Psoriasis is a chronic inflammatory disease with a complex genetic architecture. To date, the psoriasis heritability is only partially explained. However, there is increasing evidence that the missing heritability in psoriasis could be explained by multiple genetic variants of low effect size from common genetic pathways. The objective of this study was to identify new genetic variation associated with psoriasis risk at the pathway level.

We genotyped 598,258 single nucleotide polymorphisms in a discovery cohort of 2,281 case-control individuals from Spain. We performed a genome-wide pathway analysis using 1,053 reference biological pathways. A total of 14 genetic pathways ($P_{FDR} \leq 2.55 \times 10^{-5}$) were found to be significantly associated with psoriasis risk. Using an independent validation cohort of 7,353 individuals from the UK, a total of 6 genetic pathways were significantly replicated ($P_{FDR} \leq 3.46 \times 10^{-5}$). We found genetic pathways that had not been previously associated with psoriasis risk such as retinol metabolism ($P_{combined} = 1.84 \times 10^{-4}$), the transport of inorganic ions and amino acids ($P_{combined} = 1.57 \times 10^{-5}$), and post-translational protein modification ($P_{combined} = 1.57 \times 10^{-7}$). In the latter pathway, MGAT5 showed a strong network centrality, and its association with psoriasis risk was further validated in an additional case-control cohort of 3,429 individuals ($P < 0.05$). These findings provide insights into the biological mechanisms associated with psoriasis susceptibility.

INTRODUCTION

Psoriasis is a common chronic inflammatory disease of the skin that affects approximately 2% of the worldwide population (Nestle et al., 2009). In psoriasis, immune cells infiltrate the skin leading to an increased proliferation of keratinocytes (Ferenzi et al., 2000; Gudjonsson and Elder, 2007). It is a genetically complex disease with a complex mode of inheritance (Vyse and Todd, 1996). HLA class I gene HLA-C*0602 haplotype association explains the largest part of the known heritability of psoriasis (Nair et al., 2006; Strange et al., 2010).

Genome-wide association studies (GWAS) have been successful in the characterization of the genetic architecture of many complex human diseases (Manolio, 2010). To date, more than 15 GWAS have been performed using large psoriasis cohorts from Caucasian and Asian populations and have collectively identified more than 50 susceptibility loci for psoriasis (Bowes et al., 2015; Tsoi et al., 2015; Yin et al., 2015; Zuo et al., 2015). Despite progress in characterizing psoriasis genetic etiology, loci outside the HLA region only explain less than 25% of the estimated psoriasis heritability (Tsoi et al., 2012; Yin et al., 2014).

Recent research has shown that the missing heritability of complex human diseases can be explained by common genetic variants, rare variants or a combination of genetic, epigenetic, and environmental interactions (Gibson, 2012). From these, common genetic variants could explain more...
than 60% of the heritability of the most prevalent autoimmune diseases (Golan et al., 2014). Importantly, most of these common genetic variants are characterized by having low effect sizes (Park et al., 2010).

Although GWAS based on single markers have successfully identified disease-susceptibility variants, this strategy is not adequate to identify genetic variants with low effect sizes that are genuinely associated with disease risk (Du et al., 2012). In single-marker GWAS, a large number of genetic variants are tested for association with a complex trait. To avoid false positive results, a stringent genome-wide significant threshold must be used (Johnson et al., 2010). This conservative threshold, however, does not allow the identification of modest effect risk loci, unless extremely large samples sizes of cases and controls are used (Wang et al., 2010). Importantly, single-marker GWAS consider only the individual effect of each single nucleotide polymorphism and ignore the joint effect of multiple causal genetic variants as well as the biological context where disease genes operate (Zhang et al., 2010).

Functionally related genes have been shown to collectively contribute to disease susceptibility, including those loci that do not reach individually the genome-wide significant threshold (Zhong et al., 2010). Recently, new methods that are able to analyze genetic associations at the pathway level have been developed (Gui et al., 2011). Pathway-based approaches are robust statistical methodologies that integrate genetic and biological knowledge to test whether sets of functionally related genes are jointly associated with a complex trait (Ramanan et al., 2012). Therefore, pathway-based methods increase the statistical power of the association analysis by reducing the number of association tests that must be performed and allow a functional interpretation of the results (Wu et al., 2010).

