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Title: Genetic and environmental influences on stability and change in baseline levels of C-reactive protein: a longitudinal twin study

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Corresponding Author: Dr. Harold Snieder,

Corresponding Author's Institution:

First Author: Arthur A Sas

Order of Authors: Arthur A Sas; Ahmad Vaez; Yalda Jamshidi; Ilja M Nolte; Zoha Kamali; Tim D Spector; Harriette Riese, PhD; Harold Snieder

**Abstract:** Background and aims: Cross-sectional twin and family studies report a moderate heritability of baseline levels of C-reactive protein (CRP) ranging from 0.10 to 0.65 for different age ranges. Here, we investigated the stability and relative impact of genetic and environmental factors underlying serum levels of CRP, using a longitudinal classical twin design.

**Methods:** A maximum of 6,201 female twins from the TwinsUK registry with up to three CRP measurements (i.e. visit 1 [V1], visit 2 [V2] and visit 3 [V3]) over a 10 year follow-up period were included in this study. Structural equation modeling was applied to dissect the observed phenotypic variance into its genetic and environmental components. To estimate the heritability of CRP as well as its genetic and environmental correlations across different time points, a trivariate model was used. **Results:** Natural log (ln) CRP levels significantly increased from V1 to V2 ( $p=4.4 \times 10^{-25}$ ) and between V1 and V3 ( $p=1.2 \times 10^{-15}$ ), but not between V2 and V3. The median (IQR) follow-up time between V1 and V3 was 9.58 (8.00–10.46) years. Heritability estimates for CRP were around 50% and constant over time (0.46–0.52). Additionally adjustment for BMI did not meaningfully change the heritability estimates (0.49–0.51). The genetic correlations between visits were significantly smaller than one, ranging from 0.66 to 0.85.

**Conclusions:** The present study provides evidence for stable heritability estimates of CRP of around 50% with advancing age. However, between-visit genetic correlations are significantly lower than 1 indicating emergence of new genetic effects on CRP levels with age.

### Highlights

- Heritability estimates of C-reactive Protein (CRP) are around 50% and remain stable with advancing age.
- Adjustment for body mass index did not change heritability estimates of CRP.
- New genetic effects on CRP levels emerge with advancing age.

1 **Running title:** Genetic and environmental influences on stability of CRP

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4 **Genetic and environmental influences on stability and change in baseline**

5 **levels of C-reactive protein: A longitudinal twin study**

6  
7 Arthur A. Sas<sup>1\*</sup>, Ahmad Vaez<sup>1,2\*</sup>, Yalda Jamshidi<sup>3</sup>, Ilja M. Nolte<sup>1</sup>, Zoha Kamali<sup>4</sup>, Tim  
8 D. Spector<sup>5</sup>, Harriëtte Riese<sup>6</sup>, Harold Snieder<sup>1</sup>

9 \* Equal contributions

10  
11 <sup>1</sup>Department of Epidemiology, University of Groningen, University Medical Center  
12 Groningen, PO Box 30001, 9700 RB, Groningen, The Netherlands

13 <sup>2</sup>Research Institute for Primordial Prevention of Non-Communicable Disease, Isfahan  
14 University of Medical Sciences, Isfahan, Iran.

15 <sup>3</sup>Cardiogenetics Lab, Human Genetics Research Center, St. George's University of  
16 London, London SW17 0RE, United Kingdom

17 <sup>4</sup>Department of Genetics and Molecular Biology, Isfahan University of Medical  
18 Sciences, Isfahan, Iran.

19 <sup>5</sup>Department of Twin Research & Genetic Epidemiology, King's College, St. Thomas  
20 Campus, London SE1 7EH, United Kingdom

21 <sup>6</sup>Interdisciplinary Center Psychopathology and Emotion regulation, Department of  
22 Psychiatry, University of Groningen, University Medical Center Groningen, CC72, PO  
23 Box 30001, 9700 RB, Groningen, The Netherlands.

24

25 **Address for correspondence and reprints:**

26 Harold Snieder

27 Department of Epidemiology

28 University Medical Center Groningen

29 PO Box 30.001

30 9700 RB Groningen

31 The Netherlands

32 Phone: +31 50 361 0887

33 Fax: +31 50 36 14493

34 Email: h.snieder@umcg.nl

35

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41

42 **Abstract**

43 **Background and aims:** Cross-sectional twin and family studies report a moderate  
44 heritability of baseline levels of C-reactive protein (CRP) ranging from 0.10 to 0.65 for  
45 different age ranges. Here, we investigated the stability and relative impact of genetic  
46 and environmental factors underlying serum levels of CRP, using a longitudinal  
47 classical twin design.

48 **Methods:** A maximum of 6,201 female twins from the TwinsUK registry with up to  
49 three CRP measurements (i.e. visit 1 [V1], visit 2 [V2] and visit 3 [V3]) over a 10 year  
50 follow-up period were included in this study. Structural equation modeling was  
51 applied to dissect the observed phenotypic variance into its genetic and environ-  
52 mental components. To estimate the heritability of CRP as well as its genetic and  
53 environmental correlations across different time points, a trivariate model was used.

54 **Results:** Natural log (ln) CRP levels significantly increased from V1 to V2 ( $p=4.4 \times 10^{-25}$ )  
55 and between V1 and V3 ( $p=1.2 \times 10^{-15}$ ), but not between V2 and V3. The median  
56 (IQR) follow-up time between V1 and V3 was 9.58 (8.00-10.46) years. Heritability  
57 estimates for CRP were around 50% and constant over time (0.46-0.52). Additionally  
58 adjustment for BMI did not meaningful change the heritability estimates (0.49–0.51).  
59 The genetic correlations between visits were significantly smaller than one, ranging  
60 from 0.66 to 0.85.

