

FULL PAPER

The PTPN22 R620W polymorphism associates with RF positive rheumatoid arthritis in a dose-dependent manner but not with HLA-SE status

AT Lee¹, W Li¹, A Liew¹, C Bombardier², M Weisman³, EM Massarotti⁴, J Kent⁵, F Wolfe⁶, AB Begovich⁷ and PK Gregersen¹

¹North Shore-LIJ Research Institute, Manhasset, NY, USA; ²University of Toronto, Toronto, Canada; ³Cedars Sinai Hospital, Los Angeles, CA, USA; ⁴Tufts New England Medical Center, Boston, MA, USA; ⁵Abbott Laboratories, Abbott Park, IL, USA; ⁶National Data Bank for Rheumatic Diseases, Wichita, KS, USA; ⁷Celera Diagnostics, Alameda, CA, USA

We have recently described the association between rheumatoid arthritis and a coding single-nucleotide polymorphism in the intracellular protein tyrosine phosphatase, PTPN22. The disease-associated polymorphism, 1858 C/T (rs2476601), encodes an amino-acid change (R620W) in one of four SH3 domain binding sites in the PTPN22 molecule. We have now extended our initial studies to address three questions: (1) Is the association with rheumatoid arthritis limited to rheumatoid factor (RF) positive disease? (2) Does homozygosity for PTPN22 R620W substantially increase disease susceptibility? (3) Is there an interaction between PTPN22 and the rheumatoid arthritis (RA)-associated HLA-DRB1 shared epitope alleles? A total of 1413 Caucasian rheumatoid arthritis patients and 1401 Caucasian controls were genotyped. The results support the view that PTPN22 was strongly and preferentially associated with RF positive disease (OR = 1.75, 95% CI 1.46–2.10, $P = 1.3 \times 10^{-9}$). The PTPN22 risk allele was not significantly associated with RF negative disease (OR = 1.19, 95% CI 0.92–1.53, $P = 0.18$), although a very weak association cannot be completely excluded. There was a strong dose effect on disease risk; two copies of the PTPN22 R620W allele more than doubles the risk for RF positive RA (OR = 4.57, 95% CI 2.35–8.89). There was no evidence of a genetic association between PTPN22 and HLA susceptibility alleles.

Genes and Immunity (2005) 6, 129–133. doi:10.1038/sj.gene.6364159
Published online 23 December 2004

Keywords: rheumatoid arthritis; PTPN22; RF-positive; genetic association; HLA; risk allele

Introduction

Rheumatoid arthritis (RA) affects up to 1% of the adult population worldwide, making it the most common systemic autoimmune disease. It is two to three times more prevalent in women than in men and is characterized by immune-cell-mediated destruction of the joint architecture. Long-term prognosis is generally poor with joint destruction affecting approximately 80% of affected individuals within 20 years.^{1–3}

Rheumatoid factor (RF), which is an immunoglobulin directed against the Fc portion of IgG, is found in over 70% of patients with RA; high RF may confer a worse prognosis. RA patients who are RF positive tend to have more aggressive erosive bone and joint disease than those who are RF negative.^{4,5} The presence of RF may precede clinical diagnosis by many years, indicating that its production may be associated with an early key event in the autoimmune response.^{6–9} It is presently unclear to

what extent RF positive disease differs from RF negative disease in terms of disease pathogenesis, and aside from differences in HLA associations, there are no clearly established genetic differences between the two sub-phenotypes of RA.

In a recent case-control association study, Begovich *et al*¹⁰ found a strong association of the PTPN22 1858C/T (rs2476601) single-nucleotide polymorphism (SNP) with RA. The presence of the minor 1858T allele increased the relative risk for RA almost two-fold. The carrier frequency of the 1858T risk allele in Caucasians with RA was 28% while this allele was present in approximately 17% of Caucasian controls. In this original report, the predominant association appeared to be with RF positive RA, although the number of subjects with RF negative disease was too low to reach a definitive conclusion. We have now examined this issue in a large set of both RF positive and RF negative rheumatoid arthritis patients and controls. We document that PTPN22 is specifically associated with RF positive RA, and displays a marked dose effect. Interestingly, despite the association of HLA-DRB1 with rheumatoid factor production in our RA population, there is no evidence of an association between PTPN22 and the RA associated HLA-DRB1 alleles.

