORs were calculated and 95% CIs were approximated using Woolf's method [18], with Haldane's correction [19]. Haplotypes were generated using GENE-HUNTER software [20]. We then carried out multi-locus tests of association testing haplotypes of SNPs using the Transmission Disequilibrium Test and haplotype relative risk. *P* values were adjusted with Bonferroni correction [21] for each independent test performed.

Results

Power calculation

Using the previously reported frequencies for the rs6822844-T allele in Dutch RA patients (14.1%) and in controls (18.5%) [12], association analysis of 434 trio families provides an 80% power to reach statistical significance (*P* < 0.05).

Hardy-Weinberg equilibrium check

The five SNPs in the sample investigated were in Hardy-Weinberg equilibrium in the control group.

Test for association in RA trio families

A total of 1,302 European individuals from 434 trio families (one RA case and both parents) were analyzed. Three hundred and eight families were of French origin and 126 were from other continental Western European countries. With the rs6822844 (G/T) SNP we observed deviation from Mendel's first law, with a nonsignificant undertransmission of 45.3% of the T allele to the patients in the 434 families (*P* = 0.24) (Table 2). No significant association with RA was detected for the four other SNPs (Table 2).

Since RA is a heterogeneous disease and distinct subsets of patients are characterized by the presence of autoantibodies such as rheumatoid factor and anti-citrullinated protein antibodies, we also performed a stratified analysis for the presence or absence of rheumatoid factor or anti-citrullinated protein antibodies; no associations were detected (data not shown). Furthermore, the same tests were performed in subgroups stratified for the presence of the *HLA-DRB1* shared epitope, *PTPN22*-1858T and *TRAF1/CS* rs10818488-A variant, and no associations were detected (data not shown). One of the advantages of family trio data is that they provide perfectly matched controls for each patient investigated. Each patient chromosome, transmitted by a given parent, is perfectly matched for the population of origin with the untransmitted chromosome of each heterozygous parent. The genotype relative risk

Table 2
Family-based association of the chromosome 4q27 gene region with rheumatoid arthritis: Transmission Disequilibrium Test

4q27 region SNP	Allele	Transmitted allele	Untransmitted allele	Transmission ^a (%)	P value
rs4505848	A	185	189	49.5	0.84
rs11732095	A	96	78	55.2	0.17
rs6822844	T	97	114	45.3	0.24
rs4492018	G	183	174	51.3	0.63
rs1398553	G	182	182	50	1

^aPercentage of transmission from heterozygous parents.

analysis of the rs4505848 (A/G) SNP showed an association of the heterozygous AG genotype with RA ($P = 0.04$, OR = 1.32, 95% CI = 1.01 to 1.72) (Table 3). The significance threshold was not reached, however, after Bonferroni correction. In the four other SNPs, no association with RA was detected (Table 3). In concordance with previous findings in the Dutch population, we observed an undertransmission of the rs6822844-T allele and the rs6822844-GT genotype, but without significance (Tables 2 and 3).

We further investigated the five SNP (rs4505848, rs11732095, rs6822844, rs4492018 and rs1398553) haplotypes of the *KIAA1109/Tenr/IL2/IL21* gene region. These seven haplotypes of the combined five SNPs of the block have a pooled frequency of 96%. We observed a nonsignificant undertransmission of the AATGG haplotype ($P = 0.19$) (Table 4), which was reported as associated with RA in the Dutch study [12]. No relative risk was detected for any haplotype genotype (data not shown). There were no differences between families of French origin and other continental Western Europe families, as well as no differences between paternal transmission and maternal trans-

mission for all statistical analyses performed (data not shown).

Discussion

A recent case-control study in the Dutch population has shown association of RA, celiac disease and type 1 diabetes with the 4q27 gene region. In that study the rs6822844-T allele was reported to be a perfect proxy for a haplotype that is highly associated with autoimmune diseases with frequencies of 0.13 in cases versus 0.19 in North European controls [12].