61 **Conclusions:** The present study provides evidence for stable heritability estimates of  
62 CRP of around 50% with advancing age. However, between-visit genetic correlations  
63 are significantly lower than 1 indicating emergence of new genetic effects on CRP  
64 levels with age.

65 **Keywords:** aging, longitudinal, twins, heritability, C-reactive protein

## 67 Introduction

68 The link between ageing and inflammation is well established. Low levels of microbial  
69 exposition early in life is known to promote the development of more competent  
70 immune pathways and regulatory processes. Such effective anti-inflammatory  
71 networks may counterbalance proinflammatory pathways (and CRP levels) activated  
72 by chronic diseases such as obesity and atherosclerosis [1]. Furthermore, ageing is  
73 known to be associated with a gradual dysregulation of inflammatory pathways  
74 resulting in an elevation of inflammatory factors [2–5]. It has been demonstrated that  
75 chronic low grade inflammation predisposes to many chronic, age-related diseases,  
76 such as those of the pulmonary and cardiovascular system [6–9]. We have  
77 previously demonstrated the role of age as a moderator of the genetic and  
78 environmental influences on baseline levels of inflammatory markers.[10].

79 An important, well established inflammatory marker is C-reactive protein  
80 (CRP). Its baseline levels are considered to reflect systemic inflammation.

81 Considering the relationship of increased baseline CRP levels with a variety of  
82 disorders, including cancer [11], bipolar disorder [12], cardiovascular diseases [13–  
83 15], type 2 diabetes [16], and all-cause mortality [17], regulation of baseline CRP  
84 levels are of particular interest. In this context, baseline CRP levels have shown to be  
85 influenced by a variety of environmental and genetic factors. However, their relative  
86 importance and exact extent to which these factors account for the total variance in  
87 CRP level remains unknown [18].

88 Heritability studies aim to estimate the relative influence of heritable and  
89 environmental factors on a trait [19]. Twin and family studies in a wide variety of  
90 populations with different age ranges showed a moderate heritability of baseline CRP

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2 91 levels, with heritability estimates ranging from 0.10 to 0.65 [20–40] (Supplementary  
3 Table 3).

4 93 CRP levels have been shown to be fairly stable over time. DeGoma et al. [41]  
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6 94 analyzed serial CRP measures of 255 participants to evaluate the intraindividual  
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8 95 variability of CRP over a median follow up period of 4.7 years. The multivariable-  
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10 96 adjusted intraclass correlation coefficient (ICC) of CRP was estimated as 0.62. The  
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12 97 intraindividual variability of CRP was also investigated by Wu et al. [42], using CRP  
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14 98 levels of 56,218 Chinese adults over a two-year follow-up time. The ICC of CRP was  
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16 99 reported as 0.55 for men and 0.60 for women. Interestingly, the stability of CRP  
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21 100 gradually increased with age. However, twin and family studies mentioned above  
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23 101 used single CRP measurement for their heritability calculation rather than longitudinal  
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25 102 measurements. Limited by this cross-sectional design, heritability estimates for CRP  
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27 103 as reported above only provide a snapshot at one particular point in time, potentially  
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29 104 providing at least a partial explanation for the wide variety of heritability estimates  
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32 105 reported in the literature [20–40].  
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36 106 To the best of our knowledge, no longitudinal twin studies on CRP levels have  
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38 107 been conducted to date. The aim of this study was to evaluate the heritabilities and  
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40 108 the extent to which genetic and environmental influences contribute to the stability or  
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42 109 change of CRP over time in a large population of adult females using a classical twin  
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45 110 design, including up to three CRP measurements over a ten year follow-up period.  
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## 51 112 **Material and Methods**

### 52 113 *Subjects*

53 114 The study was conducted in 6,201 women from the Twins UK registry. Details of the  
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55 115 Twins UK registry have been published before [43]. Zygosity was determined by  
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116 questionnaire supplemented by DNA fingerprinting in cases with disputed or  
117 uncertain zygosity. CRP measurement follow-up was performed up to 3 times, giving  
118 6,201 measurements in visit 1 (1,457 monozygotic (MZ) pairs, 1,584 dizygotic (DZ)  
119 pairs and 119 singletons), 2,251 measurements in visit 2 (452 MZ-pairs, 632 DZ-  
120 pairs and 83 singletons) and 528 measurements in visit 3 (139 MZ-pairs, 112 DZ-  
121 pairs and 26 singletons).

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### 123 *C-reactive protein analysis*

124 High sensitive CRP was measured by latex-enhanced nephelometry on a Siemens  
125 (formally Behring) Prospec Nephelometer. The intra-assay precision expressed as  
126 coefficient of variation (CV) of this method is around 3.5% CV at 1.5 mg/l and 3.1% at  
127 12 mg/l and is expected to be <2% CV across the linear range of the assay.

