

## EXTENDED REPORT

# Fibrinogen and factor XIII A-subunit genotypes interactively influence C-reactive protein levels during inflammation

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## ABSTRACT

**Objective** Fibrinogen is a target of autoimmune reactions in rheumatoid arthritis (RA). Fibrin(ogen) derivatives are involved in inflammatory processes and the generation of a stable fibrin network is necessary for sufficient inflammation control. As the density and stability of fibrin networks depend on complex interactions between factor XIII A (F13A) and fibrinogen genotypes, the authors studied whether these genotypes were related to C-reactive protein (CRP) levels during acute-phase reactions.

**Methods** Association between  $\alpha$ -fibrinogen (FGA),  $\beta$ -fibrinogen (FGB) and F13A genotypes with CRP levels was tested in two cohorts with longitudinal CRP measurements. Discovery and replication cohorts consisted of 288 RA (913 observations) and 636 non-RA patients (2541 observations), respectively.

**Results** Genotype FGB –455G>A (rs1800790) was associated with CRP elevations ( $\geq 10$  mg/l) in both cohorts (RA, OR per allele 0.69,  $p=0.0007/P_{\text{adj}}<0.015$ ; non-RA, OR 0.70,  $p=0.0004/p_{\text{adj}}<0.02$ ; combined, OR 0.69,  $p<10^{-5}/p_{\text{adj}}=0.001$ ). Genotype F13A 34VV (rs5985) was conditional for the association of FGB –455G>A with CRP as indicated by a clear restriction on F13A 34VV individuals and a highly significant heterogeneity between F13A 34VV and F13A 34L genotypes ( $p<10^{-5}$ ,  $p_{\text{adj}}=0.001$ ). In both cohorts, mean CRP levels significantly declined with ascending numbers of FGB –455A alleles. Genotype FGA T312A (rs6050) exhibited opposite effects on CRP compared with FGB –455G>A. Again, this relation was dependent on F13A V34L genotype.

**Conclusion** Novel genetic determinants of CRP completely unrelated to previously known CRP regulators were identified. Presumably, these haemostatic gene variants modulate inflammation by influencing fibrin crosslinking. These findings could give new perspectives on the genetic background of inflammation control.

Fibrin crosslinking by factor XIII is of crucial importance not only for haemostasis but also for inflammation control.<sup>1</sup> Several aspects of inflammatory processes including chemotaxis,<sup>2 3</sup> cell adhesion and migration<sup>4-7</sup> as well as the antimicrobial host defence<sup>1 8-10</sup> are dependent on or at least significantly influenced by fibrin(ogen) and its derivatives. In this context fibrin(ogen) functions as a modulator of different types of cell–cell interactions.<sup>7 11 12</sup> Finally, fibrinogen is involved in the pathogenesis

of rheumatoid arthritis (RA),<sup>13-15</sup> and it is a target of anticitrullinated protein antibodies (ACPA), which characterise a clinically more severe subset of RA.<sup>16</sup>

The architecture of the fibrin gel is a major determinant of clot rigidity and fibrinolysis resistance.<sup>17</sup> It depends on complex interactions between environmental and genetic factors.<sup>18</sup> There are two well-characterised determinants of the fibrin gel architecture. On the one hand, the fibrin gel structure is critically influenced by fibrinogen levels, which themselves exhibit a relevant genetic background.<sup>17-19</sup> Therefore, variants located in the fibrinogen gene loci, for example,  $\beta$ -fibrinogen (FGB) –455G>A (rs1800790) or  $\alpha$ -fibrinogen (FGA) T312A (rs6050), contribute to different amplitudes of fibrinogen level changes during acute-phase reactions.<sup>20-22</sup> On the other hand, the factor XIII subunit A (F13A) genotype, particularly the variant F13A V34L (rs5985), plays a major role in decisively modulating the aforementioned relation between fibrinogen concentration and fibrin gel structure.<sup>18</sup> In the current study, we hypothesised that genetic variants of fibrinogen and F13A that are known to alter fibrin crosslinking could concomitantly influence inflammatory processes. For functional reasons, the assessment of gene–gene interactions was a particular focus of the analyses.<sup>18 23</sup>

## PATIENTS AND METHODS

Initially, the relation between fibrinogen and F13A genotypes on C-reactive protein (CRP) levels was assessed in a RA cohort. A replication of the results was achieved in a separate cohort under differing inflammatory conditions (non-RA cohort). To allow for an observational assessment of genetic determinants of CRP levels, which are influenced by different factors, repeated measurement of CRP levels is necessary.<sup>24 25</sup> Therefore, analyses were restricted to patients, for whom measurements of CRP levels on at least two independent times of presentation were available. Ethical approval for this study was obtained from the local ethics committee. Written informed consent was obtained from each patient. Data have been analysed anonymously.

