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Letters to the Editor

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A common functional variant of endoplasmic reticulum aminopeptidase 2 (*ERAP2*) that reduces major histocompatibility complex class I expression is not associated with ankylosing spondylitis

SIR, The strong genetic association between AS and HLA-B27 has defied explanation for nearly 40 years. However, the additional discovery of a strong association between AS and *ERAP1* (endoplasmic reticulum aminopeptidase 1), a gene that almost certainly operates in the trimming of peptides for optimal binding to MHC class I molecules, has rekindled hopes of rapid advances in this field [1]. It has been suggested that another aminopeptidase, endoplasmic reticulum aminopeptidase 2 (ERAP2), may act in concert with ERAP1, trimming residues inefficiently removed by ERAP1 [2]. The association described recently between *ERAP2* and Crohn's disease, which shares many clinical and genetic overlaps with AS [3], suggests that *ERAP2* is worthy of further study in AS. An experiment of nature allows us to do this relatively simply.

ERAP2 has evolved under balancing selection, similar to the MHC, and includes a high-frequency variant (~50%) that influences antigen presentation [4]. A single nucleotide polymorphism (SNP), rs2248374 (A to G), located within the 5′ canonical splice site of exon 10, results in an alternatively spliced *ERAP2* mRNA that is degraded by nonsense-mediated decay (NMD). Homozygosity for the minor G allele (carried by ~25% of the population) results in failure to express ERAP2 protein; in turn this genotype is also associated with reduced surface MHC Class I expression on human B cells [4]. Such a dramatic phenotypic effect provides a great opportunity for studying the potential role of *ERAP2* in AS, analogous to using a knockout model.

We therefore specifically tested for differences in frequency of rs2248374 in 470 sporadic AS cases and 420 healthy, ethnically matched blood donors to determine whether this functionally important variant is implicated in AS. All patients were Caucasians, of UK origin and fulfilled the modified New York criteria for AS. Eighty-four per cent of the AS patients were HLA-B27 positive. All patients gave informed consent and ethical approval was obtained (Multicentre Research Ethics Committee 98/5/23). Genotyping involved restriction fragment length polymorphism (RFLP) analysis of PCR products performed under standard conditions. Primers (forward 5′-GCATCCATGGCTAATGTGCR and reverse 5′-GTTGTGGGAAAGCCGAACTA) amplified a 370-bp product that, in the presence of the G allele, was digested to 214 and 156 bp products by *HphI*. Genotype and allele frequencies in AS cases and controls were compared using the Cochrane–Armitage test of trend and the chi-squared test, respectively. This study had 80% power under a log-additive model to detect an odds ratio (OR) of 1.3 with a population risk of AS of 0.04% and a risk allele frequency of 46% (frequency in our controls) at a significance level of 0.05. There were no significant differences between allele or genotype frequencies in AS cases and controls at rs2248374 (Table 1). Additionally, due to the potential functional significance of homozygosity at the minor G allele (i.e. no functional ERAP2 protein), we also compared the frequency of GG homozygotes in AS cases and controls, but these proved almost identical again (Table 1).

The association between *ERAP1* and AS is the second only to HLA-B27, but formal testing for additional association with the neighbouring gene, *ERAP2,* had not been previously undertaken. A study of familial AS that typed one marker within *ERAP2* reported that an *ERAP1*/*ERAP2* haplotype was over-represented in affected family members [5]. However, such studies are frequently confounded by linkage disequilibrium effects. If genetic variation at *ERAP2* were to play a role in susceptibility to AS, then a dramatic loss-of-function variant, such as rs2248374, would appear to be a prime candidate for the 'causal variant' at this locus. However, we have now excluded any significant effect arising from this important functional *ERAP2* variant. Nonetheless, the possibility of other associations between *ERAP2* and AS has not been excluded by this study. This could be done only by systematic mapping of the gene with tagging SNPs in a very large sample after controlling for the significant linkage disequilibrium between *ERAP1* and *ERAP2* would this

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TABLE 1 Genotypes, minor allele frequency (MAF), OR, 95% CI and *P*-value for the *ERAP2* SNP rs2248374

Patients and controls	Genotype counts			MAF	OR (95% CI)	*P*-value
	AA	AG	GG			
AS cases ($n = 470$)	125	245	100	0.47	1.07 (0.77, 1.14)	0.46
Controls ($n = 420$)	128	201	91	0.46		

be achievable. Our study was insufficiently powered to do this.

Rheumatology key message

- Despite its major functional effect on ERAP2 expression, rs2248374 shows no association with AS.

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Relapsing polychondritis-associated meningitis and encephalitis: response to infliximab

Sir, Relapsing polychondritis (RPC) is an uncommon systemic inflammatory disorder of unknown origin. A minority of patients with RPC develop neurological involvement (3%) [1]. The prognosis of patients with RPC complicated by meningoencephalitis (ME) is poor. The reported mortality of RPC-associated meningitis is 12% (3 out of 25 patients), and that of patients with RPC-associated encephalitis is 36.4% (4 out of 11 patients) [2–8]. Therapy with infliximab has been effective in several cases of resistant RPC. Nevertheless, the effects of anti-TNF-α therapy on RPC-associated meningitis and encephalitis have not previously been described. We report a patient with RPC and recurrent episodes of ME refractory to therapy with high-dose glucocorticoids and CYC, who had a satisfactory and long-lasting response to therapy with infliximab.

A 57-year-old male who immigrated 8 years ago from Ecuador presented with fever (up to 39°C), severe headache and two generalized seizures. During the previous 4 years, he had had one episode of erythema nodosum, and several episodes of symmetrical polyarthritis (hands and wrists), auricular chondritis, painful red eyes and dizziness, with the diagnoses of scleritis, cochlear dysfunction and neural deafness.

On admission, he was febrile (38.5°C) and confused with positive meningeal signs and a normal CT scan of the brain. Lumbar puncture (LP) disclosed 700 cells/ml, 98% lymphocytes, glucose 50 mg/dl and proteins 75 mg/dl. Cerebrospinal fluid (CSF) studies were negative for bacteria, virus, fungi and parasites or abnormal cells. Peripheral blood leucocytosis (20×10^9/μl, 90% neutrophils) and elevated acute-phase reactant proteins were observed. Kidney and liver function, ANAs, ANCAs, RF, urinanalysis and serological tests for HIV, hepatitis B virus, hepatitis C virus, CMV, EBV, treponema, rickettsias, borrelia, coxiella, brucella and echinococcus were all normal or negative. MRI of the brain showed small T2 gadolinium-enhanced lesions in the periventricular white matter of both cerebral hemispheres (Fig. 1b). The patient improved with high-dose i.v. methylprednisolone and was discharged.

During the following 20 months, he had seven admissions for ME with negative CSF studies. These episodes occurred while the patient was not taking any medications previously associated with ME, including non-steroidal anti-inflammatory agents. Although these episodes of ME improved with high-dose i.v. glucocorticoids plus