ORIGINAL RESEARCH

Predictive value of C-reactive protein for radiographic spinal progression in axial spondyloarthritis in dependence on genetic determinants of fibrin clot formation and fibrinolysis

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ABSTRACT

Objective Genetic determinants of fibrin clot formation and fibrinolysis have an impact on local and systemic inflammatory response. The aim of the present study was to assess whether coagulation-related genotypes affect the predictive value of C-reactive protein (CRP) in regards of radiographic spinal progression in axial spondyloarthritis (axSpA).

Methods Two hundred and eight patients with axSpA from the German Spondyloarthritis Inception Cohort were characterised for genotypes of α-fibrinogen, β-fibrinogen (FGB) and γ-fibrinogen, factor XIII A-subunit (F13A) and α_2-antiplasmin (A2AP). The relation between CRP levels and radiographic spinal progression defined as worsening of the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS) by ≥2 points over 2 years was assessed in dependence on the respective genetic background in logistic regression analyses.

Results Overall, CRP was associated with mSASSS progression ≥2 points: time-averaged CRP ≥10 mg/L, OR: 3.32, 95% CI 1.35 to 8.13. After stratification for coagulation-related genotypes, CRP was strongly associated with mSASSS progression in individuals predisposed to form loose, fibrinolysis-susceptible fibrin clots (FGB rs1800790GG, OR: 6.86, 95% CI 2.08 to 22.6; A2AP 6Trp, OR: 5.86, 95% CI 1.63 to 21.0; F13A 34Leu, OR: 8.72, 95% CI 1.69 to 45.1), while in genotypes predisposing to stable fibrin clots, the association was absent or weak (FGB rs1800790A, OR: 0.83, 95% CI 0.14 to 4.84; A2AP 6Arg/Arg, OR: 1.47, 95% CI 0.35 to 6.19; F13A 34Val/Val, OR: 1.72, 95% CI 0.52 to 5.71).

Conclusions Elevated CRP levels seem to be clearly associated with radiographic spinal progression only if patients are predisposed for loose fibrin clots with high susceptibility to fibrinolysis.

Key messages

What is already known about this topic?
► C-reactive protein (CRP), presence of syndesmophytes and smoking are predictors for radiographic spinal progression in axial spondyloarthritis.
► Inflammation and tissue repair are involved in pathogenesis of axial spondyloarthritis.
► Genetic determinants of fibrin clot formation and fibrinolysis influence inflammatory processes.

What does this study add?
► The predictive potential of CRP in respect of radiographic spinal progression in axial spondyloarthritis depends on genotype (constellations) influencing fibrin clot formation and fibrinolysis.
► Elevated CRP levels at baseline are associated with radiographic spinal progression in axial spondyloarthritis only if patients are predisposed for loose fibrin clots with low antifibrinolytic capacity.

How might this impact on clinical practice or future developments?
► Enhancement of prognostic evaluation of patients with axial spondyloarthritis by considering their genetic background could help to improve risk stratification and to focus treatment strategies.

INTRODUCTION

Structural damage in the spine in patients with axial spondyloarthritis (axSpA) is—together with inflammatory activity—one of the main determinants of spinal mobility and function, parameters, which are relevant for long-term outcome of disease.[1–4] Even though the pathophysiology of structural spinal damage in axSpA is not fully understood, current data suggest that initial inflammation in vertebral bodies is followed by activation of repair processes with formation of fibrous tissue, activation of osteoblasts and formation of new bone.[5] New spinal bone formation in patients with axSpA is usually assessed on

plain spinal X-rays and, therefore, referred to as radiographic spinal progression. A number of predictors of radiographic spinal progression have been identified, including already existing syndesmophytes, cigarette smoking, male sex, manual work and high inflammatory activity reflected by elevated C-reactive protein (CRP), high Ankylosing Spondylitis Disease Activity Score and active inflammatory or postinflammatory changes on MRI.[6–14]

Fibrin and factor XIII play essential roles in inflammatory processes and bone development.[15 16] Fibrin gel properties are affected by inflammation and genetic factors.[15 17 18] Reciprocally, genetic factors affecting fibrinogen synthesis (α-fibrinogen, FGA, β-fibrinogen, FGB, γ-fibrinogen, FGG), fibrin clot formation (factor XIII A-subunit/B-subunit) and fibrinolysis resistance ($α_2$-antiplasmin, A2AP) influence both local and systemic inflammatory activity.[19–21] Thus, highly frequent genotype constellations predisposing for tight, fibrinolysis-resistant fibrin clot structures attenuate systemic inflammatory response as measured by CRP and those related to looser fibrin gels, which are more susceptible for fibrinolysis, are associated with stronger CRP responses.[20 22–24]

As genetic factors affecting fibrin clot properties influence CRP responses[19] and processes with potential impact on reactive bone remodelling,[15 25–27] this topic gives a target for evaluating its effect on CRP-based prediction models in axSpA. In the GErman SPondyloarthritis Inception Cohort (GESPIC), approximately 14% of patients exhibited radiographic spinal progression over 2 years and about 2/3 of progressing patients presented with CRP elevations.[7] Currently, it is widely unclear, why some patients with unelevated CRP exhibit a progress and what differentiates progressing and nonprogressing patients with axSpA with high inflammatory activity.

The objective of this study was to investigate the performance of the above-mentioned CRP-based prediction model for radiographic spinal progression in patients with axSpA,[7] when considering the genetic background with influence on fibrin clot formation and fibrinolysis.

METHODS
Patients and clinical assessment
Patients with axSpA who completed a 2-year clinical and radiographic follow-up in GESPIC were studied.[7 28] They were included in GESPIC if they had definite axSpA, either radiographic (r-axSpA, also referred to as ankylosing spondylitis) or non-radiographic form (nr-axSpA). All patients included were of European Caucasian origin. Clinical and laboratory data were collected at baseline, and every 6 months thereafter, radiographic data (cervical spine lateral view, lumbar spine lateral and anteroposterior views) were collected at baseline and after 2 years. Treatment of patients with GESPIC has been done according to local rheumatologists, without any limitations[28] (table 1).

