Conclusions.

Our meta-analysis showed a positive association between the PADI4 gene and RA in a Japanese population. However, subsequent replication studies showed conflicting results. The aim of this study was to determine whether meta-analysis would prove the existence of the association.

Methods. PubMed was searched using the term 'PADI4' for articles from the publication of the first study to December 2005. Replication studies that tested the association between PADI4 and RA were reviewed for meta-analysis. The Breslow–Day test for homogeneity across the studies was calculated. The Mantel–Haenszel procedure was used to pool odds ratios (OR) with 95% confidence intervals (CI) to evaluate the association.

Results. Six replication studies, one from Japan and five from Europe and North America, fulfilled the selection criteria for inclusion in the meta-analysis. Homogeneity was confirmed across the replication studies. The common OR was 1.14 (95% CI = 1.07–1.21) for allelic distribution. The association was confirmed when only five replication studies in the European descent populations were combined (P = 0.0096, common OR = 1.10).

Conclusions. Our meta-analysis showed a positive association between PADI4 and RA not only in the Japanese population but also in populations of European descent.

Key words: rheumatoid arthritis, PADI4, Population genetics, Susceptibility, Meta-analysis.

Rheumatoid arthritis (RA [MIM 180300]) is one of the most common chronic systemic inflammatory diseases that cause joint destruction. It is believed to develop as a result of dysregulation of the immune system, leading ultimately to the clinical features of inflammation [1]. The aetiology of RA has been suggested to be an interaction between genetic and environmental factors.

It is recognized that early diagnosis and aggressive treatment of RA are essential for optimal outcome [2]. However, because distinguishing RA from other self-limiting conditions can be difficult, the diagnosis of RA is often delayed for months or even years. Recently, a new, powerful diagnostic test for RA based on anti-cyclic citrullinated peptide antibody (anti-CCP) was introduced and is now recognized as being the most disease-specific test [3]. In addition, it is suggested that anti-CCP may play a crucial role in the pathogenesis of RA since it appears early in the disease course, often before any clinical symptoms, and the presence of anti-CCP is associated with the severity of the disease [4].

Peptidylarginine deiminase (PADI) enzymes catalyse the conversion of arginine residues into citrulline [5]. PADI type IV, one of the four isoforms of PADI in humans, is encoded by the PADI4 gene. PADI4 mRNA is detected in haematological cells and pathological synovial tissues, and is significantly overexpressed in the blood of RA patients [6, 7]. The gene is on chromosome 1p36, a locus that has been linked to RA in European and Japanese populations [8, 9].

Because the function and location of the PADI4 gene make it a good candidate for association with RA, a candidate gene analysis of PADI4 was recently performed. The result showed a strong association between the PADI4 gene and RA [6]. The strongest association was observed for a single-nucleotide polymorphism (SNP) located in intron 3 of PADI4 (padi4_94; P = 0.000008, odds ratio [OR] = 1.97, 95% confidence interval [CI] = 1.44–2.69). Further analyses revealed a functional haplotype that affects the stability of the transcripts of PADI4, which is also associated with the presence of anti-CCP in the sera of RA patients. This association was replicated in another Japanese population (P = 0.0008) [10]. Thus, the susceptibility gene that is involved in the citrullinating pathway may have an important role in the pathogenesis of RA.

Despite the strong association between PADI4 and RA observed in Japanese populations and the supportive evidence of its pathogenetic role in the disease, the results in later studies using European populations were inconsistent [7, 11–14]. With this discrepancy, the role of PADI4 polymorphisms in the development of RA remains controversial.

Since genetic associations of common diseases are often of modest magnitude, a single study is generally underpowered to detect any association. Isolated statistical significance does not
guarantee a genetic association, and a lack of formal statistical significance does not exclude the possibility of an association [15–17]. Meta-analysis is a widely accepted tool for summarizing studies and exploring relationships. One of the major advantages of meta-analysis is to increase the sample size, which may solve the problem of lack of power.

The aim of the present study was to answer these conflicting results with a meta-analysis of all published case–control studies on PADI4 polymorphisms associated with RA.

Materials and methods

Identification of eligible studies and data extraction

The first association study of PADI4 with RA was published in August 2003. Studies included in the analysis were identified by a literature search of articles published between August 2003 and December 2005 in PubMed. To obtain the largest possible list, the single term ‘PADI4’ was used to search for appropriate articles. Manuscripts were selected if they met all the following requirements: the diagnosis of RA was established using the classification criteria of the American College of Rheumatology [18]; the study was designed using case–control samples; the distribution of PADI4 genotypes in patients and in controls was available; and the study was published as a full paper, not as a meeting abstract or review. The following information was extracted from each study: first author, year of publication, study population, the polymorphism studied, and the numbers of patients and controls for the study.

