Association of the RAVER2 gene with increased susceptibility for ulcerative colitis

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ABSTRACT

Crohn's disease (CD), ulcerative colitis (UC), systemic lupus erythematous (SLE) and autoimmune polyglandular syndromes (APS) are autoimmune diseases (ADs) that may share common susceptibility pathways. We examined ribonucleo-protein, polypyrimidine tract-binding protein (PTB)-binding 2 (RAVER2) loci for these diseases in a cohort of 39 CD cases, 67 UC cases, 93 SLE cases, 60 APS cases and 162 healthy control subjects of Tunisian origin. We genotyped 3 SNPs of RAVER2 (rs2780814, rs1333739 and rs2780889) and evaluated it genetic association with each ADs, using X2-test. For each association, odds ratio (OR) and 95% CI were calculated. We show that rs2780814 is significantly associated with UC (P = 0.00016, P corr = 0.00048, OR = 3.66 (1.82; 7.34)). We also observed a trend of possible association to SLE (P = 0.023, P corr = 0.69, OR = 2.19 (1.1; 4.36)). None of these RAVER2 SNPs were associated with CD and APS susceptibility. These findings establish RAVER2 as a new UC genetic susceptibility factor and reveal a genetic heterogeneity of the associated polymorphisms and risk alleles between ADs suggesting different immunopathological roles of RAVER2 in these diseases.

1. Introduction

Recent transcriptome analysis indicated that more than 90% of human genes undergo alternative splicing and that many mRNA isoforms appear to be regulated in a tissue-specific manner [1]. The relevance of the cell splicing control machinery becomes evident considering that more than 15% of human genetic diseases are associated with mutations in the consensus splice sites, and disruption of splicing regulatory networks contributes to development of severe diseases, including autoimmune diseases (ADs) [2–4]. Genetic susceptibility of multiple organ-specific ADs often share underlying commonalities [5,6].

Alternative splicing of pre-messenger RNA is an important post-transcriptional regulatory process that involves RNA binding proteins and splicing regulators expressed by hundreds of genes distributed in the human genome [7–11]. They belong to the SR (Ser-Arg repeat-containing domains) and hnRNP (heterogeneous-nuclear ribonucleoprotein particles) families of proteins [9,12,13]. The hnRNPs are among the most abundant proteins in the eukaryotic cell nucleus and are directly involved in DNA repair and telomere elongation; chromatin remodeling and transcription; RNA splicing and stability; export of mature RNA and translation [14,15]. Approximately 30 hnRNPs have been identified, but few of them have been thoroughly studied such as RAVER2.

RAVER2 was initially identified in database searches [16] for proteins related to Raver1, a ubiquitously expressed hnRNP that has been shown to act as a cofactor for the polypyrimidine tract-binding protein (PTB) a well characterized repressor of alternative splicing [17–19]. A recent work has analyzed the gene expression of the ribonucleoprotein RAVER2 [20], but the polymorphism of human RAVER2 has not previously been analyzed. Therefore, in this study three RAVER2 SNPs were selected according common allelic frequency in European and African population and were used to study RAVER2 association to different autoimmune diseases, including Crohn’s disease (CD) and ulcerative colitis (UC), systemic lupus erythematous (SLE) and autoimmune polyglandular syndromes (APS).

2. Patients and methods

2.1. Patients

A case-control study was performed to assess associations of the RAVER2 SNPs (rs2780814, rs1333739 and rs2780889) with IBD, SLE and APS. A total of 422 subjects of Tunisian origin, including 260 cases and 162 control subjects, were studied. Cases consisted of 107 patients with Inflammatory bowel disease (IBD) (68

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anti-TSH receptor antibodies (TRAb) (EUROIMMUN, Germany). All patients with Hashimoto thyroditis had anti thyroglobuline (TG) and/or thyroperoxidase (TPO) antibodies (Binding Site, U.K). All diabetic patients were positives for at least one β cell auto-antibody: islet cell antibody (ICA) (EUROIMMUN, Germany) and/or glutamic acid decarboxylase antibodies (GADA) (EUROIMMUN, Germany) and/or IAA2 antibodies (IA2A) (EUROIMMUN, Germany). All patients with premature ovarian failure had anti-ovarian antibodies (BIO-RAD, France).

Addison’s disease: After exclusion of other etiologies, autoimmune Addison's disease was diagnosed by adrenocortical insufficiency and the detection of anti adrenocortical antibodies (ACA) (BIO-RAD, France).

The healthy control subjects had no family history of autoimmune diseases. All cases and control subjects were informed of the purpose of the study, and their consent was obtained.