Assessment of radiographic spinal progression
Cervical and lumbar radiographs (lateral views) were scored independently by two trained readers (DP and HH) in concealed and randomly selected order according to modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS).[29] Final mSASSS was calculated as mean of two scores produced by individual readers. Meaningful radiographic spinal progression was defined as worsening of mSASSS by ≥2 points after 2 years. In addition to mSASSS, we assessed syndesmophytes on both lateral views and lumbar anteroposterior view as described previously.[7]

Genotyping
Patients were analysed for fibrinogen genotypes influencing fibrinogen levels[30–32]: FGA rs6050A>G, FGA rs2070006G>A, FGA rs2070016T>C, FGB rs1800788C>T, FGB rs1800790G>A, FGG rs1049636T>C. Variants of F13A, F13B[15 17 33] and A2AP[34 35] were characterised, which influence fibrin cross-linking and/or susceptibility to fibrinolysis: F13A Val34Leu (rs5985G>T), F13A Tyr204Phe (rs3024477A>T), F13B His95Arg (rs6003A>G), A2AP Arg6Trp (rs2070863C>T). Genotypes were determined using LightSNiP assays (TIB MOLBIOL, Berlin, Germany).

Statistics
In logistic regression analyses, radiographic spinal progression (mSASSS by ≥2 points after 2 years) was used as dependent variable. A priori, risk covariable CRP elevation was defined either (1) as time-averaged CRP level (CRP_{mean}) from up to five time points >6 mg/L[7] or (2) ≥10 mg/L.[22] Additionally, CRP levels at baseline ($CRP_{baseline}$) ≥10 mg/L were used for categorisation. Analyses were adjusted for presence of syndesmophytes at baseline, smoking, presence of definite radiographic sacroiliitis according to modified New York criteria and sex. Analyses were performed separately for subgroups defined by presence/absence of minor allele carriage of respective genotypes. ORs with 95% confidence intervals (95% CIs) were calculated and given for presence/absence of the genotype tested, respectively. Subsequently, interaction terms between CRP and the stratifying variable (ie, presence/absence of minor allele) were included in logistic regression analyses to test for interaction between both variables. OR and 95% CI of interaction terms as well as p values for homogeneity were given. Hereby, a p<0.05 indicates that the relation between CRP and radiographic spinal progression significantly differs between both genotype-defined subgroups.

For testing our hypothesis, assessment was carried out in stepwise order: Primarily, FGB rs1800790G>A genotype with strong influence on fibrinogen synthesis[36] was used for subgrouping. Second, subgrouping was performed by F13A Val34Leu,[17] A2AP Arg6Trp[35] and F13B His95Arg,[37] which modulate influence fibrin cross-linking and/or fibrinolysis resistance. Subsequently, composite prothrombotic constellations of fibrinogen and F13A Val34Leu genotypes ($P^{FGB\ rs1800790G>A}$ and P^{FGA}

Table 1 Baseline demographic and clinical characteristics of included patients with axial spondyloarthritis

Parameter	All patients (n=208)	r-axSpA (n=115)	nr-axSpA (n=93)
Age, years	37.3±10.6	36.1±11.0	38.6±10.0
Duration of symptoms, years	4.2±2.7	5.0±2.8	3.1±2.2
Male sex, n (%)	106 (51)	75 (65.2)	31 (33.3)
HLA-B27 carrier, n (%)	165 (79.3)	97 (84.3)	68 (73.9)
Peripheral arthritis, n (%)	31 (14.9)	15 (13.0)	16 (17.2)
Enthesitis*, n (%)	45 (21.6)	23 (20.5)	22 (23.9)
Uveitis ever, n (%)	45 (21.6)	27 (23.5)	18 (19.4)
Psoriasis ever, n (%)	28 (13.5)	18 (15.7)	10 (10.8)
IBD ever, n (%)	4 (1.9)	3 (2.6)	1 (1.1)
Family history of SpA, n (%)	35 (16.8)	19 (16.5)	16 (17.2)
BASDAI, points NRS (0–10)	3.9±2.1	3.8±2.2	4.1±2.0
BASFI, points NRS (0–10)	2.9±2.3	3.0±2.4	2.8±2.2
BASMI, points (0–10)	1.8±1.6	2.0±1.7	1.5±1.5
$CRP_{baseline}$, mg/L	7.48±8.6	9.64±9.9	4.82±5.6
mSASSS, points	4.3±8.4	5.9±10.3	2.3±4.3
Smoking, n (%)	63 (30.3)	39 (33.9)	24 (25.8)
Treatment with NSAIDs, n (%)	139 (66.8)	76 (66.1)	63 (67.7)
Treatment with DMARDs, n (%)	53 (25.5)	31 (26.9)	22 (23.6)
Treatment with systemic steroids, n (%)	12 (5.8)	6 (5.2)	6 (6.4)
Treatment with a TNF-α inhibitor, n (%)	5 (2.4)	4 (3.7)	1 (1.1)

*The Berlin score assesses 12 enthesitis sites of lower limbs plus optional sites elsewhere. Continuous variables are presented as mean±SD.
BASDAI, bath ankylosing spondylitis disease activity index; BASFI, bath ankylosing spondylitis functional index; BASMI, bath ankylosing spondylitis metrology index; $CRP_{baseline}$, C-reactive protein level at baseline; DMARD, disease-modifying anti-rheumatic drug; IBD, inflammatory bowel disease; mSASSS, modified Stoke Ankylosing Spondylitis Spinal Score; nr-axSpA, non-radiographic axial spondyloarthritis at baseline; NSAID, non-steroidal anti-inflammatory drug; r-axSpA, radiographic axial spondyloarthritis at baseline; TNF-α, tumour necrosis factor alpha.