Statistical analysis

The allele frequencies of PADI4 polymorphisms were determined by the allele-counting method. The point estimate of the ORs and the 95% CIs for the polymorphisms were calculated for each study using the Fisher’s exact test. Statistical analyses were performed on the distribution of alleles.

Heterogeneity of the studies was assessed on the basis of the Breslow–Day test using a significance level of 0.05. The Mantel–Haenszel procedure was used to evaluate the association between a PADI4 polymorphism and RA when the heterogeneity of the studies was denied by the Breslow–Day test. The Mantel–Haenszel method provides a common OR estimate and 95% CI, showing the population-wide impact of PADI4 polymorphism on susceptibility to RA.

To investigate the influence of the PADI4 gene on RA susceptibility in detail, two approaches were performed. Since the first positive result in a genetic association study tends to suggest a stronger genetic effect than those found in subsequent studies [19, 20], we conducted a meta-analysis excluding the first study by Suzuki et al. [6]. Secondly, a meta-analysis inclusive of European descent populations only was performed because some of the replication studies suggested that ethnic differences of PADI4 on disease susceptibility might exist.

All analyses were carried out using the R software package, version 2.0.1 (http://www.r-project.org/).

Results

Eligible studies

A total of seven manuscripts that included eight association studies concerning PADI4 and RA were initially identified through the PubMed database [6, 7, 10–14]. Two studies were excluded because one was based on pedigree data and was not a case–control study, and the other was the first positive study [6, 12]. Among the six replication studies, the populations studied were Japanese in one study [10] and were of European descent in five studies [7, 11, 13, 14] (Table 1). Because the best evidence of association reported in the first study was given by an SNP, padi4_94 (rs2240340), we restricted the present meta-analysis to the SNP. Since one study in UK did not genotype the SNP, we used the genotype data of padi4_89 (rs11203360) instead [11]. The choice was made because these SNPs were in strong linkage disequilibrium (r² = 0.97) and the minor allele frequencies of these SNPs were nearly equal in CEPH (Centre d’Etude du Polymorphisme Humain) samples according to the International HapMap project (public release 19) [21]. In total, our study included 4671 cases and 3482 controls with the genotyped padi4_94 or padi4_89.

Association between padi4_94 polymorphism and RA

The Breslow–Day test for heterogeneity was not significant for allele distributions (χ² = 8.31, P = 0.14), suggesting the homogeneity of different ethnic groups in the six replication studies. The common OR calculated with the Mantel–Haenszel procedure based on the allele distributions was 1.14 (95% CI = 1.07–1.21, P = 0.0096) (Fig. 1). Our results showed that an increased risk of RA with the minor allele was also present among European descent populations (common OR = 1.10, 95% CI = 1.02–1.19, P = 0.0096).

Discussion

Since the first positive association between PADI4 and RA was reported in a Japanese population [6], seven studies have been undertaken to replicate the association [6, 7, 10–14]. Though this association held true in the same ethnic population [10], subsequent studies in European populations rarely replicated the association [7, 11–14]. One of the UK studies showed a weak association between RA and a two-marker haplotype, but association with a single marker was not detected [7]. Only the study of the North American population observed a statistically significant association between a single PADI4 marker and RA [14].

The present meta-analysis included six replication studies on the padi4_94 polymorphism or the padi4_89 polymorphism with 8153 genotyped subjects. The test for heterogeneity accepted the combination of studies from different ethnic backgrounds. The overall data demonstrated that the PADI4 is an RA susceptibility gene across populations. The common OR for the risk allele is 1.14.

In genetic association studies, the first association study often overestimates a genetic effect [19, 20]. Indeed, the first study showed the highest OR among all studies. However, our meta-analysis excluding the first study showed a statistically significant result. This result strengthens the hypothesis that PADI4 is associated with susceptibility to RA.

Although our tests showed homogeneity between populations of Japanese and European descent, we carried out a stratified meta-analysis in which subjects were limited to populations of European descent, to supplement the recent argument that there is an ethnic difference in the contribution of genetic variance in PADI4 to the pathogenesis of RA [7, 10–13, 22]. Similar to the overall result, significant association was also observed in the populations of European descent.

