

Evidence of genetic association between TNFRSF1A encoding the p55 tumour necrosis factor receptor, and ankylosing spondylitis in UK Caucasians

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ABSTRACT

Objectives. To replicate the possible genetic association between ankylosing spondylitis (AS) and TNFRSF1A.

Methods. TNFRSF1A was re-sequenced in 48 individuals with AS to identify novel polymorphisms. Nine single nucleotide polymorphisms (SNPs) in TNFRSF1A and 5 SNPs in the neighbouring gene SCNN1A were genotyped in 1604 UK Caucasian individuals with AS and 1019 matched controls. An extended study was implemented using additional genotype data on 8 of these SNPs from 1400 historical controls from the 1958 British Birth Cohort. A meta-analysis of previously published results was also undertaken.

Results. One novel variant in intron 6 was identified but no new coding variants. No definite associations were seen in the initial study but in the extended study there were weak associations with rs4149576 ($p=0.04$) and rs4149577 ($p=0.007$). In the meta-analysis consistent, somewhat stronger associations were seen with rs4149577 ($p=0.002$) and rs4149578 ($p=0.006$).

Conclusions. These studies confirm the weak genetic associations between AS and TNFRSF1A. In view of the previously reported associations of TNFRSF1A with AS, in Caucasians and Chinese, and the biological plausibility of this candidate gene, replication of this finding in well powered studies is clearly indicated

Introduction

Ankylosing spondylitis (AS) is a highly heritable arthropathy, the polygenic nature of which is now well recognised. Defining the complete genetic aetiology of polygenic disorders, such as AS, is an important research goal since defining loci of even apparently small genetic effect can give major insights into their aetiology and pathogenesis (1). For example, the weak but robust association between rheumatoid arthritis (RA) and *CTLA4*, increasing the risk of disease by about 8 per cent (2), is indicative of the potential for therapeutic manipulation of T-cell activation through the CD28 pathway. This has been amply demonstrated by the efficacy of recombinant *CTLA4*

immunoglobulin fusion protein (abatacept) in RA (3). Genome-wide association studies (GWAS) have proved very successful at identifying novel susceptibility genes of moderate effect in AS, such as *ERAP1* and *IL23R* (4, 5). However, other loci with smaller genetic effects may require much larger data sets for discovery and validation.

The type 1 (p55) tumour necrosis factor receptor (TNFR1), encoded at *TNFRSF1A*, is a plausible candidate for involvement in AS for several reasons. First, TNFR1 has a pivotal role in TNF-related pathways leading to NF κ B-mediated activation of pro-inflammatory genes and FADD-mediated apoptosis (6); second, TNF blockade with recombinant TNF receptor/Ig fusion protein (etanercept) is a highly effective treatment (7); third, tissue-specific expression of TNFR1 is critical to the development of disease in certain animal models (8); and fourth, there is already evidence of genetic association between *TNFRSF1A* and certain inflammatory disorders, including AS (5, 9-12). Not only did the recent GWAS reported by the Triple A (Australo-Anglo-American) Spondyloarthritis Consortium (TASC) show suggestive association of AS with rs1800693 ($p=6.9 \times 10^{-5}$) at *TNFRSF1A* (5) but a weak association ($p=8.2 \times 10^{-4}$) was also reported at *TNFRSFA1* with rs4149577 in Han Chinese (9). We therefore re-sequenced the *TNFRSF1A* gene to identify new variants and then undertook a systematic re-evaluation of the genetic association with AS in a further independent UK Caucasian population sample.

Materials and methods

Re-sequencing of TNFRSF1A and detection of novel SNPs

We re-sequenced all 10 exons, exon-intron boundaries and 2100 bp of 5' flanking sequence of *TNFRSF1A*, on 96 chromosomes from 48 subjects with AS, using BigDye[®] Terminator chemistry and ABI3100 sequencing system (Applied Biosystems, Warrington, UK). Polyphred software package was used to detect SNPs (<http://droogs.washington.edu/polyphred/>). Novel SNPs were identified by comparison with known sequences and SNPs,

