

Both Baseline Clinical Factors and Genetic Polymorphisms Influence the Development of Severe Functional Status in Ankylosing Spondylitis

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Abstract

Functional severity in ankylosing spondylitis (AS) patients is variable and difficult to predict early. The aim of our study was to assess whether a combination of baseline clinical factors and genetic markers may predict the development of severe functional status in AS. We performed a cross-sectional association study on AS patients included in the Spanish National Registry of Spondyloarthropathies—REGISPONSER. Bath Ankylosing Spondylitis Functional Index (BASFI) was standardized by adjusting for disease duration since the first symptoms (BASFI/t). We considered as severe functional status the values of BASFI/t in the top of the 60th (p60), 65th (p65), 70th (p70), and 75th (p75) percentile. We selected 384 single nucleotide polymorphisms (SNPs) distributed in 190 genes to be analyzed. The study cohort included 456 patients with mean age 50.8(±10.5) years and with mean disease duration since first symptoms 24.7 (±10.1) years. Older age at disease onset and neck pain at baseline showed statistical significant association with severe BASFI/t. Polymorphisms associated in the allele frequencies test with severe BASFI/t in all classifications were: *rs2542151* (p60 [$P=.04$], p65 [$P=.04$], p70 [$P=.001$] and p75 [$P=.001$]) and *rs2254441* (p60 [$P=.004$], p65 [$P=.02$], p70 [$P=.01$] and p75 [$P<.001$]). Genotype association, after adjustment for covariates, found an association in three of the four patients' classifications for *rs2542151* and in two of the classifications for *rs2254441*. Forward logistic regression did not identify any model with a good predictive power for severe functional development. In our study we identified clinical factors and 24 polymorphisms associated with development of severe functional status in AS patients. Validation of these results in other cohorts is required.

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Introduction

Ankylosing spondylitis (AS) is a chronic progressive inflammatory disease affecting the spine and peripheral joints. It is largely confirmed that susceptibility to AS is genetically determined with *HLA-B27* as a major genetic contributor to the disease [1–3] and that environmental factors also play a role in susceptibility to the disease. In the last few years, several other genes have been reported to be involved in AS susceptibility [4–9].

Although the assessment of physical function is only one of several aspects of assessing disease severity, it is one of the most important measures of structural damage outcome in AS, as it directly influences the quality of life of patients and the economic costs of the disease [10–11]. Impairment of physical function can be subdivided into a reversible and an irreversible component. In this concept the reversible component is due to disease activity (signs and symptoms of the disease) and the irreversible component

is due to structural damage that has occurred as a consequence of the disease, such as syndesmophytes and vertebral bridging. Functional severity was found to be independently determined by both the reversible factors such as disease activity and the irreversible factors such as structural damage [12] but the loss of functional capacity in each patient was not predictable from early disease stages [13].

There is evidence that several clinical parameters such as hip involvement, disease duration, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) levels, smoking, and lower socioeconomic status are associated with worse function [13–14]. Nevertheless, much of the variability in disease functional severity in AS remains unexplained, suggesting that genetic factors could have a greater influence than environmental factors on AS progression [13].

A genetic component has been demonstrated for AS functional severity [15]. However, very little is known about the specific genes or genetic markers inside and outside the major histocompatibility gene complex (MHC) involved in the functional component of the disease [16–20].

Understanding the genetic basis of functional severity in AS would be of major value to differentiate at early stages patients at high risk of severe functional impairment and patients with a lower risk. Thus, clinicians could better select and optimize the preventive and therapeutic approach for each patient as of the time of diagnosis of the disease by objectively distributing high cost treatments.

Taking into consideration the fact that impairment of physical function may be partially controlled by the appropriate treatment, the aim of our study was to identify the baseline clinical and genetic factors that determine individual development of functional severity in AS.