## RA cohort

The discovery cohort was derived from the RA study population described previously.<sup>26</sup> Of

## Clinical and epidemiological research

373 consecutive RA patients, all 288 patients who repeatedly presented in the Department of Rheumatology and Clinical Immunology (Berlin) between 2003 and 2008 (913 observations) were included. The patients fulfilled the American College of Rheumatology criteria for classification of RA.<sup>27</sup> To account for potential confounders of CRP levels, sex, disease duration, HLA-DRB1 shared epitope (SE), erosive joint disease,<sup>28</sup> and the individual treatment course characterised by cumulative therapy intensity<sup>26</sup> were assessed and included in the analyses.

### Non-RA cohort

The results of the RA cohort were replicated in an independent study population consisting of patients with no history of autoimmune diseases (non-RA cohort). This cohort was enrolled during 2005 and 2007 at our haemostaseology outpatient department (Charité – Universitätsmedizin Berlin). During this period 1276 patients presented, and 636 patients (2541 observations), for whom measurements of CRP levels on at least two independent times of presentation were available, were included. A considerable part of the non-RA cohort consisted of women followed during pregnancy because of a history of fetal loss, placental dysfunction or venous thromboembolism. Pregnancy is known as a mild proinflammatory condition with moderately elevated CRP levels.<sup>29</sup> These are increased further in the case of disturbed placentation or other pregnancy complications.<sup>30</sup> An association of CRP levels with the risk of venous thromboembolism has been described previously.<sup>31</sup> In the case of pregnancy, CRP levels have been determined regularly with each presentation. In all other cases, CRP measurement was prompted due to overt or suspected inflammation.

### Determination of fibrinogen, F13A and HLA-DRB1 genotypes

FGB –455G>A (rs1800790), FGA T312A (rs6050) and F13A V34L (rs5985) genotypes were determined using allele-specific primer pairs, as described.<sup>32–33</sup> SE was defined by HLA-DRB1 alleles with the following constellation at the corresponding DRβ1 chain: 67Leu–69Glu–71Lys or Arg–74Ala–86Gly or Val. HLA-DRB1 typing was performed using standard techniques (Dynal, Oslo, Norway; GenoVision, Vienna, Austria; Protrans, Hockenheim, Germany).<sup>34</sup>

### Autoantibody detection and measurement of CRP and D-dimer

ACPA were quantified by a second-generation ELISA using a cut-off value of 25 arbitrary units per millilitre (Euro-Diagnostica, Malmö, Sweden). Rheumatoid factor was quantified using an ELISA-based IgM-specific technique and a cut-off value of 24 IU per ml (DLD Diagnostika, Hamburg, Germany). Plasma CRP levels as well as D-dimer levels were quantified by immunoturbidimetric methods standardised according to the International Federation of Clinical Chemistry and Laboratory Medicine (Roche Diagnostics, Mannheim, Germany).

### Statistical analyses

For univariate analyses, OR and exact 95% CI were calculated. For calculations on allele–dose dependency, the corresponding wild type was used as a reference group, and a test for linear trend of the log odds (trend test) was performed. Power calculations have been performed on each cohort separately as well as on pooled cohorts. Based on a carrier frequency of FGB –455G>A and FGA 312A of approximately 0.40, statistical power for detecting an association strength of OR 1.75 was 62%, 92%

**Table 1** Clinical characteristics of the study populations

Characteristic	RA cohort	Non-RA cohort
No of cases	288	636
Total no of observations	913	2541
Women (%)	77.1	97.6
Age, median (IQR)*	51 (39–61)	32 (28–37)
Disease duration, median (IQR)	4.5 (1–10.9)	
Erosive disease (%)	62.5	
Presence of autoantibodies (%)		
ACPA	64.2	
RF	77.2	
HLA-DRB1 shared epitope (%)		
1 copy	49.4	
2 copies	13.5	
FGB –455G>A (%)		
1 copy	34.7	35.2
2 copies	6.9	5.8
FGA T312A (%)		
1 copy	38.2	39.1
2 copies	4.9	9.3
F13A V34L (%)		
1 copy	43.4	36.5
2 copies	8.7	8.0

\*RA patients: age at disease onset; non-RA patients: age at first presentation.

