# ANKH and Susceptibility to and Severity of Ankylosing Spondylitis

FERNANDO MANUEL PIMENTEL-SANTOS, DARIO LIGEIRO, MAFALDA MATOS, ANA FILIPA MOURÃO, ELSA VIEIRA de SOUSA, PATRICIA PINTO, ANA RIBEIRO, HELENA SANTOS, ANABELA BARCELOS, FATIMA GODINHO, MARGARIDA CRUZ, JOAO EURICO FONSECA, HENRIQUE GUEDES-PINTO, HELDER TRINDADE, MATTHEW A. BROWN, and JAIME C. BRANCO, and the CORPOREA Study Group

***ABSTRACT.*** ***Objective.*** Unconfirmed reports describe association of ankylosing spondylitis (AS) with several candidate genes including *ANKH*. Cellular export of inorganic pyrophosphate is regulated by the ANK protein, and mutant mice (ank/ank), which have a premature stop codon in the 3' end of the *ank* gene, develop severe ankylosis. We tested the association between single-nucleotide polymorphisms (SNP) in these genes and susceptibility to AS in a population of patients with AS. We investigated the role of these genes in terms of functional (BASFI) and metrological (BASMI) measures, and the association with radiological severity (mSASSS).

***Methods.*** Our study was conducted on 355 patients with AS and 95 ethnically matched healthy controls. AS was defined according to the modified New York criteria. Four SNP in *ANKH* (rs27356, rs26307, rs25957, and rs28006) were genotyped. Association analysis was performed using Cochrane-Armitage and linear regression tests for dichotomous and quantitative variables. Analyses of Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), BASFI, and mSASSS were controlled for sex and disease duration.

***Results.*** None of the 4 markers showed significant single-locus disease associations ($p > 0.05$), suggesting that *ANKH* was not a major determinant of AS susceptibility in our population. No association was observed between these SNP and age at symptom onset, BASDAI, BASFI, BASMI, or mSASSS.

***Conclusion.*** These results confirm data in white Europeans that *ANKH* is probably not a major determinant of susceptibility to AS. *ANKH* polymorphisms do not markedly influence AS disease severity, as measured by BASMI and mSASSS. (First Release Nov 15 2011; J Rheumatol 2012;39:131–4; doi:10.3899/jrheum.110681)

*Key Indexing Terms:*
ANKYLOSING SPONDYLITIS GENETIC PREDISPOSITION MORBIDITY

*From CEDOC, Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Lisbon; Instituto de Biotecnologia e Bioengenharia, Centro de Genómica e Biotecnologia, da Universidade de Trás-os-Montes e Alto Douro (IBB/CGB-UTAD), Vila Real; Serviço de Reumatologia, Centro Hospitalar Lisboa Ocidental (CHLO), Hospital de Egas Moniz EPE, Lisbon; Departamento Genética, Centro de Histocompatibilidade do Sul, Lisbon; Unidade de Investigação em Reumatologia, Instituto de Medicina Molecular (IMM), Faculdade de Medicina da Universidade de Lisboa, Lisbon; Serviço de Reumatologia, Centro Hospitalar de Lisboa Norte, Hospital de Santa Maria EPE, Lisbon; Unidade de Reumatologia, Centro Hospitalar Vila Nova Gaia/Espinho EPE, Vila Nova Gaia; Serviço de Reumatologia, Centro Hospitalar Alto Minho, Hospital Conde de Bertiandos EPE, Ponte de Lima; Serviço de Reumatologia, Instituto Português de Reumatologia, Lisbon; Serviço de Reumatologia, Hospital Infante D. Pedro EPE, Aveiro; Serviço de Reumatologia, Hospital Garcia de Orta EPE, Almada; Unidade de Reumatologia, Centro Hospitalar das Caldas da Rainha, Caldas da Rainha, Portugal; and University of Queensland Diamantina Institute, Princess Alexandra Hospital, Woolloongabba, Australia.*