In addition, a study from the Wellcome Trust Case Control Consortium identified this 4q27 region in a search for risk factors for type 1 diabetes [22]. In a follow-up study, some support for this association with type 1 diabetes was provided [23]. The last meta-analysis of data from three genome-wide association studies of type 1 diabetes – testing 305,090 SNPs in 3,561 type 1 diabetes cases and 4,646 controls of European ancestry – obtained further support for 4q27 type 1 diabetes association ($P = 1.9 \times 10^{-8}$), indicating that this is a true type 1 diabetes locus [24]. The same region was studied in inflammatory bowel diseases such as Crohn's disease and ulcerative colitis. Four SNPs

Table 3
Family-based association of the chromosome 4q27 gene region with rheumatoid arthritis: genotype relative risk

SNP	Genotype	RA cases (n)	Controls (n)	Odds ratio (95% confidence interval)	P value
rs4505848	GG	38	51	0.72 (0.46 to 1.12)	0.18 (GG vs. AA + AG)
	AG	213	183	1.32 (1.01 to 1.72)	0.04 (AG vs. GG + AA) ^a
	AA	182	199	0.85 (0.65 to 1.11)	0.28 (AA vs. AG + GG)
rs11732095	GG	7	9	0.77 (0.28 to 2.09)	0.8 (GG vs. AA + AG)
	AG	87	101	0.83 (0.6 to 1.15)	0.28 (AG vs. GG + AA)
	AA	340	324	1.23 (0.9 to 1.68)	0.23 (AA vs. AG + GG)
rs6822844	TT	8	11	0.72 (0.29 to 1.81)	0.64 (TT vs. GG + TG)
	TG	99	110	0.87 (0.64 to 1.19)	0.43 (TG vs. TT + GG)
	GG	327	313	1.18 (0.87 to 1.6)	0.32 (GG vs. TT + TG)
rs4492018	GG	235	232	1.03 (0.79 to 1.35)	0.89 (GG vs. AA + AG)
	AG	174	171	1.03 (0.78 to 1.35)	0.89 (AG vs. GG + AA)
	AA	25	31	0.79 (0.46 to 1.36)	0.49 (AA vs. AG + GG)
rs1398553	GG	204	214	0.91 (0.7 to 1.19)	0.54 (GG vs. AA + AG)
	AG	195	175	1.2 (0.92 to 1.57)	0.20 (AG vs. GG + AA)
	AA	35	45	0.77 (0.49 to 1.21)	0.30 (AA vs. AG + GG)

^aP value was not significant after Bonferroni correction.

Table 4
Family-based association for the seven haplotypes^a: Transmission Disequilibrium Test

Haplotype	Transmitted	Untransmitted	Transmission ^b (%)	P value
GAGGA	113	112	50	0.95
AAGAG	133	126	52	0.69
AATGG	76	93	45	0.19
AAGGG	110	102	52	0.58
AGGGG	67	67	50	1
GAGGG	81	64	56	0.16
AAGGA	53	43	55	0.31

^ars4505848 (G/A) – rs11732095 (G/A) – rs6822844 (T/G) – rs4492018 (G/A) – rs1398553 (G/A). ^bPercentage of transmission from heterozygous parents.

were genotyped in three cohorts concerning 4,782 cases (3,194 ulcerative colitis, 1,588 Crohn's disease) and 2,616 controls. All four SNPs were strongly associated with ulcerative colitis and moderately associated with Crohn's disease [25]. The 4q27 locus was also reported to be associated with celiac disease [26]. Recent evidence is also provided of a role for this region in psoriasis and psoriatic arthritis [27]. Finally, a genetic association with systemic lupus erythematosus and two SNPs located within the *IL-21* gene was found in a case-control study (1,318 cases and 1,318 controls) [28]. All of these studies provide evidence that 4q27 seems to be a common locus for multiple forms of autoimmune diseases.

In our family-based study, compared with the Dutch study, we have detected a similar undertransmission of the rs6822844-T allele, the rs6822844-GT genotype and the AATGG haplotype, but without significance. Furthermore, we observed evidence of association between RA and the rs4505848-AG genotype, but no significance was found after Bonferroni correction. These results exclude the 4q27 locus from being a major RA genetic region, but a minor association should be not excluded and needs to be tested in a larger RA trio sample. Furthermore the already cited last RA meta-analysis, suggesting a 4q27 true-positive association, was performed in a large number of individuals. For the rs4572894 SNP tested, there was only a 2% variation between RA and non-RA minor allele frequency (27% versus 29%, respectively) with a *P* value of 1.1% [13]. The putative effect of the 4q27 locus in RA is therefore very small and difficult to replicate in our RA family sample. In addition, a two-stage genome-wide association study of RA in the Spanish population did not found 4q27 as a RA susceptibility gene region [29].

The long region of linkage disequilibrium at chromosome 4q27 contains several genes: testis nuclear RNA-binding protein, a gene encoding a protein of unknown function (KIAA1109), and genes encoding the IL-2 and IL-21 cytokines. Testis nuclear RNA-binding protein is

expressed primarily in the testis and KIAA1109 transcripts are ubiquitous, hence their roles in autoimmunity are not particularly compelling. Both IL-2 and IL-21 belong to the type 1 cytokine family, share a large degree of homology, and possess pleiotropic functions in immune cells [26]. These two genes are both plausible functional candidates as genetic modifiers of autoimmunity, and thus have particular interest to RA.

