Variants in linkage disequilibrium with the late cornified envelope gene cluster deletion are associated with susceptibility to psoriatic arthritis

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

ABSTRACT

Objective A common deletion mapping to the psoriasis susceptibility locus 4 on chromosome 1q21, encompassing two genes of the late cornified envelope (LCE) gene cluster, has been associated with an increased risk of psoriasis vulgaris (PsV). One previous report found no association of the deletion with psoriatic arthritis (PsA), suggesting it may be a specific risk factor for PsV. Given the genetic overlap between PsA and PsV, a study was undertaken to investigate whether single nucleotide polymorphisms (SNPs) mapping to this locus are risk factors for PsA in a UK and Irish population.

Methods Three SNPs with prior evidence of association with susceptibility to PsV were genotyped in 1057 patients with PsA using Sequenom iPLEX chemistry and genotype frequencies compared with data available for 5575 healthy controls. Two of the SNPs, rs4112788 and rs4085613, were reported to be highly correlated with the LCE deletion. The third SNP, rs6701216, was previously reported to be associated with PsV in a US population.

Results Alleles tagging the deletion for both rs4112788 and rs4085613 were found to be enriched in cases compared with controls (69% vs 65%) and significantly associated with increased susceptibility to PsA ($p_{\text{trend}} = 0.001$, OR 1.19 and $p_{\text{trend}} = 0.001$, OR 1.18, respectively). No association was observed with rs6701216.

Conclusions The evidence presented here supports LCE deletion as a risk factor for PsA in a UK and Irish population. It suggests that this locus is a risk factor within a shared aetiological pathway that contributes to psoriatic skin disease in both PsV and PsA.

INTRODUCTION

Psoriatic arthritis (PsA) is an inflammatory joint condition associated with psoriasis vulgaris (PsV); it is estimated that up to 30% of patients with PsV also have articular disease. PsA is considered to be a complex disease with contributions from both environmental and genetic risk factors.1 The genetic component is estimated to be strong, with a sibling recurrence RR ($\alpha_s$) of 47 in a UK population.2 Genetic investigations have revealed a number of risk factors that are common to both PsV and PsA. These established risk loci, HLA-Cw6, IL12B and IL23R, do not allow them to be genetically discriminated.3-4 A number of studies have recently identified genetic variants mapping to chromosome 1q21 within the previously identified psoriasis susceptibility locus 4 as being associated with susceptibility to PsV. A genome-wide scan for copy number variation identified an association between a 32.2 kb deletion ($\text{LCE3C}_{\text{LCE3B}}\text{-del}$) that encompasses two genes of the late cornified envelope (LCE) gene cluster ($\text{LCE3B}$ and $\text{LCE3C}1$), with increased susceptibility to PsV in multiple populations.5 This deletion was found to be highly correlated with the single nucleotide polymorphism (SNP) rs4112788 ($r^2=0.93$) with the major allele (C) tagging $\text{LCE3C}_{\text{LCE3B}}\text{-del}$. This association is further supported by evidence from an independent SNP genome-wide association study (GWAS) for PsV that reported association to rs4085613 mapping to this locus.6 The SNPs rs4112788 and rs4085613 are in high linkage disequilibrium (LD) ($r^2>0.95$) and, as such, are not considered to be independently associated. A further independent GWAS investigating susceptibility to PsV identified an association to rs6701216 that maps to the $\text{LCE1C}$ gene,7 which represents an effect independent of rs4112788 and rs4085613 as there is little LD ($r^2<0.05$) between the variants.8 From these genetic studies alone it is not clear whether the deletion itself or the SNPs in LD with it are primarily responsible for the association seen, and functional studies will be required to determine the mechanism for the disease predisposition.

