Case-control Association Study of Autoimmunity Associated Variants in PDCD1 and Juvenile Idiopathic Arthritis.

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Abstract: Purpose: Variants in the gene encoding Programmed Cell Death-1 (PDCD1) have been associated with susceptibility to Systemic Lupus Erythematosus and other autoimmune diseases. Given that clinically distinct autoimmune phenotypes share common genetic susceptibility factors, variants in PDCD1 were tested for a possible association with Juvenile Idiopathic Arthritis (JIA).

Methods: Four Single Nucleotide Polymorphisms (SNPS) in the PDCD1 gene were genotyped and analyzed: rs7421861, rs11568821, rs10204525, and rs7568402 in 834 cases and 855 controls of Northern European ancestry. Each variant was examined for possible associations with JIA and then analyzed for association with JIA categories.

Results: PDCD1 variants showed no association with JIA in the cohort overall (rs7421861 \( p=0.63 \), rs11568821 \( p=0.13 \), rs10204525 \( p=0.31 \), and rs7568402 \( p=0.45 \)). Stratification by JIA categories indicated a significant association between systemic JIA and PDCD1 rs7568402 (OR=0.53, \( p=0.0027 \)), which remained significant after 10,000 permutations, but was not replicated in an independent multi-ethnic systemic JIA cohort. A nominal association between enthesitis-related arthritis and rs11568821 was also observed (OR=0.22, \( p=0.012 \)).

Conclusion: Unlike other multiple autoimmune disease associated genetic variants, there was no association between PDCD1 variants and JIA or JIA categories.

Keywords: Juvenile arthritis, genetics, PDCD1, association, juvenile idiopathic arthritis, single nucleotide polymorphisms.

1. INTRODUCTION

Juvenile Idiopathic Arthritis, the most common cause of chronic arthritis in children, encompasses seven clinically heterogeneous categories of arthritis [1]. While the etiology of JIA is unknown, both genetic and environmental factors are believed to influence susceptibility to JIA [2,3]. Relatives of children with JIA have increased prevalence of autoimmunity [4]. Dense genotyping of over 2800 subjects by the international JIA Immunochip Consortium identified 17 variants associated with JIA [5]. However, the entire Immunochip content was estimated to explain only 18% of the risk of JIA, suggesting that other variants also likely play a role in JIA susceptibility.

Functional variants of the gene PDCD1 which encode the programmed cell death molecule (PD-1), have been associated with systemic lupus erythematosus, rheumatoid arthritis, and progression of multiple sclerosis [6-10]. A meta-analysis reported association between PDCD1 variants and RA, ankylosing spondylitis and type 1 diabetes [11]. To our knowledge, PDCD1 variants have not been previously examined for association with JIA. Given that, clinically distinct autoimmune phenotypes share common genetic susceptibility factors, variants in PDCD1 were tested for a possible association with JIA.

2. SUBJECTS AND METHODS

We genotyped 1689 subjects of Northern European Ancestry, (834 cases and 855 controls). Cases with JIA according to the International League of Associations for Rheumatology criteria were enrolled at Pediatric Rheumatology Clinics at the University of Utah and Children’s Healthcare of Atlanta under protocols approved by the Institutional Re-
view Boards. Controls were healthy volunteers from Utah who reported no history of autoimmunity at enrollment through the use of a questionnaire as previously reported [12, 13].

3. GENOTYPING

DNA was isolated from peripheral blood using the Puregene DNA purification kit from Qiagen (Valencia, CA). Subjects were genotyped for four SNPs in the PDCD1 locus (rs7421861, rs11568821, rs10204525, and rs7568402) using Taqman pre-designed SNP genotyping assays (Applied Biosystems, Foster City, CA) according to the manufacturer’s protocols. SNP rs7421861 is located on intron 1, which harbors many splicing control components and regulatory elements [14]. In SNP rs11568821 (also referred to as PD1.3), an A allele disrupts the binding of a Runx1 transcription factor and impairs the inhibitory effect of PD-1. SNP rs10204525 (also referred to as PD 1.6) and rs7568402 are located in the 3’ untranslated region, and may affect the inflammatory cytokines levels via modulating polyadenylation.

To ensure quality control, ~9% of the samples were genotyped in duplicate and were found to be concordant. The genotyping success rate was > 99% of the samples for all 4 SNPs. We have successfully used this method in the past to investigate variants in TNFA, MIF and PTPN22 loci for association with various JIA categories [15].

