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Chapter 3

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

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

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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 6201 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 × 10^{-25}) and between V1 and V3 (p=1.2 × 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.

1. Introduction

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

An important, well established inflammatory marker is C-reactive protein (CRP). Its baseline levels are considered to reflect systemic inflammation. Considering the relationship of increased baseline CRP levels with a variety of disorders, including cancer

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[11], bipolar disorder [12], cardiovascular diseases [13–15], type 2 diabetes [16], and all-cause mortality [17], regulation of baseline CRP levels are of particular interest. In this context, baseline CRP levels have shown to be influenced by a variety of environmental and genetic factors. However, their relative importance and exact extent to which these factors account for the total variance in CRP level remain unknown [18].

Heritability studies aim to estimate the relative influence of heritable and environmental factors on a trait [19]. Twin and family studies in a wide variety of populations with different age ranges showed a moderate heritability of baseline CRP levels, with heritability estimates ranging from 0.10 to 0.65 [20–40] (Supplementary Table 3).

CRP levels have been shown to be fairly stable over time. DeGoma et al. [41] analyzed serial CRP measures of 255 participants to evaluate the intraindividual variability of CRP over a median follow-up period of 4.7 years. The multivariable-adjusted intraclass correlation coefficient (ICC) of CRP was estimated as 0.62. The intraindividual variability of CRP was also investigated by Wu et al. [42], using CRP levels of 56,218 Chinese adults over a two-year follow-up time. The ICC of CRP was reported as 0.55 for men and 0.60 for women. Interestingly, the stability of CRP gradually increased with age. However, twin and family studies mentioned above used single CRP measurement for their heritability calculation rather than longitudinal measurements. Limited by this cross-sectional design, heritability estimates for CRP as reported above only provide a snapshot at one particular point in time, potentially providing at least a partial explanation for the wide variety of heritability estimates reported in the literature [20–40].

To the best of our knowledge, no longitudinal twin studies on CRP levels have been conducted to date. The aim of this study was to evaluate the heritabilities and the extent to which genetic and environmental influences contribute to the stability or change of CRP over time in a large population of adult females using a classical twin design, including up to three CRP measurements over a ten-year follow-up period.

2. Material and methods

2.1. Subjects

The study was conducted in 6201 women from the Twins UK registry. Details of the Twins UK registry have been published before [43]. Zygosity was determined by questionnaire supplemented by DNA fingerprinting in cases with disputed or uncertain zygosity. CRP measurement follow-up was performed up to 3 times, giving 6201 measurements in visit 1 (1457 monozygotic (MZ) pairs, 1584 dizygotic (DZ) pairs and 119 singletons), 2251 measurements in visit 2 (452 MZ-pairs, 632 DZ-pairs and 83 singletons) and 528 measurements in visit 3 (139 MZ-pairs, 112 DZ-pairs and 26 singletons).

2.2. C-reactive protein analysis

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

2.3. Analytical approach

Natural log (ln) transformation was necessary for the CRP data to obtain a better approximation of the normal distribution. Secondly, lnCRP was adjusted for age. This is a common procedure in twin analyses because age can spuriously introduce a shared environmental effect if there is a significant correlation between the phenotype and age, because twins are always of the same age. Next, covariate analysis was performed, testing for: current smoking, body mass index (BMI), current oral contraceptive (OC) use and current hormone replacement therapy (HRT). It was our goal to test for a limited number of important covariates (i.e., age and BMI), rather than a more extensive list of potential covariates with more moderate effect sizes. This choice is unlikely to have biased our heritability estimates, because the potential effects of these covariates, in as far as they represent environmental influences, will have ended up in the estimate of the Unique Environmental variance components E). No significant contribution to CRP variance was found for smoking, OC and HRT ($p > 0.05$), the covariate models used were: 1) Age and 2) Age + BMI. That is, lnCRP was adjusted for age in model 1 and for both age and BMI in model 2 after which the residuals were used in the model fitting. Models were fitted to the raw data using normal theory maximum likelihood allowing inclusion of incomplete data, for example, when data were only available in one twin of a pair or in a limited number of visits.

