Confirmation of TNIP1 and IL23A as susceptibility loci for psoriatic arthritis

John Bowes,1 Gisela Orozco,1 Edward Flynn,1 Pauline Ho,1,2 Rasha Brier,1 Helena Marzo-Ortega,3 Laura Coates,3 Ross McManus,4 Anthony W Ryan,4 David Kane,5 Eleanor Korendowych,6 Neil McHugh,6 Oliver FitzGerald,7 Jonathan Packham,8,9 Ann W Morgan,3 Ian N Bruce,1,2 Anne Barton1,2

ABSTRACT

Objectives To investigate a shared genetic aetiology for skin involvement in psoriasis and psoriatic arthritis (PsA) by genotyping single-nucleotide polymorphisms (SNPs), reported to be associated in genome-wide association studies of psoriasis, in patients with PsA.

Methods SNPs with reported evidence for association with psoriasis were genotyped in a PsA case and control collection from the UK and Ireland. Genotype and allele frequencies were compared between PsA cases and controls using the Armitage test for trend.

Results Seven SNPs mapping to the IL1RN, TNIP1, TNFAIP3, TSC1, IL23A, SMARTC4 and RNF114 genes were successfully genotyped. The IL23A and TNIP1 genes showed convincing evidence for association (rs2066808, p = 9.1 x 10–7; rs17728338, p = 3.5 x 10–5; respectively) whilst SNPs mapping to the TNFAIP3, TSC1 and RNF114 genes showed nominal evidence for association (rs610604, p = 0.03; rs1076160, p = 0.03; rs495337, p = 0.0025). No evidence for association with IL1RN or SMARTC4 was found but the power to detect association was low.

Conclusions SNPs mapping to previously reported psoriasis loci show evidence for association to PsA, thus supporting the hypothesis that the genetic aetiology of skin involvement is the same in both PsA and psoriasis.

Psoriatic arthritis (PsA) and psoriasis are both complex diseases with genetic and environmental contributions to their aetiology. Estimates suggest that the genetic contribution to PsA may be higher than psoriasis yet most work has been done on the latter given its greater population prevalence.1

Both diseases are characterised by psoriasis skin involvement; if the psoriasis is aetiologically identical, then all confirmed psoriasis susceptibility loci should also be associated with PsA. Of the psoriasis susceptibility loci studied to date, the HLA Cw0602 allele and variants mapping to the IL23R and IL12B genes have been associated with PsA.2–4 It might be postulated that additional PsA susceptibility loci exist. This is supported by evidence that the IL13 gene locus is associated with PsA, but not psoriasis alone.5,6 Furthermore, the recently identified TRAF3IP2 gene locus shows stronger effect sizes in some,7,8 but not all,9 PsA populations compared with psoriasis, suggesting that it too may represent a PsA-specific locus. If the skin manifestations of both diseases represent a common phenotypic endpoint arising from different aetiologies, some differences in susceptibility loci would be expected. The identification of risk loci specific to psoriasis would provide compelling evidence that the genetic basis of the skin component of the two diseases was different. There has been some evidence reported to support this possibility. Variants within the LCE gene locus were reported to be associated with psoriasis but not PsA in a German cohort,10 but a subsequent study in a population from the British Isles showed association with PsA suggesting it is involved in psoriasis per se.11

Genome-wide association studies (GWAS) have resulted in the identification of further psoriasis susceptibility loci.12,13 This provides an opportunity to explore further the overlap in genetic susceptibility between psoriasis and PsA. If the genetic liability towards skin involvement is shared between the two diseases, then loci identified in psoriasis GWAS would also show evidence of association of the susceptibility with PsA.

METHODS

Patient samples
One thousand and fifty-seven genomic DNA samples were collected from Caucasian PsA patients recruited from the UK and Ireland (385 UK and 172 Ireland), the details of which have previously been described14–16 and phenotype details are summarised in supplementary table 4 (available online only). PsA was defined as the presence of both psoriasis and peripheral inflammatory arthritis irrespective of the rheumatoid factor status. All subjects provided informed consent and the study was approved by the North West Multicentre Research Ethics Committee (MREC 99/8/84).