Pathway-based analyses have been recently performed to study the genetic basis of cancer subtypes using either selected candidate pathways, but also at a genome-wide scale (Chen et al., 2014; Koster et al., 2014). Although the genome-wide pathway analysis can have a high computational cost, this approach is able to identify novel genetic pathways associated with disease risk. The identification of new pathways associated with disease risk could increase the probability to develop new therapeutic strategies in complex diseases such as psoriasis. To date, however, the genome-wide pathway analysis approach has not been performed in psoriasis.

To gain a better understanding of the genetic risk basis of psoriasis, we performed a genome-wide pathway analysis on a large multicenter cohort of patients with psoriasis. In this study, we analyzed the association of 1,053 reference biological pathways using 1,263 patients with psoriasis and 1,558 controls from Spain. Using an independent cohort of 2,178 cases and 5,175 controls from the UK, we then performed a validation study of the significantly associated pathways in the discovery cohort. With this approach, we identified genetic pathways that had not been previously associated with psoriasis risk such as retinol metabolism, transport of inorganic ions and amino acids, and post-translational protein modification. These results provide important insights into the genetic etiology of psoriasis.

<p>| Table 1. Pathways associated with psoriasis risk and validated in the replication stage. |
| --- | --- | --- | --- | --- | --- | --- | --- |</p>
<table>
<thead>
<tr>
<th>Pathway</th>
<th>Database and Gene</th>
<th>SNPs</th>
<th>p</th>
<th>p*</th>
<th>FDR</th>
<th>FDR</th>
<th>p*</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory response</td>
<td>Biocarta</td>
<td>29</td>
<td>628</td>
<td>&lt;5.99 x 10^-8</td>
<td>2.45 x 10^-5</td>
<td>2.45 x 10^-5</td>
<td>2.45 x 10^-5</td>
<td>2.45 x 10^-5</td>
</tr>
<tr>
<td>Natural killer T cell</td>
<td>Biocarta</td>
<td>29</td>
<td>638</td>
<td>&lt;5.99 x 10^-8</td>
<td>2.45 x 10^-5</td>
<td>2.45 x 10^-5</td>
<td>2.45 x 10^-5</td>
<td>2.45 x 10^-5</td>
</tr>
<tr>
<td>DNA repair</td>
<td>Biocarta</td>
<td>112</td>
<td>2,050</td>
<td>1.33 x 10^-7</td>
<td>1.17 x 10^-7</td>
<td>1.17 x 10^-7</td>
<td>1.17 x 10^-7</td>
<td>1.17 x 10^-7</td>
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<tr>
<td>Amino acid transport across the plasma membrane</td>
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<td>31</td>
<td>1,025</td>
<td>2.00 x 10^-7</td>
<td>1.24 x 10^-7</td>
<td>1.24 x 10^-7</td>
<td>1.24 x 10^-7</td>
<td>1.24 x 10^-7</td>
</tr>
<tr>
<td>Transport to the Golgi and post-translational modification</td>
<td>Biocarta</td>
<td>188</td>
<td>5,965</td>
<td>2.00 x 10^-7</td>
<td>1.24 x 10^-7</td>
<td>1.24 x 10^-7</td>
<td>1.24 x 10^-7</td>
<td>1.24 x 10^-7</td>
</tr>
<tr>
<td>Transport of inorganic ions and amino acids</td>
<td>Biocarta</td>
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<td>1,577</td>
<td>3.33 x 10^-7</td>
<td>2.15 x 10^-7</td>
<td>2.15 x 10^-7</td>
<td>2.15 x 10^-7</td>
<td>2.15 x 10^-7</td>
</tr>
<tr>
<td>Asparagine N-linked glycosylation</td>
<td>Biocarta</td>
<td>81</td>
<td>2,760</td>
<td>4.00 x 10^-7</td>
<td>2.11 x 10^-7</td>
<td>2.11 x 10^-7</td>
<td>2.11 x 10^-7</td>
<td>2.11 x 10^-7</td>
</tr>
<tr>
<td>Transport of inorganic ions and amino acids</td>
<td>Biocarta</td>
<td>94</td>
<td>4,010</td>
<td>4.00 x 10^-7</td>
<td>2.11 x 10^-7</td>
<td>2.11 x 10^-7</td>
<td>2.11 x 10^-7</td>
<td>2.11 x 10^-7</td>
</tr>
<tr>
<td>Retinol metabolism</td>
<td>KEGG</td>
<td>64</td>
<td>5,182</td>
<td>2.00 x 10^-7</td>
<td>2.55 x 10^-5</td>
<td>2.55 x 10^-5</td>
<td>2.55 x 10^-5</td>
<td>2.55 x 10^-5</td>
</tr>
</tbody>
</table>

