Investigation of type 1 diabetes and coeliac disease susceptibility loci for association with juvenile idiopathic arthritis

Anne Hinks, Paul Martin, Edward Flynn, Steve Eyre, Jon Packham, Anne Barton; Childhood Arthritis Prospective Study (CAPS), UKRAG Consortium, BSPAR study group, Jane Worthington, Wendy Thomson

ABSTRACT

Background There is strong evidence suggesting that juvenile idiopathic arthritis (JIA) shares many susceptibility loci with other autoimmune diseases.

Objective To investigate variants robustly associated with type 1 diabetes (T1D) or coeliac disease (CD) for association with JIA.

Methods Sixteen single-nucleotide polymorphisms (SNPs) already identified as susceptibility loci for T1D/CD were selected for genotyping in patients with JIA (n=1054) and healthy controls (n=3129). Genotype and allele frequencies were compared using the Cochrane–Armitage trend test implemented in PLINK.

Results One SNP in the LPP gene, rs1464510, showed significant association with JIA (p_{trend}=0.002, OR=1.18, 95% CI 1.06 to 1.30). A second SNP, rs653178 in ATXN2, also showed nominal evidence for association with JIA (p_{trend}=0.02, OR=1.13, 95% CI 1.02 to 1.25). The SNP, rs7810546, in IL12A showed subtype-specific association with enthesitis-related arthritis (ERA) subtype (p_{trend}=0.005, OR=1.88, 95% CI 1.2 to 2.94).

Conclusions Evidence for a novel JIA susceptibility locus, LPP, is presented. Association at the SH2B3/ATXN2 locus, previously reported to be contributing to JIA susceptibility, is confirmed. In addition, a subtype-specific association of IL12A with ERA is identified. All findings will require validation in independent JIA cohorts.

INTRODUCTION

In the past few years huge advances have been made in the understanding of the genes contributing to autoimmune disease susceptibility. It is also becoming clear that, despite the apparent clinical and phenotypic differences of the autoimmune diseases, they share a number of genetic risk factors. Of note, the genetic overlap between type 1 diabetes (T1D), coeliac disease (CD) and rheumatoid arthritis (RA) (Eyre et al, submitted).

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease of childhood. It is defined as chronic inflammation of the synovial joints, with unknown aetiology, that starts before the age of 16 and persists for at least 6 weeks. Familial clustering of other autoimmune diseases in JIA families is well established, with an increased prevalence of autoimmunity in first- and second-degree relatives of patients.

There is already strong evidence suggesting that JIA shares many susceptibility loci with other autoimmune diseases such as RA, T1D and multiple sclerosis. Therefore, the aim of this study was to test single-nucleotide polymorphisms (SNPs) robustly associated with T1D or CD in a large cohort of patients with JIA and controls to investigate the overlap between these diseases.

METHODS

Subjects

DNA was available for 1054 UK Caucasian patients with JIA (332 men, 715 women) as previously described. JIA cases were classified according to the International League of Associations for Rheumatology (ILAR) criteria (online supplementary table 1).

Three thousand one hundred and twenty-nine healthy UK Caucasian control DNA samples were available as described previously. All individuals were recruited with ethical approval and provided informed consent (North-West Multi-Centre Research Ethics Committee (MREC) 99/8/84) and the University of Manchester Committee on the Ethics of Research on Human Beings).

SNP selection

SNPs that showed robust evidence for association (p<5×10^{-7}) with T1D and/or CD and had not been investigated previously were identified. In total 16 SNPs were selected for genotyping.

Genotyping

All SNPs were genotyped, in UK JIA cases and controls, using the Sequenom iPLEX MassARRAY platform according to manufacturers instructions (Sequenom, San Diego, California, USA, http://www.sequenom.com, accessed 9 July 2010). A 90% sample quality control rate and 90% SNP genotyping success rate was imposed on the analysis.

