Transforming growth factor β1 gene (HSTGFB1) nucleotide T869C (codon 10) polymorphism is not associated with prevalence or severity of rheumatoid arthritis in a Caucasian population

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In a recent report in the *Annals* Sugiura and colleagues found an association between rheumatoid arthritis (RA) and a single nucleotide polymorphism (SNP) of HSTGFB1 at nucleotide (nt) +869 (T869C; nt position relative to GenBank accession X05839), a coding SNP producing a Leu→Pro substitution in codon 10.[1] The CC genotype was found in 29/155 (19%) central Japanese patients with RA compared with 33/110 (30%) controls and this was significant when the CT and TT genotypes were pooled. Codon 10 is in the signalling peptide region and the Pro (869C) allele has been associated with higher transforming growth factor β1 (TGFβ1) production. TGFβ1 is present in the synovial tissue of patients with RA, and in addition to its profibrotic activities has regulatory effects on cells important in the pathogenesis of RA, including lymphocytes, dendritic cells, macrophages, chondrocytes, and osteoblasts.[2–5] Administration of TGFβ suppresses collagen induced arthritis, whereas antibodies to TGFβ exaggerate the process.[6] Crilly and colleagues reported an increase in the high producing codon 10 Leu (nt869T) in patients with systemic sclerosis.[7] This polymorphism has also been associated with osteoporosis and with osteophytosis.[8 9]

METHODS AND RESULTS

To explore whether this polymorphism is associated with the prevalence and/or severity of RA in white patients, we used polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis in 117 unrelated patients with RA and in 140 healthy blood donors matched for ethnicity with no known past or family history of RA or associated autoimmune disease. The patients were part of a prospective cohort who had been recruited within six months of their first RA symptom and characterised with detailed clinical, laboratory, radiographic, and immunogenetic assessments.[10] Forty two patients were also examined with magnetic resonance imaging of the dominant wrist, as reported previously.[11]

Genomic DNA was amplified with PCR primers synthesised complementary to the genomic sequence,[10] with an A→T mismatch introduced at position 25 of the reverse primer (complementing HSTGFB1 nt872) to create a *Pst1* restriction enzyme recognition site with the C allele at nt869: TGFβ1-Leu10-forward 5′ ACC ACA CCA GCC CTG TTC GC 3′ and TGFβ1-Leu10-reverse 5′AGT AGC CAC AGC AGC GGT AGC AGC TGC 3′. With this change *Pst1* digests the 110 bp PCR product into 86 and 24 bp fragments only if the C allele is present. Sequenced controls for each of the three genotypes CC, CT, and TT were included to validate each digestion. To explore interactions between HSTGFB1 nt869/codon 10 and HLA-DR "shared epitope" status, HLA-DRB1 genotype was determined at high resolution by sequence based typing. Allele frequency data in 2×2 contingency tables were analysed with Fisher's exact test with the approximation of Woolf, genotype frequencies in 2×3 contingency tables were analysed with the χ^2 test, and continuous variables of RA clinical severity were compared between genotypic groups with the Mann-Whitney U test.

Table 1 TGFβ1 nt869 (codon 10) genotype and allele frequencies in RA and control subjects

	Patients with RA No (%)	Controls No (%)
TGFβ1 nt869 genotype		
TT	32 (27)	41 (29)
TC	58 (50)	73 (52)
CC	27 (23)	26 (19)
TGFβ1 nt869 allele frequency		
T	0.52	0.56
C	0.48	0.44