Abbreviations: C, combined; D, discovery cohort; E, exclusion gene; FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; P, empirical set-based p-value; R, replication cohort. Number of single nucleotide polymorphisms mapping to a particular pathway. 

Increased permutations to refine the empirical p-value (n = 10,000,000).
RESULTS
Identification of genetic pathways associated with psoriasis risk
In the discovery stage, the genome-wide pathway analysis identified a total of 26 genetic pathways significantly associated with psoriasis risk after multiple test correction ($P_{FDR} < 0.05$, Supplementary Table S1 online). The complete results of the genome-wide pathway analysis performed in the discovery study are shown in Supplementary Table S2 online.

From the 26 significantly associated pathways, we found that 14 pathways included IL12B gene. After HLA-C*0602, IL12B is one of the strongest known genetic risk factors for psoriasis. To confirm that the observed pathway associations were the result of the joint effect of multiple genes and not the result of a single risk locus strongly associated with the disease, we removed IL12B from these genetic pathways and tested again for association. After extracting IL12B, two genetic pathways—“Inflammatory response” and “Natural killer T cell”—remained significantly associated with psoriasis risk ($P_{FDR} < 0.05$). Consequently, and to avoid redundancy, only the pathway showing the highest level of significance was selected to represent each biological process. The “Transport of inorganic ions and amino acids” ($P_{combined} = 1.57 \times 10^{-7}$, Figure 1b) and “Post-translational protein modification” ($P_{combined} = 1.57 \times 10^{-7}$, Figure 1c) pathways were therefore selected from each overlapping pathway group. The “Inflammatory response” ($P_{combined} = 1.06 \times 10^{-12}$), “Natural killer T cell” ($P_{combined} = 1.06 \times 10^{-12}$), “DNA repair” ($P_{combined} = 1.10 \times 10^{-9}$), and “Retinol metabolism” ($P_{combined} = 1.84 \times 10^{-4}$) pathways did not show a significant degree of overlap and were therefore considered as independent biological processes.

Within the final group of six genetic pathways associated with disease risk and representing independent biological processes, we analyzed the association between each particular gene and psoriasis risk (Table 2). We found 37 small-effect genes that were nominally associated with psoriasis risk both in the discovery and replication cohorts ($P \leq 1.29 \times 10^{-2}$, Table 3). The complete list of genetic associations obtained from each genetic pathway is shown in Supplementary Table S3 online. The linkage disequilibrium pattern between the SNPs mapping to each genetic pathway associated with psoriasis risk is shown in Supplementary Figure S1 online.

Characterization of the genetic pathways associated with psoriasis risk
To discard the presence of redundant pathways, we evaluated the level of gene overlap between all associated pathways. From the nine validated genetic pathways, we found that the “Amino acid transport across the plasma membrane” and “Transport of inorganic ions and amino acids” pathways, as well as the “Asparagine N-linked glycosylation,” “Transport to the Golgi and subsequent modification,” and “Post-translational protein modification” pathways had a high degree of overlap between them (>95% of shared genes, Figure 1a). Consequently, and to avoid redundancy, only the pathway showing the highest level of significance was selected to represent each biological process. The “Transport of inorganic ions and amino acids” ($P_{combined} = 1.57 \times 10^{-7}$, Figure 1b) and “Post-translational protein modification” ($P_{combined} = 1.57 \times 10^{-7}$, Figure 1c) pathways were therefore selected from each overlapping pathway group. The “Inflammatory response” ($P_{combined} = 1.06 \times 10^{-12}$), “Natural killer T cell” ($P_{combined} = 1.06 \times 10^{-12}$), “DNA repair” ($P_{combined} = 1.10 \times 10^{-9}$), and “Retinol metabolism” ($P_{combined} = 1.84 \times 10^{-4}$) pathways did not show a significant degree of overlap and were therefore considered as independent biological processes.