Correspondence: Dr AT Lee, North Shore LIJ Research Institute, 350 Community Drive, Manhasset, NY 11030, USA.
E-mail: ANLEE@NSHS.EDU
Received 20 September 2004; revised 1 November 2004; accepted 2 November 2004; published online 23 December 2004

Results

In order to obtain a large enough sample size for these studies, we combined RA cases from several sources, as detailed in the Methods section. Briefly, 372 patients with longstanding RA were studied from the Wichita Arthritis Center.¹¹ In addition, patients from two recent onset cohorts were available for study. The SONORA cohort (Study of New Onset Rheumatoid Arthritis) is comprised of 1017 patients with new onset disease who were enrolled between 2000 and 2003 for a 5-year prospective, observational study.¹² All patients were enrolled within 12 months of disease onset, and are undergoing full clinical evaluation at multiple time points, including baseline and follow-up hand X-rays. DNA and serum samples from 705 of these patients were available for study. A second longitudinal cohort, being followed in the National Data Bank for Rheumatic Diseases (NBD), has enrolled new onset RA patients within 6 months of diagnosis;¹³ 336 patients from this cohort were chosen for study on the basis of having been followed for 4 years. From these three resources we assembled 1413 RA patients, of whom 968 (68.5%) were RF positive on at least one occasion, and 445 were RF negative. In the SONORA cohort, serological measurements of RF were available at baseline and 1 year. Patients seen at the Wichita Arthritis Center generally had multiple serological assays carried out over many years. Patients in the NBD inception cohort were tested only at the time of enrollment.

Genotyping data from all Caucasian RA patients in the three sample sets were combined for analysis ($n = 1413$) (Table 1). For the entire group of RA patients, the heterozygous CT genotype was present in 287 patients (20.31%, $P = 6.9 \times 10^{-4}$, OR = 1.4, 95% CI 1.15–1.70) while the TT genotype was found in 41 patients (2.9%, $P = 2.6 \times 10^{-5}$, OR = 3.68, 95% CI 1.92–7.04). The presence of the risk genotypes (CT or TT) was significantly higher in the RA cases (23.21%) compared to the control group (16.63%, $n = 1401$) ($P = 1.2 \times 10^{-5}$, OR = 1.52, 95% CI 1.26–1.83). The overall frequency of the 1858T risk allele was 13.06% in the RA cases and 8.74% in the control

group. Compared to the controls, there was significant association of the risk allele in the RA cases ($P = 2.1 \times 10^{-7}$, OR = 1.57, 95% CI 1.32–1.86) (Table 1). These results are consistent with our previous report.¹⁰

We next stratified our analysis by RF status of the case group (Table 1). In the RF positive subgroup the frequency of the risk genotype increased slightly when compared to all RA patients (25.21 vs 23.21%). In contrast, the frequency of the risk genotype decreased to 18.88% in the RF negative subgroup, a figure that is significantly different from the RF positive subgroup ($P = 8.8 \times 10^{-3}$, OR = 1.45, 95% CI 1.10–1.91). Compared to the controls, we did not observe a significant difference in risk genotype frequency in the RF negative subgroup ($P = 0.27$), but we did for the RF positive subgroup ($P = 3.1 \times 10^{-7}$). For the allele frequency comparison, the 1858T allele frequency was significantly increased at 14.36% in the RF positive subgroup compared with the control T allele frequency of 8.74% ($P = 1.3 \times 10^{-9}$, OR = 1.75, 95% CI 1.46–2.10). The risk allele frequency of the RF negative subgroup (10.22%) was not different from that of the normal controls ($P = 0.18$), but it was significantly different when compared to the RF positive subgroup ($P = 2.4 \times 10^{-3}$, OR 1.47, 95% CI 1.15–1.89).