128

### 129 *Analytical approach*

130 Natural log (ln) transformation was necessary for the CRP data in order to obtain a  
131 better approximation of the normal distribution. Secondly, lnCRP was adjusted for  
132 age. This is a common procedure in twin analyses because age can spuriously  
133 introduce a shared environmental effect if there is a significant correlation between  
134 the phenotype and age, because twins are always of the same age. Next, covariate  
135 analysis was performed, testing for: current smoking, body mass index (BMI), current  
136 oral contraceptive (OC) use and current hormone replacement therapy (HRT). It was  
137 our goal to test for a limited number of important covariates (i.e., age and BMI),  
138 rather than a more extensive list of potential covariates with more moderate effect  
139 sizes. This choice is unlikely to have biased our heritability estimates, because the  
140 potential effects of these covariates, in as far as they represent environmental



141 influences, will have ended up in the estimate of the Unique Environmental variance  
142 components E). No significant contribution to CRP variance was found for smoking,  
143 OC and HRT ( $p>0.05$ ), the covariate models used were: 1) Age and 2) Age + BMI.  
144 That is, lnCRP was adjusted for age in model 1 and for both age and BMI in model 2  
145 after which the residuals were used in the model fitting. Models were fitted to the raw  
146 data using normal theory maximum likelihood allowing inclusion of incomplete data,  
147 for example, when data were only available in one twin of a pair or in a limited  
148 number of visits.

149 Linear mixed model analysis was applied in longitudinal analyses to determine  
150 whether lnCRP differed between visits while accounting for both repeated  
151 measurements and twin relatedness by including the twin and family identification  
152 numbers as random effects in the model. Models with and without BMI as fixed effect  
153 were analyzed. The same approach was also used to test for differences in lnCRP  
154 levels between visits among those twins that returned for a second and/or a third  
155 visit. In simple cross-sectional analyses we used generalized estimating equations  
156 (GEE) to take account of the relatedness between twins. For example, to evaluate  
157 potential selective drop out over the different visits, we tested for the difference in  
158 age, BMI and lnCRP at baseline (i.e., visit 1) between twins that returned for a  
159 second or third visit and those that did not return using GEE. GEE was also used to  
160 test for differences in baseline characteristics between MZ and DZ twins.

#### 161 162 *Model fitting*

163 Structural equation modeling (SEM) was the primary method of analysis. SEM is  
164 based on the comparison of the variance-covariance matrices in MZ and DZ twin  
165 pairs and allows separation of the observed phenotypic variance into its genetic and

166 environmental components: additive (A) or dominant (D) genetic components and  
167 common (C) or unique (E) environmental components, the latter also containing  
168 measurement error. The choice to start with either D or C in the full model depends  
169 on the relation between the MZ (rMZ) and DZ (rDZ) twin correlations. A D component  
170 is implied if  $2rDZ < rMZ$  whereas a C component is indicated if  $2rDZ > rMZ$ . Dividing  
171 each of these components by the total variance yields the different standardized  
172 components of variance. For example, the narrow sense heritability ( $h^2$ ) can be  
173 defined as the proportion of the total variance attributable to additive genetic variation  
174 [19].

175 For the longitudinal analysis, a trivariate SEM or path model (also known as a  
176 Cholesky decomposition, Figure 1) was used. With this model we can estimate both  
177 the heritability of CRP at different times of measurement separately, and also the  
178 genetic ( $r_g$ ) and environmental ( $r_e$  or  $r_c$ ) correlations between different time points,  
179 giving an estimation of the (in)stability of genetic and environmental influences with  
180 advancing age. We can further test whether the genes influencing CRP are the same  
181 (i.e.  $r_g=1$ ), partly the same (i.e.  $0 < r_g < 1$ ) or entirely different (i.e.  $r_g=0$ ) at different  
182 times of measurement (and therefore different ages). If they are partly the same, this  
183 bivariate model allows quantification of the amount of overlap between genes  
184 influencing CRP at different ages by calculating the genetic correlation between the  
185 traits:  $r_g = COV_A(\text{trait 1, trait 2}) / \sqrt{V_A\text{trait1} * V_A\text{trait2}}$ .

186 Shared and unique environmental correlations can be calculated in a similar  
187 fashion [44,45]. In order to test for differences between twin 1 and twin 2, visits 1, 2  
188 and 3 and differences between MZ and DZ twins, we tested whether the means  
189 could be set equal between different twins (twin 1 and twin 2), time points (visit 1, 2

190 and 3) and zygosity groups (MZ and DZ) without a decline in model fit. A significant  
191 decline indicates that means cannot be assumed to be equal.

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193 --Insert-Figure-1- about here--

## 194 *Software*

195 All data handling and preliminary analyses were done with STATA (version 10.1,  
196 Statacorp, TX, USA). Quantitative genetic modeling was carried out using the Mx  
197 software package [46].

198 Models were fitted to the raw data using normal theory maximum likelihood  
199 allowing inclusion of incomplete data, for example, when data were only available in  
200 one twin of a pair or in a limited number of visits. Using this method, Mx yields  
201 efficient maximum likelihood estimates even in the case of missing data through  
202 calculating twice the negative log-likelihood of the data for each observation (i.e. twin  
203 pair) [46]. This procedure follows the theory described by Lange et al., [47] based on  
204 the multivariate normal probability density function of a vector of observed scores.  
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## 206 **Results**

207 In Figure 2 the distributions of lnCRP at the three visits for all twins combined are  
208 shown. lnCRP levels significantly increased from visit 1 (V1) to visit 2 (V2) ( $p=4.4 \times 10^{-25}$ )  
209 and between V1 and visit 3 (V3) ( $p=1.2 \times 10^{-15}$ ), but not between V2 and V3  
210 ( $p=0.69$ ). Adjustment for BMI did not meaningfully change these results. The median  
211 (IQR) follow-up time was 5.60 (2.87-7.56) years between V1 and V2, 6.17 (4.10-7.53)  
212 between V2 and V3 and 9.58 (8.00-10.46) between V1 and V3. When limiting the  
213 analyses to individuals who returned for all 3 visits (robustness check), results were

215 very similar. InCRP levels among the 2,251 “returners” significantly increased in the  
216 interval between V1 and V2 ( $p=1.8 \times 10^{-29}$ ), and between V1 and V3 (N= 528;  
217  $p=4.1 \times 10^{-22}$ ), but not between V2 and V3 (N= 528;  $p=0.62$ ) (Supplementary Figure  
218 1). Additionally adjusting InCRP for BMI did not meaningfully change these results.