Materials and Methods

Patients with AS

We performed a cross-sectional association study on Spanish AS patients which were recruited from 25 hospitals which participated in the Spanish National Spondyloarthropathies Registry (REGISPONSER) [21]. Patients fulfilled the modified New York Criteria for AS [22] and had at least 10 years of follow-up from the first symptoms of the disease. Baseline characteristics of the patients at the beginning of the disease were recorded as potential prognostic predictors. Specifically, clinical and demographic data, sex, age at disease onset, family history of spondyloarthropathies (SpA), initial symptoms of SpA (inflammatory low back pain, neck pain, enthesitis, dactylitis, tarsitis, sacroiliac syndrome, coxitis, lower limb arthritis, and upper limb arthritis), and the number of initial SpA symptoms.

Demographic and clinical characteristics of the AS population

The studied cohort included 456 AS patients (348 males and 108 females) with a mean age of 50.8 ± 10.5 years, 26.1 ± 9.1 years at disease onset and 34.6 ± 11.4 years at diagnosis. The average time of evolution, from disease onset, was 24.7 ± 10.1 years. *HLA-B27* was positive in 84.9% of the patients and 19.3% had a family history of spondyloarthropathies (SpA). Patients had mean BASFI at baseline 4.0 ± 2.8 , with mean BASFI/t (years) of 0.17 ± 0.13 .

Functional phenotype

To measure functional impairment we used the BASFI score, standardized by adjusting for disease duration since first symptoms, denominated here BASFI/t. BASFI is a validated index that focuses on 10 questions pertaining to function, measured on a visual analog scale (VAS). The mean of the 10 questions generates the score, with 10 denoting worst possible functional status [23].

There are no validated threshold values for classifying AS patients into mild or severe categories according to their BASFI score standardized by disease duration (BASFI/t). Therefore, to define functional severity we performed four association analyses using the χ^2 -test with different criteria. Based on the opinion of the clinicians who participated in this study, who estimated that approximately 25% to 40% of AS patients had severe functional damage, we defined as severe functional status the values of BASFI/t in the top 60th (p60), 65th (p65), 70th (p70) or 75th (p75) percentiles. The cut-off values for BASFI/t severe phenotype for each percentile were: 0.19 for p60, 0.21 for p65, 0.22 for p70, and 0.25 for p75 respectively.

HLA-B27 typing and SNP genotyping

Genomic DNA was isolated from saliva samples using the Oragene™ DNA Self-Collection kit (DNA Genotek Inc., Ottawa, Canada), according to the manufacturer's extraction protocol. All samples were tested for the presence of the HLA-B27 allele by conventional PCR using the primers reported by Olerup et al [24]. A region of 236 bp on exon 8 from the p53 gene was used as an internal control for the performance of the PCR [25].

After an extensive bibliographic search we selected 384 SNPs distributed in 190 genes to be analyzed in this study. We selected all the SNPs previously reported in Caucasians as associated with AS or with other SpA (psoriatic arthritis, juvenile idiopathic arthritis, reactive arthritis, undifferentiated arthritis, and inflammatory bowel disease-associated spondyloarthropathy). Besides those SNPs, we included some SNPs in genes described in the literature as associated to other autoimmune diseases and to bone-related disorders, since we considered them as potential candidates to be implicated in AS severity. Finally, we included tag SNPs in genes from the metabolic pathways of the two most important genes considered to be involved in AS: ERAP1 and IL-23R. The tag SNPs were selected from the HapMap CEU panel (the minor allele frequency at each locus was required to be >0.05 in Caucasian population, with an r^2 -value of <0.8 between adjacent markers). SNP genotyping was performed using the Illumina Golden gate genotyping platform (Illumina, Inc., San Diego, CA, USA) [26].

Statistical analysis

Statistical analysis was performed with SPSS v 15.0 (SPSS, Chicago, IL, USA) and SVS v 7.3.1 (Golden Helix Inc., Bozeman, Montana, USA) softwares.

All quantitative data are presented as mean and standard deviation (\pm SD) and all qualitative data as absolute frequencies and percentages. To assess the association between clinical variables and BASFI/t severe phenotype an unvaried analysis was performed using the chi-square (χ^2) test for categorical variables and the unpaired *t* test for continuous variables.