$rs2070016T>C$) were studied (online supplemental text).[20] These prothrombotic constellations of fibrinogen and F13A Val34Leu genotypes predispose for highly cross-linked, dense (so-called prothrombotic) fibrin structures and influence CRP responses.[20] Finally, additional fibrinogen genotypes were addressed.[20]

To account for measurement errors in analyses using dichotomised mSASSS change,[38] linear regression analyses were carried out with mSASSS change as continuous dependent variable that incorporates both, positive (progression) and negative (usually considered as measurement error of reading exercised blinded for time point) values of mSASSS change (online supplemental text). Performance of $CRP_{baseline} \geq 10\,mg/L$ in predicting mSASSS change was visualised in cumulative probability plots (figure 1). We used STROBE cohort reporting guidelines.[39]

Patient and public involvement

Representatives of the German patient with axSpA organisation provided advice on outcomes in GESPIC and recommend ways to facilitate study participation while minimising burden of study visits.

RESULTS
Clinical characteristics and genotype distribution

A total of 208 of 210 patients with follow-up after 2 years were genotyped and included in this study. Baseline characteristics are presented in table 1. A total of 30 patients (14.4%) demonstrated radiographic spinal progression defined as worsening of the mSASSS (≥2 points) after 2 years. Genotype distributions in the total study population as well as in r-axSpA and nr-axSpA patients are given in online supplemental table 1.

Relation of radiographic spinal progression and time-averaged CRP levels in dependence on FGB rs1800790G>A genotype

According to the predictive model described earlier,[7] we performed analyses in respect of radiographic spinal progression stratified for FGB rs1800790 A-allele carriage using time-averaged CRP levels (CRP_{mean}) categorised for >6 mg/L[7] or ≥10 mg/L[22] (table 2).

As described earlier, using the model without genotype-defined subgrouping, CRP_{mean} (>6 mg/L) was associated with radiographic spinal progression
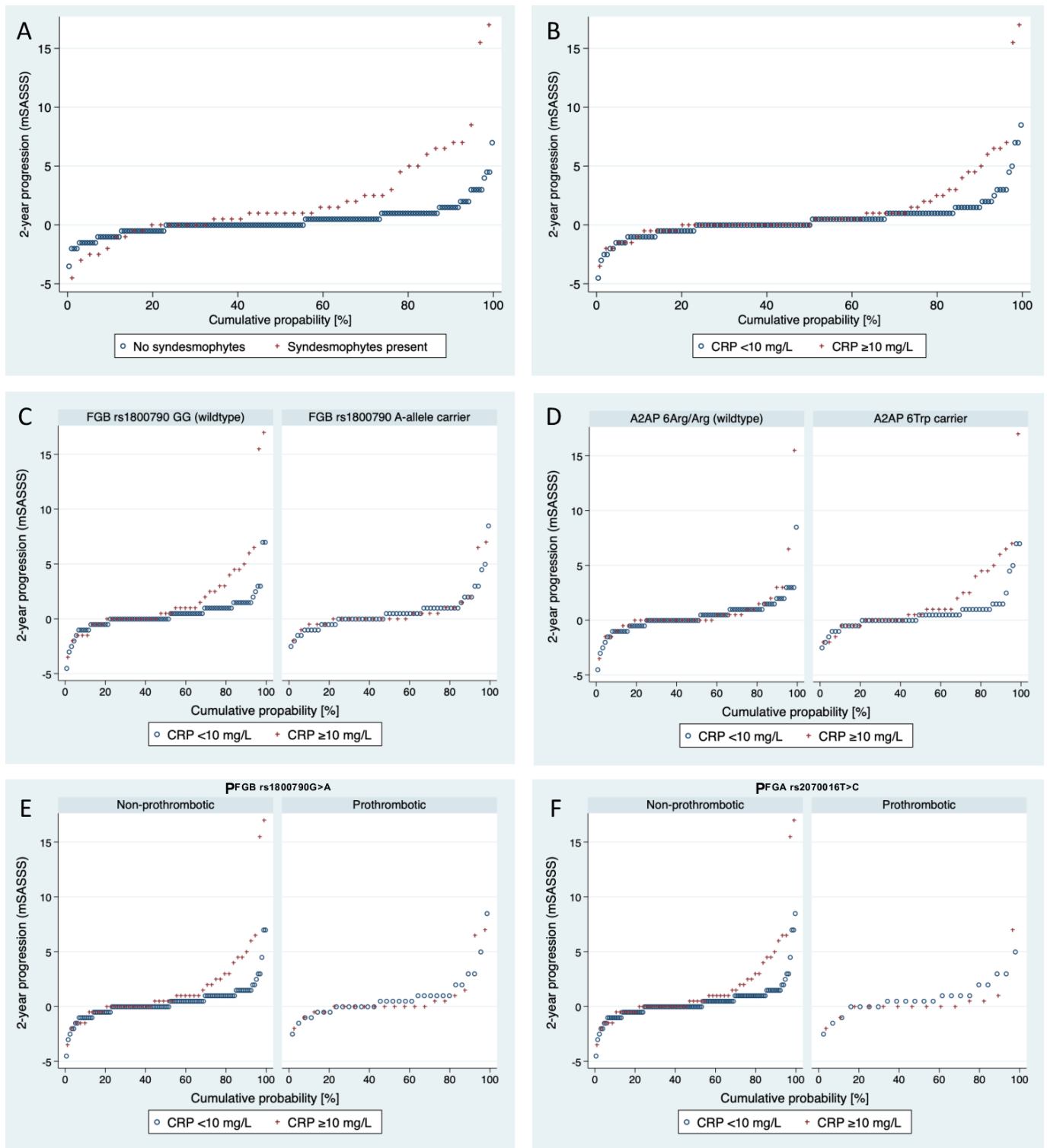
Figure 1 Cumulative probability plots of radiographic spinal progression after 2 years according to mSASSS in relation to the presence or absence of (A) syndesmophytes at baseline and (B) CRP elevations. Plots for CRP additionally are subgrouped for (C) FGB rs1800790G>A, (D) A2AP Arg6Trp, (E) P$^{FGB \ rs1800790G>A}$ and (F) P$^{FGA \ rs2070016T>C}$. A2AP, α_2-antiplasmin; CRP, C-reactive protein; FGB, β-fibrinogen; mSASSS, modified Stoke Ankylosing Spondylitis Spinal Score.