ACPA, anticitrullinated protein antibodies; F13A, factor XIIIa; FGA, α-fibrinogen; FGB, β-fibrinogen; RA, rheumatoid arthritis; RF, rheumatoid factor.

and 99% for the RA cohort, the non-RA cohort and for pooled analyses, respectively. When considering stratification for F13A V34L (carrier frequency of F13A 34L approximately 0.50) statistical power in respect of the fibrinogen genotypes and an association of OR 1.75 was: RA, 33%, non-RA, 64%, pooled, 82%. For multivariate analyses, OR and 95% CI were calculated by logistic regression analyses. Analyses on the RA cohort were adjusted for disease duration (quartiles), sex, treatment intensity, erosive joint disease and SE. The adjustment model of analyses based on the non-RA cohort comprised age (quartiles), sex, ethnicity and ongoing pregnancy including 12 weeks after delivery. To evaluate for potential interactive effects, the corresponding interaction terms were included in the logistic regression models as indicated (p for homogeneity).<sup>35</sup> In cases of repeated measurements, for univariate as well as multivariate analyses, robust clustered estimates of variances were calculated ( $p_{adj}$ ) to allow for intracluster correlation and to relax the requirement for independent observations.<sup>36</sup>

In addition to individual analysis of both cohorts, pooled analyses on both cohorts have been included. In these combined analyses, OR and 95% CI were calculated as crude as well as Mantel–Haenszel point estimates and the homogeneity between both cohorts was assessed by Breslow–Day testing.

Non-parametric comparisons of CRP levels between different genotype constellations were performed by the Mann–Whitney U test or in the case of allele–dose dependency by the Cuzick non-parametric test for trend. Adjustment for non-independent observations within clusters was performed by rank order Somers' D statistic ( $p_{adj}$ ). Statistical analyses were performed using Stata statistical software 10.1 for Macintosh (StataCorp, College Station, USA). To consider multiple testing a Bonferroni's corrected  $p < 0.0125$  was applied on the analyses of the discovery cohort.

Each polymorphism was analysed in respect of consistency with expected Hardy–Weinberg proportions by using Pearson  $\chi^2$  tests. Marker–marker linkage disequilibrium was assessed using Lewontin's D' statistic and  $r^2$  correlation statistic using Haploview 4.1 (Cambridge, USA).<sup>37</sup>

**Table 2** Allele-dose-dependent association of FGB –455G>A genotype with CRP level in RA as well as non-RA patients

	CRP $\geq 10$ mg/l			
	FGB –455GG	FGB –455GA	FGB –455AA	Trend test
	OR (reference)	OR (95% CI)	OR (95% CI)	p/p <sub>adj</sub> *
RA cohort				
Total				
Univariate	1.0	0.64 (0.48 to 0.86)	0.52 (0.29 to 0.93)	0.0007/<0.015
Multivariate	1.0	0.47 (0.30 to 0.74)	0.47 (0.16 to 1.38)	0.001/<0.03
F13A 34VV				
Univariate‡	1.0	0.56 (0.36 to 0.86)	0.28 (0.09 to 0.83)	0.0008/<0.02
Multivariate#	1.0	0.36 (0.19 to 0.71)	...†	<0.0008/0.01
F13A 34L				
Univariate‡	1.0	0.73 (0.48 to 1.09)	0.71 (0.35 to 1.44)	0.12/0.24
Multivariate§	1.0	0.59 (0.32 to 1.10)	0.73 (0.21 to 2.61)	0.14/0.33
Non-RA cohort				
Total				
Univariate	1.0	0.70 (0.54 to 0.91)	0.41 (0.21 to 0.79)	0.0004/<0.02
Multivariate	1.0	0.72 (0.56 to 0.95)	0.42 (0.22 to 0.82)	0.001/0.02
F13A 34VV				
Univariate¶	1.0	0.39 (0.27 to 0.58)	0.25 (0.10 to 0.62)	<10 <sup>-7</sup> /<0.0004
Multivariate**	1.0	0.40 (0.28 to 0.59)	0.26 (0.10 to 0.64)	<10 <sup>-6</sup> /<0.0005
F13A 34L				
Univariate¶	1.0	1.41 (0.95 to 2.09)	0.82 (0.32 to 2.13)	0.35/0.51
Multivariate**	1.0	1.47 (0.99 to 2.18)	0.74 (0.28 to 1.94)	0.38/0.50