In addition to the specific association of PTPN22 R620W with RF positive disease, there was strong evidence for a dose effect in the RF positive subgroup. As shown in Table 1, when comparing the risk profiles of the CT and the TT genotypes, the TT genotype carries approximately a three-fold risk for RF positive disease, compared with the CT genotype, with OR = 4.57 and 1.53, respectively. Compared with controls, this increase in risk of the TT genotype was highly significant ($P = 9.8 \times 10^{-7}$).

Finally, we searched for evidence of association between PTPN22 and HLA-SE genotypes within the group of RF positive patients. Both HLA DR typing and PTPN22 data were available on 658 subjects. Table 2 shows the distribution of PTPN22 genotypes among the various HLA subgroups according to the number of shared epitopes, and also stratified by the presence of the

Table 1 Genotype and allele frequencies of PTPN22 1858 T/C SNP in rheumatoid arthritis cases, stratified by the presence of rheumatoid factor

	Allele frequency			Genotype frequency				OR for Genotype vs CC		
	C	T	OR, 95% CI	CC	CT	TT	CT or TT	CT	TT	CT or TT
^a RA ($n = 1413$)	2457 (86.94%)	369 (13.06%)	1.57 (1.32–1.86) $P = 2.1 \times 10^{-7}$	1085 (76.79%)	287 (20.31%)	41 (2.9%)	328 (23.21%)	1.40 (1.15–1.70) $P = 6.9 \times 10^{-4}$	3.68 (1.92–7.04) $P = 2.6 \times 10^{-5}$	1.52 (1.26–1.83) $P = 1.2 \times 10^{-5}$
^a RF+ ($n = 968$)	1658 (85.64%)	278 (14.36%)	1.75 (1.46–2.10) $P = 1.3 \times 10^{-9}$	724 (74.29%)	210 (21.68%)	34 (3.51%)	244 (25.21%)	1.53 (1.23–1.89) $P = 6.4 \times 10^{-5}$	4.57 (2.35–8.89) $P = 9.8 \times 10^{-7}$	1.69 (1.38–2.07) $P = 3.1 \times 10^{-7}$
^a RF ($n = 445$)	799 (89.78%)	91 (10.22%)	1.19 (0.92–1.53) $P = 0.18$	361 (81.12%)	77 (17.30%)	7 (1.57%)	84 (18.88%)	1.13 (0.85–1.50) $P = 0.41$	1.89 (0.74–4.83) $P = 0.18$	1.17 (0.89–1.54) $P = 0.27$
Control ($n = 1401$)	2557 (91.26%)	245 (8.74%)		1168 (83.37%)	221 (15.77%)	12 (0.86%)	233 (16.63%)			
RF+ vs RF–			1.47 (1.15–1.89) $P = 2.4 \times 10^{-3}$					1.36 (1.02–1.82) $P = 0.037$	2.42 (1.06–5.52) $P = 0.030$	1.45 (1.10–1.91) $P = 8.8 \times 10^{-3}$

^aControls are used as reference for all comparisons, except as indicated (RF+ vs RF–).

Table 2 A 3 × 3 table showing the distribution of PTPN22 genotypes among RF+ patients stratified by the presence of 0, 1 or 2 copies of the HLA-DRB1 shared epitope (s.e.), or by DRB1*0401

	CC	CT	TT
0 SE (<i>n</i> = 167)	132 (79.04%)	29 (17.37%)	6 (3.59%)
95% CI ^a	(72.87–85.22)	(11.62–23.11)	(0.77–6.42)
1 SE (<i>n</i> = 333)	246 (73.87%)	74 (22.22%)	13 (3.90%)
95% CI ^a	(69.16–78.59)	(17.76–26.69)	(1.82–5.98)
2 SE (<i>n</i> = 158)	123 (77.85%)	29 (18.35%)	6 (3.80%)
95% CI ^a	(71.37–84.32)	(12.32–24.39)	(0.82–6.78)
DRB1*0401 ^b (<i>n</i> = 266)	196 (73.68%)	58 (21.80%)	12 (4.51%)
95% CI ^a	(68.39–78.98)	(16.84–26.77)	(2.02–7.01)

^a95% confidence interval of the proportion/frequency within each cell is given. There are no significant differences between cells corresponding to a given PTPN22 genotype.

