Edinburgh Research Explorer

IL23R Arg381Gln is associated with childhood onset inflammatory bowel disease in Scotland

Citation for published version:

Digital Object Identifier (DOI):
10.1136/gut.2007.122069

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Gut

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and/or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
IL23R Arg381Gln is associated with childhood onset inflammatory bowel disease in Scotland

The discovery of NOD2/CARD15 as the first susceptibility gene in Crohn’s disease has contributed significantly to a fundamental change in the direction of basic research in inflammatory bowel disease (IBD), triggering renewed interest in the integrity of the innate immune response in IBD and appropriate orchestration of a subsequent adaptive immune response. More widely, in all complex diseases this finding in 2001 provided a much welcomed and needed proof of principle for non-parametric linkage analysis. Another study with major implications for the pathogenesis of Crohn’s disease as well as for investigation of all complex disorders has recently been published. The North American consortium performed an association study testing 308 332 markers spanning the entire genome in 567 patients with ileal Crohn’s disease and 571 controls of non-Jewish European ancestry. Of the three markers removed due to significance after stringent Bonferroni correction, two were located in the NOD2/CARD15 gene. The third marker (rs11209026) was a non-synonymous variant in the interleukin-23 receptor (IL23R) gene on chromosome 1p31. Replication was obtained in the index paper in a Jewish ancestry case-control analysis of patients with Crohn’s disease by transmission disequilibrium testing in 883 families with offspring affected by IBD and in a combined case-control analysis of these three cohorts (IBD, p = 6.62E-19).

IL-23 is a pivotal cytokine in the differentiation of Th17 cells, especially their differentiation into Th17 T cells. Although the Th17 T cell subset has been shown to mediate chronic and autoinflammatory immune conditions in animal models, clear evidence exists for the central role of IL-23 in the development of intestinal disease. Both Crohn’s disease and ulcerative colitis commonly first present during childhood and adolescence and are associated with high disease-related and treatment-related morbidity in these young patients. Disease incidence is high in our population, and in others in Northern Europe in whom the NOD2/CARD15 contribution is small. These considerations, together with well established epidemiological data suggesting that early-onset disease has a strong genetic basis, provide a clear scientific rationale for performing molecular studies in this group.

Our aim was to assess the contribution of the Arg381Gln variant (rs11209026) of IL23R in determining susceptibility and phenotype in childhood onset IBD in Scotland. We also sought to investigate the interaction between carriage of any of the three common NOD2/CARD15 variants and carriage of this IL23R variant in determining susceptibility to Crohn’s disease.

A total of 1294 subjects comprising 358 with IBD aged 17 years at diagnosis (Table 1), 594 parents and 342 controls were genotyped for rs11209026 GA using TaqMan (7900HT sequence detection system; Applied Biosystems, Foster City, California, USA). Allelic and genotype frequency comparisons between cases and controls using χ² and transmission disequilibrium testing were applied to assess the association of IL23R rs11209026 with IBD. The three common NOD2/CARD15 variants were genotyped as previously described.

Table 1 Demographic data and inflammatory bowel disease (IBD) phenotype in patients diagnosed with IBD at <17 years of age based on Montreal guidelines for classification of Crohn’s disease/ulcerative colitis

<table>
<thead>
<tr>
<th>N</th>
<th>M/F</th>
<th>Median age at diagnosis (Q1-Q3)</th>
<th>CD/UC/IBD type</th>
<th>Unclassified</th>
</tr>
</thead>
<tbody>
<tr>
<td>358</td>
<td>205/153</td>
<td>11.1 years</td>
<td>239/88/31</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Interleukin-23 receptor (IL23R) rs11209026G/A (Arg381Gln) genotype and allelic frequencies in controls and patients with inflammatory bowel disease (IBD), Crohn’s disease (CD) and ulcerative colitis (UC) diagnosed <17 years of age

<table>
<thead>
<tr>
<th>Allele A</th>
<th>Control</th>
<th>IBD</th>
<th>p Value</th>
<th>UC</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>38/684</td>
<td>0.01</td>
<td>0.01</td>
<td>0.04</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

In cases and controls, rs11209026 was in Hardy-Weinberg equilibrium. The allelic frequency of rs11209026A differed significantly between IBD/Crohn’s disease cases and controls (2.9%/3.0% vs 5.5%, p = 0.01, OR 0.51 (95% CI 0.30 to 0.88) and p = 0.04, OR 0.53 (95% CI 0.28 to 0.98); table 2).

The GG genotype was associated with an increased risk of IBD in the disease (p = 0.01, OR 2.01 (95% CI 1.15 to 3.49) and p = 0.03, OR 1.96 (95% CI 1.03 to 3.70)). Analysis by transmission disequilibrium testing showed significant overtransmission of the G allele for IBD (p = 0.004) and Crohn’s disease (p = 0.04), with a trend towards significance in ulcerative colitis hindered by a small number of informative families with ulcerative colitis (table 3).

In Crohn’s disease there was no difference (p = 0.94) in allelic frequency between NOD2/CARD15 wild type and NOD2/CARD15 variant-carrying patients. However, owing to the small numbers of cases and controls carrying this IL23R variant, our study was not adequately powered to formally assess epistasis with NOD2/CARD15. Genotype-phenotype analysis in Crohn’s disease and ulcerative colitis based on the Montreal classification did not demonstrate any significant effect of IL23R rs11209026 specifically, we were not able to show a protective effect against ileal Crohn’s disease (p = 0.21).