--Insert-Figure-2-about here--

222 Baseline characteristics of MZ and DZ twins for the three visits are  
223 summarized in Table 1. Significant differences between MZ and DZ twins exist for  
224 age (Visit 2 and 3,  $p<0.01$ ), BMI (Visit 2,  $p<0.05$ ) and InCRP levels (Visit 1 and 2,  
225  $p<0.05$ ). In our twin models we corrected InCRP for both age and BMI.

226 Even though we optimally made use of the available follow-up measures of  
227 CRP over a ten year period, only subsamples of twins returned for the second and/or  
228 third visit. Those twins that returned for a second and/or third visit were not entirely  
229 representative of the whole sample as they were several years older, had lower BMIs  
230 and lower levels of CRP at baseline (details given in Supplementary Table 2).

231 Table 2 shows the intraclass twin correlations and results of the univariate  
232 SEM analysis of the two models for each of the three visits. For all three visits and  
233 both age adjusted, and age plus BMI adjusted InCRP values MZ twin correlations  
234 were at least about twice as large as the DZ correlations clearly indicating the  
235 importance of genetic effects on InCRP. In all models and visits, an AE-model was  
236 the best-fitting model. Heritabilities range from 0.46-0.52 (model 1) and 0.49-0.51  
237 (model 2). The heritabilities remain relatively stable over time and their confidence  
238 intervals overlap for all visits and models.

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--Insert-Table-1-about here--

Table 3 shows the results of the longitudinal trivariate analysis (Cholesky decomposition). We first tested effects of twin, visit and zygosity on the means. For model 1, mean values of twin 1 and twin 2 could be set equal within MZ and within DZ twins, but could not be set equal across visits and zygosity groups. For model 2, in which CRP was adjusted for BMI, the means could additionally be set equal across all 3 visits, but remained different between MZ and DZ twins (see also Table 1). Since CRP levels between MZ and DZ twin pairs were different we allowed the means to remain different among zygosity groups in our statistical model to ensure that these differences could not bias the variance component.

No evidence for a significant effect of genetic dominance was found as the AE model fitted best for both models. Heritability estimates for CRP were around 50% and very stable over time (0.50-0.53). Adjustment for BMI reduced heritabilities somewhat (0.45–0.49).

The genetic correlations between first and second (respectively second and third) follow-up visits were 0.82 and 0.85 (model 1), and 0.78 and 0.77 (model 2). These correlations are large, but significantly smaller than 1 based on the nonoverlapping 95% CIs indicating the emergence of new genetic effects with age. When comparing the first with the third visit, the genetic correlation dropped (0.66 for model 1 and 0.55 for model 2), indicating increasingly different genetic effects with age. Environmental correlations between first and second (respectively second and third) follow-up visits were much smaller than the genetic correlations with estimates of 0.16 and 0.27 (model 1), and 0.15 and 0.26 (model 2). When comparing the first with the third visit, the correlation remained the same (0.19).

265 As an additional sensitivity analysis we repeated the trivariate Cholesky  
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2 266 modelling using only returning subjects, i.e., twins that participated in all three visits.  
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4 267 Heritability estimates and genetic and environmental correlations showed similar  
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7 268 results (Supplementary Table 1).  
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## 12 270 **Discussion**

14 271 The present study assessed the stability of genetic and environmental influences  
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16 272 underlying baseline CRP levels, using a longitudinal classical twin design  
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19 273 incorporating up to 3 follow-up measurements over a ten year period. We were able  
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22 274 to demonstrate relative stable heritabilities with advancing age of around 50%, which  
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24 275 are in the same range as previous studies [20–40]. High genetic correlations of 0.66  
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26 276 to 0.85 between visits indicate that genes influencing CRP levels are mostly the  
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29 277 same at different ages, whereas low environmental correlations of 0.16 to 0.27 show  
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32 278 that environmental factors are largely different between visits. Genetic correlations  
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34 279 were significantly different from 1, however, also indicating emergence of some new  
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36 280 genetic effects on CRP with age.  
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41 282 --Insert-Table-2-and-3-about here--  
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46 284 The present study is, to our knowledge, the first to assess (and describe) the  
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48 285 stability of genetic and environmental influences on baseline CRP levels in a  
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51 286 longitudinal twin study. The longitudinal design with the long follow-up time of up to  
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53 287 10 years, and the relatively large sample size provided more statistical power and  
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56 288 methodological opportunities compared to previous smaller, cross-sectional studies.  
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289 We did not find evidence for genetic dominance however, in contrast to some  
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2 290 previous cross-sectional twin studies that also had large sample sizes [37,39].  
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4 291 A limitation of the present study however, is that our conclusions are not  
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7 292 generalizable to men, or subjects with diseases since only data on relatively healthy  
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10 293 women was assessed. The benefit of this homogenous sample on the other hand, is  
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12 294 that the results cannot be confounded by gender or disease since these covariates  
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14 295 have previously been shown to have significant effects [48].  
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17 296 Even though we optimally made use of the available follow-up measures of  
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19 297 CRP over a ten year period, only subsamples of twins returned for the second and/or  
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22 298 third visits. However, the Mx software package is capable of handling missing data by  
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24 299 obtaining maximum likelihood estimates and takes advantage of including all  
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26 300 available data rather than complete cases only [46]. Furthermore, a sensitivity  
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29 301 analysis including only twins for which CRP data was available for all three visits  
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31 302 yielded similar findings. As such, we believe it is unlikely that the differences between  
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34 303 returning and non-returning twins will have translated into major biases in our model  
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36 304 fitting parameter estimates.  
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39 305 An interesting feature of our study, as mentioned above, is that we are the first  
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41 306 to demonstrate relative stable heritabilities over time in a longitudinal design, even  
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43 307 though the CRP levels itself do not seem stable (higher CRP-levels are described  
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46 308 with advancing age) [2–5]. The present results show that the increase in CRP levels  
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49 309 off between V2 and V3 and partial differences in gene repertoire may well be  
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51 310 responsible for this. However, the aim of the present study was to describe stability  
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53 311 and change of (co)variance patterns over time in terms of changes in underlying  
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56 312 genetic and environmental variance components rather than explaining trends  
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2 313 in,mean-CRP-levels over time. As such, further biological explanations of this age  
3 314 trend in mean CRP remain speculative.