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

2.4. Model fitting

Structural equation modeling (SEM) was the primary method of analysis. SEM is based on the comparison of the variance-covariance matrices in MZ and DZ twin pairs and allows separation of the observed phenotypic variance into its genetic and environmental components: additive (A) or dominant (D) genetic components and common (C) or unique (E) environmental components, the latter also containing measurement error. The choice to start with either D or C in the full model depends on the relation between the MZ (rMZ) and DZ (rDZ) twin correlations. A D component is implied if 2xrDZ < rMZ whereas a C component is indicated if 2xrDZ > rMZ. Dividing each of these components by the total variance yields the different standardized components of variance. For example, the narrow sense heritability (h^2) can be defined as the proportion of the total variance attributable to additive genetic variation [19].

For the longitudinal analysis, a trivariate SEM or path model (also known as a Cholesky decomposition, Fig. 1) was used. With this model we can estimate both the heritability of CRP at different times of measurement separately, and also the genetic (r_g) and environmental (r_e or r_c) correlations between different time points, giving an estimation of the (in)stability of genetic and environmental influences with advancing age. We can further test whether the genes influencing CRP are the same (i.e. $r_g = 1$), partly the same (i.e. $0 < r_g < 1$) or entirely different (i.e. $r_g = 0$) at different times of measurement (and therefore different ages). If they are partly the same, this bivariate model allows quantification of the amount of overlap between genes influencing CRP at different ages by

calculating the genetic correlation between the traits: $r_g = COV_A$ (trait 1, trait 2)/$\sqrt{(V_A trait1 * V_A trait2)}$.

Shared and unique environmental correlations can be calculated in a similar fashion [44,45]. In order to test for differences between twin 1 and twin 2, visits 1, 2 and 3 and differences between MZ and DZ twins, we tested whether the means could be set equal between different twins (twin 1 and twin 2), time points (visit 1, 2 and 3) and zygosity groups (MZ and DZ) without a decline in model fit. A significant decline indicates that means cannot be assumed to be equal.

2.5. Software

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

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

3. Results

In Fig. 2, the distributions of lnCRP at the three visits for all twins combined are shown. lnCRP levels significantly increased from visit 1 (V1) to visit 2 (V2) (p=4.4 × 10^{-25}) and between V1 and visit 3 (V3) (p=1.2 × 10^{-15}), but not between V2 and V3 (p=0.69). Adjustment for BMI did not meaningfully change these results. The median (IQR) follow-up time was 5.60 (2.87–7.56) years between V1 and V2, 6.17 (4.10–7.53) between V2 and V3 and 9.58 (8.00–10.46) between V1 and V3. When limiting the analyses to individuals who returned for all 3 visits (robustness check), results were very similar. lnCRP levels among the 2251 "returners" significantly increased in the interval between V1 and V2 (p=1.8 × 10^{-29}), and between V1 and V3 (N = 528; p=4.1 × 10^{-22}), but not between V2 and V3 (N = 528; p=0.62) (Supplementary Fig. 1). Additionally adjusting lnCRP for BMI did not meaningfully change these results.

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

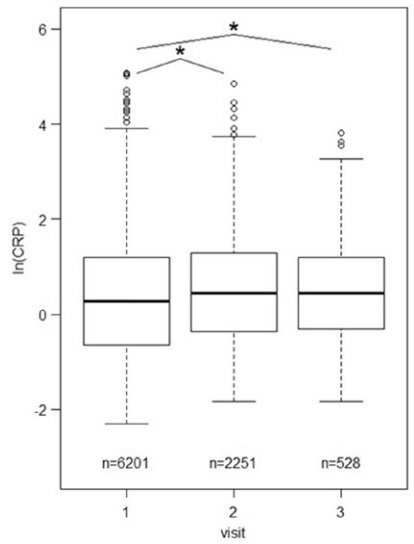

Fig. 2. Distributions of lnCRP at the three visits.
An asterix means that there is a significant difference ($p < 0.05$) in ln(CRP) between the respective visits.

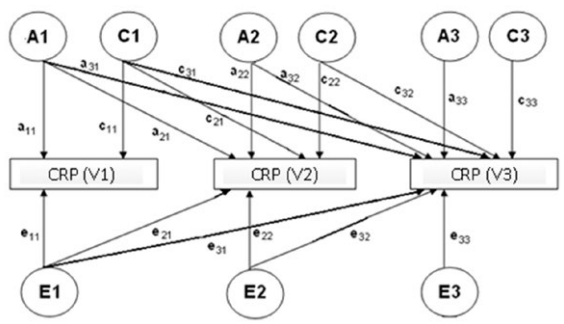

Fig. 1. Path diagram for a bivariate model.
For clarity, only one twin is depicted. A1, A2, A3 = genetic variance components; C1, C2, C3 = common environmental variance components; E1, E2, E3 = unique environmental variance components; V1, V2, V3 = Visit 1, 2 and 3; a11 through a33 = genetic path coefficients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

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

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

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 non-overlapping 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).

