Evidence to support IL-13 as a risk locus for psoriatic arthritis but not psoriasis vulgaris

John Bowes,1 Steve Eyre,1 Edward Flynn,1 Pauline Ho,1,2 Salma Salah,1 Richard B Warren,2 Helena Marzo-Ortega,4 Laura Coates,4 Ross McManus,5 Anthony W Ryan,5 David Kane,6 Eleanor Korendovych,7,8 Neil McHugh,7,8 Oliver FitzGerald,9,10 Jonathan Packham,11,12 Ann W Morgan,4 Christopher E M Griffiths,3 Ian N Bruce,1,2 Jane Worthington,1 Anne Barton1,2

ABSTRACT
Objective There is great interest in the identification of genetic factors that differentiate psoriatic arthritis (PsA) from psoriasis vulgaris (PsV), as such discoveries could lead to the identification of distinct underlying aetiological pathways. Recent studies identified single nucleotide polymorphisms (SNPs) in the interleukin 13 (IL-13) gene region as risk factors for PsV. Further investigations in one of these studies found the effect to be primarily restricted to PsA, thus suggesting the discovery of a specific genetic risk factor for PsA. Given this intriguing evidence, association to this gene was investigated in large collections of PsA and PsV patients and rheumatoid controls.

Methods Two SNPs (rs20541 and rs1800925) mapping to the IL-13 gene were genotyped in 1057 PsA and 778 type I PsV patients using the Sequenom genotyping platform. Genotype frequencies were compared to those of 5575 healthy controls. Additional analyses were performed in phenotypic subgroups of PsA (type I or II PsV and in those seronegative for rheumatoid factor).

Results Both SNPs were found to be highly associated with susceptibility to PsA (rs1800925 ptrend = 6.1×10−5 OR 1.33, rs20541 ptrend = 8.0×10−4 OR 1.27), but neither SNP was significantly associated with susceptibility to PsV.

Conclusions This study confirms that the effect of IL-13 risk locus is specific for PsA, thus highlighting a key biological pathway that differentiates PsA from PsV. The identification of markers that differentiate the two diseases raises the possibility in future of allowing screening of PsA patients to identify those at risk of developing PsA.

INTRODUCTION
Psoriasis vulgaris (PsV) is a chronic inflammatory skin disease in which up to 30% of subjects exhibit additional inflammatory articular disease.1 This has led to psoriatic arthritis (PsA) being recognised as a distinct clinical entity. Both conditions are considered to be complex diseases that are influenced by environmental and genetic factors. The genetic liability towards the susceptibility to PsA, determined using the sibling recurrence risk (λs), is estimated to be much higher (λs > 30) than that of PsV (λs = 8–12).2–4 These differences are suggestive of additional genetic susceptibility loci for PsA, although it is highly likely that environmental factors also contribute to these differences.5

A number of genetic risk loci have now been identified that are associated to both diseases; for example, variants in the HLA-Cw6, IL-12B and IL-23R regions.5–7 Interestingly, differences in the genetic basis of the two diseases have been identified; the association to HLA-Cw6 appears to be stronger for PsV than PsA (OR of 6.9 and 5.0 respectively).5 In addition, a German study found variants at the LCE locus to be associated with PsV, but not PsA.5 However, a subsequent study in a British population found single nucleotide polymorphisms (SNPs) in this region to be associated with PsA.9 Given the overlap of known genetic risk factors, and the observed differences of λs, there is great interest in the identification of risk factors that are specific for PsA. Once identified, if indeed they do exist, these specific risk factors could highlight aetiological pathways that predispose to the development of PsA and potentially lead to a better understanding of why some patients with PsV develop an inflammatory arthritis.

A number of recent studies investigating the genetic susceptibility to PsV identified association to SNPs on chromosome 5q31.10–11 This region is rich in immune-related genes and contains, among others, a cluster of four interleukin (IL) genes. There is also prior evidence that the region harbours risk loci for other common autoimmune or inflammatory diseases, including Crohn’s disease.12–13 The first of the PsV studies was a multitiered case–control study investigating 25 215 gene-centric SNPs, which found association to multiple SNPs in, or in close proximity to, the IL-13 gene.10 This study identified association to three SNPs, rs1800925 (5′ upstream), rs20541 (exonic missense, Q144R) and rs848 (3′ untranslated region), where carriage of the common alleles from any of these SNPs was found to increase the risk of PsV. The second study was a large genome-wide association study (GWAS) in samples of European ancestry, which identified and replicated association to the exonic SNP, rs20541.11 A further large GWAS reported only modest association to rs20541 (p = 0.025).14 All of these studies contained significant proportions of patients identified as also having PsA. Further investigation of this effect in one of the studies found it to be primarily restricted to those individuals with PsA, thus suggesting the discovery of a potential specific genetic risk factor for PsA.15 However, no such effect was detected in the second study, as association was detected to both diseases, and the third
study did not attempt a PsA-specific analysis.11 14 Furthermore,  
there is suggestive evidence from a Chinese study supporting  
the hypothesis that this region is PsA-specific.16

Given this intriguing suggestion of a PsA-specific effect at the  
IL-13 locus, the association to these SNPs was investigated in  
large sample collections of PsA and PsV patient samples and  
allele and genotype frequencies were compared to a common  
set of healthy controls for which genotype data were already  
available.