Analyses based on the RA cohort (288 RA patients, 913 observations) and the non-RA cohort (636 non-RA patients, 2541 observations), respectively. Univariate and multivariate analyses (RA adjusted for disease duration, sex, erosive disease, shared epitope, therapy intensity; non-RA adjusted for sex, age, pregnancy, ethnicity) as well as trend tests across FGB –455A copy numbers are given.

\*p<sub>adj</sub>, p value after adjustment for non-independent observations within clusters.

†Ellipse indicates no CRP  $\geq 10$  mg/l in the FGB –455AA genotype.

‡p for homogeneity (RA, F13A 34VV vs F13A 34L)=0.12 (univariate).

§p for homogeneity (RA, F13A 34VV vs F13A 34L)=0.07 (multivariate).

¶p for homogeneity (non-RA, F13A 34VV vs F13A 34L)<10<sup>-5</sup> (univariate).

\*\*p for homogeneity (non-RA, F13A 34VV vs F13A 34L)<10<sup>-4</sup> (multivariate).

CRP, C-reactive protein; F13A, factor XIIIa; FGB,  $\beta$ -fibrinogen; RA, rheumatoid arthritis.

## RESULTS

### Clinical characteristics and genotype distributions

The clinical characteristics of both study populations are summarised in table 1. In the RA cohort, 288 patients with a total of 913 observations (number of observations per patient: 2 (n=17), 3 (n=205), 4 (n=66)) were included. The median interval between consecutive CRP measurements was 68 days (IQR 28–173 days). All patients of the RA cohort were of Caucasian origin. In the non-RA cohort, 636 patients with a total of 2541 observations (observations per patient: 2 (n=201), 3 (n=114), 4 (n=101), 5 (n=82), >5 (n=138)) were analysed. In this population, the median interval between consecutive CRP measurements was 36 days (IQR 31–46 days). The non-RA patients visited our haemostaseology unit because of recurrent fetal losses (45%), pregnancy complications (24%), and/or venous thromboembolism (16%). Approximately 86% of the non-RA patients presented during the course of pregnancy, and 88% were of Caucasian origin.

All observed genotype distributions were consistent with Hardy-Weinberg equilibrium. There was no evidence for a linkage disequilibrium between F13A V34L and HLA-DRB1 SE (D'=0.062, r<sup>2</sup>=0.0030) as well as between FGA T312A and FGB –455G>A (D'=0.435, r<sup>2</sup>=0.02).

### Plasma CRP levels depend on FGB –455G>A in RA patients: this effect is modulated by F13A V34L genotype

In the RA cohort, the presence of the FGB –455A allele was significantly associated with a lower frequency of CRP elevations

in univariate (p=0.0003, p<sub>adj</sub>=0.01) as well as multivariate analyses (p=0.0002, p<sub>adj</sub>=0.01) (see supplementary table S1, available online only). Notably, a subanalysis among ACPA-positive RA patients usually characterised by enhanced inflammatory activity showed a strong relation between FGB –455G>A and reduced odds for CRP elevations (176 patients, 557 observations) (p=0.0001, p<sub>adj</sub>=0.003) (see supplementary table S1, available online only). It needs emphasis that the influence of FGB –455G>A on CRP elevations ( $\geq 10$  mg/l) was significantly allele-dose dependent in univariate analyses and after adjustment for possible confounders (table 2).

In the whole study population, mean plasma CRP levels declined with ascending numbers of FGB –455A alleles (p=0.0007, p<sub>adj</sub>=0.01). This effect was most impressive in ACPA-positive RA. Here, mean CRP levels declined from 20.3 mg/l (95% CI 15.6 to 25.1) for the FGB –455GG (wild-type) genotype to 13.2 mg/l (95% CI 10.4 to 16.1) and 8.6 mg/l (95% CI 3.3 to 13.8) in carriers of one and two FGB –455A alleles, respectively (p<10<sup>-5</sup>, p<sub>adj</sub>=0.0006). In ACPA-negative RA a clear relation between FGB –455G>A and CRP could not be detected.