^bIndividuals with one or two copies of DRB1*0401.

major common risk allele DRB1*0401. These subgroups were not significantly different as reflected in the overlapping confidence intervals within the relevant cells. A formal analysis of association in the RF positive patients revealed no significant associations between the PTPN22 genotypes and the presence of the SE. We also examined allelic subgroups of HLA-DR in subjects carrying only one shared epitope allele. Specifically, we stratified individuals by virtue of carrying a single copy of a 'high risk' allele (DRB1*0401 or 0404) or a 'low risk' allele (DRB1*0101 or 1001). This analysis also did not reveal any evidence of association between PTPN22 and HLA-SE genotypes (Table 2 and data not shown).

Discussion

We previously reported the strong association of the 1858C/T PTPN22 missense SNP with RA¹⁰. Herein, we confirm and expand those findings in a combined sample derived from three large independent Caucasian RA sample sets. We have further stratified our RA population by RF production and the presence of SE alleles. A statistically significant association of the PTPN22 risk allele is observed exclusively within the RF positive subgroup; minimal, if any, evidence for association was found in patients with RF negative disease. It is possible that the OR > 1 in the RF negative group may reflect a very weak association of the PTPN22 R620W allele with this disease subset. However, it seems likely that misclassification of RF positive patients into this group is a better explanation for this finding, since only one or two RF measurements were carried out for the majority of the patients under study.

The predominant association of PTPN22 R620W with RF positive RA is of considerable interest in light of the phenotype that is observed in the PEP knockout mouse (PEP is the murine homolog of human PTPN22). Hasegawa *et al*¹⁴ showed that T cells from PEP knockout mice had lowered thresholds for TCR signaling and increased numbers of effector and memory T cells. In addition, these knockout mice showed evidence of increased number of germinal centers (GC) and in-

creased immunoglobulin levels. These abnormalities in the humoral immune system appear to reflect the role of T cells in regulating B cell differentiation, since changes in B cell function were sought, but not observed in these mice. Thus, although a direct role for PTPN22 in B cell signaling is possible, the abnormal production of GC in this model is ascribed to a T cell defect. Notably, autoantibodies were not observed in these knockout mice. However, the genetic background in which these experiments were performed (B6) is not associated with autoimmunity.

The association of PTPN22 R620W with the production of RF in RA is consistent with the fact that other autoimmune diseases that have a prominent humoral component also show an association with this polymorphism. Both type 1 diabetes¹⁵ and systemic lupus erythematosus¹⁶ have been reported to associate with the PTPN22 W620 allele with similar levels of risk, and the data in SLE support a dosage effect similar to what we have observed in RA.¹⁶ In addition, we have recently shown an association with autoimmune thyroid disease (Criswell *et al*, manuscript in preparation). Strikingly, in all of these disorders, the appearance of autoantibodies can precede the development of overt clinical disease by months or years.^{17–19} Thus, it will be of considerable interest to investigate whether PTPN22 is primarily associated with the early development of these autoantibodies, or, alternatively whether the PTPN22 risk allele influences the progression to overt autoimmune disease once autoantibodies have developed. Of course, these possibilities are not mutually exclusive.

The formation of ectopic GC has been reported in both RA and autoimmune thyroiditis, as well as several other autoimmune diseases.²⁰ Excessive GC formation has also been observed in autoimmune mouse strains that exhibit type 1 diabetes and lupus-like disease.²¹ In the case of RA, the presence of GC within the inflamed synovium is not the most common phenotype, but it is present in a significant minority of patients, and is accompanied by distinct patterns of cytokine and chemokine expression.²² Rheumatoid factor is produced locally by B cells within the inflamed synovium, and sequence analysis of these immunoglobulins can show evidence of somatic mutation^{23,24}

although many synovial B cells do not show this, perhaps because they are recent immigrants.⁴ Evidence of such somatic mutation is consistent with the T cell dependent B cell differentiation that typically occurs within GC. It is not entirely clear whether the variability of synovial pathology reflects meaningful disease subsets or different stages of disease, or both. Detailed information on synovial histology is not available for analysis in large populations of patients. However, it will clearly be of great interest to examine whether the PTPN22 genotype correlates with these phenotypes, and particularly whether homozygosity for the R620W allele is associated with the more prominent appearance of ectopic GC within the rheumatoid synovium. Since CD8+ CD40L+ T cells have been implicated in the maintenance of these structures,²⁵ a specific role for PTPN22 in the regulation of these T cells should be explored in this context.