Intriguingly, unlike the previously identified psoriasis loci, $\text{LCE3C}_{\text{LCE3B}}\text{-del}$ and correlated SNPs were associated with PsV but not PsA susceptibility in a German cohort, suggesting this may be the first risk factor identified that predisposes to skin-type psoriasis only, implying that the skin disorder in PsV and PsA have differing underlying genetic aetiologies.8-9 Identification of genetic factors that differentiate the two phenotypes could help to reveal distinct aetiological pathways. Validation of such a finding in independent studies is therefore essential, and the aim of the work presented here was to investigate association with SNP markers in LD with $\text{LCE3C}_{\text{LCE3B}}\text{-del}$ in a large case–control study of subjects with PsA and controls from the UK and the Republic of Ireland (ROI).
METHODS

Patient samples
Genomic DNA was available from 1057 Caucasian patients with PsA recruited from three UK rheumatology centres and one centre in the ROI (885 patients in the UK and 172 in the ROI), details of which have been described previously. PsA 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 satisfied the CASPAR (CLAssification criteria for Psoriatic ARthritis) classification system, although some were collected prior to the introduction of this classification system.

SNP selection
We selected three SNPs for genotyping that map to the 1q21 locus (see figure 1 in online supplement). Two SNPs, rs4085613 and rs4112788, have previously been shown to be in high LD with LCE3C-LCE3B-del and were reported to be the most associated variants from two independent studies. The third SNP, rs6701216, represents a reported effect that is potentially independent of LCE3C-LCE3B-del. In addition, we genotyped 12 autosomal SNPs that were reported to be informative of geographical variation across the UK.

Control samples
Data for healthy controls were sourced from the Wellcome Trust Case-Control Consortium 2 project (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 BeadChip or Affymetrix Human SNP Array 6.0. In addition, 375 control samples were available from the ROI.

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

Statistical analysis
Statistical analyses were performed using the PLINK software package. Quality control (QC) inclusion thresholds for both individual sample and SNP genotyping missing data rates were set at >90%. Test statistics were calculated for deviation from Hardy–Weinberg equilibrium (HWE) using an exact test, the Cochran-Armitage trend test, ORs (including 95% CI) and LD (r²). The trend test was selected as the most appropriate genetic model as previous evidence in psoriasis studies suggested a dosage effect based on allele carriage, thus providing the maximal power for association testing.

The primary analysis investigated the dataset in its entirety with UK and ROI samples treated as one common population. In the interests of identifying heterogeneity between the UK and ROI cohorts, we analysed these components separately. The results were subsequently combined using an inverse variance meta-analysis under the assumption of fixed effects. Significant heterogeneity between the two groups was estimated using the Cochran Q and P statistics.

Previous work in PsV has found that the major genetic susceptibility locus, HLA-Cw*06, is associated with early onset (type I PsV, age at onset <40 years) but not late onset (type II) PsV. Stratification analysis was therefore undertaken to explore whether any associations were restricted to one subphenotype. Patients with PsA are usually but not always seronegative for rheumatoid factor. It is possible that those with a positive rheumatoid factor are actually patients with rheumatoid arthritis and co-existent psoriasis, which may bias any analysis. Subphenotype analysis was therefore also undertaken in patients with PsA seronegative for rheumatoid factor to determine whether the presence of this antibody was a significant confounder. All subphenotype analyses were performed in UK samples only. No information was available regarding the extent of psoriasis or the presence of spondyloarthritis.

RESULTS

Genotyping
All LCE SNPs were informative of geographical variation across the UK. Our interpretation of these results would be that multiple associations to these SNPs is indicative of regional ascertainment bias of case samples given that control samples are known to be collected from across the UK. This analysis was restricted to the UK samples.

Statistical analysis
For all LCE SNPs in the primary analysis the control sample groups conformed to HWE (table 1). We observed similar genotype frequencies for rs4112788 and rs4085613 and they were found to be highly correlated (D'=1.0, r²=0.99). Allele frequencies were found to be within the range of previously reported frequencies for the Caucasian population. We observed enrichment of deletion-correlated alleles in the case group compared with the control group (69% vs 65% for both SNPs). This enrichment was found to be significantly associated with PsA susceptibility under an additive model (table 1). The association remained when the analysis was restricted to the UK-only sample set (rs4112788, p=0.005; rs4085613, p=0.006) with no evidence of heterogeneity (Q=0.95, I²=0) for both SNPs. No association to rs6701216 was observed in the ROI-only analysis and deviation from HWE (p=0.02) was noted in this control group. Meta-analysis of the independent UK and ROI results supported the association (rs4112788, p=0.005; rs4085613, p=0.006) with no evidence of heterogeneity (Q=0.95, I²=0) for both SNPs. No evidence was found to support association to rs6701216.

Stratification analysis did not reveal qualitatively different associations in subgroups of patients with PsA with type I or type II PsV or in those seronegative for rheumatoid factor (table 2). It is important to note that the sample numbers were reduced owing to incomplete phenotype data.

Analysis of the 12 geographical markers revealed association to only a single SNP, rs3873375 (p_trend=1.53×10⁻⁷) (see table 1 in online supplement).

DISCUSSION
We have found that SNPs previously reported to be in high LD with LCE3C-LCE3B-del on chromosome 1q21 are associated
Table 1  
Summary of genotype and association results for the three SNPs mapping to the 1q21 locus

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Control</th>
<th>Case</th>
<th>HWE</th>
<th>OR (CI)</th>
<th>Control</th>
<th>Case</th>
<th>HWE</th>
<th>OR (CI)</th>
<th>p Value</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4085613</td>
<td>CC</td>
<td>2374 (42.6)</td>
<td>465 (47.5)</td>
<td>1.18 (1.07 to 1.31)</td>
<td>2184 (42.0)</td>
<td>375 (46.1)</td>
<td>1.16 (1.04 to 1.30)</td>
<td>190 (50.9)</td>
<td>90 (54.2)</td>
<td>1.16 (0.87 to 1.54)</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>2529 (45.4)</td>
<td>422 (43.1)</td>
<td>0.86</td>
<td>0.0014</td>
<td>2390 (46.0)</td>
<td>361 (44.4)</td>
<td>0.41</td>
<td>0.0091</td>
<td>139 (37.3)</td>
<td>61 (36.7)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>665 (11.9)</td>
<td>92 (9.4)</td>
<td>621 (12.0)</td>
<td>77 (9.5)</td>
<td>44 (11.8)</td>
<td>15 (9.0)</td>
<td>1.16</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4112788</td>
<td>CC</td>
<td>2370 (42.6)</td>
<td>465 (47.4)</td>
<td>1.19 (1.07 to 1.32)</td>
<td>2179 (42.1)</td>
<td>374 (45.9)</td>
<td>1.16 (1.04 to 1.30)</td>
<td>191 (51.1)</td>
<td>91 (54.5)</td>
<td>1.16 (0.87 to 1.54)</td>
<td>0.005</td>
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<tr>
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<td>CT</td>
<td>2529 (45.4)</td>
<td>427 (43.5)</td>
<td>0.84</td>
<td>0.0011</td>
<td>2390 (46.1)</td>
<td>366 (44.9)</td>
<td>0.41</td>
<td>0.0078</td>
<td>139 (37.1)</td>
<td>61 (36.5)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>666 (12.0)</td>
<td>90 (9.2)</td>
<td>611 (11.8)</td>
<td>75 (9.2)</td>
<td>44 (11.8)</td>
<td>15 (9.0)</td>
<td>1.16</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs6305693</td>
<td>CC</td>
<td>2274 (42.9)</td>
<td>469 (47.8)</td>
<td>0.86</td>
<td>0.0014</td>
<td>2134 (42.0)</td>
<td>374 (45.9)</td>
<td>0.41</td>
<td>0.0078</td>
<td>134 (37.4)</td>
<td>61 (36.3)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>1436 (25.8)</td>
<td>252 (25.8)</td>
<td>0.24</td>
<td>0.52</td>
<td>1344 (25.9)</td>
<td>210 (26.0)</td>
<td>0.14</td>
<td>0.98</td>
<td>92 (24.7)</td>
<td>42 (25.0)</td>
</tr>
</tbody>
</table>

Analysis of the complete dataset is presented in the primary results. Results for the UK and ROI are presented individually and combined via inverse variance meta-analysis. Genotype counts are presented with frequencies in parentheses. The C alleles of rs4112788 and rs4085613 are in high linkage disequilibrium with the LCE3C_LCE3B-del. HWE, Hardy–Weinberg equilibrium (reported in controls only); IV, inverse variance; ROI, Republic of Ireland; SNP, single nucleotide polymorphism; Q, Cochran’s Q statistic.

This study has two potential limiting factors. The first is the risk of confounding by population stratification as the cohort is drawn from two populations of European ancestry. This issue was addressed in a number of ways. First, we reduced the potential for heterogeneity by restricting the analysis to only UK samples. Both rs4112788 and rs4085613 remained significantly associated with a marginal fall in significance attributable to the smaller sample size. No association was observed in the ROI-only analysis, conceivably due the small sample size. Second, we attempted to control for heterogeneity by employing an inverse variance meta-analysis on summary statistics from the individual UK and ROI dataset. Both SNPs retained significant evidence for association with no evidence for heterogeneity, thus supporting the use of a combined dataset. Although the Cochran Q and P test statistics are not informative of underlying population structure, they would

with PsA in a large case–control cohort recruited from the UK and ROI.

This deletion is of particular interest for psoriatic phenotypes as the genes of the LCE cluster encode stratum corneum proteins that play an important role in epidermal terminal differentiation, disruption of which has been proposed to lead to abnormal differentiation and psoriatic skin lesions. We did not find evidence to support an independent association to rs6701216. Stratification analysis by subphenotype demonstrated no qualitative difference between type I and type II psoriatic subphenotypes when considering the ORs. However, no association was observed in the type II group, which is likely to be attributable to low power owing to reduced sample numbers. A stronger effect was observed in the seronegative subphenotype, but this could be due to imprecision in the effect estimates owing to smaller sample numbers reflected by the wider CIs compared with the analysis of the whole UK group.

Our findings differ from the results of a recent study performed in a German population which found no evidence to support such an association in PsA. This discrepancy could be explained by any one of three scenarios. First, it may be a false positive (type I error) in our study. However, we have genotyped two highly correlated SNPs that demonstrate comparable results, thus making genotyping error an unlikely factor. It is also important to note that our genotype frequencies, p values and effect estimates for rs4112788 closely match the German study. While the study had 95% power to detect the maximal reported effect size, there is still potential for type II errors in such well-powered studies. This point is well illustrated with examples from a recent GWAS in the UK. This study failed to identify two robust rheumatoid arthritis risk loci—tumour necrosis factor receptor-associated factor 1 and signal transducer and activator of transcription 4—which were subsequently confirmed in the UK population only following reports from other GWAS. Third, the patient demographics of the two cohorts may be substantially different. For example, they may consist of different proportions of type I and type II psoriasis or HLA-Cw6 positivity, with association restricted to a particular subphenotype. This may be of particular importance if the evidence for an epistatic interaction between LCE3C_LCE3B-del and HLA-Cw6 holds true upon further validation. Although the clinical characteristics of the British Isles and German cohorts appear similar, we do not currently have the HLA-Cw6 data that would allow us to thoroughly explore this possibility.
Table 2  Summary of results for phenotype subgroup analysis

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Type I psoriasis (n=519)</th>
<th></th>
<th>Type II psoriasis (n=176)</th>
<th></th>
<th>Seronegative (n=228)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Count (freq)</td>
<td>p_{trend}</td>
<td>OR (CI)</td>
<td>Count (freq)</td>
<td>p_{trend}</td>
<td>OR (CI)</td>
</tr>
<tr>
<td>rs4085613</td>
<td>CC</td>
<td>234 (45.2)</td>
<td>1.16 (1.01 to 1.33)</td>
<td>rs4085613</td>
<td>CC</td>
<td>85 (48.3)</td>
<td>1.15 (0.92 to 1.45)</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>240 (46.3)</td>
<td>0.03</td>
<td>rs4085613</td>
<td>CA</td>
<td>70 (39.8)</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>44 (8.3)</td>
<td></td>
<td>rs4085613</td>
<td>AA</td>
<td>21 (11.9)</td>
<td></td>
</tr>
<tr>
<td>rs4112788</td>
<td>CC</td>
<td>233 (44.9)</td>
<td>1.16 (1.01 to 1.33)</td>
<td>rs4112788</td>
<td>CC</td>
<td>85 (48.3)</td>
<td>1.17 (0.93 to 1.47)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>243 (46.8)</td>
<td>0.03</td>
<td>rs4112788</td>
<td>CT</td>
<td>71 (40.3)</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
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<td>43 (8.3)</td>
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<td>rs4112788</td>
<td>TT</td>
<td>20 (11.4)</td>
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</table>

Control frequencies are those reported in the UK analysis reported in table 1. 
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