A test for deviation from Hardy-Weinberg equilibrium (HWE) was performed for each SNP. Pruning of the initial genotype dataset with default parameters (exclusion of SNPs with poor genotype cloud clustering, of SNPs with call-rate $<85\%$, of SNPs with severe deviation from HWE ($P < .0001$) and of samples with call rate $<85\%$) led to 456 samples and 344 SNPs [27–30]. Association test between allele and genotype frequencies and BASFI/t severe phenotype was performed by the chi-square (χ^2) test. P-values were calculated using a single-value permutation test (1000 permutations). The minor allele frequency at all loci was above 10%. Logistic regression analysis was used to discard whether the baseline clinical factors associated with severe function could be confounding for the association between BASFI/t severe phenotype and the SNPs genotypes. P values of $<.05$ were considered statistically significant and p values of $(.05 \geq p < .1)$ borderline.

Clinical factors and SNPs were then studied by means of multivariate logistic regression. Individual *P* values of the SNPs and of the clinical variables were ranked and only those most significantly associated with the severe functional phenotype were included in the multivariate analysis as potential predictors ($P < .1$ in the allele frequencies association test). The multivariate analysis was performed for all four defined classifications of functional severity (p60, p65, p70, and p75). BASFI/t was considered as dependent variable and baseline clinical variables and SNPs were included as predictors. The predictive discrimination of the models was tested both by Hosmer-Lemeshow statistic and the receiver

operating characteristic curve (ROC) with 95% confidence interval (CI). An area under the ROC curve (AUC) above 0.75 was considered as an indicator of a good predictive precision of the model.

Ethical approval

This study was approved by the Ethics Committee of “Reina Sofia” University Hospital, Córdoba and “Puerta de Hierro Majadahonda” University Hospital, Madrid, Spain. Each patient signed an informed consent form upon inclusion in REGISPON-SER-AS, in accordance with the fundamental principles set out in the Declaration of Human Rights in Helsinki.

Results

Baseline clinical variables and SNPs associated with functional severity in AS patients

Of the baseline clinical variables analyzed, the association with BASFI/t severe phenotype for neck pain and older age at disease onset was found to be statistically significant. A slight association was also found for low back pain (p60) and HLA-B27 (p65 and p70) (Table 1).

In the allele frequencies test, from the SNPs analyzed, we identified 24 polymorphisms associated with functional severe phenotype in at least one of the patient classifications. Two SNPs showed consistent association with BASFI/t and were significantly associated in all four patient classifications: rs2542151 in the protein tyrosine phosphatase non-receptor type 2 (*PTPN2*) gene [p60 ($P=.046$), p65 ($P=.006$), p70 ($P=.002$) and p75 ($P=.001$)] and rs2254441 in the proline-serine-threonine phosphatase-interacting protein 1 (*PSTPIP1*) gene [p60 ($P=.036$), p65 ($P=.017$), p70 ($P=.010$) and p75 ($P=.001$)]. Five SNPs (*rs10065172*, *rs2268624*, *rs4986790*, *rs4986791*, *rs3736228*) were associated with BASFI/t in three of the four patient classifications and eight polymorphisms (*rs6887695*, *rs17481856*, *rs2280153*, *rs1217414*, *rs4958847*, *rs2227982*, *rs17551710*, *rs1248634*) in two classifications. The other nine polymorphisms (*rs6822844*, *rs11959820*, *rs3117222*, *rs660895*, *rs1061622*, *rs13151961*, *rs27044*, *rs6254*, *rs743572*) were found significantly associated with BASFI/t for only one of the patient classifications (Table 2).

As we found that age at disease onset and neck pain at onset were the clinical factors associated with BASFI/t severe phenotype, they were entered in the logistic regression modeling as covariates. The results of the genotype frequencies test showed that the SNP *rs2542151* in the *PTPN2* gene was significantly associated to BASFI/t in three of the four patients' classifications

after adjustment for age at disease onset and neck pain (Table 2). There were other four SNPs with significant or borderline genotype associations in two of the patients' classifications after adjustment for age at disease onset and neck pain, *rs2254441* in the *PSTPIP1* gene, *rs2268624* in the *TGFB3* gene, and *rs4986790* and *rs4986791* in the TLR4 gene. The rest of SNPs were not associated to BASFI/t in the genotype test after correction for age at disease onset and neck pain at onset.