METHODS

Patient samples

Caucasian PsA patients were recruited from three UK rheu-  
matology centres and one centre in Ireland, providing a total  
of 1057 genomic DNA samples (885 UK and 172 Ireland), the  
details of which have been described previously.17–19 PsA clas-  
sification was defined as the presence of both psoriasis and  
inflammatory arthritis, regardless of rheumatoid factor status  
and all had peripheral arthritis. The majority of samples satis-  
fied the CASPAR (ClASsification criteria for Psoriatic ARthritis)  
classification system, although some were collected prior to  
the introduction of this classification system.20 This study  
was approved by the North West Multicentre Research Ethics  
Committee (MREC 99/8/84), and all subjects provided informed  
consent.

A total of 778 unrelated patients with type I psoriasis (age of  
disease onset ≤40 years) were recruited from the Dermatology  
Centre, Salford Royal NHS Foundation Trust, The University of  
Manchester, Manchester, UK. All patients gave written informed  
consent and the study was approved by the Salford and Trafford  
Local Research Ethics Committee.

SNP selection

Two of the three previously identified risk SNPs, rs1800925  
and rs20541, were selected for genotyping. The remaining  
SNP, rs848, was not included due to very high linkage disequi-  
librium (LD) with rs20541 (D’ = 1.00, r2 = 0.96 CEU HapMap  
release 22).

Control samples

Data for both SNPs were available for healthy controls from  
the Wellcome Trust Case-Control Consortium 2 project (http://  
www.wtccc.org.uk). This dataset consists of samples from the  
1958 British Birth Cohort and the UK Blood Service Collection  
genotyped on the Illumina Human1M-Duo. In addition, 375  
control samples were available from Ireland.

Genotyping

SNP genotyping of the PsA, PsV and Ireland control samples was  
performed using Sequenom’s MassARRAY system (San Diego,  
California, USA) according to the manufacturers’ specifications  
for the iPLEX chemistry using 10 ng of genomic DNA. Genotype  
cluster plots were evaluated prior to analysis to ensure satisfac-  
tory assay performance.

Statistical analysis

Data handling, quality control, association and haplotype analy- 
 
ses were performed using the PLINK software package.21 The  
dataset was filtered to exclude samples and SNPs with a missing  
data rate >10%, in conjunction with 50 other SNPs in the same  
sample collections. Test statistics were calculated for deviation  
from Hardy–Weinberg equilibrium (HWE) using an exact test,  
the Cochran–Armitage trend test, OR (including 95% CI) and  
LD (r2 and D’). Multiple logistic regression was performed to  
test for independent effects of the two SNPs using Stata (ver- 
tein 10.1). The primary PsA analysis consisted of the combined  
analysis of UK and Ireland cases and controls as a single popula- 
tion. The PsV analysis compared the UK cases to the UK con- 
 
Ireland samples.

Subphenotype analysis was performed within the PsA dataset  
based on, first, the age at onset of psoriasis (type I psoriasis  
has an onset <40 years of age, whereas type II psoriasis is defined  
as an onset >40 years of age) and, second, seronegativity for  
rheumatoid factor in an attempt to exclude those patients who  
may have PsV and coexistent rheumatoid arthritis. All subphenotype analyses were  
performed in UK samples only, where age at onset was available  
for 784 patients and rheumatoid factor status was available  
for 480 patients. Each subphenotype group was compared  
against UK controls and evidence for association was tested  
using the Armitage test for trend. No information was avail- 
 
ble regarding the extent of psoriasis or the presence of  
spondyloarthritis.

In the interests of exploring whether heterogeneity exists  
between the UK and Ireland PsA datasets and the potential con- 
founding it may introduce, data from each population were next  
analysed independently. This was followed by joint analysis  
using an inverse-variance meta-analysis under the assumption  
of fixed effects. Allelic heterogeneity between the two groups  
was estimated using the Cochran Q and I2 statistics.

RESULTS

Genotyping

Both SNPs demonstrated satisfactory clustering of genotypes  
with clear and distinct clusters. Following exclusion of samples  
falling the minimum threshold for missing data, there were  
937 PsA cases, 748 type I PsV cases and 5533 healthy controls  
(5199 UK and 334 Ireland), with a genotyping success rate of  
>99% in the remaining samples.