Control data
The data for healthy UK controls was sourced from the Wellcome Trust Case–Control Consortium 2 (WTCCC2) project (http://www.wtccc.org.uk). This project genotyped over 5000 individuals from the 1958 British Birth Cohort and the UK Blood Service Collection for the Illumina Human1M-Duo and the Affymetrix Human SNP Array 6.0. If control data for any single-nucleotide polymorphism (SNP) were not available from the WTCCC2 then it was generated in-house using DNA from 2104 1958 British Birth Cohort samples. In addition, 375 control samples were available from Ireland for genotyping.
#### Genotyping

Concordance frequencies for six SNPs, confirmed to Hardy–Weinberg equilibrium (Table S1), showed no deviation from the expected distribution for the UK and Ireland datasets. SNPs were genotyped in-house using the Illumina (San Diego, California, USA) MassArray system (San Diego, California, USA) according to the manufacturers' specifications using 10 ng of genomic DNA available at the time of study design. Four of the selected SNPs were genotyped using the PLINK package. SNPs and samples with greater than 5% missing data were excluded from the study. Test statistics were calculated for deviation from Hardy–Weinberg equilibrium using an exact test, the Cochran–Armitage trend test and variance meta-analysis under the assumption of fixed effects. Allelic heterogeneity between the two groups was estimated using the Cochran Q and $I^2$ statistics. Variance meta-analysis was performed using the online genetic power calculator.

#### Statistical analysis

Exploratory analysis of subphenotypes were performed on SNP association data. As the study population has a combined age-at-onset distribution that is defined as an onset >40 years of age and seronegativity for rheumatoid arthritis, we performed the analysis using the Cochran–Armitage trend test and the conditional logistic regression model for validation. Differences among the patient groups were tested using the Cochran Q and $I^2$ statistics. Variance meta-analysis was performed using the online genetic power calculator.

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### Table 1

Summary statistics for primary case–control analysis of seven previously identified psoriasis SNPs

<table>
<thead>
<tr>
<th>SNP</th>
<th>Locus</th>
<th>Gene *</th>
<th>Alleles</th>
<th>Cases MAF</th>
<th>Controls MAF</th>
<th>HWE</th>
<th>OR (CI)</th>
<th>p</th>
<th>Trend p</th>
<th>OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2066808</td>
<td>12q13</td>
<td>IL23A</td>
<td>G/A</td>
<td>0.04</td>
<td>0.05</td>
<td>2 (0.2)</td>
<td>68 (7.5)</td>
<td>382 (92.2)</td>
<td>0.07</td>
<td>0.84</td>
</tr>
<tr>
<td>rs17728338</td>
<td>5q33</td>
<td>TNFAIP3</td>
<td>A/G</td>
<td>0.08</td>
<td>1.00</td>
<td>5 (0.6)</td>
<td>133 (14.7)</td>
<td>765 (84.7)</td>
<td>0.05</td>
<td>0.80</td>
</tr>
<tr>
<td>rs495337</td>
<td>20q13</td>
<td>TNIP1</td>
<td>T/C</td>
<td>0.38</td>
<td>0.38</td>
<td>136 (13.9)</td>
<td>477 (48.7)</td>
<td>367 (37.4)</td>
<td>0.42</td>
<td>0.64</td>
</tr>
<tr>
<td>rs610604</td>
<td>6q23</td>
<td>SLAMRA4</td>
<td>G/T</td>
<td>0.34</td>
<td>0.02</td>
<td>91 (10.1)</td>
<td>440 (48.7)</td>
<td>372 (41.2)</td>
<td>0.32</td>
<td>0.04</td>
</tr>
<tr>
<td>rs12983316</td>
<td>19p13</td>
<td>SMARCA4</td>
<td>C/T</td>
<td>0.47</td>
<td>0.26</td>
<td>192 (21.3)</td>
<td>467 (51.9)</td>
<td>243 (26.9)</td>
<td>0.50</td>
<td>0.45</td>
</tr>
<tr>
<td>rs397211</td>
<td>2q13</td>
<td>IL1RN</td>
<td>C/T</td>
<td>0.29</td>
<td>0.22</td>
<td>82 (9.2)</td>
<td>352 (39.3)</td>
<td>462 (51.6)</td>
<td>0.31</td>
<td>0.34</td>
</tr>
</tbody>
</table>

* Proximal gene of interest.

HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.
two populations (supplementary table 3, available online only). The association of the TNIP1 SNP, rs17728338, appeared stronger in samples from PsA patients with type I psoriasis (n=478) with a much lower OR observed in the PsA samples with type II psoriasis (n=163) (OR 1.59 vs 1.12, respectively), although the CI overlap (table 2). However, given the limited sample numbers these results should be considered with caution. There were no qualitative differences in the effects of any SNPs when the analysis was limited to seronegative individuals (n=302).