In the setting of rheumatoid arthritis, RF production is thought to be dependent, at least in part, on MHC class II restricted CD4+ T cell responses.³ RA associated HLA-DR alleles bearing the shared epitope, particularly DRB1*0401 and 0404, are positively associated with RF production in patients with RA. This is also the case in our data set (data not shown). However, HLA-DRB1 shared epitope alleles are neither sufficient nor required for RF production. Certain environmental factors, such as smoking, are also associated with RF production in RA, and appear to interact with HLA-DR.²⁶ Nevertheless, our results indicate that the PTPN22 R620W polymorphism is an independent risk factor for RF positive RA, and there is no convincing evidence for an association with HLA SE alleles in this data set.

We have shown that homozygosity for the PTPN22 risk allele dramatically increases susceptibility to RF positive disease, and similar findings have been noted in systemic lupus¹⁶ and are suggested by the published data on type 1 diabetes.¹⁵ This implies that the negative regulatory effect of PTPN22 is dose-dependent, consistent with a threshold effect on TCR signaling. Others and we have shown that R620W polymorphism reduces binding of PTPN22 to the intracellular tyrosine kinase, Csk, by altering the proximal SH3 binding site in the PTPN22 molecule.^{10,15} However, an alteration of T cell function by the R620W allele has not yet been directly demonstrated in humans. The evidence of a dose effect for disease susceptibility suggests that *in vitro* functional studies will be more fruitful if they are carried out in homozygous individuals, in whom a relatively subtle change in TCR signaling thresholds may be more easily detected.

In summary, our results from three independent Caucasian RA populations reaffirm our initial findings of an association of the PTPN22 R620W polymorphism with RA. In addition, we have shown that the predominant association is with RF positive, but not RF negative, disease. There is a marked dosage effect when comparing the CT and TT risk genotypes, and there is no association between HLA SE status and PTPN22 R620W. Further investigations are needed to identify the potential role of PTPN22 in other clinical manifestations of RA. Longitudinal outcome data from the SONORA study should be particularly useful in this regard with respect to disease progression as well the response to specific biologic therapies.¹²

Patients and methods

Patients

Patient samples were obtained from the Wichita Rheumatic Disease Data Bank^{11,27,28} and The Arthritis, Rheumatism and Aging Medical Information System, National Inception Cohort of Rheumatoid Arthritis Patients¹³ ($N = 779$) and from The SONORA¹² (Study Of New Onset Rheumatoid Arthritis) Study ($N = 823$). These RA sample sets were combined and only Caucasian patients were subsequently used in the analysis. Patient samples obtained from the Wichita Rheumatic Disease Data Bank were representative of Caucasian patients followed in a rheumatology practice since 1974. Mean disease duration was greater than 10 years and mean age of onset of 45 years, 83% of this cohort were RF positive over several time points. Patient samples used in conjunction with the NDB Inception Cohort of Rheumatoid Arthritis Patients were collected as previously described were also included in this cohort.¹³ Enrollment of patients occurred within 6 months of clinical diagnosis. The mean age of this cohort was 54 years old, 65% were RF positive at time of enrollment.

Patient samples obtained from The SONORA Study were from Caucasian patients meeting the ACR criteria and enrolled within 3–12 months of diagnosis. The mean age of onset was 52 years and 64% were RF titer positive either at study enrollment or at 1-year follow-up.