For further investigate the association between clinical-genetic variables and functional severe phenotype in AS, we performed a multivariate analysis using BASFI/t (severe/mild) as dependent variable and as covariates the clinical and genetic variables most significantly associated with BASFI/t ($p<0,1$ in the allele frequencies association test). We did not find any model with a good predictive accuracy for BASFI/t for any of the patient classifications, p60, p65, p70, and p75 as none of the ROC curves of the predictive models attained an area under the curve (AUC) above 0.75.

Discussion

This is a pioneering study performed on daily clinical patients which sought the clinical and genetic factors of influence in the development of functional severity in AS. Maintaining a good functional status is the main aim of pharmacologic treatment in current clinical practice. Reliable markers that could be applied early in the disease course to identify patients with potential severe functional outcome would be of major value for clinicians to consequently select the most suitable treatment strategy. The results of our study found that older age and neck pain at disease onset and several new SNPs, particularly *rs2542151* (*PTPN2*) and *rs2254441* (*PSTPIP1*), are predictors of severe functional impairment in AS. However, the combination of the genetic and clinical factors identified in our study was not sufficient to develop a predictive model with a good accuracy for AS functional outcome; therefore, additional predictors are required.

We found neck pain and older age at disease onset as the main clinical variables associated with severe AS functional status. Neck pain at onset, which in AS may arise from either mechanical or inflammatory lesions, presented a consistent association in all four patient classifications studied [p75 ($P=.040$), p70 ($P=.002$), p65 ($P=.004$), p60 ($P=.011$)]. To the best of our knowledge, this is the first study which has found neck pain as a predictor for AS functionality. There is conflicting evidence about the role of age at disease onset as a predictor of disease severity, with some studies finding an association and others failing to support this [31–35]. Patients included in our study were young adults at disease onset, with mean age of 26.1 ± 9.1 years. Interestingly, we found a statistically significant association between older age at disease onset and BASFI/t severe phenotype (p75, p70, p65, p60; $P<.001$). This result is in accordance with a recent study which found that the likelihood of developing more severe radiographic damage was greater among patients with an older age at disease onset [36]. The authors suggested that this association could result if patients with an older age at disease onset are asymptomatic early in their illness or if they have occasional or milder symptoms, which might cause them to underestimate the duration of their disease. Supporting previous studies [36–38], we found that *HLA-B27* was poorly associated with functional severity; thus, we only identified statistically significant association in two of the percentiles studied [p65 ($P=.050$), p70 ($P=.049$)]. Surprisingly, we did not find coxitis at baseline to be associated with development of severe physical function as found in several

Table 1. Baseline clinical variables associated with functional severity in AS patients.

Clinical Variable	p value			
	BASFI/t p75	BASFI/t p70	BASFI/t p65	BASFI/t p60
Age at disease onset	<.001	<.001	<.001	<.001
Neck pain	.040	.002	.004	.011
Low back pain	NS	NS	NS	.030
HLA-B27	NS	.050	.049	NS

NS- no statistically significant.

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Table 2. SNPs associated with functional severity in AS patients.