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5 315 It has been hypothesized before that increased CRP levels with age may  
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7 316 result from increases in “low grade, systemic, chronic inflammation” (due to  
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9 317 atherosclerosis for example) [2–5]. Based on our previous findings [10], one may  
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11 318 have expected an increasingly important role for random (i.e., unique environmental)  
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13 319 components reflecting reduced homeostatic control with age in this process.  
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15 320 However, this was not supported by our recent findings.  
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19 321 The role of immunological pathways in somatic outcomes has well been  
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21 322 established, as mentioned before in the introduction. This is, for example, illustrated  
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23 323 by results on the role of microbial exposition in early life in the development of  
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25 324 immune pathways and regulatory mechanisms [1], showing that a lack of exposition  
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27 325 predisposes to “disrupted” immunological pathways and increased risk for allergic  
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29 326 disorders. In this context, the relationship between Immunglobulin-E (IgE) and CRP  
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31 327 would be of particular interest. This could be investigated in a multivariate twin study  
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33 328 assessing the phenotypic and genetic relationship between IgE, CRP and age similar  
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35 329 to our recent work on the relationship between neuroticism, CRP, fibrinogen, and IgG  
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37 330 [49,50].  
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43 331 Genome-wide association studies (GWASs) have been able to identify several  
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45 332 genomic loci associated with serum levels of CRP. These studies have used large  
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47 333 sample sizes of adult population, but have not compared (possibly different) genomic  
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49 334 effects on CRP levels with advancing age [51,52]. Our results on the other hand  
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51 335 indicate emergence of some new genetic effects on CRP with age and hence,  
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53 336 warrants the need to repeat large GWAS studies with stratifying the study population  
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55 337 for different age ranges. Post-GWAS analyses of the abovementioned CRP GWAS  
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338 results revealed different biological processes involved in CRP metabolism [53].

339 However, it is unclear whether these processes are stable with advancing age.

340 The present study provides evidence of a substantial role for genetics in the  
341 regulation of baseline CRP levels. Heritabilities are stable with advancing age, and  
342 (more interestingly) the impact of environmental components remains relatively  
343 stable too during the ten years our subjects were followed. Considering the genetic  
344 correlations were significantly smaller than 1 and reduced with follow-up time, genes  
345 regulating CRP levels at younger ages must be partly different from those at more  
346 advanced ages. These results are in contrast with previous (cross-sectional) findings  
347 of other inflammatory markers, which indicate moderation of (changing) unique  
348 environmental factors with age in the regulation of IL-1 $\beta$  and TNF- $\alpha$  levels [10].

349 In conclusion, this study emphasizes the relatively stable role of genetics in  
350 regulation of CRP levels, emphasizing its potential as a biomarker of ageing over  
351 other, more biologically reactive substances, in the various immunological pathways.  
352 Furthermore, the present study highlights the importance of a combination of both  
353 environmental factors and complex genetic pathways underlying the ageing process.  
354 Finally, even though the quantitative role of genetics in regulation of baseline CRP  
355 levels remained largely the same with age, the actual genes responsible for these  
356 effects were partly different at different ages. As such, future gene finding efforts  
357 need to take this into account, for example through investigating gene by age  
358 interaction effects.

359 **Conflict of interest**

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2 360 None of the authors report a conflict of interest

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7 362 **Author contributions**

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11 363 1) Substantial contributions to conception and design (AAS, TDS, HS), acquisition of  
12  
13 364 data (YJ, TDS), analysis and interpretation of data (AAS, AV, IMN, HR, HS).

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16  
17 365 2) Drafting the article (AAS, AV, ZK, HS) revising it critically for important intellectual  
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19 366 content (YJ, IMN, ZK, TDS, HR, HS).

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23 367 3) Final approval of the version to be published (AAS, AV, YJ, IMN, ZK, TDS, HR,  
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25 368 HS).

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603 **Figure captions**

604

605 **Figure 1**

606 Path diagram for a bivariate model. For clarity, only one twin is depicted. A1, A2, A3  
607 = Genetic variance components; C1, C2, C3 = common environmental variance  
608 components; E1, E2, E3 = unique environmental variance components; V1, V2, V3 =  
609 Visit 1, 2 and 3; a11 through a33 = genetic path coefficients (or factor loadings); c11  
610 through c33 = common environmental path coefficients (or factor loadings); e11  
611 through e33 = unique environmental path coefficients (or factor loadings).