Within the final group of six genetic pathways associated with disease risk and representing independent biological processes, we analyzed the association between each particular gene and psoriasis risk (Table 2). We found 37 small-effect genes that were nominally associated with psoriasis risk both in the discovery and replication cohorts ($P \leq 1.29 \times 10^{-2}$, Table 3). The complete list of genetic associations obtained from each genetic pathway is shown in Supplementary Table S3 online. The linkage disequilibrium pattern between the SNPs mapping to each genetic pathway associated with psoriasis risk is shown in Supplementary Figure S1 online.

Functional-based networks associated with psoriasis risk
To understand the relevance of each particular gene within the genetic pathway associated with psoriasis risk, we used biological knowledge to build the associated functional-based network (Figure 2). Using known or predicted functional
Table 2. Association results of the top five genes involved in each pathway associated with psoriasis risk

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Database</th>
<th>SNP</th>
<th>COORD</th>
<th>A1</th>
<th>A2</th>
<th>OR(^a)</th>
<th>P(^d)</th>
<th>Gene(^b)</th>
<th>SNP(^b)</th>
<th>COORD</th>
<th>A1</th>
<th>A2</th>
<th>OR(^R)</th>
<th>P(^R)</th>
<th>Gene(^R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory response</td>
<td>Biocarta</td>
<td>rs20541</td>
<td>5:131995964</td>
<td>A</td>
<td>G</td>
<td>0.72</td>
<td>4.18 x 10(^{-5})</td>
<td>IL13,IL4</td>
<td>rs2965012</td>
<td>1:218786549</td>
<td>A</td>
<td>C</td>
<td>0.83</td>
<td>7.56 x 10(^{-4})</td>
<td>TGFβ2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs11739623</td>
<td>5:131864152</td>
<td>A</td>
<td>G</td>
<td>1.21</td>
<td>1.79 x 10(^{-3})</td>
<td>IL5</td>
<td>rs2243123</td>
<td>3:159709651</td>
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<td>1.06 x 10(^{-3})</td>
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<td></td>
<td></td>
<td>rs2799083</td>
<td>1:218581617</td>
<td>G</td>
<td>A</td>
<td>1.22</td>
<td>2.82 x 10(^{-3})</td>
<td>TGFβ2</td>
<td>rs25890</td>
<td>5:311437562</td>
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<td>G</td>
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<td>1.09 x 10(^{-3})</td>
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<td>C</td>
<td>1.19</td>
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<td>IL12A</td>
<td>rs20541</td>
<td>3:159195964</td>
<td>A</td>
<td>G</td>
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<td>2.41 x 10(^{-4})</td>
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<td></td>
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<td>A</td>
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<td>G</td>
<td>0.90</td>
<td>2.94 x 10(^{-5})</td>
<td>CD4</td>
</tr>
<tr>
<td>Natural killer T cell</td>
<td>Biocarta</td>
<td>rs20541</td>
<td>5:131995964</td>
<td>A</td>
<td>G</td>
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<td>4.18 x 10(^{-5})</td>
<td>IL4</td>
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<td>A</td>
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<td>A</td>
<td>1.22</td>
<td>2.82 x 10(^{-3})</td>
<td>TGFβ2</td>
<td>rs2965012</td>
<td>1:218786549</td>
<td>A</td>
<td>G</td>
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<td>3:159709651</td>
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<td>A</td>
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<td>1.06 x 10(^{-3})</td>
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<td></td>
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<td>3:159696099</td>
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<td>G</td>
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<td>1.09 x 10(^{-3})</td>
<td>CSF2</td>
</tr>
<tr>
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<td>C</td>
<td>0.77</td>
<td>5.82 x 10(^{-5})</td>
<td>ADH1C,ADH1B</td>
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<td>16:81336356</td>
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<td>G</td>
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<td>10:94852448</td>
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<td>19:4136795</td>
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<td>A</td>
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<td>FANC1</td>
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</table>

Abbreviations: A1, minor allele; A2, major allele; COORD, SNP coordinates in build GRCh37/hg19; D, discovery cohort; KEGG, Kyoto Encyclopedia of Genes and Genomes; OR, odds ratio; P, P-value; R, replication cohort.