On the assumption that the result of the present analysis is true, the most considerable reason why independent studies among the Europeans have rarely confirmed the association is that these studies have been underpowered to detect the intended association. Each study with a European population included 194–2356 subjects. Among them, the study with a Swedish population was the most powerful [14]. Using the common OR in the present study, to calculate the statistical power at the 5% significance level with a frequency of 41% for the risk
over 3700 subjects are required to have 80% power to detect a true positive result (see Power Calculator Web site: http://calculators.stat.ucla.edu/powercalc/). In this regard, the chance of a type II error was not small enough even in the study with the largest population among the European replication studies.

An important purpose of our meta-analysis was to help direct future research. The positive association found in our meta-analysis suggests that PADI4 is associated with RA with a modest risk. Many more replication studies would be required to provide an accurate estimate of a genetic risk.

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An important purpose of our meta-analysis was to help direct future research. The positive association found in our meta-analysis suggests that PADI4 is associated with RA with a modest risk. More replication studies with many more samples from around the world will be helpful in obtaining an accurate estimate of a population-wide effect of the genetic risk. In addition, the replication studies in populations of European descent focused on limited polymorphisms, so more PADI4 polymorphisms should be investigated in the future. Even if the PADI4 gene is associated with RA irrespective of ethnicity, a particular polymorphism responsible for the association might differ in different populations. Furthermore, haplotypes have been suggested to be directly responsible for the pathogenesis of RA and therefore haplotype data pooled for a meta-analysis would be of great interest [6].

**TABLE 1. Characteristics of the association studies and distribution of padi4_94 genotypes**

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Ethnicity</th>
<th>Polymorphism studied</th>
<th>Genotypea Cases</th>
<th>Controls</th>
<th>Allele 1 vs allele 2b OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suzuki et al. [6]*</td>
<td>Japanese</td>
<td>Japanese</td>
<td>padi4_89–105</td>
<td>1/1</td>
<td>1/2</td>
<td>2/2</td>
<td>MAF 1/1</td>
</tr>
<tr>
<td>Ikari et al. [10]</td>
<td>Japanese</td>
<td>Japanese</td>
<td>padi4_94, 102, 104</td>
<td>365</td>
<td>599</td>
<td>237</td>
<td>MAF 1/2</td>
</tr>
<tr>
<td>Barton et al. [11]</td>
<td>UK</td>
<td>European</td>
<td>padi4_89, 90, 92, 104</td>
<td>243</td>
<td>341</td>
<td>145</td>
<td>MAF 2/2</td>
</tr>
<tr>
<td>Harney et al. [7]</td>
<td>UK</td>
<td>European</td>
<td>padi4_92, 94, 97, 99,</td>
<td>40</td>
<td>37</td>
<td>23</td>
<td>MAF 1/1</td>
</tr>
<tr>
<td>Martinez et al. [13]</td>
<td>Spain</td>
<td>European</td>
<td>padi4_94, 104</td>
<td>83</td>
<td>116</td>
<td>49</td>
<td>MAF 1/2</td>
</tr>
<tr>
<td>Plenge et al. [14]</td>
<td>Sweden</td>
<td>European</td>
<td>padi4_94</td>
<td>517</td>
<td>737</td>
<td>244</td>
<td>MAF 2/2</td>
</tr>
<tr>
<td>North America</td>
<td>European</td>
<td>padi4_94</td>
<td>264</td>
<td>447</td>
<td>184</td>
<td>0.46</td>
<td>MAF 1/1</td>
</tr>
</tbody>
</table>

*aActual number of individuals genotyped for padi4_94, except for the study by Barton et al. [11], which used padi4_89.

bThe major allele reported in the first study was referred to as allele 1 and the minor allele as allele 2.

The study by Suzuki et al. [6] was the first positive study that was not included in the present meta-analysis. MAF, minor allele frequency; European, European descent.

**FIG. 1.** ORs (proportional to sample size) with 95% CIs from each study testing the association of RA with the risk allele. The pooled ORs with 95% CI for overall analysis and subgroup analysis in populations of European descent were calculated with the Mantel–Haenszel method (diamonds). The first study by Suzuki et al. [6] is shown for reference only and was not included in the meta-analysis.

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**Key messages**

- **PADI4** is associated with RA with a modest risk.
- Many more replication studies would be required to provide an accurate estimate of a genetic risk.
In conclusion, pooled results for 8153 subjects demonstrated a significant association between PADI4 and RA. Subgroup analysis demonstrated a significant association within populations of European descent.

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References