The successful identification of IL23R as a novel IBD susceptibility gene has provided proof of principle for the applicability of genome-wide association studies in the genetics of complex diseases. We show for the first time that IL23R variation influences susceptibility to IBD and Crohn’s disease, but not phenotype, in an exclusively paediatric IBD cohort. The data complement the results of the initial North American study and the replication studies currently underway in the UK adult population. However, the contribution of this IL23R allele to IBD is not sufficiently strong to explain the high incidence of childhood IBD in our or other populations with a low NOD2/CARD15 contribution. Other determinants are likely to be involved in Northern Europe.13,14 Genome-wide association scanning has already provided other candidates requiring rigorous analyses.

Acknowledgements

The authors acknowledge the contribution of staff at the Medical Research Council Human Genetics Unit Edinburgh, the Wellcome Trust Clinical Research Facility Edinburgh and the referring physicians from Scottish Gastrointestinal Medicine and Surgery services. They also acknowledge the help of all patients and parents who participated in the study together with the specialist nurses, dieticians and secretaries.

www.gutjnl.com
Toward best practice.

JAK V617F missense mutation is absent in pancreatic cancer

Aberant constitutive activation of STAT3 (signal transducer and activator of transcription) leads to cellular transformation and aids tumorigenesis in pancreatic cancer and its inhibition can lead to growth arrest. The exact mechanism of this constitutive activation has not been elucidated, although downstream mediators may include ERK and p21 signaling. 1,2 Blocking JAK2 (Janus Kinase), a known upstream activator of STAT3, by AG490, a tyrophostin inhibitor, reduced phosphorylation of STAT3 and produced comparable in vitro effects, suggesting a role for JAK2 in pancreatic carcinogenesis.3 JAK2 V617F missense mutation has recently been shown by several independent groups to play an important role in myeloproliferative disorders as well as prothrombotic states such as Budd-Chiari syndrome (which may be due to latent myeloproliferative disorders). 4,5 We proposed that JAK2 V617F mutation may play a role in pancreatic cancer, in which the prothrombotic state is well recognised.

We analysed genomic DNA (all from unstained slides of representative surgical specimens) from 26 patients undergoing surgery for various pancreatic diseases (table 1) along with genomic DNA from 10 cell lines (Panc1, PaCa3, MiaPaCa2, Capan1 and 2, Suti2, AsPC1, 818,4, Hpa2, H766T) for JAK2 V617F mutation using phenol-chloroform extraction with ethanol precipitation, using appropriate positive and negative controls as previously described. 6 Briefly, all cells (n = >70%) from pancreatic tissue in cancer specimens, (no microdissection) were scraped from unstained slides into high salt buffer and samples were made up to 200 μl with protease K, RNase A and sodium dodecyl sulfate, incubated at 37°C overnight before phenol extraction and ethanol precipitation. Genomic DNA from cell lines was extracted from 70% confluent million cells using Trizol (Invitrogen Ltd, Paisley, UK) reagent digestion followed by ethanol precipitation. A highly sensitive allele-specific PCR was used to detect the JAK2 V617F mutation is present, an additional band is also expected p Value

Competing interests: None.

Table 3 Interleukin-23 receptor (IL23R) rs11209026G/A (Arg381Gln) transmission disequilibrium testing in trios with childhood onset IBD

<table>
<thead>
<tr>
<th>rs11209026G/A</th>
<th>IBD</th>
<th>CD</th>
<th>UC</th>
<th>Transmissions observed</th>
<th>Transmissions expected</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg381Gln</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL23R</td>
<td></td>
<td></td>
<td></td>
<td>50.5</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In each of the Scottish paediatric teaching hospitals and the paediatricians, practice nurses and GPs throughout Scotland whose support was invaluable.

J Van Limbergen Gastrointestinal Unit, Molecular Medicine Centre, Western General Hospital, University of Edinburgh, and Department of Paediatric Gastroenterology and Nutrition, Royal Hospital for Sick Children, Edinburgh, UK

R K Russell Department of Paediatric Gastroenterology and Nutrition, Royal Hospital for Sick Children, Edinburgh, UK

E R Nimo, H E Drummond, L Smith, G Davies Gastrointestinal Unit, Molecular Medicine Centre, Western General Hospital, University of Edinburgh, Edinburgh, UK

N H Anderson Public Health Sciences, University of Edinburgh, Edinburgh, UK

P M Gillett Department of Paediatric Gastroenterology and Nutrition, Royal Hospital for Sick Children, Edinburgh, UK

P McGrogan, K Hassan Department of Paediatric Gastroenterology, Yorkhill Hospital, Glasgow, UK

L Weaver Department of Child Health, University of Glasgow, Glasgow, UK

W M Bisset, G Mahdi Department of Paediatric Gastroenterology, Royal Aberdeen Children’s Hospital, Aberdeen, UK

D C Wilson Department of Paediatric Gastroenterology and Nutrition, Royal Hospital for Sick Children, Edinburgh, and Child Life and Health, University of Edinburgh, Edinburgh, UK

J Satsangi Gastrointestinal Unit, Molecular Medicine Centre, Western General Hospital, University of Edinburgh, Edinburgh, UK

Correspondence to: Dr Johan Van Limbergen, Molecular Medicine Centre, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK: johannvanlimbergen@hotmail.com doi: 10.1136/gut.2007.122069

JVL is funded by a Research Training Fellowship from Action Medical Research, the Gay-Ramsay-Steel-Malland or Stafford Trust and the Hazel M Wood Charitable Trust. RKR was funded by a University of Edinburgh Medical Faculty Fellowship and ERK is supported by a Wellcome Trust Programme Grant (072789/2/03/Z). Financial assistance was also provided by Schering-Plough and the GI/Nutrition Research Fund, Child Life and Health, University of Edinburgh.

www.gutjnl.com