\(^{1}\)The detailed description of the “Inflammatory response” and “Natural killer T cell” pathways corresponds to the association results after excluding the IL12B gene from the genome-wide pathway analysis.
networks are a powerful approach to represent and analyze associations between the pathway genes, functional-based networks are a powerful approach to represent and analyze the topological structure of a biologic pathway.

To characterize the network properties of the resulting functional-based networks, we determined the betweenness centrality (BC) and degree centrality (DC) statistics (Supplementary Table S4 online). These two measures are useful to identify those network elements (genes in this case) that are likely to be more influential in the structure of the network. BC and DC have been widely used to identify the genes that are more likely to be essential for pathway functionality (Hahn and Kern, 2005; Joy et al., 2005; Vallabhajosyula et al., 2009). We found that SLC7A11 from the “Transport of inorganic ions and amino acids” pathway and MGAT5 from the “Post-translational protein modification” pathway had markedly high BC values (BC ≥ 0.1). From these, MGAT5 gene also showed a much stronger DC value than SLC7A11 (DC_{MGAT5} = 19, DC_{SLC7A11} = 3).

Given the strong network centrality properties found for MGAT5 gene in the “Post-translational protein modification” pathway, we decided to further test the association of this key gene with psoriasis risk in an independent case-control cohort. Using this additional replication cohort, we significantly validated the association of MGAT5 gene with psoriasis risk (P = 1.3 × 10^{-2}; odds ratio [95% confidence interval] = 0.85 [0.74–0.96]).

### Functional analysis of MGAT5 variation

MGAT5 encodes for a key enzyme in the N-glycosylation pathway, a post-translational process that is directly implicated in T-cell activation and differentiation (Demetriou et al., 2001). To assess the functional role of MGAT5 in psoriasis pathogenesis, we evaluated the association between genetic variation at MGAT5 gene and the levels of T-cell surface glycosylation. Flow cytometry analysis of in vitro activated CD4^+ and CD8^+ T cells obtained from 27 patients with psoriasis showed an increase in N-glycosylation levels in patients carrying one or two copies of the protective allele (G) compared with homozygous individuals for the risk allele (A) (Figure 3). The increased glycosylation levels in individuals carrying at least one copy of (G) allele was observed both in activated CD8^+ and CD4^+ T cells. In CD4^+ T lymphocytes, the glycosylation level was significantly higher in GG homozygotes compared with AA homozygotes (P = 0.01, Figure 3).

### DISCUSSION

Genome-wide association analyses have successfully identified more than 50 loci associated with psoriasis susceptibility. To date, however, the genetic basis of psoriasis is still not completely understood. In this study, we have performed a genome-wide pathway analysis of psoriasis genetic risk. Using a discovery cohort from Spain and an independent cohort from the UK, we have identified and validated the association of six genetic pathways with psoriasis susceptibility. Importantly, these validated pathways include biological processes such as retinol metabolism, transport of inorganic ions and amino acids, and post-translational protein modification that had not been previously associated with psoriasis risk at the genetic level. In addition, analyzing the network properties of these validated pathways we have found that MGAT5 gene has a strong centrality in the post-translational protein modification pathway. Using an additional independent case-control cohort from Spain, we have further replicated the association of MGAT5 with psoriasis risk. Taken together,
these findings contribute to a better understanding of the genetic risk basis of psoriasis and provide important insights into the biological mechanisms associated with the disease pathogenesis.