(mSASSS ≥2 points)[7]: OR: 2.56, 95% CI 1.03 to 6.35. When analysing genotype-defined subgroups separately, this association considerably depended on underlying FGB rs1800790G>A genotypes. In FGB rs1800790GG wild types, CRP$_{mean}$ exhibited a strong association with

mSASSS progression (OR: 5.64, 95% CI 1.56 to 20.3), whereas it was completely missing in carriers of FGB rs1800790 A-allele (OR: 0.99, 95% CI 0.20 to 4.92). However, heterogeneity between both subgroups was not statistically significant (interaction term, OR: 0.28,

Table 2 Analysis of parameters associated with radiographic spinal progression (worsening of mSASSS by ≥2 points after 2 years) in axial spondyloarthritis stratified by FGB rs1800790G>A genotype

Parameters	Unstratified OR (95% CI) (n=208)	FGB rs1800790GG (wild-type) OR (95% CI) (n=124)	FGB rs1800790A (A-allele carriage) OR (95% CI) (n=84)
CRP_{mean}			
>6 mg/L (model 1)	2.56 (1.03 to 6.35)	5.64† (1.56 to 20.3)	0.99† (0.20 to 4.92)
≥10 mg/L (model 2)	3.32 (1.35 to 8.13)	6.86* (2.08 to 22.6)	0.83* (0.14 to 4.84)
Syndesmophytes			
Model 1	4.70 (1.92 to 11.5)	3.25 (1.00 to 10.6)	12.2 (2.12 to 70.5)
Model 2	4.82 (1.96 to 11.9)	3.47 (1.06 to 11.4)	12.7 (2.14 to 75.2)
Current smoking			
Model 1	2.37 (0.99 to 5.67)	1.39 (0.45 to 4.31)	6.08 (1.18 to 31.2)
Model 2	2.26 (0.93 to 5.48)	1.51 (0.48 to 4.77)	6.28 (1.19 to 33.2)

Logistic regression analyses were performed in two models using either CRP_{mean}>6 mg/L (model 1) or CRP_{mean}≥10 mg/L (model 2). Syndesmophytes at baseline and current smoking were used as covariables. All analyses were adjusted for sex and for presence of definite radiographic sacroiliitis according to modified New York criteria. Data of unstratified analysis as well as after stratification for FGB rs1800790GG and FGB rs1800790A are presented.
*P for homogeneity: 0.11.
†P for homogeneity: 0.17.
CRP_{mean}, time-average level of C-reactive protein; FGB, β-fibrinogen; mSASSS, modified Stoke Ankylosing Spondylitis Spinal Score.

95% CI 0.04 to 1.76, p for homogeneity: 0.17). In respect of presence of syndesmophytes at baseline or current smoking as predictors of radiographic progression an opposite effect could be found. In both cases, associations with radiographic progression were restricted on carriers of the FGB rs1800790 A-allele (syndesmophytes, OR: 12.2, 95% CI 2.12 to 70.5; current smoking, OR: 6.08, 95% CI 1.18 to 31.2).

Based on CRP_{mean}≥10 mg/L, the strength of association between CRP_{mean} and mSASSS progression in FGB rs1800790GG wild-types was further increased (OR: 6.86, 95% CI 2.08 to 22.6) and heterogeneity between both subgroups was more pronounced compared with a cut-off level of CPR_{mean}>6 mg/L (interaction term, OR: 0.22, 95% CI 0.03 to 1.42, p for homogeneity: 0.11) (table 2). Results of linear regression analyses are given in online supplemental table 2. In following sections, analyses were based on CRP_{mean}≥10 mg/L.

Analyses on relation between time-averaged CRP levels and radiographic spinal progression in dependence on F13A, A2AP and F13B genotypes

We tested whether variability of other genes related to fibrin cross-linking and/or fibrinolysis resistance influences association between CRP_{mean} (≥10 mg/L) and radiographic spinal progression. Interestingly, for these genotypes, similar effects as for FGB rs1800790G>A could be found in the prognostic model (table 3).

Carriers of the respective minor allele(s) of F13A Val34Leu, A2AP Arg6Trp or F13B His95Arg exhibited a strong, highly significant relation between elevated CRP_{mean} (≥10 mg/L) and radiographic progression. In contrary, in corresponding wild-type associations of CRP_{mean} and mSASSS progression were clearly diminished.

Results of linear regression analyses are given in online supplemental table 2.

Relation of radiographic spinal progression and time-averaged CRP levels in dependence on potentially prothrombotic genotype constellations

As the effect of fibrinogen and factor XIII genetics on inflammatory processes results from their interactive influence on fibrin clot formation, we assessed the performance of the predictive model in dependence of prothrombotic genotype constellations $P^{FGB\ rs1800790G>A}$ and $P^{FGA\ rs2070016T>C}$, which are associated with inflammation.[20]

For both genotypes, in absence of the prothrombotic constellation, CRP_{mean} (≥10 mg/L) was strongly associated with mSASSS progression and in presence of the prothrombotic constellation, this association was considerably diminished (table 4) ($P^{FGB\ rs1800790G>A}$: interaction term, OR: 0.13, 95% CI 0.02 to 0.98, p for homogeneity: 0.048; $P^{FGA\ rs2070016T>C}$: interactions term, OR: 0.15, 95% CI 0.02 to 1.39, p for homogeneity: 0.094).