As there is strong evidence supporting a modulatory effect of the F13A V34L genotype on fibrin network formation,<sup>18 20</sup> we further tested whether the effect of FGB –455G>A on CRP elevations ( $\geq 10$  mg/l) was interrelated with the F13A V34L genotype (table 2). Indeed, the relation between FGB –455A allele number and CRP was restricted to F13A 34VV genotype (138 RA patients, 441 observations) in univariate and multivariate

**Table 3** Interactive influence of FGB –455G>A and F13A V34L genotype on plasma CRP level in the non-RA cohort

CRP (mg/l)	Carriage of FGB –455A versus FGB –455GG			
	Univariate	Trend test	Multivariate	Trend test
	OR (95% CI)	p/P <sub>adj</sub> *	OR (95% CI)	p/P <sub>adj</sub> *
Total				
≤5	1.0 (reference)		1.0 (reference)	
5–10	0.74 (0.60 to 0.90)		0.77 (0.62 to 0.94)	
10–15	0.71 (0.50 to 1.00)		0.75 (0.53 to 1.06)	
>15	0.52 (0.36 to 0.75)	<10 <sup>-5</sup> /0.009	0.54 (0.37 to 0.78)	<0.0001/<0.02
F13A 34VV				
≤5	1.0 (reference)		1.0 (reference)	
5–10	0.58 (0.45 to 0.77)		0.61 (0.46 to 0.81)	
10–15	0.39 (0.24 to 0.64)		0.41 (0.25 to 0.68)	
>15	0.27 (0.16 to 0.46)	<10 <sup>-10</sup> †/0.0001	0.28 (0.17 to 0.47)	<10 <sup>-9</sup> ‡/<0.0002
F13A 34L				
≤5	1.0 (reference)		1.0 (reference)	
5–10	0.97 (0.72 to 1.32)		0.99 (0.72 to 1.34)	
10–15	1.42 (0.86 to 2.35)		1.45 (0.87 to 2.41)	
>15	1.20 (0.69 to 2.10)	0.29†/0.63	1.23 (0.70 to 2.17)	0.35‡/0.56

Analyses based on 636 patients and 2541 observations. Results of univariate and multivariate analyses (adjusted for sex, age, pregnancy and ethnicity) as well as trend tests across ordinal CRP level categories are given.

\*p<sub>adj</sub>, p value after adjustment for non-independent observations within clusters.

†p for homogeneity (F13A 34VV vs F13A 34L) <10<sup>-6</sup> (univariate).

‡p for homogeneity (F13A 34VV vs F13A 34L) <10<sup>-6</sup> (multivariate).

CRP, C-reactive protein; F13A, factor XIIIa; FGB, β-fibrinogen; RA, rheumatoid arthritis.

analysis, suggesting an interaction of these genotypes in modulating the extent of inflammation. HLA-DRB1 SE did neither modulate the relation between FGB –455G>A and CRP nor was it related to CRP levels directly.

Finally, we tested whether fibrinogen and F13A genotypes were associated with erythrocyte sedimentation rate or markers of disease severity (ie, Steinbrocker score or disease activity score in 28 joints).<sup>38</sup> In ACPA-positive RA, FGB –455G>A exhibited a non-significant trend for lower erythrocyte sedimentation rate levels (Mann–Whitney U test, p=0.19). There was no relation between these genotypes and the Steinbrocker score or disease activity score in 28 joints (data not shown).

#### Plasma CRP levels depend on FGB –455G>A in non-RA patients: this effect is modulated by F13A V34L genotype

In order to replicate the above findings and to assess whether they were restricted to RA, we further tested the genetic markers in a non-RA cohort. In complete agreement with the results of the RA cohort, in the non-RA cohort the FGB –455G>A genotype exhibited a highly significant trend towards lower CRP level categories (table 3), and again, this effect was clearly allele–dose dependent (table 2). The CRP-modulating effect of FGB –455G>A exhibited a striking restriction to carriers of the F13A 34VV (wild-type) genotype indicating a highly significant interaction between FGB –455G>A and F13A V34L (p<10<sup>-5</sup>, table 2; p<10<sup>-6</sup>, table 3). The non-parametric comparison of CRP levels in dependence on the FGB –455A copy number (total, p<10<sup>-5</sup>, p<sub>adj</sub><0.01; F13A 34VV, p<10<sup>-11</sup>, p<sub>adj</sub><10<sup>-4</sup>; F13A 34L carriers, p=0.40, p<sub>adj</sub>=0.49) further corroborated the results.