As an additional sensitivity analysis we repeated the trivariate Cholesky modeling using only returning subjects, i.e., twins that participated in all three visits. Heritability estimates and genetic and environmental correlations showed similar results (Supplementary Table 1).

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

	MZ		DZ		p-value
	N	Age (years)	N	Age (years)	
Visit 1	2955	49.1 ± 13.4	3246	48.3 ± 12.4	ns
Visit 2	934	57.9 ± 10.1	1317	56.0 ± 10.3	<0.01
Visit 3	292	65.6 ± 8.1	236	61.4 ± 9.7	<0.01
	N	BMI (kg/m^2)	N	BMI (kg/m^2)	
Visit 1	2955	25.4 ± 4.6	3246	25.6 ± 4.7	ns
Visit 2	934	25.7 ± 4.2	1317	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	2955	1.20 (0.48–3.15)	3246	1.44 (0.58–3.47)	<0.05
Visit 2	934	1.45 (0.68–3.39)	1317	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

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. BMI, body Mass Index; CRP, C-reactive protein; DZ, dizygotic twins; MZ, monozygotic twins; N, number of subjects; ns, not significant.
Data are given in mean ± SD for age and BMI and median (IQR) for CRP.

4. Discussion

The present study assessed the stability of genetic and environmental influences underlying baseline CRP levels, using a longitudinal classical twin design, incorporating up to 3 follow-up measurements over a ten-year period. We were able to demonstrate relative stable heritabilities with advancing age of around 50%, which are in the same range as previous studies [20–40]. High genetic correlations of 0.66–0.85 between visits indicate that genes influencing CRP levels are mostly the same at different ages, whereas low environmental correlations of 0.16–0.27 show that environmental factors are largely different between visits. Genetic correlations were significantly different from 1, however, also indicating emergence of some new genetic effects on CRP with age.

The present study is, to our knowledge, the first to assess (and describe) the stability of genetic and environmental influences on baseline CRP levels in a longitudinal twin study. The longitudinal design, with the long follow-up time of up to 10 years, and the relatively large sample size provided more statistical power and methodological opportunities compared to previous smaller, cross-sectional studies. We did not find evidence for genetic dominance, however, in contrast to some previous cross-sectional twin studies that also had large sample sizes [37,39].

A limitation of the present study, however, is that our conclusions are not generalizable to men, or subjects with diseases since only data on relatively healthy women was assessed. The benefit of this homogenous sample, on the other hand, is that the results cannot be confounded by gender or disease since these covariates have previously been shown to have significant effects [48].

Even though we optimally made use of the available follow-up measures of CRP over a ten-year period, only subsamples of twins returned for the second and/or third visits. However, the Mx software package is capable of handling missing data by obtaining maximum likelihood estimates and takes advantage of including all available data rather than complete cases only [46]. Furthermore, a sensitivity analysis including only twins for which CRP data was available for all three visits yielded similar findings. As such, we believe it is unlikely that the differences between returning and non-returning twins will have translated into major biases in our model fitting parameter estimates.

An interesting feature of our study, as mentioned above, is that we are the first to demonstrate relative stable heritabilities over time in a longitudinal design, even though the CRP levels itself do not seem stable (higher CRP-levels are described with advancing age) [2–5]. The present results show that the increase in CRP levels off between V2 and V3 and partial differences in gene repertoire may well be responsible for this. However, the aim of the present study was to describe stability and change of (co)variance patterns over time in terms of changes in underlying genetic and environmental variance components rather than explaining trends in mean-CRP-levels over time. As such, further biological explanations of this age trend in mean CRP remain speculative.

It has been hypothesized before that increased CRP levels with age may result from increases in "low grade, systemic, chronic inflammation" (due to atherosclerosis for example) [2–5]. Based on our previous findings [10], one may have expected an increasingly

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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)	Best Fitting model	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)

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

important role for random (i.e., unique environmental) components reflecting reduced homeostatic control with age in this process. However, this was not supported by our recent findings. The role of immunological pathways in somatic outcomes has well been established, as mentioned before in the introduction. This is, for example, illustrated by results on the role of microbial exposition in early life in the development of immune pathways and regulatory mechanisms [1], showing that a lack of exposition predisposes to "disrupted" immunological pathways and increased risk for allergic disorders. In this context, the relationship between Immunoglobulin-E (IgE) and CRP would be of particular interest. This could be investigated in a multivariate twin study assessing the phenotypic and genetic relationship between IgE, CRP and age similar to our recent work on the relationship between neuroticism, CRP, fibrinogen, and IgG [49,50].

