Association of Variants at 1q32 and STAT3 with Ankylosing Spondylitis Suggests Genetic Overlap with Crohn’s Disease

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Abstract

Ankylosing spondylitis (AS) is a common inflammatory arthritic condition. Overt inflammatory bowel disease (IBD) occurs in about 10% of AS patients, and in addition 70% of AS cases may have subclinical terminal ileitis. Spondyloarthritis is also common in IBD patients. We therefore tested Crohn’s disease susceptibility genes for association with AS, aiming to identify pleiotropic genetic associations with both diseases. Genotyping was carried out using Sequenom and Applied Biosystems TaqMan and OpenArray technologies on 53 markers selected from 30 Crohn’s disease associated genomic regions. We tested genotypes in a population of unrelated individual cases (n = 2,773) and controls (n = 2,215) of white European ancestry for association with AS. Statistical analysis was carried out using a Cochran-Armitage test for trend. Association was detected at chr1q32 near KIF21B (rs11584383, P = 1.6 × 10⁻¹⁰, odds ratio (OR) = 0.74, 95% CI: 0.68–0.82). Association with disease was also detected for 2 variants within STAT3 (rs6503695, P = 4.6 × 10⁻⁴, OR = 0.86 (95% CI: 0.79–0.93); rs744166, P = 2.6 × 10⁻⁷, OR = 0.84 (95% CI: 0.77–0.91)). Association was confirmed for IL23R (rs11465804, P = 1.2 × 10⁻⁵, OR = 0.45 (95% CI: 0.35–0.54)), and further associations were detected for 4 loci. IL23R and detects suggestive association with another 4 loci. STAT3 is a key signaling molecule within the Th17 lymphocyte differentiation pathway and further enhances the case for a major role of this T-lymphocyte subset in ankylosing spondylitis. Finally these findings suggest common aetiopathogenic pathways for AS and Crohn’s disease and further highlight the involvement of common risk variants across multiple diseases.

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Introduction

Ankylosing spondylitis (AS) is one of a group of common inflammatory rheumatic diseases known as spondyloarthritides in which involvement of the spine and sacroiliac joints is prominent [1]. Heritability of the disease assessed by twin studies has been determined to be >90% [2]. Apart from the well known HLA-B27 association, recent genetic studies have identified ERAPI, IL23R and 2 intergenic regions at chr2p15 and chr21q22 as genes/loci associated with AS [3,4]. However, these alleles only explain a fraction of the overall genetic risk for AS, and other loci are also expected to contribute to susceptibility.
Author Summary

Ankylosing spondylitis is a common inflammatory arthritis primarily affecting the spine and pelvis. The disease is highly heritable (heritability >90%), and the major genetic allele for the disease, HLA-B27, contributes approximately half the genetic risk for the condition. Ankylosing spondylitis and inflammatory bowel disease (Crohn’s disease and ulcerative colitis) frequently occur together in the same families and individuals, suggesting that they share common risk factors. We tested whether genes associated with Crohn’s disease are also associated with ankylosing spondylitis and confirmed that the two diseases share associations at chromosome 1q32 near KIF21B, STAT3, IL12B, CDKAL1, LRRK2/MUC19, and chromosome 13q14. These associations were present even in ankylosing spondylitis cases with no clinical inflammatory bowel disease. These findings greatly expand our understanding of why these conditions co-occur and provide further evidence of pleiotropy in human disease pathology. As the genes IL23R, STAT3, and IL12B all influence Th17 lymphocyte differentiation/activation, this provides further evidence implicating the Th17 lymphocyte subset in the pathogenesis of ankylosing spondylitis.