#### Plasma CRP levels are influenced by FGA T312A in RA as well as in non-RA patients: this effect is restricted to F13A 34VV individuals

In F13A 34VV (wild-type) individuals, the FGB –455G>A genotype is negatively associated with CRP elevations in RA as well as in non-RA patients (see tables 2 and 3 as well as

supplementary table S1, available online only). Fibrinogen levels and fibrin gel formation are differentially influenced by the fibrinogen genotypes FGB –455G>A and FGA T312A.<sup>18,21,22</sup> We therefore tested the association of FGA T312A with CRP in both cohorts. In F13A 34VV individuals, FGA T312A was positively associated with CRP elevations (≥10 mg/l) in the non-RA as well as the RA cohort. The OR per FGA T312A allele were nearly identical in both cohorts (non-RA, OR 1.50, p<0.002, p<sub>adj</sub><0.05; RA, OR 1.49, p=0.02, p<sub>adj</sub><0.09). The F13A 34VV genotype was conditional for this relation, ie, in F13A 34L-positive individuals an association between FGA T312A and CRP could be detected neither in the RA nor in the non-RA cohort. The results of these analyses were in complete accordance with those of non-parametric comparisons of CRP levels. In both cohorts, the mean CRP levels increased with each FGA T312A allele. This effect was especially pronounced in F13A 34VV-positive RA patients, in whom mean CRP levels increased from 12.3 mg/l (95% CI 9.7 to 14.9) for the FGA T312T (wild-type) genotype to 21.9 mg/l (95% CI 12.7 to 31.0) and 21.0 mg/l (95% CI 11.4 to 30.5) in carriers of one and two FGA T312A alleles, respectively (p<10<sup>-4</sup>, p<sub>adj</sub><0.01).

#### Association of FGB –455G>A and FGA T312A with CRP levels: pooled analysis on RA and non-RA cohorts

The relations of FGB –455G>A and FGA T312A genotypes on CRP elevations (≥10 mg/l) were assessed in a pooled analysis on both cohorts (table 4). Again, FGB –455G>A and FGA T312A exhibited oppositely directed associations with CRP elevations (≥10 mg/l). For both genotypes the relations significantly differed between F13A 34VV and F13A 34L individuals, with the F13A 34VV genotype being conditional for the association. Importantly, there was no evidence of heterogeneity between the RA cohort and the non-RA cohort as indicated by Breslow–Day tests for homogeneity (table 4). These findings could also be confirmed when analysing the relation of FGB –455G>A and FGA T312A to categorical CRP levels (≤5 mg/l, 5–10 mg/l, 10–15 mg/l, >15 mg/l) (data not shown).

**Table 4** Allele-dose-dependent association of FGB -455G>A and FGA T312A genotype on plasma CRP levels: pooled analyses on RA as well as non-RA cohort

	CRP $\geq 10$ mg/l			
	FGB -455G>A*		FGA T312A†	
	Crude	Pooled (M-H)	Crude	Pooled (M-H)
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Total				
0 copy	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
1 copy	0.67 (0.56 to 0.82)	0.67 (0.55 to 0.82)	1.05 (0.87 to 1.26)	1.14 (0.94 to 1.38)
2 copies	0.50 (0.33 to 0.76)	0.46 (0.30 to 0.72)	0.91 (0.63 to 1.31)	1.13 (0.78 to 1.65)
p/p <sub>adj</sub> trend	<10 <sup>-5</sup> /0.001	<10 <sup>-6</sup> /0.0006	1.0/1.0	0.23/0.40
p <sub>BD</sub>	0.98		0.19	
F13A 34VV				
0 copy	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
1 copy	0.46 (0.35 to 0.61)	0.45 (0.34 to 0.61)	1.31 (1.01 to 1.70)	1.49 (1.14 to 1.96)
2 copies	0.25 (0.13 to 0.51)	0.26 (0.13 to 0.52)	1.64 (1.04 to 2.58)	2.11 (1.31 to 3.40)
p/p <sub>adj</sub> trend	<10 <sup>-10</sup> / $<0.00002$	<10 <sup>-9</sup> / $<0.00002$	0.008/0.07	0.0001/0.009
p <sub>BD</sub>	0.32		0.98	
F13A 34L				
0 copy	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
1 copy	1.01 (0.77 to 1.31)	1.02 (0.77 to 1.34)	0.83 (0.63 to 1.08)	0.83 (0.63 to 1.10)
2 copies	0.93 (0.54 to 1.58)	0.75 (0.42 to 1.32)	0.38 (0.19 to 0.75)	0.42 (0.20 to 0.85)
p/p <sub>adj</sub> trend	0.88/0.92	0.59/0.70	<0.01/0.04	0.01/0.06
p <sub>BD</sub>	0.08		0.25	