Statistical analysis

Power calculations were performed using QUANTO based on the effect sizes reported in previous studies of these SNPs in other autoimmune diseases. Calculations assumed a log-additive model and an α value corrected for the number of SNPs analysed. Genotype and allele frequencies were compared between cases with JIA and controls using PLINK. Comparisons were expressed as ORs and their 95% CIs as well as the Cochrane–Armitage test for trend. JIA is a phenotypically heterogeneous disease and can be...
Association analysis of T1D and CD associated SNPs in UK JIA case–control cohort

Table 1

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<th>SNP</th>
<th>Gene</th>
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<th>Position</th>
<th>p</th>
<th>trend</th>
<th>OR (95% CI)</th>
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DISCUSSION

The emerging overlap of autoimmune disease susceptibility loci makes many of the newly discovered T1D and CD loci appealing candidates for investigation as possible JIA susceptibility loci. There is already some evidence for the overlap of JIA and T1D susceptibility loci, with confirmed association of genes such as the major histocompatibility complex, PTPN22, IL2RA, IL2/21 region and CCR5 with both diseases. 5 10–12 By investigating confirmed CD and T1D loci in JIA we have identified a novel locus conferring susceptibility to JIA. This SNP lies within the Lim domain containing preferred translocation partner in lipoma (LPP) gene. SNPs in LPP show validated association with CD. 13 14 Interestingly, the rs1464510 SNP does not appear to be associated with T1D 2 and studies in RA show conflicting results: in one large UK population study, no association was detected (p trend =0.62, OR=1.01, 95% CI 0.94 to 1.08) (Eyre et al, submitted) while in a Dutch population, modest evidence for association was reported (p=0.012, OR=1.14, 95% CI 1.03 to 1.26). 8

LPP has a number of known functions; it is important in cell migration, cell adhesion and acts as a versatile scaffolding and adaptor protein. 15 Recently, LPP was identified as a substrate of the protein-tyrosine phosphatase 1B, a negative regulator of multiple signalling pathways downstream of receptor tyrosine kinases and functionally linked to Ras signalling. 16 Further work will be required to determine which of the multiple potential pathways with which the protein interacts is affected by the variants that predispose to autoimmune disease.

We also found weak evidence for association of a SNP, rs653178, in the SH2B3/ATXN2 gene region with JIA, though it did not remain significant after correction for multiple testing.

RESULTS

Genotype counts for all SNPs were in Hardy–Weinberg equilibrium (p>0.05) in the control cohort. Three SNPs (rs12708716, rs45450798 and rs229541) failed SNP quality control thresholds and were excluded from further analysis, leaving 13 SNPs for analysis. A Bonferroni correction of 13 was applied to correct for the number of tests, resulting in a p value of 0.0017 for significance (table 1). One SNP in the LPP gene associated with CD, rs1464510, showed significant association with JIA at the corrected threshold (p trend =0.002, OR=1.18, 95% CI 1.06 to 1.30) (table 1). A second SNP, rs653178, in the ATXN2 gene associated with CD, was nominally associated with JIA (p trend =0.02, OR=1.13, 95% CI 1.02 to 1.25) but this was not statistically significant after correction for multiple testing (p trend =corrected=0.26).

All SNPs were examined for differences in allele frequencies across the ILAR subtypes. The SNP, rs17610546, in IL12A associated with CD, showed a significant allele frequency difference (p=0.03) between the subtypes and therefore this SNP was reanalysed stratifying by ILAR subtype. The enthesitis-related arthritis (ERA) subtype showed strong association with IL12A SNP (p trend =0.005, OR=1.88, 95% CI 1.2 to 2.94). There was no evidence for association with any other subtype.
We had >80% power to detect an effect at studies for CD and T1D, which had much larger sample sizes. This is owing to the limited power for many of these loci. This is important to note that the numbers in this subgroup are small (n=61) so this finding should be interpreted with caution and will require validation in an independent cohort. To date the SNP and the SNP tested in our study (r^2 = 0.5). Hence, both OR plots for CD and T1D susceptibility genes across the different autoimmune diseases, JIA, RA, T1D and CD, are shown in figures 1 and 2, respectively.

In conclusion we have identified novel association of the LPP gene and show weak but confirmatory association of the ATXN2/SH2B3/c1orf50 region with JIA in a UK cohort. In addition, we report an ERA subtype-specific association with the IL12A gene. Investigation of all these loci in independent JIA case-control cohorts followed by meta-analysis will be required to confidently confirm or refute these as JIA susceptibility loci. Nonetheless, the approach of targeting variants associated with other autoimmune diseases is already yielding insights into the genetic complexity underlying susceptibility to this serious childhood disease.

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**References**