Table 1 Summary of genotype and association results for the two SNPs mapping to the IL-13 gene vicinity for UK/Ireland cases and controls

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Controls (n = 5533)</th>
<th>PsA cases (n = 937)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Count/Frq</td>
<td>RAF</td>
</tr>
<tr>
<td>rs1800925</td>
<td>CC</td>
<td>3715 (67.1)</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>1619 (29.3)</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>180 (3.3)</td>
<td>0.82</td>
</tr>
<tr>
<td>rs20541</td>
<td>GG</td>
<td>3749 (67.8)</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>1804 (29.0)</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>175 (3.2)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Genotype counts are presented with frequencies in parentheses.  
HWE, Hardy–Weinberg equilibrium (reported in controls only); RAF, risk allele frequency.
Table 2  Summary of genotype and association results for the two SNPs mapping to the IL-13 gene vicinity for PsV and UK controls samples

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Controls (n = 5199)</th>
<th>PsV cases (n = 743)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Count/Frq</td>
<td>RAF</td>
</tr>
<tr>
<td>rs1800925</td>
<td>CC</td>
<td>3480 (66.9)</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>1531 (29.4)</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>169 (3.3)</td>
<td>0.82</td>
</tr>
<tr>
<td>rs20541</td>
<td>GG</td>
<td>3506 (67.4)</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>1521 (29.3)</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>168 (3.2)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Genotype counts are presented with frequencies in parentheses. HWE, Hardy–Weinberg equilibrium (reported in controls only); n, number; RAF, risk allele frequency.

Statistical analysis

Neither SNP demonstrated deviation from HWE in the combined control group. Both SNPs demonstrated similar genotype frequencies; however, only moderate LD was observed (r² = 0.28, D' = 0.49). Significant association with susceptibility to PsA was found for both SNPs (rs1800925 ptrend = 6.1×10⁻⁵, rs20541 ptrend = 8.0×10⁻⁴), where the major allele increased the risk of disease susceptibility (table 1). No significant association was found to type I PsV with either SNP (table 2). Direct comparison of the two disease groups demonstrated significant differences for both SNPs (rs1800925 p = 0.015; rs20541 p = 0.004).

Association to rs1800925 remained significant after adjusting for rs20541 (p = 0.0065; OR 0.80 (0.69 to 0.94)) in the multiple logistic model. However, association to rs20541 did not reach significant statistical significance on adjustment for rs1800925 genotypes (p=0.10; OR 0.88 (0.75 to 1.03)). The haplotype formed by the two risk alleles (CG) was significantly enriched in cases compared to controls (79.4% vs 74.6%) and is more significantly associated than single-point analysis of either SNP (p = 7.55×10⁻⁵; OR 1.32) (supplementary table 2).

Finally, the association was investigated within phenotypic and population-based subgroups within the PsA samples. Both SNPs were found to be associated in the type 1 and seronegative subgroups (table 3). There were no qualitative differences between the effect sizes observed in any of the subgroups, although the statistical evidence for association was weakened due to the smaller sample sizes.

Both SNPs remained associated upon independent analysis of the UK PsA dataset, with effect sizes similar to those observed in the primary analysis, and no significant deviation from HWE in the controls (supplementary table 1). Only rs18900925 was significantly associated in the Ireland dataset. The failure to detect association to rs20541 may well be attributable to decreased power due to the small sample numbers in this dataset. Neither SNP significantly deviated from HWE in the Ireland controls. Effect estimates for the meta-analysis were consistent with those reported for the primary analysis, with no evidence for allelic heterogeneity between the two populations (rs1800925 Q = 0.22, I² = 32.2; rs20541 Q = 0.68, I² = 0) (supplementary table 1).

DISCUSSION

The results presented here confirm the findings of Duffin et al, who described association of the IL-13 locus with PsA, but not with PsV. This represents one of the first replicated examples of a differential association between these two closely related phenotypes.

Although the present results support the observation made by Duffin et al that the effect of rs1800925 is limited to PsA, they contradict the findings reported by Nair et al. That PsV GWAS contained a significant proportion of PsA cases in the primary scan and validation stages, where upon subgroup analysis there was significant association to both PsA and PsV.