There is increasing interest in the genetics community in the study of genetic findings from related diseases to identify pleiotropic genes, as an efficient method to identify further disease-associated variants. Findings from genome-wide association (GWA) studies have identified susceptibility genes common to different diseases, particularly in autoimmune conditions [5]. For example, variants in PTPN22 are associated with rheumatoid arthritis (RA), type-1 diabetes (T1D) and Crohn’s disease (CD). Thus far, only the gene IL23R (associated with AS) has also been found to be associated with inflammatory bowel disease (IBD) and psoriasis, although the three conditions commonly occur in the same patients, and are familial. About 10% of AS patients have overt IBD, and in addition about 70% of AS cases have subclinical terminal ileitis [6]. Gut inflammation is frequent in patients with spondylarthritides, and one-quarter of patients who have chronic spondyloarthritis have some features of CD [7]. Spondyloarthritides is also common in IBD patients. Axial and peripheral arthritis can occur in up to 30% of patients with IBD [8]. The prevalence of axial involvement in IBD is 10–20% for sacroiliitis and 3–12% for spondylitis [9], while radiographic evidence of sacroiliitis is reported in 10–51% of patients with IBD [10]. A study of families of AS probands (n = 205) and of healthy controls (n = 1,352) in the Icelandic population demonstrated evidence to support a common genetic component for AS and IBD [11]. In addition to confirming the known familiality of both conditions, this study demonstrated a risk ratio of 3.0 and 2.1 in 1st and 2nd-degree relatives respectively, for the occurrence of AS in families of probands with IBD, and with the occurrence of IBD in families of patients with AS. It therefore seems likely that common pathogenic pathways may act in the development of both diseases and may be major players in chronic inflammatory disorders. We therefore sought to investigate CD risk variants for association with AS in order to explain the co-occurrence of both conditions.

Results

This study has 80% power to detect an additive association (P = 0.05) with odds ratios of 1.15–1.23 for markers with minor allele frequencies of 0.5–0.1 respectively. These values are based on a disease prevalence of 0.4% in the general population and linkage disequilibrium between markers of r² = 0.8.

In phase 1 of the genotyping experiment MAIs for 53 markers genotyped on cases were compared to MAF calculated from historical controls from the 1958 BBC (genotyped and imputed SNPs). Eight SNPs were excluded from further analysis; two variants failed to meet imputation QC thresholds, four were excluded based on their MAF and two failed the call rate criterion. Of the remaining markers, 23 SNPs achieved a nominal P value of 0.1 (Table S1). This is significantly more markers than would be expected by chance (P = 4.8 x 10^{-10}). Of these, one failed assay design and 22 were taken forward for further genotyping in phase 2 of the experiment. A further marker failed QC thresholds in phase 2 and was excluded from further analysis.

Considering the combined phase 1 and 2 data, experiment-wise association (P < 0.05) was observed with seven genomic regions (eight variants) and nominal association (P = 0.05) was detected for 18 markers (Table 1). Associations were tested in both the overall cohort, and comparing cases with no clinical IBD (n = 2613) with healthy controls. The frequency of AS cases with coexistent IBD in our dataset is 5.8%. No significant difference was noted in any of the findings of having excluded cases with clinical IBD, supporting these associations being relevant to the pathogenesis of AS, rather than just coexistent IBD.

The strongest association was identified at genome-wide significance for rs11584383 at chr1q32 (P = 1.6 x 10^{-10}) with OR = 0.74 (95% CI: 0.68–0.82). This marker is downstream of and flanked by KIF21B and the putative open reading frame C1orf106. Convincing evidence of association was also identified for STAT3. Association in STAT3 was detected at experiment-wise significance for 2 markers (rs744166, P = 2.6 x 10^{-5}, OR = 0.84 (95% CI: 0.77–0.91); rs6503695, P = 4.6 x 10^{-4}, OR = 0.86 (95% CI: 0.79–0.93)). Another 2 variants within the gene showed more modest association (rs2306580, P = 0.023; rs4103200, P = 1.9 x 10^{-5}) but rs4103200 demonstrated nominal association in both phases of the genotyping experiment.