4. STATISTICAL ANALYSIS

Prior to association analysis, we tested each variant to confirm that it was in Hardy-Weinberg equilibrium (HWE). We tested each SNP for an additive association with JIA and JIA categories by using logistic regression, adjusting for gender. From the models, we calculated the allelic odds ratios (OR) and 95% confidence intervals (CI). We used permutations to adjust for multiple hypothesis testing. The permutation procedure we implemented allowed us to take into account effects of gender on JIA, as well as preserve linkage disequilibrium patterns of the SNPs. We performed 10,000 permutations under the null hypothesis of no association between genotype and JIA. We performed all analyses using the statistical programming language R. We also attempted to replicate in silico the association observed between PDCD1 SNP rs7568402 and systemic JIA in a large multi-ethnic systemic JIA case control association study of 982 children with systemic JIA and 8010 healthy controls [16, 17]. The final cases and controls used in the meta-analysis were derived after the removal of individuals with ancestral dissimilarities within their strata. Moreover, association testing in each stratum was performed by logistic regression, adjusted for ancestry-informative principal components and gender. This resulted in extremely well matched cases and controls in each geographically defined stratum, as demonstrated by genomic control inflation factors [16]. Some of the systemic JIA cases examined in the present study were also included among the U.S. stratum of the large multi-ethnic systemic association study.

5. RESULTS

The median age of onset for the cases was 6.6 years and 70% of the subjects were female; 7% of the cases were diagnosed with systemic JIA, 8% with RF positive polyarticular JIA, 26% with RF negative polyarticular JIA, 47% with oligoarticular JIA, 7% with Enthesitis related arthritis (ERA), and 6% with other subtypes. Of the control subjects 60% were female.

All four variants were in HWE in the controls (p>0.10). Using logistic regression, we observed that PDCD1 variants showed no association with JIA as a combined phenotype (Table 1). However, after stratification two variants were found to be associated with JIA categories (Table 2). PDCD1 SNP rs11568821 was nominally associated with ERA JIA (OR=0.22, p=0.012) in our cohort, but this association was not significant after permutation procedure. Additionally, PDCD1 SNP rs7568402 was nominally associated with systemic JIA in our cohort (OR=0.53, p=0.0027). After the permutation procedure, this association remained significant (p=0.047). In silico replication of this finding was attempted in a large multi-ethnic systemic JIA case control association study of 982 children with systemic JIA and 8010 healthy controls [16, 17]. However, an association between any of the PDCD1 variants and systemic JIA was not found in the large combined cohort or any of the individual strata that made up the cohort (Table 3).

6. DISCUSSION

JIA is a complex trait and there is substantial evidence for genetic predisposition to JIA. Many of the variants associated with JIA have also shown to be associated with other autoimmune disorders. While some genes such as PTPN22 and STAT4 are associated with multiple autoimmune pheno-

<table>
<thead>
<tr>
<th>Variant</th>
<th>Cases</th>
<th>MAF</th>
<th>Controls</th>
<th>MAF</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7568402</td>
<td>815</td>
<td>0.43</td>
<td>845</td>
<td>0.44</td>
<td>0.95 (0.82-1.09)</td>
<td>0.45</td>
</tr>
<tr>
<td>rs10204525</td>
<td>830</td>
<td>0.13</td>
<td>855</td>
<td>0.12</td>
<td>1.12 (0.90-1.38)</td>
<td>0.31</td>
</tr>
<tr>
<td>rs11568821</td>
<td>810</td>
<td>0.095</td>
<td>845</td>
<td>0.11</td>
<td>0.84 (0.67-1.05)</td>
<td>0.13</td>
</tr>
<tr>
<td>rs7421861</td>
<td>819</td>
<td>0.33</td>
<td>852</td>
<td>0.33</td>
<td>1.04 (0.90-1.20)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

MAF = minor allele frequency.
OR = odds ratio; 95% CI: 95% confidence interval.
types, others such as NOD2 are associated with specific immune mediated phenotypes such as inflammatory bowel disease. Since variants in PDCD1 had been associated with SLE, RA and multiple sclerosis, we chose to examine the PDCD1 gene for an association with JIA as well. To our knowledge, PDCD1 variants have not been previously investigated for an association with JIA. While the JIA ImmunoChip Consortium identified 17 variants associated with oligoarticular and polyarticular JIA [5], the ImmunoChip array had sparse coverage of variants near the PDCD1 locus located at the telomeric end of the long arm on chromosome 2, (2q37).

We discovered that PDCD1 variants showed no association with JIA as a whole, but after stratification by JIA categories, rs11568821 was nominally associated with ERA JIA in our cohort but this association did not withstand permutation. The SNP rs7568402, which was associated with systemic JIA in our cohort as it withstood correction by permutation (OR=0.53, p=0.0027), has previously not been associated with other autoimmune phenotypes. However the initial association was not replicated in a large multi-ethnic cohort of systemic JIA patients suggesting our results might reflect a false positive association.

Table 2. Case control analysis of PDCD1 variants and JIA categories.

