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Original Article

IL-1A gene variation in relation to cytokine levels and clinical characteristics in ankylosing spondylitis

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Abstract

Objective: Variations in the IL-1 alpha (IL-A) gene increase the risk for ankylosing spondylitis (AS), but the pathway underlying this association is not fully understood. As IL-1A is primarily a regulatory cytokine, we investigated the influence of IL-1A gene variation on disease severity and cytokine expression in AS.

Methods: This was a cross sectional study of tumor necrosis factor inhibitors (TNFi)-naïve AS patients (n=334, 90% B27 +, age 45 years) fulfilling the modified New York criteria. We recorded demographics, clinical findings, spinal mobility, Bath AS Functional Index (BASFI), and routine lab findings. IL-1A genotyping for three AS-associated single nucleotide polymorphism (SNP; rs2856836, rs17561 and rs1894399) was performed using Taqman RT-PCR, with TNF, IL-6, IL-17A, and IL-23 levels measured using ELISA. Genotypic associations included logistic regression analysis for genotype (codominant model) and global haplotype (threshold 5%) associations with cytokine levels and clinical features.

Results: The three variants were in near complete linkage disequilibrium and formed two only common haplotypes (ACC 67%, GAT 33%). The levels for TNF, IL-6, IL-17A, IL-23, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were similar across genotypes and haplotypes (all p-values >0.4) as were the measures for spinal mobility and BASFI. The TAQ haplotype showed a borderline significant trend with reduced heart disease and mortality during follow-up.

Conclusion: IL-1A gene cluster variations do not have an impact on the clinical disease measures or cytokine levels in AS, suggesting that IL-1A has no direct role in AS.

Keywords: Ankylosing spondylitis, IL1A, SNP, spinal function, cytokines

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Introduction

A complicated and chronic spinal inflammatory disease, ankylosing spondylitis (AS) affects 0.2-0.6% of the population (1). Despite a strong association with HLA-B27, the exact etiology of AS remains unclear. Additional genetic and environmental factors are considered essential for disease development (2, 3). Genome-wide association studies have demonstrated that three single nucleotide variations (SNP) in the IL-1 alpha (IL-1A) gene cluster independently associate with an increased susceptibility for AS (4-6). The contribution of these three SNPs to AS susceptibility was confirmed in other populations (7-10), but the mechanistic functional effect underlying this and other gene associations remains poorly understood. IL-1A is a constitutively produced cytokine present within cells as an active precursor protein, and following danger signaling by cell necrosis, it can initiate sterile inflammation by stimulating TNF and IL-6 as well as acute phase protein production. As translational research for SNP's with a high frequency and a strong disease association has been recommended (2, 11, 12). We investigated whether three IL-1A gene variations have an impact on clinical disease activity, cytokine expression, and loss of spinal function in AS patients.

Methods

Patients

Ankylosing spondylitis patients for whom full blood was available were selected from an existing disease registry for AS in Northern Norway. The registry has been described in detail previously with all patients fulfilling the modified New York (NY) criteria (13). Clinical data were gathered simultaneously with biological samples, demographic data, prior disease evolution, and questionnaire-based evaluation of function (Bath AS Functional Index [BASFI]), complete spinal examination and standard laboratory investigations at study visit. All patients were tumor necrosis factor inhibitors (TNFi) naïve at the time of study, and follow-up data post research visit were gathered from electronic records. The regional ethics committee approved the study protocol (REK Nord 2012/1589), and patients provided written informed consent.

Whole blood was collected from patients at the time of study, and DNA was later isolated from peripheral blood mononuclear cells in stored samples (-20°C) using the Puregene® blood core kit A (Qiagen) according to the manufacturers' protocol. Samples were quantitated using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies). ILA genotyping was then performed using commercial Taqman (Applied Biosystems, Foster City, CA, USA)-based RT-PCR allelic discrimination assays for three non-synonymous SNPs: IL-1A gene cluster (rs2856836-A/G, rs17561 (+4845 A/C), and rs1894399 (+1893 C/T).