Variants or near CDKAL1 (rs6908425, P = 1.1 x 10^{-4}, OR = 0.82 (95% CI: 0.74–0.91)), IL12B (rs10045431, P = 5.2 x 10^{-5}, OR = 0.83 (95% CI: 0.76–0.91)), LRRK2/MUC19 (rs11175593, P = 9.9 x 10^{-5}, OR = 1.92 (95% CI: 1.38–2.67)) and at chr13q14 within C1orf106 (rs3764147, P = 5.9 x 10^{-4}, OR = 1.19 (95% CI: 1.08–1.31)) also demonstrated experiment-wise significant association in this study. The function of CDKAL1 is poorly understood but the gene has been associated with type-2 diabetes [12,13], and could also possibly be a psoriasis risk locus [14].

Other nominal associations detected in this study include 3 variants within ATG16L1 and single SNPs at MSTI1, JAK2, AKX2-3 and chr8q24. Further studies will be required to robustly establish the significance of these findings. No association was observed at TNFSF15 (rs4263839, P = 0.3), consistent with previous findings in AS [3,4], contrasting with findings in undifferentiated spondyloarthritis [15], in which suggestive but not genome-wide significant association has been reported (rs4979459, P = 4.9 x 10^{-5}). The peak IBD and spondyloarthritides associations are with different SNPs that lie 83kb apart, and thus if this gene is truly spondyloarthritis-associated, there is likely that different genetic variants are associated with IBD and spondyloarthritides.