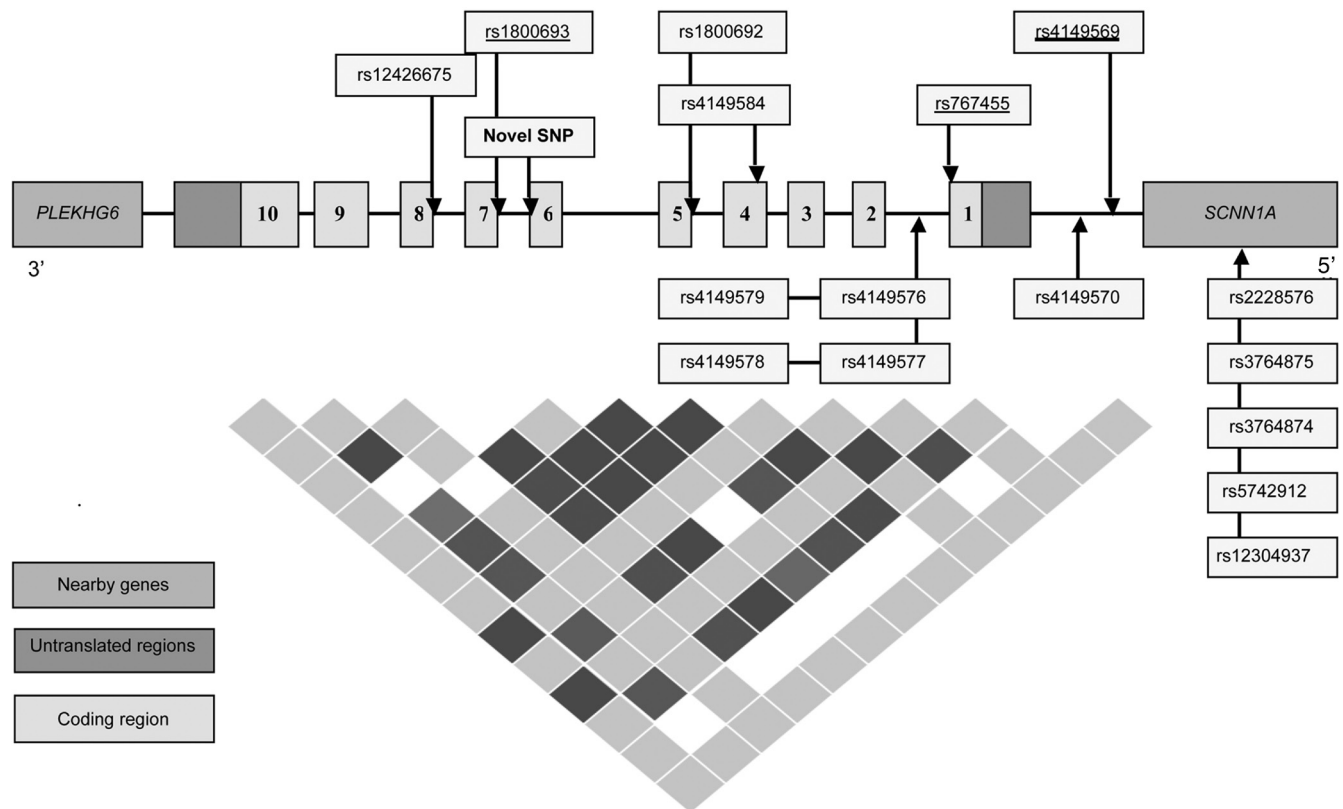


Fig. 1. Schematic of TNFRSF1A structure with detected known and novel SNPs with their relative locations (above). Underlined are SNPs tagged by rs4149576 and not genotyped ($r^2 > 0.8$). rs4149569 is within a repeat sequence and not genotyped. Hapmap tagging SNPs with their relative locations ($r^2 > 0.8$, below). LD plot for TNFRSF1A region from HapMap (Phase 3).

(<http://www.ensembl.org>, GRCh37; <http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp>, GRCh37, genome build 37.1; <http://genome.ucsc.edu/>, GRCh37/hg19). RFLP analysis was used to confirm the presence of novel SNPs.

Case-control study and meta-analysis

In "Study A", we genotyped 14 SNPs in a new sample of 1604 UK Caucasians with AS and 1019 age and ethnically-matched controls not included in the previous GWAS (5). All cases fulfilled the modified New York criteria for AS (13). The study was approved by the UK multicentre research ethics committee (MREC project number: 98/5/23). The 14 SNPs included one novel SNP in *TNFRSF1A*, eight previously known SNPs in *TNFRSF1A*, including 7 tagging SNPs ($r^2 > 0.8$), and 5 SNPs in the final 3 kilobases of the upstream gene *SCNN1A*, encoding sodium channel, non-voltage-gated 1 alpha (Fig. 1). Four more 5' SNPs in