RF determination

RF values were determined by latex fixation (RF + >25) for the Wichita Rheumatic Disease Data Bank and NDB Inception Cohort of Rheumatoid Arthritis. For patients of the NDB Inception Cohort of Rheumatoid Arthritis, RF status was based on a single baseline time point. Patients from the Wichita Rheumatic Disease Data Bank had multiple RF measurements, if all were negative; the patient was categorized RF negative. RF values for SONORA samples were measured by laser nephelometry at baseline and at 1 year. If either were positive (>40), the patient was categorized as RF positive.

Genotyping

After receiving informed consent, DNA was isolated by standard methodologies from peripheral whole-blood samples. Genotyping of the PTPN22 SNP (rs2476601, 1858C→T, R620W) was performed using a PSQ HS 96A Pyrosequencer. Briefly, 2 ng of DNA was amplified by PCR in a 10 μ l reaction using the following primers: forward 5'-GTTGCGCAGGCTAGTCTTGA-3', reverse 5'-GCT GCT CCG GTT CAT AGA TT GGATAG-CAACTGCTCCAAGG-3', Univ1_B 5'-Biotin-GCT GCT CCG GTT CAT AGA TT-3'. The addition specific sequences to the 5' end of the reverse primer (shown in italics) allowed the use of a biotinylated universal primer Univ1_B. These primers were used at a ratio of 1:9 (reverse:universal primer). PCR conditions were as follows: 95°C – 12 min, 50 \times (95°C – 45 s, 56.4°C – 45 s, 72°C – 45 s), 72°C – 10 min, 4°C forever. The amplicon was denatured with NaOH, separated, washed and neutralized. The sequencing primer 5'AAATGATTCAGGTGTCC3' was used in combination with appropriate pyrosequencing substrates and enzymes according to the manufacturer's instructions to detect the polymorphism.

HLA typing was performed as previously described.²⁹

Statistical analysis

All 2-by-2 contingency tables are analyzed by the Pearson's χ^2 test. The statistical program used is S-PLUS version 3.4 (MathSoft, Inc.), and the subroutine *chisq.test* was applied. Fisher's exact test led to similar results (R version 1.8.0, subroutine *fisher.test*), thus these were not shown here. The formula for the 95% confidence interval for the odds ratio (OR) is due to Woolf.³⁰

Acknowledgements

We would like to acknowledge all RA patients who participated in each of the RA studies. We thank Lyn Maguire for support of the SONORA Project and Marlena Kern for her commitment to SONORA and all the projects within Robert S Boas Center for Genomics and Human Genetics, TL Bugawan and E Trachtenberg for HLA typing, A Chokkalingham and V Carlton for valuable comments on this manuscript, and Abbott Laboratories for financial support of the SONORA study. PKG is supported in part by NIH grants RO1 AR44422 and NO1-AR-2-2263.