This study also confirms the IL23R association with AS at rs11465804 (P = 1.2 x 10^{-5}, OR = 0.65 (95% CI: 0.54–0.79)). In a recent study, IL23R association with AS was confirmed with a peak signal within the gene at rs11290926 (P = 9.1 x 10^{-11}) [4]. However, evidence of association at rs11465804 (3,432 bp away from rs11290926) is much weaker, and was previously reported at...
| Marker   | Chr | Position | Candidate       | Minor allele | Phase 1 Case MAF | Phase 1 Control MAF | Phase 1 P value | Phase 2 Case MAF | Phase 2 Control MAF | Phase 2 P value | Combined odds ratio(95% CI) | Combined P value | Combined excluding IBD P value |
|----------|-----|----------|----------------|--------------|------------------|--------------------|-----------------|------------------|--------------------|-----------------|--------------------------------|----------------|--------------------------------|
| rs11465804 | 1   | 67475114 | IL23R          | G            | 0.042            | 0.061              | 4.4 × 10⁻³      | 0.035            | 0.053              | 3.9 × 10⁻³      | 0.65 (0.54–0.79)             | 1.2 × 10⁻⁵        | 5.8 × 10⁻⁵                          |
| rs6679677  | 1   | 111405331| TPNP22        | A            | 0.074            | 0.092              | 3.1 × 10⁻²      | 0.095            | 0.106              | 0.23            | 0.88 (0.76–1.01)             | 6.4 × 10⁻²        | 0.14                          |
| rs11584383 | 1   | 199202489| Intergenic      | C            | 0.26             | 0.319              | 7.9 × 10⁻⁶      | 0.242            | 0.296              | 6.7 × 10⁻³      | 0.74 (0.68–0.82)             | 1.6 × 10⁻¹0       | 1.0 × 10⁻¹0                         |
| rs12994997 | 2   | 233838342| ATG16L1       | G            | 0.456            | 0.493              | 1.1 × 10⁻²      | 0.471            | 0.476              | 0.72            | 0.91 (0.84–0.99)             | 2.4 × 10⁻²        | 4.2 × 10⁻²                           |
| rs2241880  | 2   | 23384107 | ATG16L1       | T            | 0.453            | 0.491              | 8.8 × 10⁻³      | 0.465            | 0.477              | 0.43            | 0.9 (0.83–0.98)              | 1.0 × 10⁻²        | 2.1 × 10⁻²                           |
| rs3792106  | 2   | 233855479| ATG16L1       | A            | 0.395            | 0.435              | 5.7 × 10⁻³      | 0.406            | 0.413              | 0.66            | 0.9 (0.83–0.98)              | 1.1 × 10⁻²        | 1.5 × 10⁻²                           |
| rs3924462  | 3   | 49499240 | MST1          | C            | 0.406            | 0.442              | 1.2 × 10⁻²      | 0.435            | 0.453              | 0.26            | 0.91 (0.84–0.99)             | 5.0 × 10⁻²        | 7.8 × 10⁻²                           |
| rs3181219  | 5   | 158684717| IIL12B        | T            | 0.1              | 0.118              | 5.0 × 10⁻²      | 0.109            | 0.116              | 0.43            | 0.88 (0.78–1)               | 5.3 × 10⁻²        | 5.5 × 10⁻²                           |
| rs1433048  | 5   | 15868423 | IIL12B        | G            | 0.165            | 0.191              | 2.2 × 10⁻²      | 0.172            | 0.176              | 0.72            | 0.9 (0.81–1)               | 5.0 × 10⁻²        | 3.9 × 10⁻²                           |
| rs10045431 | 5   | 158747111| IIL12B        | A            | 0.257            | 0.302              | 6.7 × 10⁻⁴      | 0.259            | 0.287              | 3.7 × 10⁻²      | 0.83 (0.76–0.91)             | 5.2 × 10⁻³        | 1.1 × 10⁻⁴                           |
| rs6908425  | 6   | 20836710 | CDKAL1        | T            | 0.194            | 0.229              | 4.0 × 10⁻³      | 0.196            | 0.216              | 3.2 × 10⁻²      | 0.82 (0.74–0.91)             | 1.1 × 10⁻⁴        | 1.0 × 10⁻⁴                           |
| rs2301436  | 6   | 167357978| CCR6          | G            | 0.507            | 0.469              | 8.0 × 10⁻³      | 0.448            | 0.461              | 0.39            | 1.03 (0.95–1.12)             | 0.44            | 0.44                            |
| rs1456893  | 8   | 50240218 | Intergenic     | G            | 0.291            | 0.318              | 5.2 × 10⁻²      | 0.306            | 0.334              | 4.5 × 10⁻²      | 0.89 (0.82–0.97)             | 1.1 × 10⁻²        | 1.2 × 10⁻²                           |
| rs10758669 | 9   | 4971602  | JAK2          | C            | 0.353            | 0.327              | 6.5 × 10⁻²      | 0.37             | 0.343              | 7.5 × 10⁻²      | 1.14 (1.05–1.24)             | 2.9 × 10⁻⁴        | 2.3 × 10⁻⁴                           |
| rs11190140 | 10  | 101281583| NNX2-3        | C            | 0.518            | 0.482              | 1.3 × 10⁻²      | 0.494            | 0.509              | 0.34            | 1.11 (1.02–1.2)              | 1.3 × 10⁻³        | 3.5 × 10⁻³                           |
| rs1175593  | 12  | 38888207 | LRRK2/MUCT19  | T            | 0.02             | 0.013              | 5.3 × 10⁻²      | 0.025            | 0.02             | 0.01           | 5.8 × 10⁻⁴                  | 1.92 (1.38–2.67)  | 9.9 × 10⁻⁵                           |
| rs3764147  | 13  | 43355925 | Intergenic     | G            | 0.235            | 0.202              | 4.3 × 10⁻³      | 0.237            | 0.241              | 0.72            | 1.19 (1.08–1.31)             | 5.9 × 10⁻⁴        | 9.1 × 10⁻⁴                           |
| rs2063580  | 17  | 37745206 | STAT3         | C            | 0.065            | 0.085              | 9.8 × 10⁻³      | 0.07             | 0.076              | 0.43            | 0.83 (0.71–0.96)             | 2.3 × 10⁻²        | 2.6 × 10⁻²                           |
| rs6503695  | 17  | 37750359 | STAT3         | C            | 0.329            | 0.352              | 9.7 × 10⁻²      | 0.32             | 0.37              | 0.9           | 0.86 (0.79–0.93)             | 4.6 × 10⁻⁴        | 3.4 × 10⁻⁴                           |
| rs4103200  | 17  | 37760591 | STAT3         | C            | 0.258            | 0.284              | 4.5 × 10⁻²      | 0.254            | 0.284              | 2.4 × 10⁻²      | 0.96 (0.79–0.95)             | 1.9 × 10⁻³        | 2.0 × 10⁻³                           |
| rs744166   | 17  | 37767727 | STAT3         | C            | 0.399            | 0.437              | 7.3 × 10⁻³      | 0.395            | 0.446              | 7.9 × 10⁻⁴      | 0.84 (0.77–0.91)             | 2.6 × 10⁻⁴        | 3.8 × 10⁻⁴                           |