Genome-wide association studies (GWASs) have been able to identify several genomic loci associated with serum levels of CRP. These studies have used large sample sizes of adult population, but have not compared (possibly different) genomic effects on CRP levels with advancing age [51,52]. Our results, on the other hand, indicate emergence of some new genetic effects on CRP with age and hence, warrants the need to repeat large GWAS studies with stratifying the study population for different age ranges. Post-GWAS analyses of the abovementioned CRP GWAS results revealed different biological processes involved in CRP metabolism [53]. However, it is unclear whether these processes are stable with advancing age.

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

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

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

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

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.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

Substantial contributions to conception and design (AAS, TDS, HS), acquisition of data (YJ, TDS), analysis and interpretation of data (AAS, AV, IMN, HR, HS).

Drafting the article (AAS, AV, ZK, HS) revising it critically for important intellectual content (YJ, IMN, ZK, TDS, HR, HS).

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

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2017.08.008.

References

[1] T.W. McDade, Early environments and the ecology of inflammation, Proc. Natl. Acad. Sci. U. S. A. 109 (2) (2012) 17281–17288, http://dx.doi.org/10.1073/pnas.1202244109.
[2] C. Franceschi, M. Bonafè, S. Valensin, F. Olivieri, M. De Luca, E. Ottaviani, G. De Benedictis, Inflamm-aging. An evolutionary perspective on immunosenescence, Ann. N. Y. Acad. Sci. 908 (2000) 244–254.
[3] C. Franceschi, M. Capri, D. Monti, S. Giunta, F. Olivieri, F. Sevini, M.P. Panourgia, L. Invidia, L. Celani, M. Scurti, E. Cevenini, G.C. Castellani, S. Salvioli, Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans, Mech. Ageing Dev. 128 (2007) 92–105, http://dx.doi.org/10.1016/j.mad.2006.11.016.