SNP Name	Gene Symbol	Risk Allele	Allele frequencies test, p value [§]			
			BASFI/t p75	BASFI/t p70	BASFI/t p65	BASFI/t p60
rs2542151	<i>PTPN2</i>	C	0.001*	0.002*	0.006*	0.046 [#]
rs2254441	<i>PSTPIP1</i>	A	0.001*	0.010 [†]	0.017 [#]	0.036 [#]
rs2268624	<i>TGFB3</i>	G	0.002*	0.017*	0.032 [#]	NS
rs4986790	<i>TLR4</i>	G	0.008 [†]	0.009 [†]	0.037 [#]	NS
rs4986791	<i>TLR4</i>	A	0.011*	0.006*	0.031 [#]	NS
rs10065172	<i>IRGM</i>	G	NS	0.014 [#]	0.012 [#]	0.006*
rs3736228	<i>LRP5</i>	A	0.049 [#]	0.051 [#]	0.056 [#]	NS
rs6887695	<i>IL12B</i>	C	NS	0.038 [#]	0.037 [#]	NS
rs17481856	<i>ERAP1</i>	G	NS	0.035 [#]	NS	0.038 [#]
rs2280153	<i>WISP3</i>	G	NS	NS	0.032 [#]	0.014*
rs1217414	<i>PTPN22</i>	G	NS	0.049 [#]	NS	0.034 [†]
rs4958847	<i>IRGM</i>	G	NS	NS	0.042 [#]	0.027 [#]
rs2227982	<i>PDCD1</i>	A	NS	0.019 [†]	0.044 [#]	NS
rs17551710	<i>COL6A1</i>	A	0.043 [#]	NS	NS	0.048 [#]
rs1248634	<i>DLG5</i>	A	NS	0.047 [#]	0.043 [#]	NS
rs6822844	<i>IL21</i>	A	NS	NS	NS	0.031 [#]
rs11959820	<i>PPARBC1B</i>	C	NS	NS	NS	0.017*
rs3117222	<i>LOC646702</i>	G	NS	NS	NS	0.018*
rs660895	<i>HLA-DRB1</i>	G	NS	NS	0.044 [†]	NS
rs1061622	<i>TNFRSF1B</i>	A	NS	NS	NS	0.050 [#]
rs13151961	<i>KIAA1109</i>	G	NS	NS	NS	0.042 [#]
rs27044	<i>ERAP1</i>	C	NS	NS	NS	0.020*
rs6254	<i>PTH</i>	A	0.025 [#]	NS	NS	NS
rs743572	<i>CYP17A1</i>	A	0.032 [#]	NS	NS	NS

[§]- The p-values shown correspond to the allele frequencies test corrected by a single-value permutation test.

The symbols represent the results of the genotype frequencies association test before and after a logistic regression analysis using the age at disease onset and neck pain as covariates:

*Significant genotype association ($p < 0.05$) after logistic regression analysis with adjustment for age at disease onset and neck pain;

[†]Borderline genotype association ($0.05 \geq p < 0.1$) after logistic regression analysis with adjustment for age at disease onset and neck pain;

[#]Significant genotype association ($p < 0.05$) was lost after logistic regression analysis with adjustment for age at disease onset and neck pain.

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previous studies that sought clinical predictors of functional disability in AS [39–41].

Recent genome-wide association studies have provided valuable evidence supporting the involvement of genetic factors in the pathogenesis and prognosis of autoimmune diseases. The two major SNPs found in this study to be associated with development of severe physical function are located in the autoinflammatory genes *PTPN2* and *PSTPIP1*. Our study found a consistent association between AS functional severity and the C allele of the SNP *rs2542151* in the *PTPN2* gene. *PTPN2* is a remarkable gene, since it appears to influence most cells involved in the development of the immune system [42]. *PTPN2* encodes the T cell protein tyrosine phosphatase TCPTP, a key negative regulator of inflammatory responses. Abnormalities in tyrosine phosphorylation have been found to be involved in the pathogenesis of numerous human diseases, such as developmental defects, neoplastic disorders, immunodeficiency, and autoimmunity [43]. The C allele of the SNP *rs2542151* at *PTPN2* gene, which we found to influence AS functional severity, was previously found to be associated with other autoimmune diseases, such as Crohn's disease, type 1 diabetes, and rheumatoid arthritis [44–45]. The fact that the SNP *rs2542151* in *PTPN2* is associated with different

aspects of autoimmune diseases suggests that these diseases, including AS, could share common pathogenic mechanisms.