Influence of genetic variants in FGB, FGA and FGG on relation between time-averaged CRP levels and radiographic spinal progression

For FGB rs1800788C>T (wild-type, OR: 5.91, 95% CI 1.52 to 23.0; T-allele carriers, OR: 1.83, 95% CI 0.47 to 7.15; p for homogeneity: 0.50), FGA rs6050A>G (wild-type, OR: 4.76, 95% CI 1.48 to 15.3; G-allele carriers, OR: 2.23, 95% CI 0.40 to 12.4; p for homogeneity: 0.16), FGA rs2070016T>C (wild type, OR: 4.10, 95% CI 1.43 to 11.8; C-allele carriers, OR: 1.22, 95% CI 0.15 to 9.72; p for homogeneity: 0.66), again, a tendency for heterogeneity between wild-types and minor allele carriers in respect

Table 3 Analysis of parameters associated with radiographic spinal progression (worsening of mSASSS by ≥2 points after 2 years) in axial spondyloarthritis stratified by A2AP Arg6Trp, F13A Val34Leu or F13B His95Arg genotype

Genotype	CRP$_{mean}$≥10 mg/L OR (95% CI)	Syndesmophytes OR (95% CI)	Current smoking OR (95% CI)
A2AP Arg6Trp			
6Arg/Arg (n=116)	1.47‡ (0.35 to 6.19)	4.80 (1.16 to 19.9)	6.59 (1.73 to 25.1)
6Trp (n=93)	5.86‡ (1.63 to 21.0)	5.76 (1.55 to 21.4)	0.74 (0.17 to 3.11)
F13A Val34Leu			
34Val/Val (n=117)	1.72* (0.52 to 5.71)	4.65 (1.41 to 15.3)	1.08 (0.33 to 3.52)
34Leu (n=91)	8.72* (1.69 to 45.1)	9.47 (1.76 to 51.0)	5.15 (1.10 to 24.0)
F13B His95Arg			
95His/His (n=142)	2.36† (0.66 to 8.43)	15.1 (3.31 to 69.3)	4.11 (1.26 to 13.4)
95Arg (n=67)	6.84† (1.51 to 30.9)	2.61 (0.56 to 12.1)	1.14 (0.25 to 5.25)

Logistic regression analyses were performed after stratification as indicated using CRP$_{mean}$≥10 mg/L, syndesmophytes at baseline and current smoking. All analyses were adjusted for sex and for presence of definite radiographic sacroiliitis according to modified New York criteria.
*P for homogeneity: 0.21.
†P for homogeneity: 0.30.
‡P for homogeneity: 0.18.
A2AP, α2-antiplasmin; CRP$_{mean}$, time-averaged level of C-reactive protein; F13A, factor XIII A-subunit; F13B, factor XIII B-subunit; mSASSS, modified Stoke Ankylosing Spondylitis Spinal Score.

of association of CRP$_{mean}$ (≥10 mg/L) and mSASSS worsening was found.

Relation of radiographic spinal progression and CRP level at baseline

From a clinical and research perspective, prognostic information yielded from baseline data on course of structural damage progression would be of interest.

In the total study population, CRP$_{baseline}$ elevation (≥10 mg/L) tended to be associated with mSASSS progression (OR: 2.25, 95% CI 0.91 to 5.56). In FGB rs1800790GG wild types, this association was highly significant (OR: 5.71, 95% CI 1.69 to 19.3) and completely missing in FGB rs1800790 A-allele carriers (OR: 0.38, 95% CI 0.06 to 2.53) with relevant heterogeneity between both subgroups (interaction term, OR: 0.13, 95% CI 0.02 to 0.92, p for homogeneity: 0.041) (table 5). The same holds true, when stratifying for absence (OR: 4.98, 95% CI 1.57 to 15.7) and presence (OR: 0.26, 95% CI 0.03 to 2.34) of P$^{FGB\ rs1800790G>A}$ (interaction term, OR: 0.09, 95% CI 0.01 to 0.72, p for homogeneity: 0.024) (table 5).

For A2AP Arg6Trp (wild type, OR: 0.63, 95% CI 0.14 to 2.86; minor allele carrier, OR: 4.51, 95% CI 1.24 to 16.5; interaction term, OR: 5.66, 95% CI 0.88 to 36.6, p for homogeneity: 0.069) and P$^{FGA\ rs2070016T>C}$ (nonprothrombotic, OR: 4.63, 95% CI 1.59 to 13.8; prothrombotic, OR: 0.14, 95% CI 0.005 to 3.85; interaction term, OR: 0.063, 95% CI 0.005 to 0.86, p for homogeneity: 0.038) considerable heterogeneity between both subgroups could be found, as well.

Table 4 Analyses of parameters associated with radiographic spinal progression (worsening of mSASSS by ≥2 points after 2 years) in axial spondyloarthritis stratified by the prothrombotic genotype constellations P$^{FGB\ rs1800790G>A}$ and P$^{FGA\ rs2070016T>C}$

Genotype	CRP$_{mean}$≥10 mg/L OR (95% CI)	Syndesmophytes OR (95% CI)	Current smoking OR (95% CI)
P$^{FGB\ rs1800790G>A}$			
Absent (n=156)	6.31† (2.09 to 19.0)	4.41 (1.49 to 13.1)	1.88 (0.65 to 5.41)
Present (n=52)	0.50† (0.06 to 4.00)	12.4 (1.53 to 100.8)	3.71 (0.50 to 27.2)
P$^{FGA\ rs2070016T>C}$			
Absent (n=172)	5.58* (1.97 to 15.8)	5.42 (1.90 to 15.4)	1.53 (0.56 to 4.22)
Present (n=36)	0.40* (0.02 to 6.38)	16.3 (0.78 to 338.9)	9.76 (0.83 to 114.5)

Logistic regression analyses were performed after stratification for absence or presence of the indicated prothrombotic genotype constellation using CRP$_{mean}$≥10 mg/L, syndesmophytes at baseline and current smoking. All analyses were adjusted for sex and for presence of definite radiographic sacroiliitis according to modified New York criteria.
*P for homogeneity: 0.094.
†P for homogeneity: 0.048.
CRP$_{mean}$, time-average level of C-reactive protein; FGA, α-fibrinogen; FGB, β-fibrinogen; mSASSS, modified Stoke Ankylosing Spondylitis Spinal Score.