Retinol has been demonstrated to inhibit inflammatory processes in dermatological diseases (Balato et al., 2013). In particular, retinol inhibits the regulatory activity of the nuclear factor kappa B (NFKB) in the skin (Austenaa et al., 2004). NFKB is an established transcriptional factor that regulates multiple proinflammatory genes that are key in psoriasis pathogenesis like tumor necrosis factor and interleukin-17 (Goldminz et al., 2013). The NFKB signaling pathway has also been associated with the regulation of the proliferation of epidermal keratinocytes (Tsuruta, 2009). These findings are consistent with the elevated levels of NFKB that have been found in lesional and non-lesional psoriatic...
Previous studies have found that a translational modification pathway has been found to be necessary for the immune system tolerance to self-antigens (Hayashi et al., 2013). The transport of amino acids into T cells is essential to maintain the increased production of proinflammatory cytokines associated with psoriasis risk includes the transport of inorganic ions and amino acids pathway associated with psoriasis (Khananshvili, 2013; Vig and Kinet, 2009). Accordingly, the expression of amino acid transporters has been shown to contribute to autoimmune and inflammatory diseases (Lang et al., 2014). In particular, the intracellular transport of calcium is crucial for controlling the expression of proinflammatory genes in immune cells (Jaeger et al., 2008). These results therefore suggest that genetic variation in the transport of amino acids in activated T cells can increase the risk to develop psoriasis by modulating T-cell functionality.

The post-translational protein modification pathway is responsible for the N-linked glycosylation of the asparagine residues in the HLA molecules (Rudd et al., 2001). This post-translational modification pathway has been found to be necessary for the immune system tolerance to self-antigens (Ryan and Cobb, 2012). Previous studies have found that a deficient or aberrant asparagine glycosylation can induce autoimmune diseases (Green et al., 2007). Also, post-translationally modified autoantigens have been associated with psoriasis (Iversen et al., 2011). In patients with psoriasis, the peptide glycosylation activity has been found to be markedly increased in comparison with healthy controls (Damasiewicz-Bodzek and Wielkoszynski, 2012). Furthermore, specific post-translational modifications on glycoproteins expressed on the surface of T lymphocytes have been shown to target these cells to the inflamed skin (Fuhlbrigge et al., 1997). Therefore, genetic variation in the post-translational protein modification pathway could perturb the glycosylation processes that are crucial to maintain the immune system tolerance.

MGAT5 encodes for a key enzyme in the N-glycosylation pathway. This pathway has been directly implicated in T-cell activation and autoimmunity (Demetriou et al., 2001). Recent research has found an association between MGAT5 glycosylation activity and multiple sclerosis etiology both in experimental models and in humans (Grigorian and Demetriou, 2011; Mkhikian et al., 2011). In this study, we have found that the MGAT5 is a key gene in the post-translational protein modification pathway associated with psoriasis. Subsequently, we found that genetic variation at MGAT5 is associated with the level of glycosylation of in vitro activated T cells. This result is consistent with previous findings showing that deficiency of MGAT5 glycosylation activity reduces the T-cell activation threshold and, consequently, promotes the triggering of autoimmune diseases (Demetriou et al., 2001). Further studies evaluating the implication of the T-cell surface glycosylation in clinically relevant outcomes in psoriasis such as skin severity are warranted.

The association of psoriasis risk with the inflammatory response and the natural killer T-cell pathways involves more than 10 immune-related genes, including IL12B. In a recent pathway analysis study using association results of a meta-analysis for psoriasis risk (Tsoi et al., 2015a), these two pathways were also found to be associated. These findings, however, were not validated using an independent cohort. Our study, therefore, provides strong confirmation of the implication of these two genetic pathways in the risk of psoriasis. Also, the permutation-based approach used in our study allowed to control for the potential bias associated with the presence of strong linkage disequilibrium patterns within genes. Our results indicate that the association of these pathways is not only driven by IL12B gene, but it is the result of the joint contribution of other small-effect genes in these pathways. One of these genes is CXCR4, which encodes for a chemokine receptor from the natural killer T-cell pathway (Colantonio et al., 2002). Although CXCR4 gene has not been previously associated with psoriasis risk in single-marker GWAS, CXCR4 chemokine has been shown to reduce keratinocyte proliferation and, consequently, the expansion of psoriatic plaques by regulating the proliferative cytokine signals that are activated in psoriatic lesions (Takekoshi et al., 2013). In addition, the inflammatory angiogenesis of psoriatic skin that leads to vascular remodeling has been recently shown to be modulated by CXCR4 chemokine (Zgraggen et al., 2014). Using the pathway analysis, we can therefore identify small-effect genes like CXCR4 that cannot be
detected by single-marker GWAS but that are biologically implicated in key processes of the disease pathophysiology.