Table 1. Characteristics of AS patients (n=334) included in the study. Numbers indicate median values or percentages

Age (years)	45.4 (12.6)
Disease duration (years)	23.1 (12.2)
Male gender (%)	233 (70)
Diagnostic delay (years)	9.6 (7.5)
HLA-B27 pos. (%)	303 (91)
Morning stiffness (hours)	1.5 (1.2)
BASFI (SD)	3..4 (2.1)
Schober's test (cm)	3.5 (1.5)
Chest expansion (cm)	4.4 (2.1)
Finger-floor distance (cm)	15.5 (14.6)
Lateral movement (cm)	5.7 (1.2)
Occiput-wall distance (cm)	3.3 (6.5)
Loss of height (cm)	2.6 (3.8)
Any extra spinal manifestation	194 (46)
Psoriasis (%)	28 (8.4)
IBD (%)	20 (6)
Uveitis (%)	109 (32.6)
Arthritis (%)	37 (11)

Serum cytokine levels for TNF, IL-6, Il-17A, and IL-12/23 were measured from stored serum aliquots (-20°C) using a quantitative sandwich immunoassay (Human TNF-alpha DuoSet, R&D Systems, Minneapolis, USA). Results presented are the average of duplicate runs in each lot with levels below the limit of detection, which were assigned the lower limit of detection for computational purposes.

Statistical analysis

Geno and haplotype estimation was done through the SNPStats software program (14) by applying logistic regression analysis for genotypic (codominant model) and global haplotype (frequency threshold 5%) associations with clinical features and cytokine levels. The European cohort of the 1000 genes project (http://www.internationalgenome.org/data) was used as control groups for SNP allele frequencies. Values given are means (± standard deviation [SD]) and counts (percentage) unless otherwise indicated. As most data were skewed, statistical analyses performed using the Statistical Package of Social Sciences version 23.0 (IBM Corp.; Armonk, NY, USA) software for nonparametric techniques (Chi-square, Mann Whitney U test [MWU], Spearman's rank correlation coefficient [Rs]). Two-sided p-values corresponding to alpha levels ≤5% were considered statistically significant.

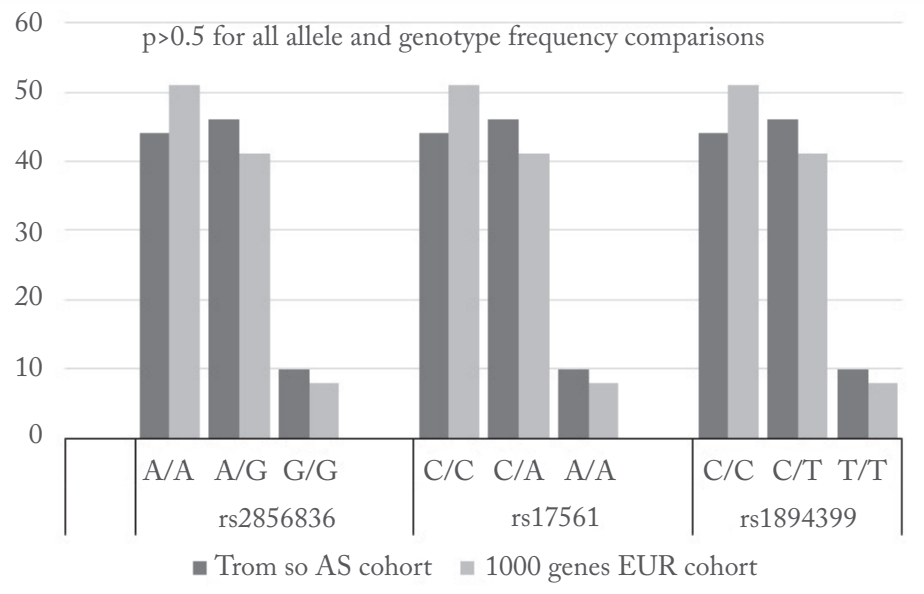

Figure 1. IL-1A gene variations distribution in AS patients and European controls in the 1000 genes project