IL-2 exerts its effects on many cell types, the most prominent of which is the T lymphocyte. Accordingly, a major function of IL-2 is to promote proliferation and expansion of both antigen-specific clones of CD4⁺ and CD8⁺ T cells as well as to induce production of other cytokines. In CD4⁺ T cells, IL-2 plays a nonredundant role in the development of CD4⁺ CD25⁺ regulatory T cells. Accumulating evidence supports CD4⁺ CD25^{high} regulatory T cells playing an essential role in controlling and preventing autoimmunity [30]. In addition, the *IL2* receptor (*CD25*) susceptibility locus has recently been reported to be associated with RA [22].

IL-21 is involved in both cell-mediated and humoral responses and has a pleiotropic effect on a variety of immune and nonimmune cells. In RA, the synovial fluid and tissue have enhanced inflammatory responses to IL-21 and elevated IL-21 receptor expression. In two animal models – collagen-induced arthritis and adjuvant-induced arthritis – treatment with an IL-21-blocking agent ameliorated disease and/or reversed established disease, and also lowered levels of IL-6 and IL-17 [31].

Conclusions

In the present study, using a family-based approach, we have provided a trend for the association of the *KIAA1109/Tenr/IL2/IL21* gene region with RA in European descent populations. Nevertheless, we failed to replicate a significant association of this region in our RA family sample. Further investigation of this region, including detection and testing of all variants, is required to confirm RA association.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

VHT carried out the molecular genetic studies. VHT and EP-T carried out the design of the study and drafted the manuscript. VHT, EP-T, BP and CP performed analysis of the data. PM, AB, RW, PB, HA, CV, MF, DP-S, SB, JD, TRR, PVR, LvdP, AL-V, TB and FC contributed to the recruitment of families and to the acquisition of clinical data. All authors read and approved the final manuscript.

Authors' information

The European Consortium on Rheumatoid Arthritis Families initiated with funding from the European Commission (BIOMED2) is coordinated by FC.

Acknowledgements

The present work was supported by Association Française des Polyarthritiques, Société Française de Rhumatologie, Association Rhumatisme et Travail, European Union for AutoCure, Association Polyarthritique, Groupe Taitbout, Genopole®. VHT's work was supported by the Foundation for Science and Technology, Portugal (grant SFRH/BD/23304/2005). The authors are grateful to the RA patients, their family and rheumatologists for their participation in this study. The authors thank Dr Sandra Lasbleiz and Dr Pierre Fritz for reviewing clinical data.