612

613 **Figure 2**

614 Distributions of lnCRP at the three visits. An asterisk means that there is a significant  
615 difference ( $p < 0.05$ ) in ln(CRP) between the respective visits.

616

617 **Supplementary figure 1**

618 Distributions of paired differences in lnCRP between two visits. An asterisk means that  
619 the paired difference is significantly different from zero ( $p < 0.05$ ).



Figure 1  
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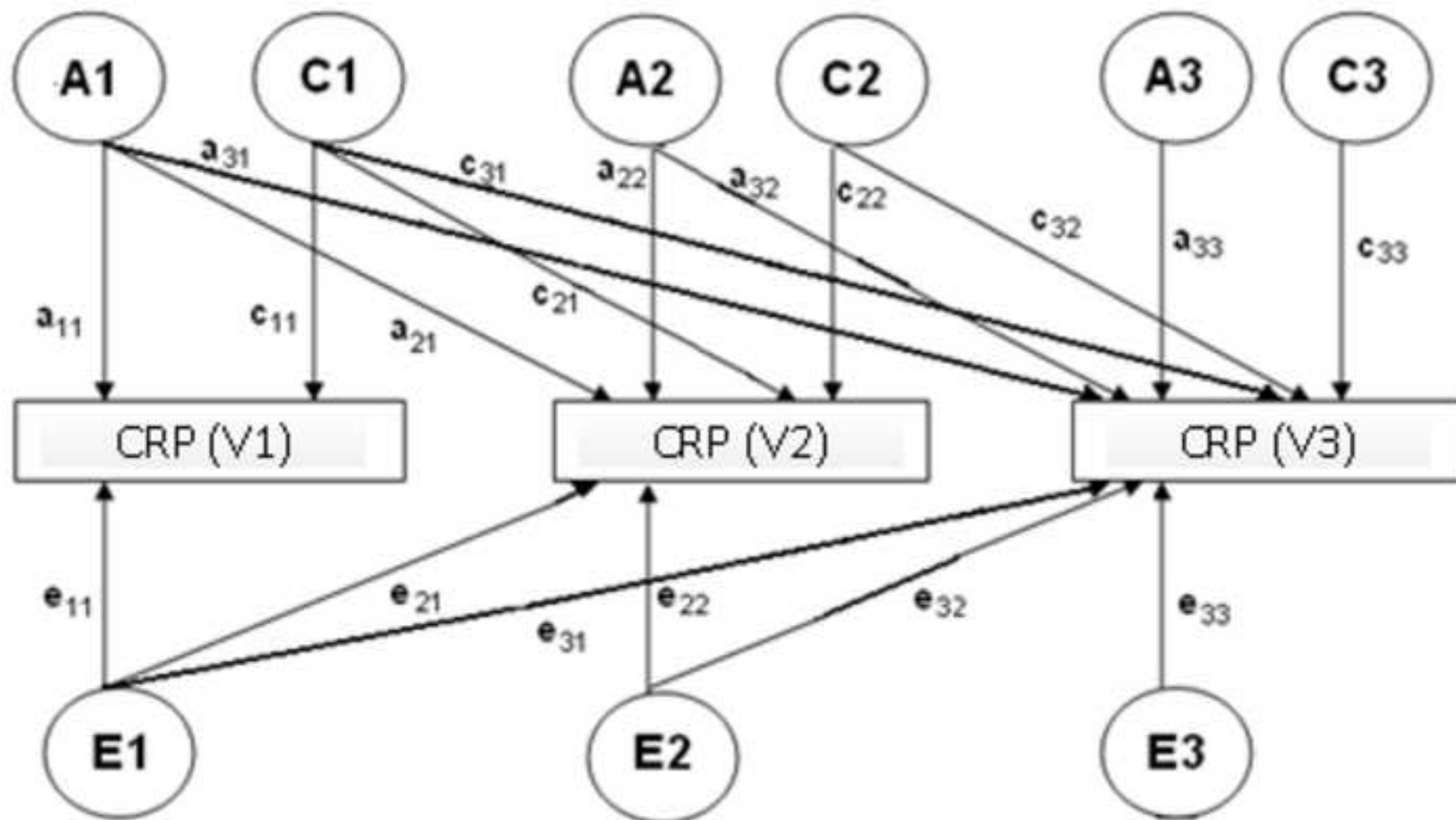