Another consistent association between poor physical function of AS patients and SNPs was found for the A allele of the polymorphism *rs2254441* in the *PSTPIP1* gene. *PSTPIP1* is a cytoskeleton-associated adaptor protein that regulates innate and adaptive immune responses. Although this pathway has been traditionally related to diseases associated with pyoderma gangraenousum, such as aseptic abscesses syndrome and chronic inflammatory bowel disease (IBD) [46], the SNP *rs2254441* has been recently reported as associated with psoriatic juvenile idiopathic arthritis, a disease included in the spondyloarthropathies group together with AS and IBD [47].

In addition to SNPs in *PTPN2* and *PSTPIP1* genes, a milder association with BASFI/t severe phenotype was observed for SNPs in the immunity-related GTPase family M protein (*IRGM*), transforming growth factor beta 3 (*TGFB3*), Toll-like receptor 4 (*TLR4*), and LDL receptor related protein 5 (*LRP5*) genes. *IRGM* is a human immunity-related GTPase which confers autophagic defense against intracellular pathogens such as *Mycobacterium tuberculosis* (BCG) and *Salmonella typhimurium*. This gene's polymorphism *rs10065172*, which we found associated with AS functional

severity, has been associated with increased tuberculosis risk in some populations [42] and is a well-established risk factor for IBD [43].

In addition to SNPs in *PTPN2* and *PSTPIP1* genes, a milder association with BASFI/t severe phenotype was seen for SNPs in the transforming growth factor beta 3 (*TGFB3*) and Toll-like receptor 4 (*TLR4*) genes. Regarding *TGFB3*, high serum levels of this protein have been linked to osteoporosis risk [48] and polymorphisms in *TGFB3* have been found to be associated with ossification of the posterior longitudinal ligament of the spine (OPLL) in the Japanese population [49]. To the best of our knowledge this is the first report which linked risk from allele G of rs2268624 in *TGFB3* with development of functional severity in AS.

The association of the SNPs rs4986790 and rs4986791 in the *TLR4* gene could represent the involvement of the innate immune system in the progression of AS. Dysregulation of Toll-like receptor (TLR)-related pathways, specifically upregulation of TLR4 and TLR5, has been reported in AS [50].

Of particular interest was to find an association between severe functional impairment and rs3736228 in *LRP5* gene, since a recently published study in the Chinese population has found several other SNPs in *LRP5* to be associated with AS susceptibility [47]. The SNP rs3736228 was also identified to be associated with gender dependent bone mass formation and may be implicated in the adaptation of bone to mechanical load in humans [48]. As far as we are concerned, this is the first report in which a link between polymorphisms involved in bone formation and AS functional severity is identified.

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The strength of this study lies in the large number of clinically well characterized AS patients. However, our study has some limitations. First, we performed a cross-sectional study in which we did not analyze the treatment administered to patients as a possible factor that influences physical outcome, since we did not have reliable data about which patients were on a specific therapy prior to their inclusion in the study. The lack of information about the treatment could have introduced some bias. Secondly, in spite of the clinical and genetic factors found to influence AS functional prognosis, we could not achieve a good predictive model for development of severe functional status by combining these factors. Thus, further research in this area in other cohorts or in prospective studies is needed to confirm which of these genetic markers in combination with clinical factors could identify an accurate predictive model for AS functional severity.

In conclusion, our results confirm that severe functional status in AS is associated with both clinical and genetic factors. We found that older age and neck pain at disease onset, lymphoid tyrosine phosphatase (*PTPN2*)-rs2542151, proline-serine-threonine phosphatase interacting protein 1 (*PSTPIP1*)-rs2254441 polymorphisms, are potential predictors of the development of severe functional status in AS patients. Validation of these results in other cohorts is required.

Author Contributions

Conceived and designed the experiments: RS AS JS ECE AB JM. Performed the experiments: NB MS AM DT MA. Analyzed the data: RS NB AS MS DT MA ECE JM. Contributed reagents/materials/analysis tools: AS ECE JM JS EC AB. Wrote the paper: RS AS NB ECE JM.

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