[4] S. Vasto, G. Candore, C.R. Balistreri, M. Caruso, G. Colonna-Romano, M.P. Grimaldi, F. Listi, D. Nuzzo, D. Lio, C. Caruso, Inflammatory networks in ageing, age-related diseases and longevity, Mech. Ageing Dev. 128 (2007) 83–91, http://dx.doi.org/10.1016/j.mad.2006.11.015.
[5] G. Pawelec, R.B. Effros, C. Caruso, E. Remarque, Y. Barnett, R. Solana, T cells and aging (update february 1999), Front. Biosci. J. Virtual Libr. 4 (1999) D216–D269.
[6] H. Bruunsgaard, M. Pedersen, B.K. Pedersen, Aging and proinflammatory cytokines, Curr. Opin. Hematol. 8 (2001) 131–136.
[7] R.B. Schnabel, K.L. Lunetta, M.G. Larson, J. Dupuis, I. Lipinska, J. Rong, M.-H. Chen, Z. Zhao, J.F. Yamamoto, J.B. Meigs, V. Nicaud, C. Perret, T. Zeller, S. Blankenberg, L. Tiret, J.F. Keaney, R.S. Vasan, E.J. Benjamin, The relation of genetic and environmental factors to systemic inflammatory biomarker concentrations, Circ. Cardiovasc. Genet. 2 (2009) 229–237, http://dx.doi.org/10.1161/CIRCGENETICS.108.804245.
[8] J. Danesh, R. Collins, P. Appleby, R. Peto, Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies, JAMA 279 (1998) 1477–1482.
[9] G. Luc, J.-M. Bard, I. Juhan-Vague, J. Ferrieres, A. Evans, P. Amouyel, D. Arveiler, J.-C. Fruchart, P. Ducimetiere, PRIME Study Group, C-reactive protein, interleukin-6, and fibrinogen as predictors of coronary heart disease: the PRIME Study, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 1255–1261, http://dx.doi.org/10.1161/01.ATV.0000079512.66448.1D.
[10] A.A. Sas, Y. Jamshidi, D. Zheng, T. Wu, J. Korf, B.Z. Alizadeh, T.D. Spector, H. Snieder, The age-dependency of genetic and environmental influences on serum cytokine levels: a twin study, Cytokine 60 (2012) 108–113, http://dx.doi.org/10.1016/j.cyto.2012.04.047.
[11] K.H. Allin, S.E. Bojesen, B.G. Nordestgaard, Baseline C-reactive protein is associated with incident cancer and survival in patients with cancer, J. Clin. Oncol. 27 (2009) 2217–2224, http://dx.doi.org/10.1200/JCO.2008.19.8440.
[12] D. De Berardis, C.M. Conti, D. Campanella, A. Carano, M. Scali, A. Valchera, N. Serroni, A.M. Pizzorno, A. D'Albenzio, M. Fulcheri, F. Gambi, R. La Rovere, C. Cotellessa, R.M. Salerno, F.M. Ferro, Evaluation of C-reactive protein and total serum cholesterol in adult patients with bipolar disorder, Int. J. Immunopathol. Pharmacol. 21 (2008) 319–324.
[13] J. Danesh, J.G. Wheeler, G.M. Hirschfield, S. Eda, G. Eiriksdottir, A. Rumley, G.D.O. Lowe, M.B. Pepys, V. Gudnason, C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease, N. Engl. J. Med. 350 (2004) 1387–1397, http://dx.doi.org/10.1056/NEJMoa032804.
[14] H.D. Sesso, J.E. Buring, N. Rifai, G.J. Blake, J.M. Gaziano, P.M. Ridker, C-reactive protein and the risk of developing hypertension, JAMA J. Am. Med. Assoc. 290 (2003) 2945–2951, http://dx.doi.org/10.1001/jama.290.22.2945.
[15] S. Kaptoge, E. Di Angelantonio, G. Lowe, M.B. Pepys, S.G. Thompson, R. Collins, J. Danesh, C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis, Lancet 375 (2010) 132–140, http://dx.doi.org/10.1016/S0140-6736(09)61717-7.
[16] A. Dehghan, I. Kardys, M.P.M. de Maat, A.G. Uitterlinden, E.J.G. Sijbrands, A.H. Bootsma, T. Stijnen, A. Hofman, M.T. Schram, J.C.M. Witteman, Genetic variation, C-reactive protein levels, and incidence of diabetes, Diabetes 56 (2007) 872–878, http://dx.doi.org/10.2337/db06-0922.
[17] T.B. Harris, L. Ferrucci, R.P. Tracy, M.C. Corti, S. Wacholder, W.H. Ettinger Jr., H. Heimovitz, H.J. Cohen, R. Wallace, Associations of elevated Interleukin-6 and C-reactive protein levels with mortality in the elderly, Am. J. Med. 106 (1999) 506–512, http://dx.doi.org/10.1016/S0002-9343(99)00066-2.
[18] S. Kathiresan, M.G. Larson, R.S. Vasan, C.-Y. Guo, P. Gona, J.F. Keaney, P.W.F. Wilson, C. Newton-Cheh, S.L. Musone, A.L. Camargo, J.A. Drake, D. Levy, C.J. O'Donnell, J.N. Hirschhorn, E.J. Benjamin, Contribution of clinical correlates and 13 C-reactive protein gene polymorphisms to interindividual variability in serum C-reactive protein level, Circulation 113 (2006) 1415–1423, http://dx.doi.org/10.1161/CIRCULATIONAHA.105.591271.
[19] J. van Dongen, P.E. Slagboom, H.H.M. Draisma, N.G. Martin, D.I. Boomsma, The continuing value of twin studies in the omics era, Nat. Rev. Genet. 13 (2012) 640–653, http://dx.doi.org/10.1038/nrg3243.
[20] J.S. Pankow, A.R. Folsom, M. Cushman, I.B. Borecki, P.N. Hopkins, J.H. Eckfeldt, R.P. Tracy, Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study, Atherosclerosis 154 (2001) 681–689.
[21] M.A. Vickers, F.R. Green, C. Terry, B.M. Mayosi, C. Julier, M. Lathrop, P.J. Ratcliffe, H.C. Watkins, B. Keavney, Genotype at a promoter polymorphism of the interleukin-6 gene is associated with baseline levels of plasma C-reactive protein, Cardiovasc. Res. 53 (2002) 1029–1034.
[22] M.A. Austin, C. Zhang, S.E. Humphries, W.L. Chandler, P.J. Talmud, K.L. Edwards, D.L. Leonetti, M.J. McNeely, W.Y. Fujimoto, Heritability of C-reactive protein and association with apolipoprotein E genotypes in Japanese Americans, Ann. Hum. Genet. 68 (2004) 179–188, http://dx.doi.org/10.1046/j.1529-8817.2004.00078.x.
[23] L.G. Best, K.E. North, R.P. Tracy, E.T. Lee, B.V. Howard, V. Palmieri, J.W. Maccluer, Genetic determination of acute phase reactant levels: the strong heart study, Hum. Hered. 58 (2004) 112–116, http://dx.doi.org/10.1159/000083032.
[24] M.P.M. de Maat, E.M. Bladbjerg, J. von B. Hjelmborg, L. Bathum, J. Jespersen, K. Christensen, Genetic influence on inflammation variables in the elderly, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 2168–2173, http://dx.doi.org/10.1161/01.ATV.0000143856.01669.e7.
[25] A.J. MacGregor, J.R. Gallimore, T.D. Spector, M.B. Pepys, Genetic effects on baseline values of C-reactive protein and serum amyloid a protein: a comparison of monozygotic and dizygotic twins, Clin. Chem. 50 (2004) 130–134, http://dx.doi.org/10.1373/clinchem.2003.028258.
[26] J. Dupuis, M.G. Larson, R.S. Vasan, J.M. Massaro, P.W.F. Wilson, I. Lipinska, D. Corey, J.A. Vita, J.F. Keaney, E.J. Benjamin, Genome scan of systemic biomarkers of vascular inflammation in the Framingham Heart Study: evidence for susceptibility loci on 1q, Atherosclerosis 182 (2005) 307–314, http://dx.doi.org/10.1016/j.atherosclerosis.2005.02.015.
[27] L.A. Lange, K. Burdon, C.D. Langefeld, Y. Liu, S.R. Beck, S.S. Rich, B.I. Freedman, K.B. Brosnihan, D.M. Herrington, L.E. Wagenknecht, D.W. Bowden, Heritability and expression of C-reactive protein in type 2 diabetes in the Diabetes Heart Study, Ann. Hum. Genet. 70 (2006) 717–725, http://dx.doi.org/10.1111/j.1469-1809.2006.00280.x.
[28] C.L. Saunders, M.C. Gulliford, Heritabilities and shared environmental effects were estimated from household clustering in national health survey data, J. Clin. Epidemiol. 59 (2006) 1191–1198, http://dx.doi.org/10.1016/j.jclinepi.2006.02.015.
[29] W. Tang, Y. Hong, M.A. Province, S.S. Rich, P.N. Hopkins, D.K. Arnett, J.S. Pankow, M.B. Miller, J.H. Eckfeldt, Familial clustering for features of the metabolic syndrome: the national heart, lung, and blood institute (NHLBI) family heart study, Diabetes Care 29 (2006) 631–636.
[30] M.A. Wörns, A. Victor, P.R. Galle, T. Höhler, Genetic and environmental contributions to plasma C-reactive protein and interleukin-6 levels–a study in twins, Genes Immun. 7 (2006) 600–605, http://dx.doi.org/10.1038/sj.gene.6364330.
[31] J. Wessel, G. Moratorio, F. Rao, M. Mahata, L. Zhang, W. Greene, B.K. Rana, B.P. Kennedy, S. Khandrika, P. Huang, E.O. Lillie, P.-A.B. Shih, D.W. Smith, G. Wen, B.A. Hamilton, M.G. Ziegler, J.L. Witztum, N.J. Schork, G.W. Schmid-Schönbein, D.T. O'Connor, C-reactive protein, an "intermediate phenotype" for inflammation: human twin studies reveal heritability, association with blood pressure and the metabolic syndrome, and the influence of common polymorphism at catecholaminergic/beta-adrenergic pathway loci, J. Hypertens. 25 (2007) 329–343, http://dx.doi.org/10.1097/HJH.0b013e328011753e.
[32] E.R. Fox, E.J. Benjamin, D.F. Sarpong, C.N. Rotimi, J.G. Wilson, M.W. Steffes, G. Chen, A. Adeyemo, J.K. Taylor, T.E. Samdarshi, H.A. Taylor, Epidemiology, heritability, and genetic linkage of C-reactive protein in African Americans (from the Jackson heart study), Am. J. Cardiol. 102 (2008) 835–841, http://dx.doi.org/10.1016/j.amjcard.2008.05.049.
[33] S. Su, H. Snieder, A.H. Miller, J. Ritchie, J.D. Bremner, J. Goldberg, J. Dai, L. Jones, N.V. Murrah, J. Zhao, V. Vaccarino, Genetic and environmental influences on systemic markers of inflammation in middle-aged male twins, Atherosclerosis 200 (2008) 213–220, http://dx.doi.org/10.1016/j.atherosclerosis.2007.12.009.
[34] R.B. Schnabel, K.L. Lunetta, M.G. Larson, J. Dupuis, I. Lipinska, J. Rong, M.-H. Chen, Z. Zhao, J.F. Yamamoto, J.B. Meigs, V. Nicaud, C. Perret, T. Zeller, S. Blankenberg, L. Tiret, J.F. Keaney, R.S. Vasan, E.J. Benjamin, The relation of genetic and environmental factors to systemic inflammatory biomarker concentrations, Circ. Cardiovasc. Genet. 2 (2009) 229–237, http://dx.doi.org/10.1161/CIRCGENETICS.108.804245.
[35] S. Su, A.H. Miller, H. Snieder, J.D. Bremner, J. Ritchie, C. Maisano, L. Jones, N.V. Murrah, J. Goldberg, V. Vaccarino, Common genetic contributions to depressive symptoms and inflammatory markers in middle-aged men: the twins heart study, Psychosom. Med. 71 (2009) 152–158, http://dx.doi.org/10.1097/PSY.0b013e31819082ef.
[36] J. Wu, J.S. Pankow, R.P. Tracy, K.E. North, R.H. Myers, M.E. Feitosa, M.A. Province, I.B. Borecki, A QTL on 12q influencing an inflammation marker and obesity in white women: the NHLBI Family Heart Study, Obes. Silver Spring Md 17 (2009) 525–531, http://dx.doi.org/10.1038/oby.2008.556.
[37] I. Rahman, A.M. Bennet, N.L. Pedersen, U. de Faire, P. Svensson, P.K.E. Magnusson, Genetic dominance influences blood biomarker levels in a sample of 12,000 Swedish elderly twins, Twin Res. Hum. Genet. 12 (2009) 286–294, http://dx.doi.org/10.1375/twin.12.3.286.
[38] G. Jermendy, T. Horváth, L. Littvay, R. Steinbach, A.L. Jermendy, A.D. Tárnoki, D.L. Tárnoki, J. Métneki, J. Osztovits, Effect of genetic and environmental influences on cardiometabolic risk factors: a twin study, Cardiovasc. Diabetol. 10 (2011) 96, http://dx.doi.org/10.1186/1475-2840-10-96.
[39] M. Neijts, J. van Dongen, C. Kluft, D.I. Boomsma, G. Willemsen, E.J.C. de Geus, Genetic architecture of the pro-inflammatory state in an extended twin-family design, Twin Res. Hum. Genet. 16 (2013) 931–940, http://dx.doi.org/10.1017/thg.2013.58.
[40] A.A. Sas, F.V. Rijsdijk, J. Ormel, H. Snieder, H. Riese, The relationship between neuroticism and inflammatory markers: a twin study, Twin Res. Hum. Genet. 17 (2014) 177–182, http://dx.doi.org/10.1017/thg.2014.19.
[41] E.M. DeGoma, B. French, R.L. Dunbar, M.A. Allison, E.R. Mohler, M.J. Budoff, Intraindividual variability of C-reactive protein: the multi-ethnic study of atherosclerosis, Atherosclerosis 224 (2012) 274–279, http://dx.doi.org/10.1016/j.atherosclerosis.2012.07.017.
[42] S. Wu, Y. Li, C. Jin, P. Yang, D. Li, H. Li, C. Shen, Intra-individual variability of high-sensitivity C-reactive protein in Chinese general population, Int. J. Cardiol. 157 (2012) 75–79, http://dx.doi.org/10.1016/j.ijcard.2010.12.019.
[43] A. Moayyeri, C.J. Hammond, D.J. Hart, T.D. Spector, The UK adult twin registry (TwinsUK resource), Twin Res. Hum. Genet. 16 (2013) 144–149, http://dx.doi.org/10.1017/thg.2012.89.
[44] M. Neale, L. Cardon, Methodology for Genetic Studies of Twins and Families, Springer Science & Business Media, 1992.
[45] J.M. McCaffery, H. Snieder, Y. Dong, E. de Geus, Genetics in psychosomatic medicine: research designs and statistical approaches, Psychosom. Med. 69