Table 5 Analyses of parameters (including baseline CRP levels) associated with radiographic spinal progression (worsening of mSASSS by ≥2 points after 2 years) in axial spondyloarthritis stratified by the FGB rs1800790G>A or P[FGB rs1800790G>A]

Genotype	CRP$_{baseline}$ ≥10 mg/L OR (95% CI)	Syndesmophytes OR (95% CI)	Current smoking OR (95% CI)
FGB rs1800790G>A			
GG (wild-type) (n=124)	5.71† (1.69 to 19.3)	4.84 (1.44 to 16.3)	1.49 (0.47 to 4.72)
A-allele carrier (n=84)	0.38† (0.06 to 2.53)	14.2 (2.31 to 87.2)	6.69 (1.24 to 36.2)
P[FGB rs1800790G>A]			
Absent (n=156)	4.98* (1.57 to 15.7)	5.3 (1.76 to 16.0)	2.00 (0.70 to 5.67)
Present (n=52)	0.26* (0.03 to 2.34)	13.3 (1.69 to 104.1)	3.91 (0.48 to 31.6)

Logistic regression analyses were performed after stratification for absence or presence of the indicated genotype constellation using CRP$_{baseline}$ ≥10 mg/L, syndesmophytes at baseline and current smoking. All analyses were adjusted for sex and for presence of definite radiographic sacroiliitis according to modified New York criteria.
*P for homogeneity: 0.024.
†P for homogeneity: 0.041.
CRP$_{baseline}$, level of C-reactive protein at baseline; FGB, β-fibrinogen; mSASSS, modified Stoke Ankylosing Spondylitis Spinal Score.

The relation between CRP$_{baseline}$ and mSASSS change in genotype-defined subgroups is visualised in cumulative probability plots (figure 1).

Results of analyses on a possible direct relation between the genotypes tested and radiographic spinal progression or CRP levels are given in online supplemental text and online supplemental table 3.

DISCUSSION

Pathology of axSpA is characterised by prolonged and/or increased inflammatory response at the interface between cartilage and bone, presumably triggered and/or perpetuated by mechanical stress and finally resulting in dysbalanced bone formation, that is the main pathologic process behind radiographic spinal progression.[40] Until now, only CRP is used for risk stratification on top of already existing structural damage (syndesmophytes) in clinical practice.[7] When conjointly considering syndesmophytes, current smoking and CRP elevations, presence of syndesmophytes is strongly associated with radiographic spinal progression, while CRP and current smoking moderately contribute to prognostic risk assessment (table 2).

Our study was triggered by two facts. (1) Fibrin(ogen) derivatives and proteins related to fibrin clot formation and fibrinolysis modulate inflammation and tissue repair[15 16] and (2) genetic variants of gene loci, which influence fibrinogen synthesis,[30 32] fibrin clot formation/structure[17] or susceptibility to fibrinolysis[17 34 35 41 42] and also influence the magnitude of inflammatory activity.[20 22 24] Thus, we hypothesised that the genetic background of fibrinogen and related proteins could modify the predictive value of CRP in respect of radiographic spinal progression.

Data presented here are best comprehensible, when considering that genotype constellations predisposing for loose, fibrinolysis-susceptible fibrin clot structures are permissive for stronger CRP responses and that, vice versa, in genotype constellations resulting in tight, fibrinolysis-resistant so-called prothrombotic clot structures; CRP responses are comparatively diminished.[20 22 24] Consequently, local inflammation in vertebral bodies in patients with the latter genetic background might not be associated with a strong systemic CRP response. A lack of predictive value of CRP for radiographic spinal progression in this patient subgroup is a sequel.

In our study population, genotypes predisposing for increased fibrinogen synthesis (FGB rs1800790 A-allele carriage) (table 2), strong fibrinolysis resistance (carriage of A2AP 6Arg/Arg wild-type) (table 3) or for prothrombotic clot structures (P[FGB rs1800790G>A], P[FGA rs2070016T>C]) (table 4) abrogated completely association of CRP with radiographic spinal progression (mSASSS change) (figure 1). All these genotypes are known to diminish inflammatory activity.[20 22 24] On the other hand, in individuals carrying genotypes related to lower fibrinogen synthesis (FGB rs1800790GG wild-types), higher susceptibility to fibrinolysis (A2AP 6Trp carriers, F13A 34Leu carriers) and/or less crosslinked, loose fibrin clot structures elevated CRP levels were strongly associated with radiographic spinal progression (tables 2–4 and figure 1). These genotype constellations are permissive for stronger CRP responses,[20 22] and in those patients elevated CRP can be considered as good predictor of subsequent structural damage progression. Associations in these cases were considerably stronger compared with the association without considering the genetic background. Thus, our findings seem consistently to indicate that in axSpA, the predictive value of CRP elevation in respect of increased radiographic spinal progression may be limited to individuals, which are not genetically predisposed to form so-called prothrombotic fibrin clot structures under proinflammatory triggers.

We have analysed 10 genotypes and the sample size of GESPIC is rather low. For this reason, results of statistical testing should be interpreted carefully. However, the fact that results derived from genotypes from four chromosomal locations (FGB/FGA, chromosome 4; F13A,

chromosome 6; F13B, chromosome 1 and A2AP, chromosome 17) were in agreement with the one tested hypothesis, in our view seems to support the assumption.

In our study population, even a single baseline CRP level (≥10 mg/L) was predictive for a meaningful radiographic progression, when the permissive genetic background was present. This finding could be relevant for prediction of progression and risk stratification in clinical practice and research.