Pooled analyses on RA (288 patients, 913 observation) and non-RA cohort (636 patients, 2541 observations). Crude as well as Mantel-Haenszel (M-H) point estimates of OR and 95% CI are given.

\*p/p<sub>adj</sub> for homogeneity (F13A 34VV vs F13A 34L) for FGB <10<sup>-5</sup>/=0.001.

†p/p<sub>adj</sub> for homogeneity (F13A 34VV vs F13A 34L) for FGA=0.0001/=0.006.

CRP, C-reactive protein; F13A, factor XIIIa; FGA,  $\alpha$ -fibrinogen; FGB,  $\beta$ -fibrinogen; p<sub>adj</sub>, p value after adjustment for non-independent observations within clusters; p<sub>BD</sub>, Breslow-Day testing for homogeneity of OR between both cohorts; RA, rheumatoid arthritis.

### Relation of FGB -455G>A and FGA T312A genotypes to D-dimer levels in non-RA patients

We tested whether fibrinogen genotypes were associated with D-dimer levels in 607 non-RA patients (2069 observations). There was a significant correlation between the FGB -455A copy number and lower D-dimer levels (p<0.04), which persisted after adjusting for potential confounders (CRP, pregnancy, ethnicity, age and history of venous thromboembolism) (p<sub>adj</sub><0.04). The carriage of FGA 312A did not influence D-dimer levels significantly.

### DISCUSSION

In this report, we describe a modulating effect of fibrinogen genotypes on plasma CRP levels that is significantly dependent on the F13A V34L genotype. The involved genotypes are highly frequent and are known to exert an important effect on the haemostatic system predominantly by alteration of fibrin gel formation.<sup>17-19</sup> While weak associations of these genotypes with atherothrombosis<sup>18</sup> and venous thromboembolism<sup>39</sup> have been described previously, there are no data indicating a relation to the risk of RA. The results identified in a discovery cohort of RA patients were confirmed in an independent replication cohort of non-RA patients. In both cohorts, the measures of association strength were of striking comparability and there was no statistical evidence for heterogeneity between them. Replication in a non-RA cohort indicates that the relation between fibrinogen and F13A genotypes to CRP is independent of ACPA positivity and HLA-DRB1 SE-specific immune reactions.

As the data presented in our report are reminiscent of the relation between fibrinogen concentration and fibrin clot density in dependence on the F13A V34L genotype,<sup>18</sup> regulation of fibrin crosslinking should be described in more detail. The