SNPs mapping to the TNFAIP3, TSC1 and RNF144 genes showed borderline evidence for association at a nominal p value threshold of 0.05 and the remaining two SNPs showed no evidence for association with PsA in this dataset (table 1).

**DISCUSSION**

We have confirmed that two loci, IL23A and TNIP1, associated with psoriasis in multiple populations, are also associated with PsA. Support for another three loci, TNFAIP3, TSC1 and RNF144, is provided by evidence of association at nominal p value thresholds of 0.05 although confirmation of association with PsA will be required in other, large datasets.

SNPs mapping to the final two loci tested, SMARCA4 and IL1RN, did not show any evidence of association with PsA, which might indicate that they are exclusively associated with psoriasis. However, the power to detect association with these loci was poor in the sample size tested (20% and 17%, respectively). Interestingly, the risk allele in both the psoriasis GWAS and the current data is the same and the strength and direction of association are similar. Association of TSC1, SMARCA4 and IL1RN with psoriasis was reported by Nair et al., although association was found in two datasets (p<0.05), the strength of association failed to reach genome-wide significance levels in the combined dataset of nearly 13 000 samples. No other studies have yet replicated the association of SMARCA4, casting doubt on whether it truly represents a psoriasis susceptibility locus. However, an independent family-based study in a French population has shown association of the IL1RN gene with psoriasis, albeit with a different SNP (rs315934; r² with rs397211=0.08). It may be, therefore, that confirmed evidence for association with PsA will be demonstrated in a larger dataset or by meta-analysis in the future.

Strengths of this study include the large sample size tested, the majority of whom were from the UK. Previous studies have shown minimal population stratification across the UK thus minimising this potential source of bias in genotyping. Differences in genetic substructure between the UK and Irish population have been noted previously, but we controlled for this by analysing the data from each of the populations separately followed by meta-analysis.

Some limitations of the study are apparent, particularly the lack of power to exclude association with PsA confidently for psoriasis loci with small effect sizes. In addition, we have tested a single SNP at each locus based on previous reports of confirmed association with psoriasis. However, this is unlikely to be the causal variant and association at any given locus may be complex. For example, several SNPs mapping to the IL23R and IL12B genes show independent associations with psoriasis susceptibility; fine mapping of the associated regions will be required to identify all associated variants and to identify those most likely to be functionally important. A further limitation is the fact that the psoriasis GWAS contain a large proportion of patients with PsA, which makes comparison of the genetic aetiology of the two diseases very difficult. Studies comparing the genetic aetiology of the two diseases would be
greatly facilitated by a psoriasis cohort reviewed by a rheumatologist to exclude patients with inflammatory arthritis. It should also be noted that we have used a broad definition of PsA, including patients with psoriasis and a peripheral inflammatory arthritis, rather than the recently validated CASPAR criteria.21 The latter include x-ray changes as part of the criteria and this was not available for many subjects as cohort collections were started before the introduction of these criteria. However, the majority of patients did satisfy these criteria, when x-ray data were available. Finally, a number of GWAS of psoriasis have been published recently in European and Asian populations providing a further opportunity to explore whether psoriasis susceptibility loci are also associated with PsA.8 9 12 22 23 However, this information was not available at the time of study design and so the novel psoriasis loci identified from those scans still require investigation for association with PsA.

It is expected that epistasis between genetic loci may result in larger effect sizes. Indeed, the first demonstration of this was recently reported in that association of ERAP1 with psoriasis was only observed in individuals carrying the HLA Cw*0602 allele.8 No interaction was detected between either of the HLA Cw*0602 markers and HLA Cw*0602 (data not shown). However, interaction analyses would be best explored in larger sample collections that would afford greater power than that provided in the current study.

In summary, our data largely support the thesis that psoriasis susceptibility loci are also associated with PsA; in particular, we show definitive confirmation that IL23A and TNIP1 and supportive evidence that the TNFAIP3, TSC1 and RNF114 genes are associated with susceptibility to PsA as well as psoriasis.

Acknowledgements This study makes use of data generated by the Wellcome Trust Case–Control Consortium. The authors are grateful for access to DNA from the 1958 Birth Cohort.

Funding The authors acknowledge the support of the NIHR Manchester Biomedical Research Centre and NIHR Leeds Musculoskeletal Biomedical Research Unit. JB, INB and AB are funded by Arthritis Research UK (grant 17552). EF is supported by the European Community’s Sixth Framework Programme AutoCure funding. This work was funded by the Arthritis Research UK. Funding for the project was provided by the Wellcome Trust under awards 076113 and 098547.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the North West Multicentre Research Ethics Committee (MREC 99/8/84).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES