PTPN22 is associated with susceptibility to psoriatic arthritis but not psoriasis: evidence for a further PsA-specific risk locus

John Bowes,1 Sabine Loehr,2 Ashley Budu-Aggrey,1,3 Steffen Uebe,2 Ian N Bruce,1,3,4 Marie Feletr,5 Helena Marzo-Ortega,6 Philip Helliwell,6 Anthony W Ryan,7 David Kane,8 Eleanor Korendowych,9 Gerd-Marie Alenius,10 Emiliano Giardina,11 Jonathan Packham,12 Ross McManus,7 Oliver FitzGerald,13 Matthew A Brown,14 Frank Behrens,15 Harald Burkhardt,15 Neil McHugh,9 Ulrike Huffmeier,2 Pauline Ho,1,4 Andre Reis,2 Anne Barton1,3,4

INTRODUCTION
Psoriatic arthritis (PsA) is a chronic inflammatory arthritis associated with psoriasis; it has a higher estimated genetic component than psoriasis alone, however most genetic susceptibility loci identified for PsA to date are also shared with psoriasis. Here we attempt to validate novel single nucleotide polymorphisms selected from our recent PsA Immunochip study and determine specificity to PsA.

METHODS
A total of 15 single nucleotide polymorphisms were selected (PImmunochip <1×10−4) for validation genotyping in 1177 cases and 2155 controls using TaqMan. Meta-analysis of Immunochip and validation data sets consisted of 3139 PsA cases and 11 078 controls. Novel PsA susceptibility loci were compared with data from two large psoriasis studies (WTCCC2 and Immunochip) to determine PsA specificity.

RESULTS
We found genome-wide significant association to rs2476601, mapping to PTPN22 (p=1.49×10−9, OR=1.32), but no evidence for association in the psoriasis cohort (p=0.34) and the effect estimates were significantly different between PsA and psoriasis (p=3.2×10−6). Additionally, we found genome-wide significant association to the previously reported psoriasis risk loci; NOS2 (rs4795067, p=5.27×10−9).

CONCLUSIONS
For the first time, we report genome-wide significant association of PTPN22 (rs2476601) to PsA susceptibility, but no evidence for association to psoriasis.
a significance threshold of $p < 1 \times 10^{-4}$. Genotyping was performed using the Life Technologies TaqMan chemistry on the QuantStudio genotyping platform at the University of Erlangen, Germany. Sample and SNPs with low call rates (<0.9) were excluded prior to analysis. All genotype cluster plots were manually reviewed and SNPs were screened for deviation from Hardy-Weinberg equilibrium in control samples (Bonferroni corrected $p < 3.3 \times 10^{-3}$).

**Statistical analysis**

Association testing was performed using logistic regression implemented in PLINK and meta-analysis of Immunochip and validation summary statistics was performed, weighting SNPs by inverse-variance and assuming fixed effects, using the software package METAL.

For loci not previously reported as being associated with psoriasis susceptibility we investigated PsA-specificity using two large psoriasis studies. First, we tested association to psoriasis using genotype data from WTCCC2 and association summary statistics from the largest psoriasis study to date, consisting of 10,586 psoriasis cases and 22,806 controls, from ImmunoBase (http://www.immunobase.org). Second, we directly compared PsA and psoriasis genotypes, with PsA coded as cases and psoriasis coded as controls. Sex differentiated associations were investigated by analysing men and women separately and comparing differences in effect estimates using Cochranes’s Q statistic using Immunochip genotype data.

To control for phenotype misclassification with rheumatoid arthritis (RA), we included a genetic risk score (GRS) comprised of the 41 non-HLA RA susceptibility SNPs reported in the RA Immunochip study, weighted by odds ratio (OR), as a covariate and recalculated the PsA Immunochip summary statistics.

**RESULTS**

Following quality control of the validation genotype data a total of 13 SNPs for 1177 cases and 2155 controls was available for analysis. Meta-analysis of the validation samples with

<table>
<thead>
<tr>
<th>rs</th>
<th>chr</th>
<th>bp</th>
<th>Gene</th>
<th>Risk/non-risk</th>
<th>Immunochip (cases=1962, controls=8923)</th>
<th>Validation (cases=1177, controls=2155)</th>
<th>Meta-analysis (cases=3139, controls=11 078)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RAF</td>
<td>p Value</td>
<td>OR</td>
<td>p Value</td>
</tr>
<tr>
<td>rs2476601</td>
<td>1</td>
<td>114,377,568</td>
<td>PTPN22</td>
<td>A/G</td>
<td>0.10</td>
<td>1.29E-05</td>
<td>1.28</td>
</tr>
<tr>
<td>rs495067</td>
<td>17</td>
<td>26,106,675</td>
<td>NOS2</td>
<td>G/A</td>
<td>0.34</td>
<td>1.94E-07</td>
<td>1.21</td>
</tr>
<tr>
<td>rs984971</td>
<td>2</td>
<td>63,224,521</td>
<td>KCNH7</td>
<td>G/A</td>
<td>0.36</td>
<td>3.62E-06</td>
<td>0.84</td>
</tr>
<tr>
<td>rs1306395</td>
<td>2</td>
<td>61,076,272</td>
<td>LINCO118</td>
<td>C/T</td>
<td>0.43</td>
<td>2.99E-05</td>
<td>0.86</td>
</tr>
<tr>
<td>rs7552167</td>
<td>1</td>
<td>24,518,643</td>
<td>IFNL1</td>
<td>A/G</td>
<td>0.14</td>
<td>1.53E-05</td>
<td>0.79</td>
</tr>
<tr>
<td>rs8106664</td>
<td>19</td>
<td>10,128,630</td>
<td>SLCA4A2</td>
<td>G/T</td>
<td>0.23</td>
<td>3.28E-06</td>
<td>0.81</td>
</tr>
<tr>
<td>rs2392581</td>
<td>7</td>
<td>38,573,234</td>
<td>AMPH</td>
<td>G/A</td>
<td>0.42</td>
<td>6.90E-05</td>
<td>0.87</td>
</tr>
<tr>
<td>rs8103241</td>
<td>19</td>
<td>13,122,612</td>
<td>NFIX</td>
<td>G/A</td>
<td>0.46</td>
<td>9.08E-05</td>
<td>0.87</td>
</tr>
<tr>
<td>rs1133071</td>
<td>9</td>
<td>32,455,674</td>
<td>DDX58</td>
<td>C/T</td>
<td>0.30</td>
<td>3.36E-05</td>
<td>1.17</td>
</tr>
<tr>
<td>rs6713082</td>
<td>2</td>
<td>62,516,544</td>
<td>B3GNT2</td>
<td>A/C</td>
<td>0.24</td>
<td>4.59E-05</td>
<td>1.18</td>
</tr>
<tr>
<td>rs2298428</td>
<td>22</td>
<td>21,882,892</td>
<td>YDIC</td>
<td>T/C</td>
<td>0.18</td>
<td>4.38E-05</td>
<td>1.20</td>
</tr>
<tr>
<td>rs8016947</td>
<td>14</td>
<td>35,832,666</td>
<td>NFKBIA</td>
<td>T/G</td>
<td>0.44</td>
<td>9.65E-05</td>
<td>0.87</td>
</tr>
<tr>
<td>rs7895120</td>
<td>10</td>
<td>129,064,193</td>
<td>DOCK1</td>
<td>T/C</td>
<td>0.14</td>
<td>5.29E-05</td>
<td>0.80</td>
</tr>
</tbody>
</table>

bp, base position; chr, chromosome; I2, heterogeneity index for ORs; Q, Cochranes’s Q statistic for heterogeneity of ORs; RAF, risk allele frequency;
Immunochip data resulted in a combined data set of 3139 PsA cases and 11 078 controls. We identified genome-wide significance to two loci; NOS2 (rs4795067, p=5.27×10^{-8}) and PTPN22 (rs2476601, p=1.49×10^{-8}) (table 1). Association to NOS2 has previously been reported to psoriasis; however no such association has been made to PTPN22 (figures 1 and 2). Interestingly we observe a higher effect estimate for rs2476601 in men compared with women (1.31 vs 1.22, respectively) as previously reported for this SNP in PsA, however this difference is not statistically significant (Q=0.52). We also observe a much lower minor allele frequency for rs2476601 in the Italian population which is consistent with previous studies demonstrating a North-East to South-West gradient for minor allele frequency (MAF) across continental Europe.

As SNPs at the PTPN22 locus have not previously been reported to be associated to psoriasis susceptibility we investigated this further in two large psoriasis data sets. First we analysed genotyped data from the WTCCC2 psoriasis study, excluding known PsA samples (cases n=1784, controls n=5175), for rs2476601 and found no evidence for association (p=0.34). Second we searched summary statistics from the largest psoriasis study to date (cases n=10 588, controls n=22 806) using the ImmunoBase database and again found no evidence for association of rs2476601 to psoriasis susceptibility (p=0.49). Using genotype data from the PsA Immunochip study and WTCCC2 we directly compared the effect estimates for rs2476601 in PsA and psoriasis using multinomial logistic regression and we found the estimates to be significantly different (p=3.2×10^{-4}). A direct comparison of genotypes for PsA (n=1962) and psoriasis (n=1784) found significant association to an increased risk of PsA (p=4.4×10^{-4}, OR=1.3).

Given that rs2476601 is a genetic risk factor for RA we were concerned that the observed p value in the discovery study was a false positive due to phenotype misclassification caused by the presence of unidentified RA samples in the case cohort. However, we found the association to rs22476601 in the PsA Immunochip data was unaffected by the inclusion of the RA-GRS (p=1.29×10^{-4} vs p_{GRS}=1.30×10^{-4}).

**DISCUSSION**

In this study we present evidence for association of rs2476601 to susceptibility of PsA exceeding the threshold recognised as genome-wide significant (p<5×10^{-8}) for the first time. In addition we used genotype data and summary statistics from two large psoriasis studies to demonstrate that this locus is differentially associated to PsA and not psoriasis per se. We also confirm association of PsA with a previously reported psoriasis locus, NOS2, bringing the total number of confirmed, genome-wide significant, PsA loci to 10 including 4 that are PsA-specific (HLA-B, chromosome 5q31, PsA-specific variants within IL23R and now PTPN22). Studies have shown that PTPN22 is a potent inhibitor of T cell activation and it is possible that the effect may differ between T cell subpopulations. For example we have shown that CD8+ T cells are important for PsA, while this has not been reported in psoriasis.

Strengths of the current study include the large sample sizes used, which allowed us to confirm association at accepted genome-wide thresholds. Previous studies of this locus in PsA have been limited by small sample size; results have either shown weak evidence for association, weak association in men only or no evidence for association at all. Indeed, our previous attempts to investigate rs2476601 and PsA susceptibility failed to find any evidence of association. This previous study had approximately 60% power to detect an effect of the size estimated in the current study. The absence of association for rs2476601 in the Italian cohort of this study is attributed to reduced power due to the much lower MAF (figure 2). Previous investigations of the rs2476601 PTPN22 variant with psoriasis have consistently reported no evidence for association, but some have found association to other variants in the region, for example rs3789604 (RSBN1) or haplotypes spanning PTPN22. However, in the largest psoriasis genetic association study performed to date, no association was detected to either rs2476601 or rs3789604 (p=0.49 and p=1.00, respectively). Indeed, a direct comparison of psoriasis and PsA confirmed that the rs2476601 association is PsA-specific, making it the fourth such locus to be identified.

In contrast to the previous reports, the study presented here is performed in a large cohort of 3139 cases and 11 078 controls, includes independent validation and, for the first time, reports confirmed association with susceptibility to PsA exceeding genome-wide significance (p=1.49×10^{-9}). The identification of PsA-specific loci is vital in terms of understanding the different pathways involved, which may require different treatments, and for future screening strategies to identify subjects at risk of developing PsA in patients with psoriasis.

The SNP rs2476601, has been found to be associated with multiple autoimmune diseases including RA, where the association is predominantly found in anti-citrullinated protein antibody (ACPA)-positive subjects, although association in the ACPA-negative subgroup has been reported. One possibility, therefore, is that the association with PsA could be due to the inclusion of patients with RA and coincidental psoriasis in the PsA cohort. Unfortunately, ACPA or rheumatoid factor status was not available for many samples. A strength of the current study, however, is that we used a GRS of known RA loci, which

**Figure 2** Forest plot of effect estimates for rs2476601 from the Immunochip, validation and meta-analysis. Rows are labelled by study group and include MAF, p values, ORs and 95% CIs. Reported MAF is estimated from control group, for Immunochip cohort this is estimated from UK controls. CI, confidence interval; minor allele frequency; OR, odds ratio.

<table>
<thead>
<tr>
<th>Study</th>
<th>MAF</th>
<th>P-value</th>
<th>OR [95% CI]</th>
<th>Meta-analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunochip</td>
<td>0.1</td>
<td>1.29e-05</td>
<td>1.29[1.14:1.42]</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>0.1</td>
<td>9.51e-04</td>
<td>1.50[1.18:1.90]</td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>0.12</td>
<td>5.48e-03</td>
<td>1.40[1.10:1.78]</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>0.03</td>
<td>0.435</td>
<td>1.32[0.66:2.72]</td>
<td></td>
</tr>
</tbody>
</table>

![Forest plot of effect estimates for rs2476601 from the Immunochip, validation and meta-analysis. Rows are labelled by study group and include MAF, p values, ORs and 95% CIs. Reported MAF is estimated from control group, for Immunochip cohort this is estimated from UK controls. CI, confidence interval; minor allele frequency; OR, odds ratio.](image-url)
has been previously shown to adequately control for potential phenotype misclassification, to explore this possible confounder and found that the association with PsA remained statistically significant even after this adjustment. 10

In conclusion we report for the first time genome-wide signifi- cant association of the rs2476601 variant in the PTPN22 gene with susceptibility to PsA consistent with reports in many other autoimmune diseases. In addition, we use genotype data from a large psoriasis study to demonstrate that rs2476601 is differentially associated to PsA and not psoriasis.

**Author affiliations**

1Arthritis Research UK Centre for Epidemiology, Centre for Musculoskeletal Research, Institute for Inflammation and Repair, Manchester Academic Health Science Centre, The University of Manchester, Manchester, UK
2Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany
3NIHR Manchester Musculoskeletal Biomedical Research Unit, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK
4The Kellgren Centre for Rheumatology, Central Manchester Foundation Trust, NIHR Manchester Biomedical Research Centre, Manchester, UK
5Monash University, Melbourne, Victoria, Australia
6NIHR-Leeds Musculoskeletal Biomedical Research Unit, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK
7Department of Clinical Medicine, Institute of Molecular Medicine, Trinity College Dublin, Dublin, Ireland
8Adelaide and Meath Hospital and Trinity College Dublin, Dublin, Ireland
9Royal National Hospital for Rheumatic Diseases and Department Pharmacy and Pharmacology, University of Bath, Bath, UK
10Department of Public Health and Clinical Medicine, Rheumatology, University Hospital, Umeå, Sweden
11Department of Biopathology, Centre of Excellence for Genomic Risk Assessment in Multifactorial and Complex Diseases, School of Medicine, University of Rome ‘Tor Vergata’ and Fondazione PT’Policlinico Tor Vergata’, Rome, Italy
12Rheumatology Department, Haywood Hospital, Health Services Research Unit, Institute of Science and Technology in Medicine, Keele University
13Department of Rheumatology, St. Vincent’s University Hospital, UCD School of Medicine and Medical Sciences and Conway Institute of Biomedical and Biomedical Research, University College Dublin, Dublin, Ireland
14The University of Queensland Diamantina Institute, Translational Research Institute, Princess Alexander Hospital, Woolloongabba, Brisbane, Queensland, Australia
15Division of Rheumatology and Fraunhofer IHE-Project Group Translational Medicine and Pharmacology, Goethe University, Frankfurt, Germany

**Acknowledgements** The authors acknowledge the assistance given by IT Services and the use of the Computational Shared Facility (CSF) at The University of Manchester. The authors thank Arthritis Research UK for their support (grant ref 20385) and the NIHR Manchester Musculoskeletal Biomedical Research Unit. This report includes independent research funded by the National Institute for Health Research Biomedical Research Unit Funding Scheme. The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the Department of Health. The authors gratefully acknowledge the contribution of patients and staff of the Early Swedish Psoriatic Arthritis Registry (SwepSA).

**Contributors** AB devised the study concept and design. JB performed statistical analysis. JB and AB wrote the manuscript. SL and UH performed validation genotyping and contributed to statistical analysis. AB-A and SU contributed to the statistical analysis. FB, HB and AR contributed to interpretation of findings. IH, HM-O, Phe, AWR, DK,EK, G-MA, EG, JP, RM, OF, NM, Pho, MAB and MF contributed data to the discovery phase. All authors contributed to and approved the manuscript.

**Funding** Frankfurt: the German Federal Ministry of Education and Research ArthroMark (project 4, 01 EC 1009C), the Federal State of Hesse (LOEWE-project: IHE Fraunhofer Project Group Translational Medicine & Pharmacology at the Goethe University), HB received funding from Pfizer Pharma, Germany (Forschungsförderpreis Rheumatologie 2012); Support for the Australian component of the study was received from Abbvie. MAB is funded by a National Health and Medical Research Foundation (Australia) Senior Principal Research Fellowship.

**Competing interests** None declared.

**Ethics approval** All samples were collected with approval from the respective local ethical committee: the medical faculties of the Universities of Erlangen and Münster, the University of Tor Vergata of Rome and the Umeå University, Sweden.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** All summary statistics for data generated in this study are presented in table 1. Further information can be obtained by contacting the authors.

**Open Access** This is an Open Access article distributed in accordance with the terms of the Creative Commons Attribution (CC BY 4.0) license, which permits others to distribute, remix, adapt and build upon this work, for commercial use, provided the original work is properly cited. See: http://creativecommons.org/licenses/by/4.0/
PTPN22 is associated with susceptibility to psoriatic arthritis but not psoriasis: evidence for a further PsA-specific risk locus

John Bowes, Sabine Loehr, Ashley Budu-Aggrey, Steffen Uebe, Ian N Bruce, Marie Feletar, Helena Marzo-Ortega, Philip Helliwell, Anthony W Ryan, David Kane, Eleanor Korendowych, Gerd-Marie Alenius, Emiliano Giardina, Jonathan Packham, Ross McManus, Oliver FitzGerald, Matthew A Brown, Frank Behrens, Harald Burkhardt, Neil McHugh, Ulrike Huffmeier, Pauline Ho, Andre Reis and Anne Barton

*Ann Rheum Dis* published online April 28, 2015

Updated information and services can be found at: [http://ard.bmj.com/content/early/2015/04/28/annrheumdis-2014-207187](http://ard.bmj.com/content/early/2015/04/28/annrheumdis-2014-207187)

These include:

**Supplementary Material**

Supplementary material can be found at: [http://ard.bmj.com/content/suppl/2015/04/28/annrheumdis-2014-207187.DC1.html](http://ard.bmj.com/content/suppl/2015/04/28/annrheumdis-2014-207187.DC1.html)

**References**

This article cites 22 articles, 5 of which you can access for free at: [http://ard.bmj.com/content/early/2015/04/28/annrheumdis-2014-207187#BIBL](http://ard.bmj.com/content/early/2015/04/28/annrheumdis-2014-207187#BIBL)

**Open Access**

This is an Open Access article distributed in accordance with the terms of the Creative Commons Attribution (CC BY 4.0) license, which permits others to distribute, remix, adapt and build upon this work, for commercial use, provided the original work is properly cited. See: [http://creativecommons.org/licenses/by/4.0/](http://creativecommons.org/licenses/by/4.0/)

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**

Articles on similar topics can be found in the following collections

- Open access (456)
- Degenerative joint disease (4236)
- Musculoskeletal syndromes (4526)
- Genetics (894)

**Notes**

To request permissions go to: [http://group.bmj.com/group/rights-licensing/permissions](http://group.bmj.com/group/rights-licensing/permissions)

To order reprints go to: [http://journals.bmj.com/cgi/reprintform](http://journals.bmj.com/cgi/reprintform)

To subscribe to BMJ go to: [http://group.bmj.com/subscribe/](http://group.bmj.com/subscribe/)