The discrepancy in findings could exist for a number of reasons; first, it could result from a false-negative (type II error) in the PsV arm of the present study. However, the study has greater than 80% power to detect an effect of similar magnitude to that reported by Nair et al. A type II error is therefore unlikely, but not impossible. Second, it is conceivable that a false-positive (type I error) occurred in the PsV arm of the study performed by Nair et al. Although this is a well-powered study, its samples are drawn from multiple populations (USA, Canada, Germany and France) and collections (contributions from nine research groups) that may well lead to population stratification and confound results. In support of this possibility, it is interesting to note that the control allele frequencies across the nine individual cohorts genotyped varied from 0.76 to 0.81 for the rs20541 and rs484 SNPs. Indeed, the paper does not report association to rs1890025, the main effect observed in this study and that performed by Duffin et al. The effect of possible confounding may be further exacerbated by the presence of unidentified PsA cases as most of the PsV patients included were not reported as having had a rheumatological diagnosis, and many of these patients had not been reported as having had a rheumatological diagnosis.

In spite of these limitations, this remains the best powered study to date and, as such, its results should not be dismissed casually. On the contrary, the authors should...
be commended for organising a consortium with sufficient power to detect novel susceptibility loci. In light of the contradicting results, further studies in independent sample collections will be required to validate the differential association of the locus with PsA but not PsV.

The presence of unclassified PsA patients within a PsV sample collection represents a significant source of confounding for studies attempting to identify PsA-specific risk loci. This could lead to false-positive associations in PsV samples at PsA specific loci and potentially invalidate any attempts to identify such loci. Such studies would greatly benefit from a PsV sample collection screened by a rheumatologist to exclude PsA as a comparator group. In the study presented here, the PsV samples were not screened by a rheumatologist; however, the lack of association with PsV would suggest that only a limited proportion of these samples have unclassified PsA.

From the data presented here and in other published studies, it is unclear as to which variant is causal or if there are multiple independent effects. The present results confirm a strong association to rs1800925; however, the results for an independent effect at rs20541 are inconclusive. Only rs1800925 remained significant in the multiple logistic model, suggesting that this represents the main effect. However, an independent effect at rs20541 cannot be ruled out when the effect estimates are taken into consideration, indeed the data are suggestive of independent effect at this locus. It is likely that a statistically significant association to rs20541 was not achieved in this model due to a lack of power. Haplotype analysis found the CG haplotype to be more associated than either SNP individually. However, the haplotype results should be considered with caution given the observed evidence for historical recombination between the two SNPs (D' = 0.49). Larger sample sizes than available for this study would be required to robustly confirm or rule out any potential independent effect for rs20541. The three SNPs implicated to date each have potential functional impact: rs20541 is a missense SNP that maps to exon 4 of IL-13 and results in the substitution of a glycine for arginine (Q144R), rs1800925 maps to 1 kb of the 5' of the coding region and rs848 is located within the 3' untranslated region. However, it is possible that these SNPs could tag an unknown causal variant, therefore fine mapping will be required to identify a suitable short-list of candidate SNPs to take forward for functional evaluation.

In conclusion, the present study supports the PsA-specific association to SNPs at chromosome 5q31 and potentially highlights a key pathway that is distinct for joint inflammation in psoriatic arthritis.

Acknowledgements The authors acknowledge the support of the NIHR Manchester Biomedical Research Centre and NIHR Leeds Musculoskeletal Biomedical Research Unit.

Funding JB, IB and AB are funded by Arthritis Research UK (arc grant; 17552). EF is supported by the European Community’s Sixth Framework Programme AutoCure funding. This study makes use of data generated by the Wellcome Trust Case–Control Consortium. A full list of the investigators who contributed to the generation of the data is available from http://www.wtccc.org.uk. Funding for the project was provided by the Wellcome Trust under award 076113 and 085475.

Competing interests None.

Ethics approval This study was conducted with the approval of the MREC 99/8/84.

Provenance and peer review Not commissioned; externally peer reviewed.

Author affiliations
1 Arthritis Research UK Epidemiology Unit, Manchester Academic Health Science Centre, The University of Manchester, Manchester, UK
2 The Kellogg Centre for Rheumatology, Central Manchester Foundation Trust, NIHR Manchester Biomedical Research Centre, Manchester, UK
3 Dermatology Sciences, Salford Royal NHS Foundation Trust, Manchester Academic Health Science Centre, The University of Manchester, Manchester, UK
4 NIHR-LEeds Musculoskeletal Biomedical Research Unit, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK
5 Department of Clinical Medicine, Institute of Molecular Medicine, Trinity College Dublin, Dublin, Ireland
6 Adelaide and Meath Hospital and Trinity College Dublin, Dublin, Ireland
7 Royal National Hospital for Rheumatic Diseases, Bath, UK
8 Department of Pharmacy and Pharmacology, University of Bath, Bath, UK
9 Department of Rheumatology, St Vincent’s University Hospital, Dublin, Ireland
10 UCD School of Medicine and Medical Sciences and Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland
11 Haywood Hospital, Stoke on Trent, Staffordshire, UK
12 Arthritis Research UK Primary Care Centre, Keele University, Staffordshire, UK

REFERENCES