The study was approved by the local ethics committee.

2.2. Methods

2.2.1. Genotyping methods

Genomic DNA was extracted from whole blood samples using a standard protease K digestion and phenol/chloroform extraction procedure. Genotyping was performed using primer extension chemistry and mass spectrometric analysis (iPlex assay, Sequenom, San Diego, CA) on the Sequenom MassArray at the Instituto Gulbenkian de Ciência, Oeiras, Portugal. We genotyped 3 SNPs in RAVER2 (rs2780814, rs1333739 and rs2780889). The SNP markers under study were in Hardy-Weinberg equilibrium. All information about the selected SNPs was extracted from dbSNP public database, built 126 and gene information from the genome browser Ensembl, release 61. For quality control purposes these SNPs were also typed using a panel of 8 HapMap DNA samples that have shown the expected genotypes according to the HapMap Project information.

Assay design was performed according to manufacturer’s instructions, whereby the genomic sequence containing the SNP was amplified by multiplex PCR reactions. The amplified product was cleaned using shrimp alkaline phosphatase and used for allele specific primer extension reaction according the MassEXTEND protocol. The reaction mixture was then spotted onto a SpectroCHIP microarray and subjected to the MALDI-TOF mass spectrometry. SpectroTYPEr software identifies the SNP-specific peaks and automatically assigns the genotype calls.

2.2.2. Data analysis

Quality control criteria excluded SNPs with call rate lower than 90% in cases and controls. Genotype frequencies were in Hardy-Weinberg equilibrium for all the typed SNPs (P > 0.05) of the Tunisian control population. Case-control association analysis was performed for each SNP. Calculations of allelic and genotypic associations of SNPs with susceptibility to IBD were performed using a home-made program written in R language (http://www.r-project.org). The results were verified with the Khi2 test. A result is considered statistically significant when P < 0.05. Bonferroni’s correction was carried out when single SNP analysis yielded significant results. Relative risks were calculated as odds ratio (OR)
Table 2 | Genotypic and allelic association analysis of the RAVER2 SNP polymorphisms in patients with CD, UC, SLE, and APS

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotypes/ Alleles</th>
<th>Controls (N=210)</th>
<th>UC (N=128)</th>
<th>SLE (N=55)</th>
<th>APS (N=43)</th>
</tr>
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<tbody>
<tr>
<td>rs2780814</td>
<td>CC</td>
<td>55 (26.2)</td>
<td>11 (28.2)</td>
<td>5 (9.1)</td>
<td>7 (16.3)</td>
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<tr>
<td></td>
<td>CT</td>
<td>78 (37.3)</td>
<td>20 (51.3)</td>
<td>26 (47.3)</td>
<td>37 (86.0)</td>
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<tr>
<td></td>
<td>TT</td>
<td>19 (9.1)</td>
<td>8 (20.5)</td>
<td>23 (41.8)</td>
<td>15 (34.9)</td>
</tr>
<tr>
<td>rs1333739</td>
<td>AA</td>
<td>38 (18.2)</td>
<td>14 (35.3)</td>
<td>14 (25.5)</td>
<td>11 (25.6)</td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>42 (20.2)</td>
<td>12 (30.8)</td>
<td>19 (34.5)</td>
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</tr>
<tr>
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<td>19 (34.5)</td>
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<td>12 (30.8)</td>
<td>26 (47.3)</td>
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<td>15 (34.9)</td>
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<tr>
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<td>8 (20.5)</td>
<td>23 (41.8)</td>
<td>15 (34.9)</td>
</tr>
</tbody>
</table>

CD: Crohn's disease; UC: ulcerative colitis; IBD: inflammatory bowel diseases; SLE: systemic lupus erythematosus; APS: autoimmune polyendocrinopathy syndrome; CI, confidence interval; OR, odds ratio. Significant associations are highlighted in bold.

a Two-tailed P values were calculated by the Bonferroni correction.

b Corrected P values (Pcorr) were calculated using D’ and r-square and a LD plot was generated using Haploview version 4.2.

c Calculated using D’ and r-square and a LD plot was generated using Haploview version 4.2.

3. Results

The RAVER2 SNPs rs2780814 and rs1333739 and rs2780889 were genotyped with a success call rate of 96.2%, 94.7%, 96.4%, respectively, and were within Hardy–Weinberg equilibrium in both cases and controls (P > 0.05). We observed moderate linkage disequilibrium among the three SNPs (rs2780814 vs rs1333739, D’ = 1, r² = 0.58; rs1333739 vs rs2780889, D’ = 0.95, r² = 0.75; rs2780889 vs rs2780814, D’ = 0.76, r² = 0.40) (Fig. 1).