Figure2  
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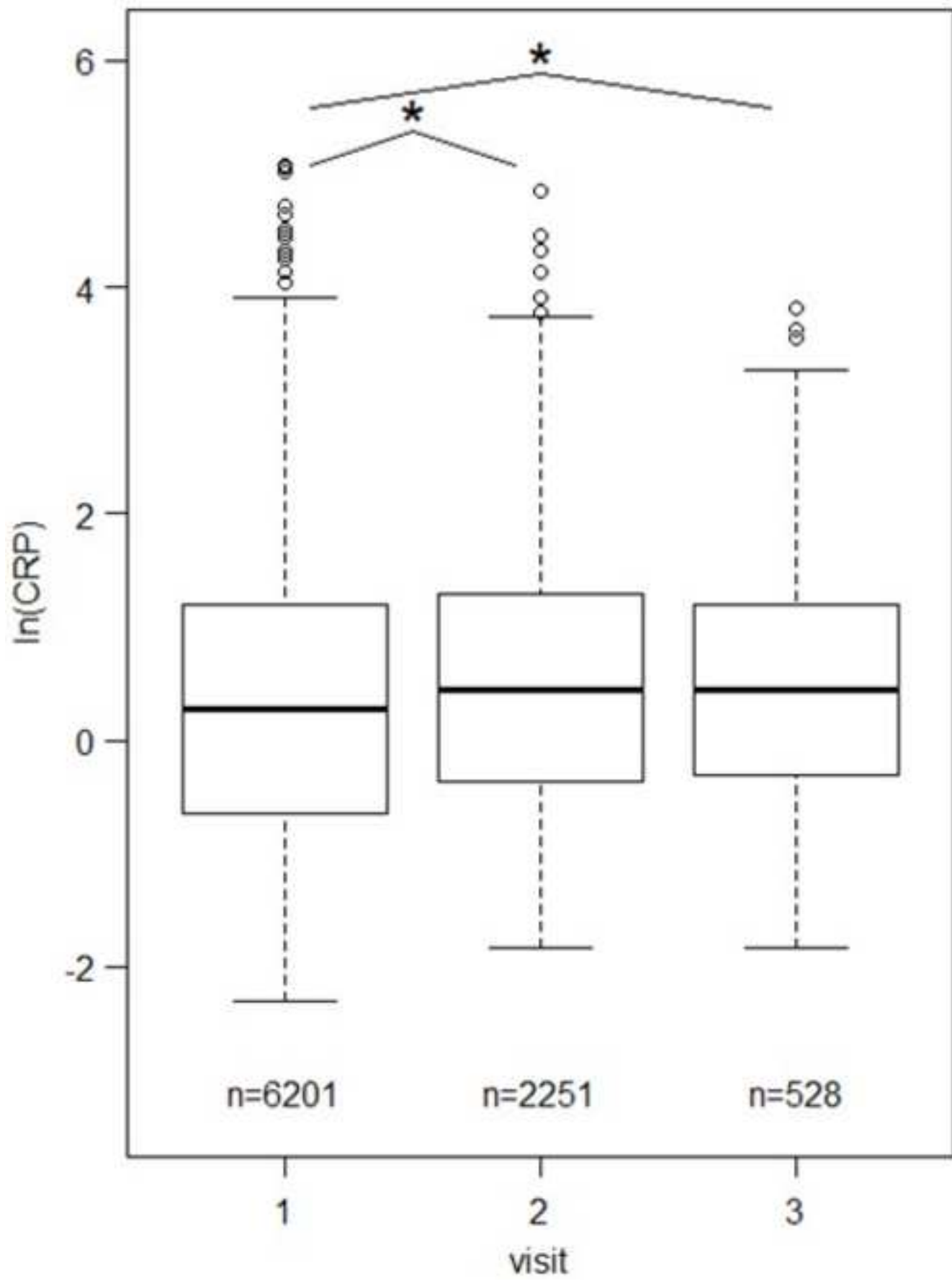
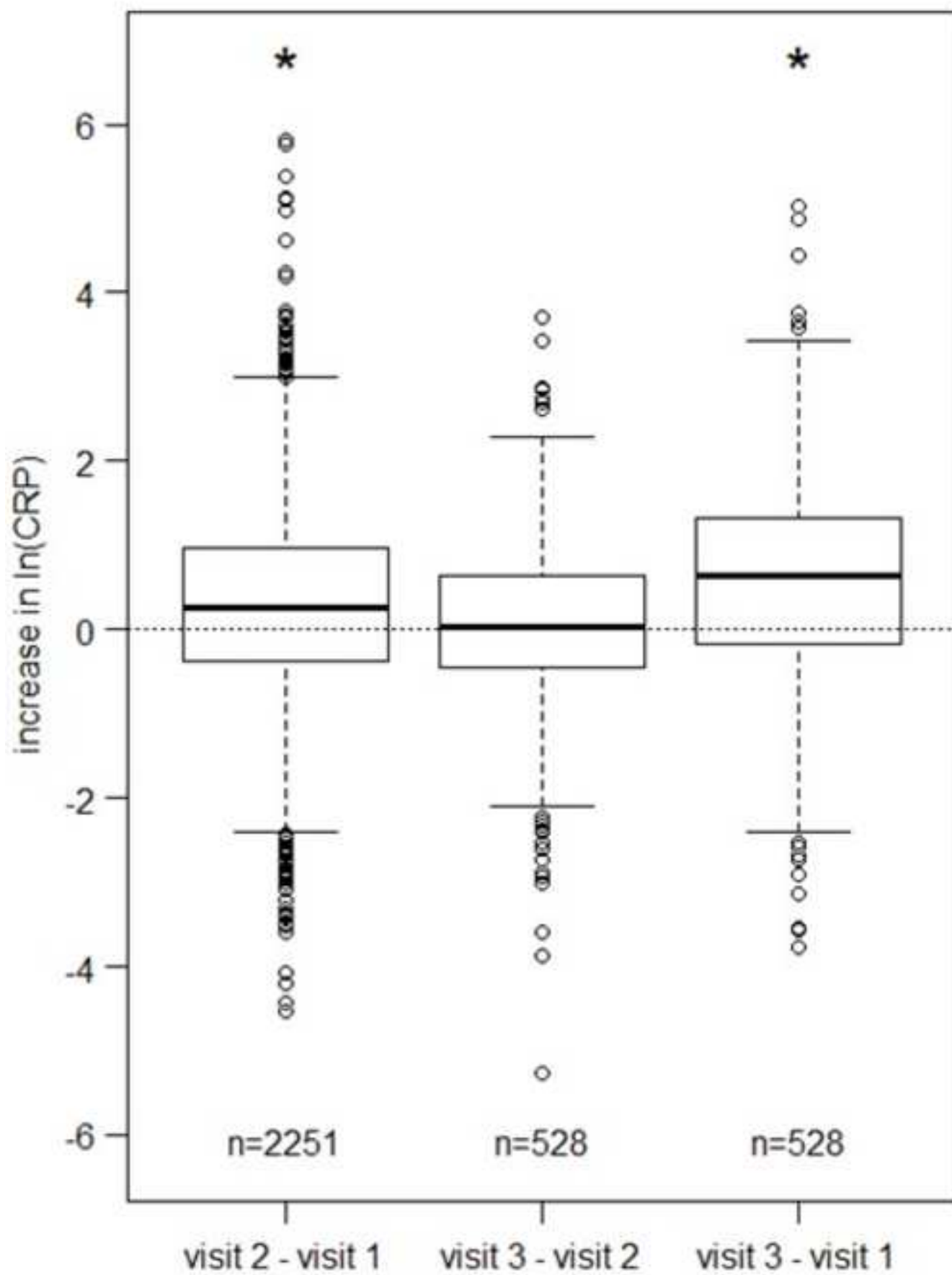