Positions based on NCBI reference sequence build 36.3. MAF, minor allele frequency; Chr, chromosome. Phase 1 involved 1230 cases and 1295 controls, and phase 2 21545 cases and 920 controls. doi:10.1371/journal.pgen.1001195.t001
Discussion

The study presented here has identified new loci associated with AS. The strongest of these associations was within an intergenic region at chr1q32, near the gene KIF21B. The protein encoded by this gene belongs to a family of kinesin motor proteins. Kinesins are used for the transport of essential components along axonal and dendritic microtubules by neurons. KIF5A has been associated with rheumatoid arthritis, type-1 diabetes, and is close to a locus recently reported to be associated with multiple sclerosis [16]. It is possible that KIF5A is not the key associated gene at this chromosome 12q13-14 locus. However, if confirmed as the true disease-susceptibility gene for these autoimmune diseases, this would strongly suggest alternate functions for the kinesin protein family.

The association with IL12B is of particular interest given the associations of IL23R and STAT3 with AS. This gene has also been shown to be associated with psoriasis in Caucasian and Chinese populations [18,19]. IL12B encodes the p40 subunit common to both IL-12 and IL-23 and again highlights the involvement of Th17 cells in disease development. Another marker (rs14330491) within the gene was also nominally associated (P<0.05) in the combined analysis of the study. It is not clear at the LRRK2/MUC19 locus which is the key associated gene with Crohn’s disease, although a recent study suggests that LRRK2 is the more likely to be truly disease-associated [20]. LRRK2, a member of the leucine-rich repeat kinase family, is thought to be involved in the process of autophagy. MUC19 encodes a mucin involved in epithelial lining protection; altered intestinal permeability has long been thought to be important in the pathogenesis of AS.

This study also provides further evidence of pleiotropic effects in human disease pathology. A notable example of this is the association of PTEN with several autoimmune conditions including RA, T1D, CD and SLE. One of the 1st AS risk loci identified, IL23R, is also associated with both forms of IBD (ulcerative colitis and Crohn’s disease) as well as psoriasis. In this study we provide further evidence for previously and newly identified pleiotropic genes in autoimmune diseases. Given the delicate nature of the immune system and the tight control of the different cell populations it is not surprising that risk alleles of important immune response genes may be associated across a number of different conditions. These findings support the use of study designs focusing on genes previously identified as being associated with related conditions as being an efficient method for identifying further genetic disease-associations.

This study of genes associated with Crohn’s disease has identified definite genome-wide significant association with AS of SNPs at chromosome 1q32 near KIF21B, and experiment-wise association at five other novel-AS loci including STAT3, IL12B, CDEKAL1, LRRK2/MUC19, and at chr13q14. This confirms that genes play an important part in the co-familiality of Crohn’s disease and AS, and highlights the value of studies of potentially pleiotropic genes in related diseases.