SCNN1A had been genotyped in the TASC study but showed no evidence of association and were not therefore included in this study. Ten SNPs were genotyped by iPLEX technology (MassArray, Sequenom) and four SNPs (rs4149576, rs1800692, rs12426675, novel SNP IVS6-32C→A) by KASPar technology (KBiosciences, Hoddesdon, Herts, UK). Genotypes were tested for Hardy-Weinberg equilibrium ($p < 0.05$ was considered statistically significant but none needed to be excluded from the study). Cochran-Armitage test of trend was used for case-control analyses; p -values < 0.05 were considered statistically significant. We extended this study, ("Study B"), by including control data on between 1400 and 1475 controls (depending on the SNP) from the 1958 British Birth Cohort (BBC) (<http://www.b58cgene.sgu.ac.uk/October2008>). Case control data were available for two SNPs (rs4149577 and rs4149578) from the 2010 TASC study (5). "Study A" data and the TASC discovery set data for these two SNPs

were therefore combined in a meta-analysis (excluding the 1958 BBC control data from the "Study B" because of overlap with the TASC controls). Mantel-Haenszel test was used to calculate fixed effects pooled odds ratio, chi-square and p -values using StatsDirect software (version 2.6.6 03/02/2008). Cochran Q p -values were calculated to establish the combinability of the studies. An insignificant p -value (> 0.05) indicates low heterogeneity between studies, validating the use of a fixed-effects meta-analysis approach. Quanto software was used for power calculations assuming a log-additive mode of inheritance and a disease prevalence of 0.004, using unmatched cases and controls (version 1.2.4 May 2009). The PHASE program was used to derive haplotypes from the genotype data from "Study A" (SNPs rs4149584, rs4149579, rs4149578, rs4149577, rs4149576 and rs4149570). Differences in the frequencies of the predicted haplotypes between cases and controls were tested using a chi-square test.

Table I. The UK case-control study and the extended study results for the TNFRSF1A region. SNPs with $p < 0.05$ are shown in bold.

STUDY			(Study A) UK case-control study (Cases ~1604, controls ~1019)					(Study B) Extended UK case-control study (Including 1958 BBC controls ~1420, total controls ~2439)				
SNP	Gene	Position	<i>p</i> -value	OR (95% CI)	MAF controls	MAF cases	Power (%)	<i>p</i> -value	OR (95% CI)	MAF controls	Power (%)	
rs12426675	TNFRSF1A	Chr12:6439470	0.3	0.93 (0.80-1.08)	0.20	0.21	25					
Novel SNP	TNFRSF1A	Chr12:6439909	0.2	0.43 (0.12-1.46)	0.003	0.001	31					
rs1800692	TNFRSF1A	Chr12:6442346	0.9	1.01 (0.90-1.13)	0.43	0.42	7					
rs4149584	TNFRSF1A	Chr12:6442643	0.3	0.78 (0.51-1.21)	0.016	0.02	36	0.1	0.76 (0.54-1.07)	0.015	57	
rs4149579	TNFRSF1A	Chr12:6447357	0.2	0.87 (0.71-1.08)	0.075	0.084	37	0.3	0.91 (0.78-1.08)	0.08	32	
rs4149578	TNFRSF1A	Chr12:6447437	0.2	0.87 (0.71-1.06)	0.084	0.095	40	0.7	0.97 (0.83-1.13)	0.09	10	
rs4149577	TNFRSF1A	Chr12:6447522	0.2	1.08 (0.96-1.21)	0.5	0.48	32	0.007	1.13 (1.03-1.24)	0.51	89	
rs4149576	TNFRSF1A	Chr12:6449115	0.1	0.92 (0.81-1.03)	0.41	0.39	42	0.04	0.90 (0.82-0.99)	0.42	70	
rs4149570	TNFRSF1A	Chr12:6451590	0.4	0.95 (0.85-1.06)	0.42	0.43	21	0.1	0.93 (0.85-1.02)	0.41	43	
rs2228576	SCNN1A	Chr12:6457062	0.7	1.03 (0.91-1.16)	0.36	0.35	12	0.8	1.01 (0.92-1.11)	0.36	7	
rs3764875	SCNN1A	Chr12:6458084	1.0	1.00 (0.89-1.13)	0.35	0.35	5	0.8	1.01 (0.92-1.11)	0.36	7	
rs3764874	SCNN1A	Chr12:6458274	0.2	0.97 (0.85-1.11)	0.22	0.23	11					
rs5742912	SCNN1A	Chr12:6458350	0.4	0.84 (0.56-1.26)	0.019	0.022	24					
rs12304937	SCNN1A	Chr12:6458703	0.1	1.50 (0.91-2.45)	0.016	0.01	47					

(OR: odds ratio; CI: confidence interval; MAF: minor allele frequency; BBC: British birth cohort (<http://www.b58cgene.sgu.ac.uk/>)).