In this study, we have also found a significant association between the DNA repair genetic pathway and psoriasis risk. Together with the dysregulation of immune system processes, the epidermal hyperproliferation is another well-known biological process implicated in the psoriasis pathophysiology (Wolf et al., 2012). The application of ultraviolet radiation in psoriasis skin lesions to induce apoptosis in aberrantly proliferating keratinocytes has proved to be a successful treatment for the clearance of plaque psoriasis in approximately 70% of patients (Weatherhead et al., 2011). The ultraviolet radiation induces DNA damage that promotes the transcription of the DNA repair pathway genes (Roos and Kaina, 2006). Consequently, the enzymatic machinery of the pathway repairs the DNA damage and also triggers the cell death by activating the p53 apoptotic signaling (Lavin et al., 2005). Therefore, these results suggest that genetic variation in the DNA repair pathway promotes an inefficient activation of the p53 apoptotic signaling that leads to an increased keratinocyte proliferation, as well as an inefficient response to ultraviolet therapy in patients with psoriasis.

Although the pathway-based analysis is a powerful approach to identify small-effect genetic variants associated with disease risk, this methodology is not exempt of limitations. Intergenic SNPs across the whole genome that map physically far away from genes were not included in this study. These genetic variants could be known risk loci (e.g., rs12188300 is associated with psoriasis risk and is located at >20Kb from IL12B gene) or may regulate the expression of genes through cis- and trans-expression quantitative trait loci mechanisms (Gilad et al., 2008). Also, some SNPs might not be functionally related to the closest genes. With the increasing regulatory information derived from expression quantitative trait loci and epigenomic data (Bernstein et al., 2010; Martens and Stunnenberg, 2013; Raney et al., 2011), intergenic SNPs could be integrated in the pathway-based analysis in the next few years.

The complex linkage disequilibrium structure of the HLA region together with the strong association with the susceptibility to multiple common diseases has been shown to generate false positive results in pathway-based methods (Wang et al., 2010). Following recent studies, in this study we removed the SNPs mapping to this locus to perform the present pathway analysis (Chen et al., 2014). As a result, known pathways associated with psoriasis risk that include genes from the HLA region, like the NFKB pathway, were not analyzed in this study. Importantly, however, in this study we have found and validated the association between genetic pathways related to IL12 signaling, an established genetic risk pathway for psoriasis and psoriasis risk. Also, within the associated pathways there are known risk genes for psoriasis (e.g., REV3L and IL4) within the DNA repair and inflammatory response pathways, respectively. Together, these results confirm the accuracy of the present pathway-based approach to identify relevant genetic variation associated with psoriasis risk.

The present genome-wide pathway analysis has two important strengths. First, we used PLINK software (Boston, MA) to identify genetic pathways associated with psoriasis risk. This pathway analysis method uses genotype data in contrast to the methodologies that are only based on association statistics. An important limitation of these latter methodologies is that they do not account for the linkage disequilibrium between SNPs. This can result in highly biased results and a significant increase in false positive results (Wang et al., 2010). Instead, the pathway analysis approach that we used, although can be computationally costly, efficiently overcomes these biases by maintaining the correct linkage disequilibrium patterns between SNPs. Finally, compared with previous pathway-based studies in other complex diseases, we have performed a two-stage pathway analysis in two large cohorts from different populations. Using an independent population, we have validated genetic pathways associated with psoriasis risk.

In conclusion, using a genome-wide pathway analysis approach we have identified to our knowledge previously unreported genetic pathways associated with psoriasis risk. These biological pathways include retinol metabolism, transport of inorganic ions and amino acids, and post-translational protein modification. The results of this study represent an important contribution to the characterization of the genetic risk basis of psoriasis.

**MATERIALS AND METHODS**

**Study population**

A total of 1,263 patients with psoriasis and 1,558 controls were recruited for the discovery stage (Supplementary Table S5 online). An independent case-control cohort of 7,353 individuals from the UK was used to validate the significantly associated pathways in the discovery cohort. An independent cohort of 1,381 patients with psoriasis and 2,048 controls from Spain was used to replicate the association between MGAT5 gene and psoriasis risk (Supplementary Materials, Supplementary Table S6 online).