References

- Firestein GS: **Evolving concepts of rheumatoid arthritis [review].** *Nature* 2003, **423**:356-361.
- Helm-van Mil AH Van der, Wesoly JZ, Huizinga TW: **Understanding the genetic contribution to rheumatoid arthritis.** *Curr Opin Rheumatol* 2005, **17**:299-304.
- Huizinga TW, Amos CI, Helm-van Mil AH van der, Chen W, van Gaalen FA, Jawaheer D, Schreuder GM, Wener M, Breedveld FC, Ahmad N, Lum RF, de Vries RR, Gregersen PK, Toes RE, Criswell LA: **Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins.** *Arthritis Rheum* 2005, **52**:3433-3438.
- Begovich AB, Carlton VE, Honigberg LA, Schrodli SJ, Chokkalingam AP, Alexander HC, Ardlie KG, Huang Q, Smith AM, Spoerke JM, Conn MT, Chang M, Chang SY, Saiki RK, Catanese JJ, Leong DU, Garcia VE, McAllister LB, Jeffery DA, Lee AT, Batliwalla F, Remmers E, Criswell LA, Seldin MF, Kastner DL, Amos CI, Sninsky JJ, Gregersen PK: **A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis.** *Am J Hum Genet* 2004, **75**:330-337.
- Kurreeman FA, Padyukov L, Marques RB, Schrodli SJ, Seddighzadeh M, Stoeken-Rijsbergen G, Helm-van Mil AH van der, Allaart CF, Verduyn W, Houwing-Duistermaat J, Alfredsson L, Begovich AB, Klareskog L, Huizinga TW, Toes RE: **A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis.** *PLoS Med* 2007, **4**:e278.
- Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B, Liew A, Khalili H, Chandrasekaran A, Davies LR, Li W, Tan AK, Bonnard C, Ong RT, Thalamuthu A, Pettersson S, Liu C, Tian C, Chen W, Carulli JP, Beckman EM, Altshuler D, Alfredsson L, Criswell LA, Amos CI, Seldin MF, Kastner DL, Klareskog L, Gregersen PK: **TRAF1-C5 as a risk locus for rheumatoid arthritis: a genome-wide study.** *N Engl J Med* 2007, **357**:1199-1209.
- Kurreeman FA, Rocha D, Houwing-Duistermaat J, Vrijmoet S, Teixeira VH, Migliorini P, Balsa A, Westhovens R, Barrera P, Alves H, Vaz C, Fernandes M, Pascual-Salcedo D, Michou L, Bombardieri S, Radstake T, van Riel P, Putte L van de, Lopes-Vaz A, Prum B, Bardin T, Gut I, Cornelis F, Huizinga TW, Petit-Teixeira E, Toes RE: **Replication of the tumor necrosis factor receptor-associated factor 1/complement component 5 region as a susceptibility locus for rheumatoid arthritis in a European family-based study.** *Arthritis Rheum* 2008, **58**:2670-2674.
- Thomson W, Barton A, Ke X, Eyre S, Hinks A, Bowes J, Donn R, Symons D, Hider S, Bruce IN, Wellcome Trust Case Control Consortium, Wilson AG, Marinou I, Morgan A, Emery P, YEAR Consortium, Carter A, Steer S, Hocking L, Reid DM, Wordsworth P, Harrison P, Strachan D, Worthington J: **Rheumatoid arthritis association at 6q23.** *Nat Genet* 2007, **39**:1431-1433.
- Plenge RM, Cotsapas C, Davies L, de Bakker PI, Maller J, Pe'er I, Burt NP, Blumenstiel B, DeFelice M, Parkin M, Barry R, Winslow W, Healy C, Graham RR, Neale BM, Izmailova E, Roubenoff R, Parker AN, Glass R, Karlson EW, Maher N, Hafler DA, Lee DM, Seldin MF, Remmers EF, Lee AT, Padyukov L, Alfredsson L, Cobllyn J, et al.: **Two independent alleles at 6q23 associated with risk of rheumatoid arthritis.** *Nat Genet* 2007, **39**:1477-1482.
- Lee HS, Remmers EF, Le JM, Kastner DL, Bae SC, Gregersen PK: **Association of STAT4 with rheumatoid arthritis in the Korean population.** *Mol Med* 2007, **13**:455-460.
- Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, de Bakker PI, Le JM, Lee HS, Batliwalla F, Li W, Masters SL, Booty MG, Carulli JP, Padyukov L, Alfredsson L, Klareskog L, Chen W, Amos CI, Criswell LA, Seldin MF, Kastner DL, Gregersen PK: **STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus.** *N Engl J Med* 2007, **357**:977-986.
- Zhernakova A, Alizadeh BZ, Bevova M, van Leeuwen MA, Coenen MJ, Franke B, Franke L, Posthumus MD, van Heel DA, Steege G van der, Radstake TR, Barrera P, Roep BO, Koeleman BP, Wijmenga C: **Novel association in chromosome 4q27 region with rheumatoid arthritis and confirmation of type 1 diabetes point to a general risk locus for autoimmune diseases.** *Am J Hum Genet* 2007, **81**:1284-1288.
- Raychaudhuri S, Remmers EF, Lee AT, Hackett R, Guiducci C, Burt NP, Gianniny L, Korman BD, Padyukov L, Kurreeman FA, Chang M, Catanese JJ, Ding B, Wong S, Helm-van Mil AH van der, Neale BM, Cobllyn J, Cui J, Tak PP, Wolbink GJ, Crusius JB, Horst-Bruinsma IE van der, Criswell LA, Amos CI, Seldin MF, Kastner DL, Ardlie KG, Alfredsson L, Costenbader KH, Altshuler D, et al.: **Common variants at CD40 and other loci confer risk of rheumatoid arthritis.** *Nat Genet* 2008, **40**:1216-1223.
- Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey L, Kaplan S, Liang M, Luthra H, Medsger T, Mitchel D, Neustadt D, Pinals R, Schaller J, Sharp J, Wilder R, Hunder G: **The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis.** *Arthritis Rheum* 1988, **31**:315-324.
- Garnier S, Dieudé P, Michou L, Barbet S, Tan A, Lasbleiz S, Bardin T, Prum B, Cornelis F: **IRF5 rs2004640-T allele, the new genetic factor for systemic lupus erythematosus, is not associated with rheumatoid arthritis.** *Ann Rheum Dis* 2007, **66**:828-831.
- Spielman M, McGinnis E, Ewens W: **Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM).** *Am J Hum Genet* 1993, **52**:506-526.
- Lathrop GM: **Estimating genotype relative risks.** *Tissue Antigens* 1983, **22**:160-162.
- Woolf B: **On estimating the relation between blood group and disease.** *Ann Hum Genet* 1955, **19**:251-253.
- Haldane JB: **The estimation and significance of the logarithm of a ratio of frequencies.** *Ann Hum Genet* 1956, **20**:309-311.
- Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES: **Parametric and nonparametric linkage analysis: a unified multipoint approach.** *Am J Hum Genet* 1996, **58**:1347-1363.
- Bonferroni CE: **Teoria statistica delle classi e calcolo delle probabilità.** *Pubblicazioni del R Istituto Superiore di Scienze Economiche e Commerciali di Firenze* 1936, **8**:3-62.
- Wellcome Trust Case Control Consortium: **Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls.** *Nature* 2007, **447**:661-678.
- Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, Bailey R, Nejentsev S, Field SF, Payne F, Lowe CE, Szeszkó JS, Hafler JP, Zeitels L, Yang JH, Vella A, Nutland S, Stevens HE, Schuilburg H, Coleman G, Maisuria M, Meadows W, Smink LJ, Healy B, Burren OS, Lam AA, Ovington NR, Allen J, Adlem E, Leung HT, et al.: **Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes.** *Nat Genet* 2007, **39**:857-864.