Results

There was moderate functional impairment with a BASFI score of 3.5 (±2.1) in this AS cohort with longstanding disease (duration, 23±7.7 years) and otherwise unremarkable characteristics were noted (Table 1). The allele and genotype frequencies for the investigated SNPs in this Caucasian cohort were comparable to those reported for the European individuals in the 1000 genes project (Figure 1), with allele and genotype distribution independent of HLA B27 status (all p>0.2). The three SNPs were in near complete linkage disequilibrium (D' 0.999, r2 0.996) and formed two common (>1 %) haplotypes (A/C/C 0.68, T/A/G 0.32). With a single SNP sufficient for tagging, the clinical and serological associations for these haplotypes are presented through SNP rs2856836. There was no significant interaction for rs2856836 with disease presentation (gender, age, and HLA-B27 positivity, coexisting conditions), with disease activity measures, or levels for IL-6, TNF, IL-17A, IL-23, and C-reactive protein (CRP) at the research visit (Table 2). There was also no genotypic influence of rs2856836 on a variety of measures for loss of spinal function (Table 3). Similarly, there was no association for IL-1A genotypes with methotrexate use (n= 32) or subsequent initiation of TNFi therapy (n=128) during the mean follow-up of 85 months (all p>0.2). There was however a trend toward lower risk for heart disease (ischemia or failure; n=25; odds ratio [OR]: 0.55, confidence interval [CI]: 0.27-1.11, p=0.09) and necessity for a disability pension (n=90; OR: 0.72, CI: 0.49-1.05, p=0.09) with the TAG haplotype, but no effect was seen on the risk for cancer (n=16) or death (n=18; Table 3).

Discussion

While a large number of non-MHC genes have now been implicated in disease susceptibility for AS, only limited studies have been conducted on the functional impact of such gene alterations. In this large translation cohort study, we found no direct impact of IL-1A gene variations on disease manifestations or serological markers of inflammation in AS patients.

IL-1A is passively released from dying cells and functions as a danger signal for the activation of the immune system. Following the release into the extracellular environment, IL-1A binds to the IL-1R receptor on target cells and promotes transcription of proinflammatory genes (11). The three IL-1A SNP in this study have the potential to influence IL-1A protein function as rs17651 is a missense variation leading to conformational and possibly functional protein changes through amino acid substitution, while rs2856836 (3 prime UTR variant) and rs1894399 (intron variant) can impact the gene and protein

Table 2. IL-1A gene cluster variations and association with clinical characteristics in AS patients

	rs2856836				Haplotype	
	A/A	A/G	G/G	p	TAG vs CCA	p
	Odds Ratio / mean value				Odds ratio / mean difference (confidence interval)	
Demographics						
HLA*B27 pos	1	1.66	1.34	0.44	1.35 (0.74-2.43)	0.33
Male gender, age diagnosis (years)	1	1.26	1	0.64	1.09 (0.91-1.69)	0.65
	23	23.1	24	0.82	0.33 (-0.12-2.26)	0.64
Disease activity						
BASFI	3.59	3.46	3.52	0.9	-0.07 (-0.47-0.33)	0.74
MHAQ	0.46	0.42	0.52	0.36	0 (-0.07-0.07)	0.65
Dougados Funct. Index ASDAS-CRP	1.5	1.4	1.4	0.43	-0.06 (-0.16-0.04)	0.78
	1.91	1.78	1.88	0.33	-0.06 (-0.19-0.06)	0.34
Laboratory markers						
Hemoglobin (g/dL)	13.6	14.2	13.8	0.01	0.26 (0.01-0.05)	0.04
CRP (mg/L)	13.2 21	10.3	10.9 23	0.33	-2.02 (-5.08-1.04)	0.2
ESR (mm)		16		0.09	-1.82 (-5.62-1.98)	0.32
IL-6 (pg/µL)	78	106	37	0.35	-3.3 (-47.72-41.06)	0.88
TNF (pg/µL)	151	180	116	0.62	-1.1 (-80.07-78.1)	0.9
IL-17AA (pg/µL)	140	215	104	0.09	14.8 (-43.53-73.17)	0.62
IL-12/23 (pg/µL)	243	270	131	0.71	-26 (-170.83-117.69)	0.7

Table 3. IL-1A gene cluster associations with spinal function loss and other comorbidities