In general, our results can be discussed in two ways. It could be assumed that only in individuals not genetically predisposed for prothrombotic fibrin clot structures, CRP-responses are sufficiently strong to reflect the magnitude of inflammation load in the axial skeleton. In other words, in patients predisposed for prothrombotic fibrin clots, CRP-responses could be diminished, hereby hampering the predictive value of CRP in respect of disease progress.

Otherwise, it could be hypothesised that loose fibrin gel structures with low anti-fibrinolytic potential are more permissive for cell migration into inflamed tissues, hereby modifying clinical course, allowing locally for stronger cytokine release and systemically for a more prominent CRP response. Hereby, genetics related to fibrin clot formation and fibrinolysis would be directly involved in processes of pathological inflammation and/or tissue repair in axSpA. This interpretation would help understand the missing association between these genotypes and CRP levels in patients with GESPIC (online supplemental table 3). FGB rs1800790G>A, P$^{FGB\ rs1800790G>A}$ and P$^{FGA\ rs2070016T>C}$ have been described to be strongly associated with CRP levels.[20 22] Additionally, in patients with rheumatoid arthritis, both composite genotype constellations were related to clinical disease activity.[20] If these genotype constellations in patients with axSpA would directly influence processes involved in pathogenesis, missing associations between genotypes and CRP could result from a direct influence of these genetic variants on clinical course in axSpA.

In respect of the inverse predictive behaviour of CRP and syndesmophytes/smoking in genotype-defined subgroups (tables 2–5), there are two possible explanations. Overall, CRP, smoking and syndesmophytes with some overlap are predictors for radiographic progression. In individuals with diminished CRP-response due to predisposition for tight fibrin gels, CRP does not adequately reflect inflammatory burden and consequently has reduced predictive value for progression. In these individuals, association strength between syndesmophytes/smoking with progression could increase due to the decreased predictive value of CRP. Another explanation could assume that individuals with a different genetic background behave biologically different.

The presented findings can be interpreted in a way that CRP cannot be considered without any limitation as a good predictive marker of radiographic spinal progression in the entirety of patients with axSpA or in a more conservative wording the findings could help to explain the clinical course in progressing patients without CRP elevations. The predictive potential of CRP in respect of radiographic spinal progression seems to be considerably increased, if a genetic background predisposing to loose, fibrinolysis-susceptible fibrin clots is given, and nearly absent otherwise. Further studies should address the question whether the same can also be true for CRP as predictor of treatment response in axSpA.

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REFERENCES

1 Machado P, Landewé R, Braun J, et al. Both structural damage and inflammation of the spine contribute to impairment of spinal mobility in patients with ankylosing spondylitis. *Ann Rheum Dis* 2010;69:1465–70.

2 Landewé R, Dougados M, Mielants H, et al. Physical function in ankylosing spondylitis is independently determined by both disease activity and radiographic damage of the spine. *Ann Rheum Dis* 2009;68:863–7.

3 Wanders A, Landewé R, Dougados M, et al. Association between radiographic damage of the spine and spinal mobility for individual patients with ankylosing spondylitis: can assessment of spinal mobility be a proxy for radiographic evaluation? *Ann Rheum Dis* 2005;64:988–94.

4 Poddubnyy D, Listing J, Haibel H, et al. Functional relevance of radiographic spinal progression in axial spondyloarthritis: results from the German spondyloarthritis inception cohort. *Rheumatology* 2018;57:703–11.

5 Poddubnyy D, Sieper J. Mechanism of new bone formation in axial spondyloarthritis. *Curr Rheumatol Rep* 2017;19:55.

6 Poddubnyy D, Haibel H, Listing J, et al. Cigarette smoking has a dose-dependent impact on progression of structural damage in the spine in patients with axial spondyloarthritis: results from the German spondyloarthritis inception cohort (GESPIC). *Ann Rheum Dis* 2013;72:1430–2.

7 Poddubnyy D, Haibel H, Listing J, et al. Baseline radiographic damage, elevated acute-phase reactant levels, and cigarette smoking status predict spinal radiographic progression in early axial spondylarthritis. *Arthritis Rheum* 2012;64:1388–98.

8 Poddubnyy D, Protopopov M, Haibel H, et al. High disease activity according to the ankylosing spondylitis disease activity score is associated with accelerated radiographic spinal progression in patients with early axial spondyloarthritis: results from the German spondyloarthritis inception cohort. *Ann Rheum Dis* 2016;75:2114–8.

9 van Tubergen A, Ramiro S, van der Heijde D, et al. Development of new syndesmophytes and bridges in ankylosing spondylitis and their predictors: a longitudinal study. *Ann Rheum Dis* 2012;71:518–23.

10 Ramiro S, Stolwijk C, van Tubergen A, et al. Evolution of radiographic damage in ankylosing spondylitis: a 12 year prospective follow-up of the OASIS study. *Ann Rheum Dis* 2015;74:52–9.

11 Ramiro S, van der Heijde D, van Tubergen A, et al. Higher disease activity leads to more structural damage in the spine in ankylosing spondylitis: 12-year longitudinal data from the OASIS cohort. *Ann Rheum Dis* 2014;73:1455–61.

12 Ramiro S, Landewé R, van Tubergen A, et al. Lifestyle factors may modify the effect of disease activity on radiographic progression in patients with ankylosing spondylitis: a longitudinal analysis. *RMD Open* 2015;1:e000153.

13 Machado PM, Baraliakos X, van der Heijde D, et al. Mri vertebral corner inflammation followed by fat deposition is the strongest contributor to the development of new bone at the same vertebral corner: a multilevel longitudinal analysis in patients with ankylosing spondylitis. *Ann Rheum Dis* 2016;75:1486–93.

14 Baraliakos X, Heldmann F, Callhoff J, et al. Which spinal lesions are associated with new bone formation in patients with ankylosing spondylitis treated with anti-TNF agents? a long-term observational study using MRI and conventional radiography. *Ann Rheum Dis* 2014;73:1819–25.