The nt869 allele and genotype frequencies were in Hardy-Weinberg equilibrium and did not differ between patients and controls (table 1). Median age of onset did not differ according to HSTGFB1 nt869 genotype (TT 53 years, TC 48, CC 56; NS). Within the prospective cohort of patients with early RA no difference was found according to HSTGFβ1 nt869 genotype for measures of disease severity over a two year period of follow up: IgM rheumatoid factor status (positive at >40 IU/ml in 68% of CC genotype patients with RA *v* 66% with CT and TT), erythrocyte sedimentation rate (27 and 28 mm/1st h at baseline, 21 and 20 mm/1st h at two years), C reactive protein level (10 and 13 mg/l at baseline, 6 and 9 mg/l at two years), Health Assessment Questionnaire (0.50 and 0.60 at baseline, 0.20 and 0.20 at two years), disease activity scores (3.22 and 3.46 at baseline, 2.06 and 2.16 at two years), or the prescribing of disease modifying antirheumatic drugs (44% and 51% at baseline, 73% and 73% at two years). Radiographic erosions were present in 24/116 (21%) patients with RA at baseline, 50/112 (45%) at one year, and 61/111 (55%) at two years' follow up and did not differ according to TGFβ1 nt869 genotype. Radiographic data were incomplete for one, five, and six patients, respectively, owing to pregnancy or withdrawal from the study. Wrist magnetic resonance erosion, tendon, synovial, and contrast enhancement scores did not differ according to nt869 genotype. Stratifying the RA cohort according to HLA-DRB1 shared epitope (DRB1*0101, 0401, 0404, 0405, 0408, 1001, and 1402) status did not show any additional relationships. The signal sequence polymorphism at codon 25 (Arg→Pro; nt G915C) was also examined using PCR-RFLP (*Sau96I*),[12] but similarly showed no association with RA prevalence or severity (data not included).

DISCUSSION

In this study of Caucasian patients with RA we did not confirm the association recently reported[1] with HSTGFB1 nt869/codon 10 in Japanese patients. This discrepancy may be due to the different ethnic populations studied; Japanese

patients with RA have different HLA-DR associations (DRB1*0405, 0410) and, possibly, their non-HLA component is also different from that of Caucasian subjects.

This study cannot exclude a weak association. RA is a genetically complex disease, and it is likely that most of the non-HLA genes contributing to the approximately 50% genetic component in RA are individually very weak. To exclude a 4% difference between patients and controls (allele frequencies 0.48 and 0.44, respectively, in our sample of 257 subjects) with 80% power at an α level of 0.05 would require testing over 2400 subjects in each group and 90% power would need 3200 in each group, beyond the scope of any published RA association genetics study. In addition, this study cannot exclude the possibility of an effect of other polymorphisms on RA, or an effect that may only be manifest when the nt869 SNP is present on the background of a particular extended haplotype. Several additional polymorphisms have been identified within or close to HSTGFB1, and further detailed study would be needed to exclude fully a contribution from this gene.

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Acute, non-obstructive, sterile cholecystitis associated with etanercept and infliximab for the treatment of juvenile polyarticular rheumatoid arthritis

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Etanercept and infliximab are soluble tumour necrosis factor α (TNFα) antibodies. Etanercept is approved for the treatment of polyarticular juvenile rheumatoid arthritis (JRA), and infliximab is in the process of being approved. We report the first case in which the same patient developed a non-obstructive, sterile cholecystitis with etanercept (Enbrel) and later with infliximab (Remicade).

CASE REPORT

A 15 year old white female patient with polyarticular JRA, treated with a combination of 14 mg/m^2 methotrexate once a week intramuscularly and 2 g sulfasalazine a day, did not show any remission. Sulfasalazine was stopped, and 0.4 mg/kg etanercept twice a week subcutaneously was added. With this combination treatment the patient was in full remission after 4 weeks, but at 12 weeks her disease flared. Etanercept was increased to 0.5 mg/kg twice a week subcutaneously. Two weeks later she developed upper abdominal pain, nausea, and weight loss; she could not attend school. Gastroscopy showed a minimally active duodenitis and antrum gastritis. Histologically a helicobacter infection was proved. The abdominal sonography showed a thickened gall bladder with a halo sign. The laboratory tests showed normal lipase, aspartate aminotransferase, and alanine aminotransferase, but increased bilirubin (20 µmol/l). Despite eradication treatment against *Helicobacter pylori*, the symptoms were unchanged. A puncture of the gall bladder did not help to prove any infectious agent. Shortly after stopping etanercept, the abdominal symptoms resolved, and ultrasound showed a decrease of the thickness of the gallbladder wall.

Her arthritis flared, so infliximab was started at a dose of 3 mg/kg, and given at 0, 2, 4, 8, 14, and 20 weeks. Again her arthritis improved quickly. At week 20 the same upper abdominal pain and increase of bilirubin occurred as during etanercept treatment. The abdominal sonography again showed thickening of the gallbladder wall. Infliximab had to be stopped. A cholecystectomy was conducted, and the