References

- McInnes IB. Rheumatoid arthritis. From bench to bedside. *Rheum Dis Clin N Am* 2001; **27**: 373–387.
- Gabriel SE. The epidemiology of rheumatoid arthritis. *Rheum Dis Clin N Am* 2001; **27**: 269–281.
- Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature* 2003; **423**: 356–361.
- Dorner T, Egerer K, Feist E, Burmester GR. Rheumatoid factor revisited. *Curr Opin Rheumatol* 2004; **16**: 246–253.
- Newkirk MM. Rheumatoid factors: what do they tell us? *J Rheumatol* 2002; **29**: 2034–2040.
- Davidson A, Bridges Jr SL. Autoimmunity. In: St Clair EW, Pisetsky DS, Haynes BF (eds). *Rheumatoid Arthritis*. Lippincott Williams and Williams: Philadelphia, 2004, pp 197–212.
- Halldorsdottir HD, Jonsson T, Thorsteinsson J, Valdimarsson H. A prospective study on the incidence of rheumatoid arthritis among people with persistent increase of rheumatoid factor. *Ann Rheum Dis* 2000; **59**: 149–151.
- Rantapaa-Dahlqvist S, de Jong BA, Berglin E et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003; **48**: 2741–2749.
- Nielen MM, van Schaardenburg D, Reesink HW et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004; **50**: 380–386.
- Begovich AB, Carlton VE, Honigberg LA et al. 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.
- Baugh JA, Chitnis S, Donnelly SC et al. A functional promoter polymorphism in the macrophage migration inhibitory factor (MIF) gene associated with disease severity in rheumatoid arthritis. *Genes Immun* 2002; **3**: 170–176.
- Bombardier C, Deaton R, Gregersen P, Massarotti E, Formica C, Weisman M. Pattern of DMARD use in a North American cohort of patients with early rheumatoid arthritis (SONORA). *Arthritis Rheum* 2002; **46**: S344 (abstract).
- Fries JF, Wolfe F, Apple R et al. HLA-DRB1 genotype associations in 793 white patients from a rheumatoid arthritis inception cohort: frequency, severity, and treatment bias. *Arthritis Rheum* 2002; **46**: 2320–2329.
- Hasegawa K, Martin F, Huang G, Tumas D, Diehl L, Chan AC. PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. *Science* 2004; **303**: 685–689.
- Bottini N, Musumeci L, Alonso A et al. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet* 2004; **36**: 337–338.
- Kyogoku C, Langefeld CD, Ortmann WA et al. Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. *Am J Hum Genet* 2004; **75**: 504–507.
- Arbuckle MR, McClain MT, Rubertone MV et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* 2003; **349**: 1526–1533.
- Hoppu S, Ronkainen MS, Kulmala P, Akerblom HK, Knip M, Childhood Diabetes in Finland Study Group. GAD65 antibody isotypes and epitope recognition during the prediabetic process in siblings of children with type I diabetes. *Clin Exp Immunol* 2004; **136**: 120–128.
- Strieder TG, Prummel MF, Tijssen JG, Endert E, Wiersinga WM. Risk factors for and prevalence of thyroid disorders in a cross-sectional study among healthy female relatives of patients with autoimmune thyroid disease. *Clin Endocrinol (Oxford)* 2003; **59**: 396–401.
- Weyand CM, Kurtin PJ, Goronzy JJ. Ectopic lymphoid organogenesis: a fast track for autoimmunity. *Am J Pathol* 2001; **159**: 787–793.
- Luzina IG, Atamas SP, Storrer CE et al. Spontaneous formation of germinal centers in autoimmune mice. *J Leukocyte Biol* 2001; **70**: 578–584.
- Weyand CM, Goronzy JJ. Ectopic germinal center formation in rheumatoid synovitis. *Ann NY Acad Sci* 2003; **987**: 140–149.
- Haberman AM, William J, Euler C, Shlomchik MJ. Rheumatoid factors in health and disease: structure, function, induction and regulation. *Curr Dir Autoimmun* 2003; **6**: 169–195.
- Randen I, Thompson KM, Pascual V et al. Rheumatoid factor V genes from patients with rheumatoid arthritis are diverse and show evidence of an antigen-driven response. *Immunol Rev* 1992; **128**: 49–71.
- Kang YM, Zhang X, Wagner UG et al. CD8T cells are required for the formation of ectopic germinal centers in rheumatoid synovitis. *J Exp Med* 2002; **195**: 1325–1336.
- Mattey DL, Dawes PT, Clarke S et al. Relationship among the HLA-DRB1 shared epitope, smoking, and rheumatoid factor production in rheumatoid arthritis. *Arthritis Rheum* 2002; **47**: 403–407.
- Choi HK, Hernan MA, Seeger JD, Robins JM, Wolfe F. Methotrexate therapy and mortality in patients with rheumatoid arthritis: a prospective study. *Lancet* 2002; **359**: 1173–1177.
- Wolfe F, Michaud K, Gefeller O, Choi HK. Predicting mortality in patients with rheumatoid arthritis. *Arthritis Rheum* 2003; **48**: 1530–1542.
- Jawaheer D, Li W, Graham RR et al. Dissecting the genetic complexity of the association between human leukocyte antigens and rheumatoid arthritis. *Am J Hum Genet* 2002; **71**: 585–594.
- Woolf B. On estimating the relationship between blood group and disease. *Ann Hum Genet* 1955; **19**: 251–253.