15 Muszbek L, Bereczky Z, Bagoly Z, et al. Factor XIII: a coagulation factor with multiple plasmatic and cellular functions. *Physiol Rev* 2011;91:931–72.

16 Weisel JW. Fibrinogen and fibrin. *Adv Protein Chem* 2005;70:247–99.

17 Lim BCB, Ariëns RAS, Carter AM, et al. Genetic regulation of fibrin structure and function: complex gene-environment interactions may modulate vascular risk. *Lancet* 2003;361:1424–31.

18 Undas A, Ariëns RAS. Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases. *Arterioscler Thromb Vasc Biol* 2011;31:e88–99.

19 Hoppe B, Dörner T. Coagulation and the fibrin network in rheumatic disease: a role beyond haemostasis. *Nat Rev Rheumatol* 2012;8:738–46.

20 Hoppe B, Schwedler C, Edelmann A, et al. Fibrinogen, factor XIII and α_2-antiplasmin genotypes are associated with inflammatory activity and anti-citrullinated protein antibodies. *Thromb Res* 2020;191:90–6.

21 Luyendyk JP, Schoenecker JG, Flick MJ. The multifaceted role of fibrinogen in tissue injury and inflammation. *Blood* 2019;133:511–20.

22 Hoppe B, Häupl T, Skapenko A, et al. Fibrinogen and factor XIII a-subunit genotypes interactively influence C-reactive protein levels during inflammation. *Ann Rheum Dis* 2012;71:1163–9.

23 Raghu H, Cruz C, Rewerts CL, et al. Transglutaminase factor XIII promotes arthritis through mechanisms linked to inflammation and bone erosion. *Blood* 2015;125:427–37.

24 Kovar FM, Marsik C, Jilma B, et al. The fibrinogen -148 C/T polymorphism influences inflammatory response in experimental endotoxemia in vivo. *Thromb Res* 2007;120:727–31.

25 Altieri DC. Regulation of leukocyte-endothelium interaction by fibrinogen. *Thromb Haemost* 1999;82:781–6.

26 Petzelbauer P, Zacharowski PA, Miyazaki Y, et al. The fibrin-derived peptide Bbeta15-42 protects the myocardium against ischemia-reperfusion injury. *Nat Med* 2005;11:298–304.

27 Lanir N, Ciano PS, Van de Water L, et al. Macrophage migration in fibrin gel matrices. II. Effects of clotting factor XIII, fibronectin, and glycosaminoglycan content on cell migration. *J Immunol* 1988;140:2340–9.

28 Rudwaleit M, Haibel H, Baraliakos X, et al. The early disease stage in axial spondylarthritis: results from the German spondyloarthritis inception cohort. *Arthritis Rheum* 2009;60:717–27.

29 Creemers MCW, Franssen MJAM, van't Hof MA, et al. Assessment of outcome in ankylosing spondylitis: an extended radiographic scoring system. *Ann Rheum Dis* 2005;64:127–9.

30 Jacquemin B, Antoniades C, Nyberg F, et al. Common genetic polymorphisms and haplotypes of fibrinogen alpha, beta, and gamma chains affect fibrinogen levels and the response to proinflammatory stimulation in myocardial infarction survivors: the AIRGENE study. *J Am Coll Cardiol* 2008;52:941–52.

31 Kolz M, Baumert J, Gohlke H, et al. Association study between variants in the fibrinogen gene cluster, fibrinogen levels and hypertension: results from the MONICA/KORA study. *Thromb Haemost* 2009;101:317–24.

32 Reiner AP, Carty CL, Carlson CS, et al. Association between patterns of nucleotide variation across the three fibrinogen genes and plasma fibrinogen levels: the coronary artery risk development in young adults (cardia) study. *J Thromb Haemost* 2006;4:1279–87.

33 Pruissen DMO, Slooter AJC, Rosendaal FR, et al. Coagulation factor XIII gene variation, oral contraceptives, and risk of ischemic stroke. *Blood* 2008;111:1282–6.

34 Christiansen VJ, Jackson KW, Lee KN, et al. The effect of a single nucleotide polymorphism on human alpha 2-antiplasmin activity. *Blood* 2007;109:5286–92.

35 Uitte de Willige S, Miedzak M, Carter AM, et al. Proteolytic and genetic variation of the alpha-2-antiplasmin C-terminus in myocardial infarction. *Blood* 2011;117:6694–701.

36 Tybjaerg-Hansen A, Agerholm-Larsen B, Humphries SE, et al. A common mutation (G-455--> A) in the beta-fibrinogen promoter is an independent predictor of plasma fibrinogen, but not of ischemic heart disease. A study of 9,127 individuals based on the Copenhagen City Heart Study. *J Clin Invest* 1997;99:3034–9.

37 Reiner AP, Heckbert SR, Vos HL, et al. Genetic variants of coagulation factor XIII, postmenopausal estrogen therapy, and risk of nonfatal myocardial infarction. *Blood* 2003;102:25–30.

38 Sepriano A, Ramiro S, Landewé R, et al. Percentage of progressors in imaging: can we ignore regressors? *RMD Open* 2019;5:e000848.

39 von Elm E, Altman DG, Egger M, et al. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Epidemiology* 2007;18:800–4.

40 Sieper J, Poddubnyy D. Axial spondyloarthritis. *Lancet* 2017;390:73–84.

41 Ajjan R, Lim BCB, Standeven KF, et al. Common variation in the C-terminal region of the fibrinogen beta-chain: effects on fibrin structure, fibrinolysis and clot rigidity. *Blood* 2008;111:643–50.

42 Collet JP, Park D, Lesty C, et al. Influence of fibrin network conformation and fibrin fiber diameter on fibrinolysis speed: dynamic and structural approaches by confocal microscopy. *Arterioscler Thromb Vasc Biol* 2000;20:1354–61.