<table>
<thead>
<tr>
<th>Category</th>
<th>rs7568402</th>
<th>rs10204525</th>
<th>rs11568821</th>
<th>rs7421861</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>OR (95% CI)</td>
<td>p-value</td>
<td>n</td>
</tr>
<tr>
<td>Systemic</td>
<td>58</td>
<td>0.53 (0.35-0.80)</td>
<td>0.0027*</td>
<td>59</td>
</tr>
<tr>
<td>RF-Positive</td>
<td>64</td>
<td>0.88 (0.59-1.28)</td>
<td>0.50</td>
<td>63</td>
</tr>
<tr>
<td>RF-Negative</td>
<td>208</td>
<td>1.05 (0.84-1.31)</td>
<td>0.70</td>
<td>213</td>
</tr>
<tr>
<td>ERA</td>
<td>56</td>
<td>0.83 (0.55-1.23)</td>
<td>0.35</td>
<td>56</td>
</tr>
<tr>
<td>Oligoarticular</td>
<td>382</td>
<td>1.00 (0.84-1.20)</td>
<td>0.99</td>
<td>389</td>
</tr>
<tr>
<td>Controls</td>
<td>845</td>
<td></td>
<td></td>
<td>855</td>
</tr>
</tbody>
</table>

OR = odds ratio; 95% CI: 95% confidence interval.
*p value = 0.047 after 10,000 permutations.

Table 3. Minor allele frequencies of PDCD1 rs7568402 in our cohort compared to a multi-ethnic systemic JIA cohort*.

<table>
<thead>
<tr>
<th>Population</th>
<th>Cases n</th>
<th>Controls n</th>
<th>Case MAF</th>
<th>Control MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>58</td>
<td>845</td>
<td>0.30</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Meta analyses results

<table>
<thead>
<tr>
<th>Population</th>
<th>Cases n</th>
<th>Controls n</th>
<th>Case MAF</th>
<th>Control MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States*</td>
<td>243</td>
<td>1718</td>
<td>0.38</td>
<td>0.44</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>202</td>
<td>4097</td>
<td>0.45</td>
<td>0.43</td>
</tr>
<tr>
<td>Germany</td>
<td>115</td>
<td>193</td>
<td>0.44</td>
<td>0.46</td>
</tr>
<tr>
<td>Italy</td>
<td>49</td>
<td>59</td>
<td>0.43</td>
<td>0.41</td>
</tr>
<tr>
<td>Turkey</td>
<td>49</td>
<td>94</td>
<td>0.35</td>
<td>0.32</td>
</tr>
<tr>
<td>Brazil</td>
<td>48</td>
<td>62</td>
<td>0.44</td>
<td>0.43</td>
</tr>
<tr>
<td>Argentina</td>
<td>33</td>
<td>115</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Canada</td>
<td>17</td>
<td>427</td>
<td>0.44</td>
<td>0.40</td>
</tr>
</tbody>
</table>

MAF = minor allele frequency.
*p value = 0.047 after 10,000 permutations.

PDCD1 encodes the inhibitory immunoreceptor PD-1, which belongs to the immunoglobulin superfamily [18]. PD-1 plays an essential role in cell-mediated immunity, and is expressed on T-cells, B-cells and activated monocytes. The programmed death ligand (PD-L) binds to the PD-1 receptor on the cells. The role of the PD-1/PD-L pathway in the prevention of autoimmune diseases has been extensively investigated due to the role of PD-1 in the regulation of autoimmunity and in self-tolerance [19, 20]. Activated T-cells express receptors that mediate inhibitory signals from antigen presenting cells. Triggering of receptors on T and B cells and on endothelial and epithelial cells cause release of the two ligands PD-L1 and PD-L2. These ligands inhibit the
immune response by binding to the PD-1, therefore preventing the differentiation of self-antigen specific inflammatory T-cells. Thus, variants in PDCD1 that could potentially alter the function or expression of PD-1, could result in autoimmunity and hence make this an attractive candidate to examine for associations.

Although our JIA cohort is relatively large, individual categories of JIA were still modest in number. While our cases and controls came from different centers, they were all of Northern European ancestry and did not show any evidence of population stratification in other studies that have utilized these cohorts [5, 21]. We addressed the issue of multiple comparisons by using a permutation procedure. It should be noted that some of our cases, but not controls were also included in the US stratum of the meta-analysis, but overall the sJIA cohorts did not show any trend towards an association.

A modest association cannot be excluded as our sample size, while considerable was still underpowered for some categories of JIA. Investigations of other cohorts of JIA for genetic association with PDCD1 variants, as well as meta-analyses would be of interest to definitely exclude PDCD1 as a candidate associated with JIA susceptibility. In summary, PDCD1 was found not to be associated with JIA as a whole, or with any of the JIA categories.

COMPETING INTERESTS
S.P. was supported by grants from The National Institute of Arthritis and Musculoskeletal and Skin Diseases (R01-AR060893), The Marcus Foundation Inc. and The Arthritis Foundation. M.J.O. was supported by the Intramural Research Program of the National Institute of Arthritis and Musculoskeletal and Skin Diseases (Z01-AR041198). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Arthritis and Musculoskeletal and Skin Diseases or the National Institutes of Health.

DISCLOSURE
Part of this article has been reported in the author’s thesis ‘Investigation of Associations between Autoimmunity Associated Variants in PDCD-1 and Juvenile Idiopathic Arthritis Categories’.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
This study was approved by the Institutional Review Boards of the participating institutions.

HUMAN AND ANIMAL RIGHTS
No Animals were used in this study. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2008.

CONSENT FOR PUBLICATION
Human subjects used in the study provided informed consent to participate in the study.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS
Declared none.

REFERENCES