fibrinogen level variation in the course of an acute-phase reaction is influenced by the fibrinogen genotype.<sup>20</sup> In this context, FGB -455G>A and FGA T312A favour higher and lower fibrinogen levels, respectively.<sup>21,22</sup> Fibrin crosslinking in turn is critically dependent on fibrinogen levels, and this relation is decisively modulated by the F13A V34L genotype.<sup>17,18</sup> In the case of the F13A 34VV genotype, the density and stability of the fibrin network strongly increases with increasing fibrinogen concentrations. In contrast, the fibrin clot structure becomes gradually less dependent on fibrinogen levels with ascending numbers of F13A 34L alleles.<sup>18</sup> In our study, the F13A 34VV genotype was also clearly conditional for the influence of FGB -455G>A and FGA T312A on CRP levels, and both fibrinogen genotypes were clearly inversely related to CRP. For example, the FGB -455G>A genotype, known to predispose to enhanced fibrinogen synthesis during inflammation,<sup>20</sup> exhibited significantly lower CRP levels (see tables 2-4 as well as supplementary table S1 available online only), while the opposite relation could be identified for FGA T312A (table 4). These relations were restricted to the F13A 34VV genotype, ie, to individuals who are prone to form tight fibrin clot structures during phases of elevated fibrinogen levels and vice versa.<sup>18</sup> The architecture of the fibrin gel matrix controls migratory processes.<sup>6,40</sup> Differences in fibrin crosslinking could thus be an explanation for the significant interaction between fibrinogen and F13A genotypes on CRP levels, which we describe in this study. Briefly, a predisposition to high fibrin clot densities during inflammation seems to be related to a less intense CRP elevation (see supplementary figure S1, available online only). The relation between FGB -455G>A and lower D-dimer levels identified in the non-RA cohort could be explained by increased fibrin clot density and consequently fibrinolysis resistance, and these data are completely compatible

## Clinical and epidemiological research

with this hypothesis. Alternatively, a genotype-dependent differential release of chemotactic or otherwise activating fibrin(ogen) derivatives<sup>2 10 41</sup> could give another explanation of our findings. The fact that the relation between FGB -455G>A and CRP is especially pronounced in ACPA-positive RA (see supplementary table S1, available online only) could be explained by increased inflammatory activity in this RA subset.

Most if not all previously described genetic traits that affect CRP levels were located either within the CRP locus itself or in genes related to proinflammatory cytokine pathways.<sup>42–45</sup> Of note, previous studies did not provide any indication for a dependency of CRP levels on genetic variants of haemostatic factors. The fibrinogen variant FGB -148C>T (rs1800787), which is in strong linkage disequilibrium with FGB -455G>A, has been implicated in a reduction of tumour necrosis factor alpha (TNF $\alpha$ ) and interleukin 6 responses after lipopolysaccharide exposure—effects on CRP levels have not been described.<sup>46</sup> It is widely accepted that single-locus association studies have a high likelihood to fail in the identification of risk factors, which exhibit significant interactive effects.<sup>23</sup> Therefore, published data, which until now were derived from locus-by-locus analyses, were presumably incapable of elucidating interactive effects like those described in our report.

Measurement of CRP levels is an important and practical tool for monitoring disease activity in RA and a number of other inflammatory diseases.<sup>47</sup> Recent data indicate that in RA enhanced CRP levels serve as an independent prognostic factor for progressive joint damage independent of the underlying treatment.<sup>48 49</sup> Therefore, the data of our study could be of clinical value, as they could help in characterising patients with respect to their presumptive CRP response, thereby allowing for an adaptation of CRP-directed clinical decision models based on the patient's genetic background.<sup>42</sup>

There are a few limitations of our study that should be recognised. First of all, our discovery cohort is small-sized, which results in a low statistical power, especially when considering analyses on stratified data. Therefore, it is of importance that the results could be confirmed in a larger replication cohort. Second, our study is primarily focused on CRP levels as a surrogate marker of inflammation. Further studies should include additional markers or mediators of inflammation to get more insight into the mechanisms underlying our findings. Finally, the described mechanisms that could potentially explain the results of our study are hypothetical. Even though the findings on the relation between fibrinogen genotypes and D-dimer levels are compatible with the described hypothesis, a more sophisticated analysis of circulating fibrin(ogen) fragments could additionally support this assumption. Finally, future experimental studies should help to elucidate the way fibrinogen and F13A genotypes influence inflammatory responses.

However, our findings give perspectives to elucidate a previously unknown genetic mechanism of inflammation control. Targeting the process of fibrin polymerisation and crosslinking could offer potential tools for pharmaceutical modulation of inflammatory processes.

**Contributors** BH developed the hypotheses and conceived and designed the study. GRB, SZ, TD, TH and BH coordinated sample acquisition and laboratory analyses. TD, TH and BH analysed and ASa, ASk, GRB, HSK, RT, TD, TH and BH interpreted the data. BH drafted the manuscript. ASa, ASk, GRB, HSK, RT, TD and TH critically revised the manuscript for important intellectual contents. All authors had full access to all data and approved the final version of the manuscript.

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## Fibrinogen and factor XIII A-subunit genotypes interactively influence C-reactive protein levels during inflammation

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