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*F.M. Pimentel-Santos, MD, MSc, CEDOC, Faculdade de Ciências Médicas da Universidade Nova de Lisboa; IBB/CGB-UTAD, Vila Real; Serviço de Reumatologia, CHLO, Hospital de Egas Moniz EPE; D. Ligeiro, BSc, Departamento Genética, Centro de Histocompatibilidade do Sul; M. Matos, BSc, Msc, IBB/CGB-UTAD; A.F. Mourão, MD, CEDOC, Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Serviço de Reumatologia, CHLO, Hospital de Egas Moniz EPE; E. Vieira de Sousa, MD, Unidade de Investigação em Reumatologia, Instituto de Medicina Molecular (IMM), Faculdade de Medicina da Universidade de Lisboa, Serviço de Reumatologia, Centro Hospitalar de Lisboa Norte, Hospital de Santa Maria EPE; P. Pinto, MD, Unidade de Reumatologia, Centro Hospitalar Vila Nova Gaia/Espinho EPE; A. Ribeiro, MD, Serviço de Reumatologia, CHLO, Hospital de Egas Moniz EPE; H. Santos, MD, Serviço de Reumatologia, Instituto Português de Reumatologia; A. Barcelos, MD, Serviço de Reumatologia, Hospital Infante D. Pedro EPE; F. Godinho, MD, Serviço de Reumatologia, Hospital Garcia de Orta EPE; M. Cruz, MD, Unidade de Reumatologia, Centro Hospitalar das Caldas da Rainha; J.E. Fonseca, MD, PhD, Unidade de Investigação em Reumatologia, IMM, Faculdade de Medicina da Universidade de Lisboa, Serviço de Reumatologia, Centro Hospitalar de Lisboa Norte, Hospital de Santa Maria EPE; H. Guedes-Pinto, PhD, IBB/CGB-UTAD; H. Trindade, MD, PhD, CEDOC, Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Departamento Genética, Centro de Histocompatibilidade do Sul; M.A. Brown, MD, PhD, University of Queensland Diamantina Institute, Princess Alexandra Hospital; J.C. Branco, MD, PhD, CEDOC, Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Serviço de Reumatologia, CHLO, Hospital de Egas Moniz EPE.*

*Address correspondence to Dr. F.M. Pimentel-Santos, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, CEDOC, Campo Mártires da Pátria 130, Lisbon 1169-056, Portugal.*
*E-mail: pimentel.santos@gmail.com*

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Ankylosing spondylitis (AS) is a chronic inflammatory arthropathy, with an estimated prevalence of 0.1%–0.9% in white populations[1]. Genetic factors play a major role in the risk of developing AS[2,3], and influence several measures of disease severity, including the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Functional Index (BASFI)[4,5], and Bath AS radiographic index (BASRI)[6]. Studies have shown that sex influences susceptibility and disease severity. The prevalence of AS is 2.5 times higher in men than in women[7], and women have a later onset of the disease and less thoracic and lumbar spinal radiographic severity[8]. The pathophysiological mechanisms underlying these differences remain unclear. It is unlikely that the major genetic factors involved are X-linked because there is no linkage of AS susceptibility with X-chromosome markers[9].

The *ANKH* gene is of particular interest in AS, as mice with a loss-of-function mutation in the homologous gene, *ank*, develop severe ectopic mineralization and skeletal ankylosis resembling AS[10]. Humans with gain-of-expression mutations and polymorphisms in this gene develop calcium pyrophosphate chondrocalcinosis[11,12], whereas loss-of-function mutations cause excess hydroxyapatite deposition in Jackson's craniometaphyseal dysplasia disease[13,14]. A family has recently been described with a spondyloarthropathy with some similarities to AS due to homozygosity for a loss-of-function *ANKH* mutation[15]. An initial study of *ANKH* showed no association with susceptibility to AS[16], and no association has been identified with this gene in genomewide association studies in AS to date[17,18]. However, weak positive findings have been reported by some investigators[19], and it has been suggested that the association may be more strongly observed in women[20]. Further, no study has investigated the association of *ANKH* variants with radiographic or joint metrology indices. We sought to test the association between single-nucleotide polymorphisms (SNP) in *ANKH* and susceptibility to AS in a Portuguese population. Additionally we investigated the association of *ANKH* with functional (BASFI) and metrological (Bath Ankylosing Spondylitis Metrology Index; BASMI) measures, and for association with radiological severity (modified Stoke Ankylosing Spondylitis Spine Score; mSASSS).