All the procedures were followed in compliance with the principles of the Declaration of Helsinki and all patients provided written informed consent to participate in this study. The study and the consent procedure were approved by the local Institutional Review Board of each participating center.

**DNA extraction and genome-wide genotyping**

GWAS genotyping of the 2,821 individuals from the discovery cohort was performed using Illumina Quad610 Beadchips (Illumina, San Diego, CA) (Supplementary Materials). After the quality control analysis, a final data set of 541,926 SNPs from 1,172 patients with psoriasis was available for the pathway-based analysis. The genome-wide genotyping of the patients with psoriasis from the validation stage was performed using the Illumina Human660W-Quad (Illumina) and the healthy controls were genotyped using the Illumina custom Human1.2M-Duo (Illumina) as has been previously described (Strange et al., 2010). The final data set used for the replication study included 515,703 SNPs from 2,178 patients with psoriasis. The genotyping of the MGAT5 replication cohort was performed using the Taqman real-time PCR platform (Applied Biosystems, Foster City, CA) (Supplementary Materials).

**Pathway-based analysis**

**Gene set definition.** Reference biological pathway annotation databases BioCarta (www.biocarta.com), Kyoto Encyclopedia of Genes and Genomes (Kanehisa and Goto, 2000), and Reactome (Croft et al., 2014) were used to determine the global pathways.
The statistical association analysis was performed using the PLINK set-based test (Purcell et al., 2007) (Supplementary Materials). To obtain the global statistical significance of each validated pathway, we combined the empirical P-values resulting from the discovery and replication stages using Fisher's method (Kugler et al., 2010). We tested the association of 1,053 pathways with psoriasis risk. The false discovery rate (FDR) method (Hochberg and Benjamini, 1990) was used to account for multiple testing.

**Sensitivity analysis by removing the HLA and IL12B loci.** In pathway-based analysis, the presence of a single marker with very strong effects can lead to false positive associations. In these cases, the joint contribution of the pathway genes to disease risk is masked and not adequately evaluated (Wang et al., 2010). Similar to previous studies, to avoid this type of spurious associations, we removed all SNPs mapping to the HLA region (Megabases 25.6 to 33.3 in chromosome 6) (Chen et al., 2014). In the discovery stage, we found genetic pathways in which the IL12B gene was significantly associated with disease risk at a genome-wide scale. IL12B is a well-known psoriasis risk gene that shows a large effect on disease susceptibility and, like the HLA region, could generate false positive results (Cargill et al., 2007; Nair et al., 2008; Zhu et al., 2013). Accordingly, we removed this psoriasis susceptibility locus (from 158,741,791 to 158,757,481 base pairs in chromosome 5) from the significant pathways and we repeated the analysis. We excluded 73 and 58 SNPs from the discovery and replication studies, respectively.

**Characterization of the genetic pathways associated with psoriasis risk**

Genetic pathways involved in similar biological processes may share genes. To identify pathways representing different and independent biological processes, we computed the gene overlap between each pair of genetic pathways associated with psoriasis risk (Supplementary Materials).

The statistical significance of the association between pathway genes and psoriasis risk was determined according to the most significant SNP mapping to each particular gene.

**Analysis of the functional-based networks associated with psoriasis risk**

The biological knowledge representing the functional association between gene pairs was used to build the functional-based network of each genetic pathway associated with psoriasis risk. To identify those genes that are more likely to play a central role in the genetic pathways associated with psoriasis risk, we analyzed the network statistical properties of each functional-based network (Supplementary Materials). Using the genes that were nominally associated with psoriasis risk in both discovery and replication stages, we identified the most influential gene according to the highest values of these network statistics.

**Functional analysis of MGAT5 variation**

Following the methodology previously described (Chen et al., 2009), we evaluated the association of MGAT5 psoriasis risk variant with the level of cell surface glycosylation of in vitro activated CD4+ and CD8+ T cells isolated from n = 27 patients with psoriasis (Supplementary Materials).

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

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**SUPPLEMENTARY MATERIAL**

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2015.11.026.

**REFERENCES**

BIOCARTA Pathways. [http://www.biocarta.com].


Grigorian A, Demetriou M. Mgat5 deficiency in T cells and experimental autoimmune encephalomyelitis. ISRN Neurol 2011;374314.