Materials and Methods

Cases included in the study were unrelated individuals with AS of white European ancestry from the UK, USA, Canada and Australia. The diagnosis of AS met the modified New York definition criteria [21]. Ethnically matched unrelated control individuals were selected from the 1958 British Birth Cohort (BBC) and an Oxfordshire (UK) healthy blood donor cohort. All patients gave informed, written consent, and the study was approved by the relevant ethics review committees.

SNPs chosen for the study were selected from previously reported risk loci identified in a CD GWA study [22]. Three candidate loci of high interest were studied in greater depth (STAT3, IL12B and ATG16L1). For these genes, tagging SNPs (r^2≥0.8) were selected using Tagger in haploviev (http://www.broadinstitute.org/haploviev/haploviev) and using the CEU population as a reference panel from the International HapMap Project database (http://www.hapmap.org/). Whenever assay design for selected SNPs failed, perfect proxy SNPs (r^2 = 1) were selected.

Genotyping was carried out in two phases. In phase 1, 53 SNPs from 30 distinct genomic regions were selected for genotyping in 1,230 cases. Case genotype results were compared with historical genotypes from 1,295 unrelated individuals from the 1958 British Birth Cohort, which had been typed with both the Illumina HumanHap 550 and Affymetrix SNP Array 5.0 microarrays. Whenever selected markers were missing from the control genotypes, imputation was carried out using MACH (http://www.sph.umich.edu/csg/abecasis/MACH/index.html) against the HapMap CEU dataset (reference panel). Imputed markers with low overall quality scores (Q<0.95) and/or in low LD with typed markers (r^2<0.3) were excluded from further analysis. Only genotypes with quality scores ≥0.95 were included in the study. All nominally associated markers (P<0.1) were taken forward for genotyping in phase 2 on 1,543 cases and 920 controls.

In phase 2, genotyping was performed using Sequenom (Sequenom Inc., San Diego, USA), and Applied Biosystems TaqMan and OpenArray technologies (Life Technologies, Carlsbad, USA). For Sequenom, SNPs were assayed and typed using iPLEX chemistry on a matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer. PCR reactions, cycling conditions and post-PCR extension reactions were all performed as recommended by the manufacturer. The iPLEX reaction products were desalted and spotted on SpectroChip and were processed and analysed in a compact mass spectrometer (MassARRAY Workstation). MassARRAY Typer 4.0 software was used for automated and manual genotype calling. OpenArray is a new genotyping platform technology from Applied Biosystems for medium-throughput experiment. SNPs were typed using TaqMan genotyping chemistry supported on a metal-based array. DNA samples were loaded and amplified on arrays as recommended by the manufacturer. Arrays were scanned on the OpenArray NT imager and genotypes were called using the OpenArray SNP Genotyping analysis software. Whenever assay design or the genotyping assay failed, markers were then genotyped using single TaqMan probe technology as recommended by the manufacturer.

Association statistics were calculated using PLINK software [23]. Analysis was carried out using the Cochran-Armitage test for trend, excluding markers failing the following criteria; missingness rate >0.1, minor allele frequency (MAF)<0.01 and exact Hardy-Weinberg equilibrium P<3x10^-5. We also excluded individuals with a missing genotype rate >0.1 from the analysis. Experiment-wise significance (P=9.4x10^-5) was determined by Bonferroni correction based on the total number of markers genotyped in phase 1 of the study.
Supporting Information

Table S1

| Study Details |
|---------------|
| Association study findings for phase 1 for all markers genotyped. Markers with blank case and control MAF failed genotyping. Positions based on NCBI reference sequence build 36.3. MAF, minor allele frequency; chr, chromosome. |

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Author Contributions

Conceived and designed the experiments: PD MAB. Performed the experiments: KP JH MAB. Analyzed the data: MAB. Contributed reagents/materials/analysis tools: PD LAB CF JP MW MW JDR BPW MAS WPM PR DG RDI. Wrote the paper: PD MAB.