## MATERIALS AND METHODS

*Subjects*. The study was conducted on 355 unrelated patients with AS and 95 ethnically matched healthy controls, all of Portuguese ancestry. AS was defined according to the modified New York criteria[21]. Cases were recruited from hospital outpatient departments; controls were healthy Portuguese bone marrow donors. Our study was approved by the ethics board of the study centers involved, and written informed consent was obtained from the participating individuals.

Patients completed a questionnaire self-assessment of clinical features, including the BASDAI and the BASFI. Age at disease onset was defined as the age at symptom onset, and disease duration was defined as the period of time (years) after symptom onset. Metrology investigation was performed by 1 investigator (FPS) to obtain the BASMI score. Radiological evaluation was performed using the mSASSS; all radiographs were scored independently by 2 authors (FPS, AFM). Where there was discordance between the scores, they were reevaluated by both reviewers for a consensus score. Data on current therapy were collected.

*Genotyping*. Genomic DNA from cases and controls was prepared from peripheral blood lymphocytes using standard techniques. Samples were genotyped for *ANKH* allelic variants (rs27356, rs26307, rs25957, and rs28006) that had previously been associated with AS in either men or women[20]. Taqman® SNP assays (Applied Biosystems, Foster City, CA, USA) were used for genotyping, performed according to the manufacturer's protocols. Genotyping reactions were performed with an ABI 7900HT instrument, and the allele call by analysis of allelic discrimination plots with ABI SDS 2.3 software. Replicate known and negative genotype control samples were typed in each 96-well plate.

*Statistical analysis*. SNP genotype data were assessed for missing data and for Hardy-Weinberg equilibrium in controls. Individuals with > 10% missingness were excluded. Association analysis was performed using the Cochrane-Armitage test as implemented in the PLINK program (Harvard University, Cambridge, MA, USA; Website: http://pngu.mgh.harvard.edu/~purcell/ plink/gplink.shtml). Association between SNP and the quantitative variables age of symptom onset, BASDAI, BASFI, BASMI, and mSASSS were tested by linear regression assuming an additive model using PLINK, taking into account sex and disease duration as covariates. Statistical power was tested using the Genetic Power Calculator[22].

## RESULTS

The AS cohort population (n = 355) included 224 (63.1%) men and 131 (36.9%) women with a mean age of 45.4 (SD ± 13.2) years (range 20–79 yrs) and a mean disease duration of 19.1 (SD ± 12.6) years (range 0–60 yrs), of whom 82% were HLA-B27-positive. The therapies used were similar in all patients, with the exception of nonsteroidal antiinflammatory drugs, which were used in a greater percentage of women (92.6%) than men (80.4%). Thus differences in therapy between sexes are unlikely to be an explanation of observed differences in AS activity or severity. Epidemiological data of the cases are summarized in Table 1.

All genetic markers studied were in Hardy-Weinberg equilibrium in the control group, with missing-ness rates < 10%, and there were no observations of differential missing-ness in cases and controls ($p < 0.01$). The minor allele frequencies (MAF) of the 4 SNP are presented in Table 2.

None of the 4 studied markers showed significant single-locus disease associations ($p > 0.05$), in the whole group and in subanalysis by sex, suggesting that *ANKH* gene is not a major determinant of AS susceptibility in a Portuguese population (Table 2). In addition, no association was observed between these SNP and age of symptom onset, BASDAI, BASFI, BASMI, or mSASSS when considering the whole population (Table 3). The sample size in individual sex groups was too small for a worthwhile analysis.