Results

We detected 7 sequence variants of *TNFRSF1A*, of which one was a novel SNP in intron 6 (IVS6-32C→A) and six (rs4149569, rs767455, rs4149584, rs1800692, rs1800693 and rs12426675) had previously been reported (Fig. 1). Other possible SNPs detected by the polyphred software proved to be non-polymorphic on visual inspection and RFLP analysis.

Study A: The results of this independent replication study are shown in Table I. No significant associations with AS were seen with any SNP in *TNFRSF1A* or *SCNN1A*.

Study B: Increasing the power of the study by including control data from the 1958 BBC revealed weak association with two markers, rs4149576 ($p=0.04$, OR=0.9, 95% CI 0.82-0.99) and rs4149577 ($p=0.007$, OR=1.1, 95% CI 1.03-1.24). However, no association was observed with rs4149578 ($p=0.7$). No additional significant associations were apparent after stratifying for age of onset, peripheral joint disease, presence of inflammatory bowel disease, uveitis or HLA-B27 status (data not shown). Comparison of patient and control haplotypes revealed no additional associations (data not shown). However these analyses have reduced power, for instance for rs4149577,

stratifying on presence of uveitis reduces the power to <50% to detect an OR of 1.1.

Meta-analysis: When data for the two SNPs (rs4149577 and rs4149578) common to "Study A" and the previously published TASC study were combined in a meta-analysis, a somewhat stronger association was observed with rs4149577 ($p=0.002$, OR=1.1, 95% CI 1.04-1.18, power=92%) and there was also a positive association with rs4149578 ($p=0.006$, OR=0.9, 95% CI 0.77-0.96, power=91%). The magnitude and direction of the associations are in agreement for the meta-analysis with very narrow confidence intervals for the fixed effects pooled odds ratios for rs4149577 and rs4149578. The data from the Chinese study could not be included in the meta-analysis because of significantly different minor allele frequencies between the two populations.

Discussion

Mutations in *TNFRSF1A* have already been implicated in auto-inflammatory diseases known as TNF receptor associated periodic syndromes (14) but there is no firm association of common variants of this gene with common diseases. We were able to identify one new SNP with a very low minor allele frequency and to confirm the existence of

six others in *TNFRSF1A* with minor allele frequencies in excess of 0.01. The initial replication study did not reveal significant associations with AS. However, associations with functional candidates may escape identification due to low minor allele frequencies, small effect sizes and/or low power. By including data from the 1958 BBC, which had already been typed for a subset of the markers employed in our replication study, the power to detect association for some SNPs was approximately doubled. We can have more confidence that these are true results as the 95% CIs are narrower for both the associated and non-associated SNPs.

In the extended "Study B" there was some evidence of association with two markers, rs4149576 ($p=0.04$) and rs4149577 ($p=0.007$). Of particular interest, rs4149576 tags another SNP, rs1800693 ($r^2 > 0.8$), which showed the strongest association with AS (6.9×10^{-5}) in the TASC study (5). Even though this SNP was not formally typed in this study, it still supports our results as these two SNPs, which are in strong LD, are associated with AS in two different studies. The meta-analysis of rs4149577 and rs4149578 was consistent with a real association between with *TNFRSF1A* but none of these results on their own provide conclusive

evidence of true association. The ORs for the associated SNPs in this study (0.9 and 1.1) are comparable to those found by others in the most recent reports in Crohn's disease, a related inflammatory disorder (15).

The population attributable risk (PAR) for the two associated *TNFRSF1A* SNPs in study B, rs4149576 (PAR~4%) and rs4149577 (PAR~6%), are lower than those for *ERAP1* (rs2287987 OR~0.7, PAR~26%) or *IL23R* (rs11209032 OR~1.3, PAR~9%), unsurprisingly given their lower ORs but similar to the ~6.5% that we estimated for rs1800693 from published data (5, 16). The proven efficacy of anti-TNF therapy in AS already provides strong biological evidence for the involvement of TNF-driven pathways (potentially including *TNFRSF1A*) in the pathogenesis of AS (7). Given the previously described associations of *TNFRSF1A* with other inflammatory diseases, such as IBD (11, 12) and multiple sclerosis (10), further examination of the relationship of this locus with AS is clearly merited. Testing for association in other ethnic populations has already provided additional support for this locus (9) but additional independent replication studies and meta-analyses are still required to confirm this result. Understanding the functional consequences of *TNFRSF1A* mutations could also provide further crucial information about its involvement in AS.

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