24. Cooper JD, Smyth DJ, Smiles AM, Plagnol V, Walker NM, Allen JE, Downes K, Barrett JC, Healy BC, Mychaleckyj JC, Warram JH, Todd JA: **Meta-analysis of genome-wide association study data identifies additional type 1 diabetes risk loci.** *Nat Genet* 2008, **40**:1399-1401.
25. Festen EA, Goyette P, Scott R, Annese V, Zhernakova A, Brant SR, Cho JH, Silverberg MS, Taylor KD, de Jong DJ, Stokkers PC, McGovern D, Palmieri O, Achkar JP, Xavier RJ, Duerr RH, Daly MJ, Wijmenga C, Weersma RK, Rioux JD: **Genetic variants in the region harbouring IL2/IL21 associated to ulcerative colitis.** *Gut* 2009 in press.
26. van Heel DA, Franke L, Hunt KA, Gwilliam R, Zhernakova A, Inouye M, Wapenaar MC, Barnardo MC, Bethel G, Holmes GK, Feighery C, Jewell D, Kelleher D, Kumar P, Travis S, Walters JR, Sanders DS, Howdle P, Swift J, Playford RJ, McLaren WM, Mearin ML, Mulder CJ, McManus R, McGinnis R, Cardon LR, Deloukas P, Wijmenga C: **A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21.** *Nat Genet* 2007, **39**:827-829.
27. Liu Y, Helms C, Liao W, Zaba LC, Duan S, Gardner J, Wise C, Miner A, Malloy MJ, Pullinger CR, Kane JP, Saccone S, Worthington J, Bruce I, Kwok PY, Menter A, Krueger J, Barton A, Saccone NL, Bowcock AM: **A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci.** *PLoS Genet* 2008, **4**:e1000041.
28. Sawalha AH, Kaufman KM, Kelly JA, Adler AJ, Aberle T, Kilpatrick J, Wakeland EK, Li QZ, Wandstrat AE, Karp DR, James JA, Merrill JT, Lipsky P, Harley JB: **Genetic association of interleukin-21 polymorphisms with systemic lupus erythematosus.** *Ann Rheum Dis* 2008, **67**:458-461.
29. Julià A, Ballina J, Cañete JD, Balsa A, Tornero-Molina J, Naranjo A, Alperi-López M, Erra A, Pascual-Salcedo D, Barceló P, Camps J, Marsal S: **Genome-wide association study of rheumatoid arthritis in the Spanish population: KLF12 as a risk locus for rheumatoid arthritis susceptibility.** *Arthritis Rheum* 2008, **58**:2275-2286.
30. Chistiakov DA, Voronova NV, Chistiakov PA: **The crucial role of IL-2/IL-2RA-mediated immune regulation in the pathogenesis of type 1 diabetes, an evidence coming from genetic and animal model studies [review].** *Immunol Lett* 2008, **118**:1-5.
31. Spolski R, Leonard WJ: **The Yin and Yang of interleukin-21 in allergy, autoimmunity and cancer [review].** *Curr Opin Immunol* 2008, **20**:295-301.