Figure3  
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**Table 1: General characteristics of twins by zygosity and visit number.**

	MZ		DZ		<i>p</i> -value
	N	Age (years)	N	Age (years)	
Visit 1	2,955	49.1±13.4	3,246	48.3±12.4	ns
Visit 2	934	57.9±10.1	1,317	56.0±10.3	<0.01
Visit 3	292	65.6±8.1	236	61.4±9.7	<0.01
	N	BMI (kg/m <sup>2</sup> )	N	BMI (kg/m <sup>2</sup> )	
Visit 1	2,955	25.4±4.6	3,246	25.6±4.7	ns
Visit 2	934	25.7±4.2	1,317	26.3±4.8	<0.05
Visit 3	292	26.1±4.2	236	26.3±4.4	ns
	N	CRP (mg/L)	N	CRP (mg/L)	
Visit 1	2,955	1.20 (0.48 – 3.15)	3,246	1.44 (0.58 – 3.47)	<0.05
Visit 2	934	1.45 (0.68 – 3.39)	1,317	1.61 (0.72 – 3.89)	<0.05
Visit 3	292	1.54 (0.73 – 3.18)	236	1.59 (0.73 – 3.80)	ns

Note: Differences between MZ and DZ twins were tested using GEE with adjustment for age (for BMI) and age and BMI (for CRP). CRP was transformed by natural logarithm prior to analysis. Abbreviations: BMI, Body Mass Index; CRP, C-reactive protein; DZ, dizygotic twins; MZ, monozygotic twins; N, number of subjects; n.s., not significant. Data are given in mean±SD for age and BMI and median (IQR) for CRP.

**Table 2: Intraclass correlations and parameter estimates of best fitting univariate models of lnCRP at the three visits**

Visit	Model	Intraclass correlations		Univariate Model Fitting		
		rMZ (95% CI)	rDZ (95% CI)		A (95% CI)	E (95% CI)
1	N, pairs	1457	1584			
	1	0.54 (0.50-0.58)	0.24 (0.20-0.29)	AE	0.52 (0.46 – 0.58)	0.48 (0.42 – 0.54)
	2	0.48 (0.44-0.52)	0.20 (0.16-0.25)	AE	0.51 (0.38 – 0.61)	0.49 (0.39 – 0.62)
2	N, pairs	452	632			
	1	0.50 (0.43-0.57)	0.25 (0.18-0.33)	AE	0.51 (0.45 – 0.57)	0.49 (0.43 – 0.55)
	2	0.46 (0.38-0.53)	0.24 (0.17-0.31)	AE	0.51 (0.39 – 0.62)	0.49 (0.38 – 0.61)
3	N, pairs	139	112			
	1	0.54 (0.43-0.66)	0.13 (0.00-0.31)	AE	0.46 (0.40 – 0.52)	0.54 (0.48 – 0.60)
	2	0.51 (0.39-0.64)	0.15 (0.00-0.33)	AE	0.49 (0.36 – 0.59)	0.51 (0.41 – 0.64)

Note: Model 1, adjusted for age; Model 2, adjusted for age and BMI; A, additive genetic variance component; E, unique environmental variance component.

**Table 3: Parameter estimates (95% CI) of best fitting trivariate models of lnCRP levels.**

Model	Visit	1	2	3
1	1	<b>0.53 (0.50-0.56)</b>	0.16 (0.09-0.23)	0.19 (0.06-0.31)
	2	0.82 (0.74-0.90)	<b>0.50 (0.45-0.57)</b>	0.27 (0.12-0.40)
	3	0.66 (0.51-0.81)	0.85 (0.71-0.97)	<b>0.52 (0.39-0.62)</b>
2	1	<b>0.48 (0.44-0.51)</b>	0.15 (0.08-0.22)	0.19 (0.06-0.31)
	2	0.78 (0.70-0.87)	<b>0.45 (0.40-0.52)</b>	0.26 (0.12-0.39)
	3	0.55 (0.40-0.70)	0.77 (0.61-0.92)	<b>0.49 (0.36-0.59)</b>

Note: The best fitting model for all analyses was the AE model; Genetic correlations [ $r_g$  (95% CI)] are given below the diagonal and environmental correlations [ $r_e$  (95% CI)] above the diagonal; Heritability [ $r_g$  (95% CI)] estimates are given on the diagonal in bold, Model 1, adjusted for age; Model 2, adjusted for age and BMI.