The study had 80% power to detect association with AS ($p < 0.05$, assuming a population prevalence of 0.5%, with D' = 1) with an additive OR of 1.9, and to detect association with quantitative measures (BASDAI, BASFI, BASMI, mSASSS) contributing > 3% of the trait variance.

## DISCUSSION

We analyzed 4 intronic markers previously described as asso-

*Table 1*. Characteristics of the Portuguese AS cases (n = 355). Except where indicated otherwise, values are the mean (standers deviation).

| | Males | Females | p |
|---|---|---|---|
| No. (%) | 224 (63) | 131 (37) | NA |
| Age, yrs | 45.49 (13.36) | 44.88 (12.53) | NS |
| Age of symptom onset, yrs | 25.73 (10.63) | 27.20 (10.43) | NS |
| Disease duration, yrs | 19.78 (12.17) | 17.59 (12.86) | NS |
| BASDAI | 3.75 (2.17) | 4.89 (2.28) | < 0.01 |
| BASFI | 3.77 (2.63) | 4.59 (2.69) | < 0.01 |
| BASMI | 4.26 (2.60) | 3.56 (2.24) | < 0.05 |
| mSASSS | 26.97 (24.52) | 10.16 (14.85) | < 0.05 |
| Therapy (%) | | | |
| NSAID | 182 (80.4) | 122 (92.6) | NA |
| Corticosteroids | 43 (19) | 22 (16.5) | NA |
| DMARD | 119 (52.6) | 67 (50.4) | NA |
| Anti-TNF-α | 55 (24.2) | 27 (20.6) | NA |

AS: ankylosing spondylitis; BASDAI: Bath AS Disease Activity Index; BASFI: Bath AS Functional Index; BASMI: Bath AS Metrology Index; mSASSS: modified Stoke Ankylosing Spondylitis Spine Score; NSAID: nonsteroidal antiinflammatory drugs; DMARD: disease-modifying antirheumatic drugs; NA: not applicable; NS: not significant; TNF: tumor necrosis factor.

ciated with AS in men (rs26307, rs27356) or women (rs28006, rs25957), in a study of 201 multiplex families[20]. In our study, involving unrelated patients with AS, we demonstrate that *ANKH* is not significantly associated with either susceptibility to AS or measures of its activity or severity, either in the whole group or in men or women separately. There are several possible explanations for the discrepancy of the results observed in the 2 studies: intrinsic differences between the 2 populations (North Americans vs Portuguese subjects) or differences in the patient populations (multiplex families vs unrelated individuals). Finally, both studies were underpowered to detect genes with small effects consistently, potentially leading to discrepancies between results. Despite the methodological differences (ethnicity, case ascertainment approaches, and *ANKH* marker variants analyzed), this investigation reinforces the results of another study in white Europeans[16], where no associations with disease susceptibility or phenotypic characteristics were seen. Our current study extends these previous observations, in that it is the first study to test *ANKH* associations with metrological (BASMI) and radiological (mSASSS) indices. Given the previous findings in mice and humans with loss-of-function *ANKH* mutants, we hypothesized that *ANKH* polymorphisms may contribute to spinal ossification in AS. This effect would be more easily detected by the potential influence on variables of the BASMI and mSASSS. Even considering these aspects, *ANKH* variants appeared to have no significant role in our population.

Several major ossification pathways have been identified that may play a central role in diseases characterized by bone formation, such as AS. They involve transforming growth factor-ß[23,24], bone morphogenetic proteins[25,26], and the wingless (Wnt) proteins[27,28]. Further studies investigating these pathways in larger datasets are indicated to identify genes influencing the severity and rate of ankylosis in AS.