The summary of the genotypic and allelic association analysis is given in Table 2.

The strongest association was observed with the rs2780814 between UC cases and healthy controls. The frequency of the rs2780814 T allele was 53.7% in patients with UC compared with 38.2% (Table 2) in controls (P = 0.0024, Pcorr = 0.0048, OR (95% CI) = 1.88 (1.25; 2.84)). The homozygous TT genotype was observed in 34.3% of patients with UC compared with 12.5% (Table 2) of control subjects (P = 0.00016, Pcorr = 0.00048, OR (95% CI) = 3.66 (1.82, 7.34)).

The rs2780814 polymorphism of RAVER2 showed also genotypic association (TP = 0.023, OR = 2.19 (1.1: 4.36)) in SLE patients when compared with unaffected controls. However, statistical significance was lost after Bonferroni’s correction (Pcorr = 0.069). These results suggest that the TT genotype at rs2780814 acts in a recessive mode to confer susceptibility to UC and possibly to SLE.

No genotypic or allelic association for the rs2780814 was found between Tunisian CD and APS.

No significant differences were found in the allelic and genotypic frequencies of the other polymorphisms (rs17583959 and rs2384352) studied in the autoimmune diseases under analysis.

4. Discussion

The genetic association study is a powerful tool to identify associated SNPs influencing disease susceptibility. Recent genome wide association (GWA) and candidate gene studies across human autoimmune disease revealed a shared genetic architecture [25]. However, the association with RAVER2 gene had not been evaluated in any AD.

To evaluate the impact of RAVER2 on Tunisian population, we genotyped relatively homogeneous samples with ethnically matched controls in multiple ADs. We found evidence of significant association of rs2780814 SNP in Tunisian UC but not in CD, that suggest different immunopathological mechanisms. We also observed a trend of possible association of this RAVER2 SNP with SLE. It is conceivable that the reduced sample size in our study hampered detection of existing associations with other autoimmune disease in particular studies in larger samples are required to more precisely define the role of RAVER2 in SLE susceptibility.

However, no association for RAVER2 was found between Tunisian APS and controls. The lack of association of all SNPs with APS in our study may be due to the small sample size analyzed. Therefore, genotyping of a larger number of APS samples is warranted before any definite conclusion can be drawn.

RAVER2, an additional co-factor that interacts with PTB, was identified in a search for Raver1-related proteins [16]. RAVER2 has been proposed to be a modulator of PTB activity [16,26]. PTB, also referred to as hnRNP I, which regulates tissue specific mRNA alternative splicing [27], mRNA stability [28], localization [29], and translation [30]. Yang et al. [31] have provided evidence that overexpression of hnRNP I promotes the degradation of Notch
intracellular domain (NICD) and inhibits Notch signaling and that hnRNP I plays critical roles in intestinal homeostasis. The Notch pathway, a highly conserved signaling pathway is involved in the renewal of the intestinal epithelium and regulates many other stem cell lineages during embryonic development and adult tissue homeostasis [32–34]. It is widely believed that Notch signaling maintains intestinal stem cells in a proliferative state and promotes the absorptive cell fate determination in vertebrate intestine and ablation of Notch target genes impairs intestinal epithelial homeostasis [35–37].

A number of hnRNP proteins have been described as autoantigens in several ADs such as, rheumatoid arthritis, SLE, and mixed connective tissue disease [38]. Antibodies to hnRNP A1 and HnRNP D (AUF1) have been reported in SLE [39,40]. Antibodies to hnRNP G have been described in dogs with SLE [41]. hnRNP I was described as an autoantibody target in patients with systemic sclerosis [42]. Recently, Van den Bergh et al. described antibodies to hnRNP H1 in patients with Sjogren’s syndrome [43]. Our work demonstrates suggestive associations of RAVER2 with SLE and raises the possibility that RAVER2 is part of the autoantigen complexes represented by hnRNP. No statistically significant results were found in order to sustain the hypothesis of association between RAVER2 gene polymorphism, CD and APS.

In conclusion, our study establishes RAVER2 as a new UC genetic susceptibility factor. However, further studies involving larger number of UC patients should be performed before arriving at a definitive conclusion regarding the implications of the analyzed SNP in UC susceptibility. Our findings reveal also a genetic heterogeneity of the associated polymorphisms and risk alleles between ADs suggesting different immunopathological roles of RAVER2 in these diseases.

Acknowledgments

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References