	SNP rs2856836				Haplotype	
	A/A	A/G	G/G	p	TAG vs CCA	p
	(mean value/OR vs AA)				(mean difference/OR)	
Spinal mobility	3.36	3.45	3.79	0.43	0.17 (-0.11-0.45)	0.24
Schober's test (cm)	4.19	4.62	4.17	0.25	0.16 (-0.23-0.55)	0.43
Chest expansion	3.84	3.05	3.7	0.43	0.92 (-2.4-0.24)	0.12
Occiput wall distance (cm)	15.7	15.4	14.9	0.96	-0.36 (-0.97-0.45)	0.79
Finger floor distance	36.1	37.4	38.8	0.75	1.16 (-0.82-4.16)	0.45
Spondylometry flexion Loss of height (cm)	3	2.3	2	0.25	-0.57 (-1.03-0.12)	0.11
Comorbidity						
Death (n=18)	1	0.6 0.64	0.13		0.79 (0.30-1.79)	0.55
Heart disease (n=25)	1	0.4	0.5	0.13	0.55 (0.27-1.11)	0.09
Cancer (n=16)	1	2.6	2.3	0.49	1.67 (0.6-4.64)	0.33
Work disability (n=90)	1	0.35	1.28	0.11	0.72 (0.49-1.05)	0.09

expressions. The finding that these IL-1A gene variants did not affect the initial AS presentation, the subsequent disease activity measures, or spinal function loss suggest that IL-1A pathways do not drive the processes behind these clinical features in AS. This would agree with the estimates that the attributable risk of IL-1A gene variants to AS susceptibility is around 6% (5) and with the findings that the response to treatment with monoclonal IL-1 inhibitors in AS patients has been underwhelming (15). We unexpectedly detected a trend toward lower risk for heart disease and disability in AS patients with the TAG haplotype. However, as this was based on borderline significance in post hoc

univariate analyses with relatively small numbers, the consistency of these findings will need confirmation in other longitudinal studies.

Based on the ability for IL-1A to induce TNF and IL-6 expression and the potential of the investigated SNPs to change IL-1A protein structure and function, we also investigated whether IL-1A SNPs led to aberrant expression of these inflammatory markers. Despite significant numbers of patients in the subgroup analyses, we failed to detect an impact of the three IL-1A SNP (alone or in combination) on the levels of erythrocyte sedimentation rate/CRP and found no effect on TNF/IL-6/ IL-17A, and IL-12/23 levels. As there is limited comparable data on how the IL-1A pathway might be involved in AS, there are possible explanations for why our findings do not confirm the anticipated impact of IL-1A SNP on inflammatory cytokines. IL-1A gene expression can involve simultaneous activation of related genes, such as its counterpart IL-1B, which is located in the proximally on the same gene cluster and exerts competitive agonism for IL-1R binding on target cells. Furthermore, IL-1A lies just downstream to the IL-1 receptor antagonist (IL-1RA), which is a natural IL1 antagonist that limits proinflammatory IL-1 signaling through competitive blocking of IL-1R (16). In addition, the mechanisms of IL-1A release are not fully understood and seem to be depending on cell type and the type of cell stimuli with experimental data further suggesting that IL-1A is sequestered in apoptotic blebs, thereby unable to set off a proinflammatory response (11). Finally, the examined structural nucleotide changes do not invariably lead to the postulated consequences for gene expression and/or protein function (17). AS is a genetically diverse condition and despite statistical associations reported for individual SNPs, a more complex interplay of multiple genetic alterations may be required to not only create an environment suited to disease development, but also to underwrite the diversity of the clinical disease (18, 19).

The limitations for this study lie in the selection of three specific AS-related gene variations, while we cannot exclude that these IL-1A gene variations work in concert with other genes to collectively underwrite the heterogeneity of AS. While the frequency of gene variations in this study did not differ from controls with a similar ethnic background, the lower number of participants compared to GWAS limits the power to detect smaller differences in allele frequency (4, 5). However, the long-term follow-up and complete data collection are a considerable strength of this study, as the lack of large AS cohorts with detailed clinical parameters and well-characterized disease outcome have hampered meaningful analyses of genotypes obtained in GWAS (2). Finally, the homogenous background of AS patients does not allow extrapolation of our results to dissimilar cohorts.

In conclusion, there is no discernible impact of AS-associated IL-1A gene variations on clinical disease severity or inflammatory cytokine in a large cohort of AS patients. This suggests that there is no direct role of IL-1A SNP on disease development.

Ethics Committee Approval: Ethics committee approval was received for this study from Regional Ethical Committee (REK Nord 2012/1589).

Informed Consent: Written informed consent was obtained from all the patients who participated in this study.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: The authors have no conflict of interest to declare.

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