Our results confirm previous data in white Britons that *ANKH* is not a major determinant of susceptibility to AS[16], and also demonstrate that *ANKH* variants do not have a major

*Table 2*. *ANKH* minor allele frequencies (MAF) in the Portuguese AS cohort.

| | | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|---|
| NCBI SNP Reference | Minor Allele | MAF, Cases | MAF, Controls | p for trend | OR (95% CI) | MAF, Cases | MAF, Controls | p for trend | OR (95% CI) |
| rs26307 | T | 0.21 | 0.24 | 0.43 | 1.25 (0.82–1.92) | 0.22 | 0.15 | 0.65 | 1.14 (0.73–1.78) |
| rs27356 | C | 0.22 | 0.22 | 0.29 | 1.35 (0.88–2.07) | 0.21 | 0.17 | 0.61 | 1.16 (0.75–1.81) |
| rs28006 | T | 0.31 | 0.32 | 0.68 | 0.88 (0.59–1.31) | 0.28 | 0.35 | 0.84 | 0.93 (0.62–1.41) |
| rs25957 | C | 0.31 | 0.34 | 0.70 | 0.89 (0.59–1.32) | 0.28 | 0.35 | 0.81 | 0.92 (0.61–1.39) |

AS: ankylosing spondylitis; NCBI: US National Center for Biotechnology Information; SNP: single-nucleotide polymorphism.

*Table 3*. Association between *ANKH* single-nucleotide polymorphisms and phenotypic characteristics of ankylosing spondylitis (AS).

| | Age at Disease Onset | BASDAI | BASFI | BASMI | mSASSS* |
|---|---|---|---|---|---|
| rs26307 | 0.6311 | 0.7821 | 0.8728 | 0.1211 | 0.08/0.32/0.16 |
| rs27356 | 0.6162 | 0.9569 | 0.9327 | 0.06895 | 0.14/0.28/0.22 |
| rs28006 | 0.4899 | 0.442 | 0.09126 | 0.9675 | 0.3/0.43/0.73 |
| rs25957 | 0.4577 | 0.4478 | 0.1072 | 0.577 | 0.34/0.4/0.88 |

* Men/women/total. BASDAI: Bath AS Disease Activity Index; BASFI: Bath AS Functional Index; BASMI: Bath AS Metrology Index; mSASSS: modified Stroke Ankylosing Spondylitis Spine Score.

influence on severity of AS (measured by BASDAI, BASFI, BASMI, or mSASSS) or age at disease onset.


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## APPENDIX

List of study collaborators. The CORPOREA Study Group: Centro Hospitalar de Lisboa Ocidental, Hospital de Egas Moniz EPE, Lisbon: A.F. Mourão, A.A. de Matos, C. Ribeiro, F.M. Pimentel-Santos, J. Bravo Pimentão, J.C. Branco, M. Mateus, P. Nero, P. Araújo, S. Falcão, T.L. Pinto, W. Castelão; Unidade de Investigação em Reumatologia, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa: E. Vieira de Sousa, J. Caetano-Lopes, J.E. Fonseca; Instituto Português de Reumatologia, Lisbon: C. Silva, E. Simões, H. Madeira, H. Santos, J. Vaz Patto, J. Ferreira, M. Micaelo, M.J. Mediavilla, M. Sousa; Hospital Curry Cabral EPE, Lisbon: P. Soares Branco; Hospital Garcia de Orta EPE, Almada: F. Godinho, J. Canas da Silva, S. Garcês, V. Tavares; Centro Hospitalar do Alto Minho, Hospital Conde de Bertiandos EPE, Ponte de Lima: A. Ribeiro, D. Araújo, J.A. Costa, L. Costa, M.C. Afonso, M. Bogas, S. Alcino; Centro Hospitalar de Vila Nova de Gaia/Espinho EPE, Vila Nova de Gaia: P. Pinto; Hospital Infante D Pedro EPE, Aveiro: A. Barcelos, I. Silva, C. Ambrósio; Centro Hospitalar de Oeste Norte, Centro Hospitalar das Caldas da Rainha, Caldas da Rainha: M. Cruz; Hospital de Faro EPE, Faro: G. Sequeira; Hospital Espírito Santo EPE, Évora: A.R. Cravo; Hospital Militar Principal, Lisbon: R.A. Santos.

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