(2007) 206–216, http://dx.doi.org/10.1097/PSY.0b013e31802f5dd4.
[46] M.C. Neale, S.M. Boker, G. Xie, H.H. Maes, Mx: Statistical Modeling, VCU Box 900126, Richmond, VA 23298, Department of Psychiatry, 2003.
[47] K. Lange, J. Westlake, M.A. Spence, Extensions to pedigree analysis. III. Variance components by the scoring method, Ann. Hum. Genet. 39 (1976) 485–491.
[48] A.R. Sutin, A. Terracciano, B. Deiana, S. Naitza, L. Ferrucci, M. Uda, D. Schlessinger, P.T. Costa, High neuroticism and low conscientiousness are associated with interleukin-6, Psychol. Med. 40 (2010) 1485–1493, http://dx.doi.org/10.1017/S0033291709992029.
[49] D.P. Potaczek, M. Kabesch, Current concepts of IgE regulation and impact of genetic determinants, Clin. Exp. Allergy J. Br. Soc. Allergy Clin. Immunol. 42 (2012) 852–871, http://dx.doi.org/10.1111/j.1365-2222.2011.03953.x.
[50] D.P. Potaczek, Links between allergy and cardiovascular or hemostatic system, Int. J. Cardiol. 170 (2014) 278–285, http://dx.doi.org/10.1016/j.ijcard.2013.11.029.
[51] A. Dehghan, J. Dupuis, M. Barbalic, J.C. Bis, G. Eiriksdottir, C. Lu, N. Pellikka, H. Wallaschofski, J. Kettunen, P. Henneman, J. Baumert, D.P. Strachan, C. Fuchsberger, V. Vitart, J.F. Wilson, G. Paré, S. Naitza, M.E. Rudock, I. Surakka, E.J.C. de Geus, B.Z. Alizadeh, J. Guralnik, A. Shuldiner, T. Tanaka, R.Y.L. Zee, R.B. Schnabel, V. Nambi, M. Kavousi, S. Ripatti, M. Nauck, N.L. Smith, A.V. Smith, J. Sundvall, P. Scheet, Y. Liu, A. Ruokonen, L.M. Rose, M.G. Larson, R.C. Hoogeveen, N.B. Freimer, A. Teumer, R.P. Tracy, L.J. Launer, J.E. Buring, J.F. Yamamoto, A.R. Folsom, E.J.G. Sijbrands, J. Pankow, P. Elliott, J.F. Keaney, W. Sun, A.-P. Sarin, J.D. Fontes, S. Badola, B.C. Astor, A. Hofman, A. Pouta, K. Werdan, K.H. Greiser, O. Kuss, H.E. Meyer zu Schwabedissen, J. Thiery, Y. Jamshidi, I.M. Nolte, N. Soranzo, T.D. Spector, H. Völzke, A.N. Parker, T. Aspelund, D. Bates, L. Young, K. Tsui, D.S. Siscovick, X. Guo, J.I. Rotter, M. Uda, D. Schlessinger, I. Rudan, A.A. Hicks, B.W. Penninx, B. Thorand, C. Gieger, J. Coresh, G. Willemsen, T.B. Harris, A.G. Uitterlinden, M.-R. Järvelin, K. Rice, D. Radke, V. Salomaa, K. Willems van Dijk, E. Boerwinkle, R.S. Vasan, L. Ferrucci, Q.D. Gibson, S. Bandinelli, H. Snieder, D.I. Boomsma, X. Xiao, H. Campbell, C. Hayward, P.P. Pramstaller, C.M. van Duijn, L. Peltonen, B.M. Psaty, V. Gudnason, P.M. Ridker, G. Homuth, W. Koenig, C.M. Ballantyne, J.C.M. Witteman, E.J. Benjamin, M. Perola, D.I. Chasman, Meta-analysis of genome-wide association studies in >80 000 subjects identifies multiple loci for C-reactive protein levels, Circulation 123 (2011) 731–738, http://dx.doi.org/10.1161/CIRCULATIONAHA.110.948570.
[52] S. Naitza, E. Porcu, M. Steri, D.D. Taub, A. Mulas, X. Xiao, J. Strait, M. Dei, S. Lai, F. Busonero, A. Maschio, G. Usala, M. Zoledziewska, C. Sidore, I. Zara, M. Pitzalis, A. Loi, F. Virdis, R. Piras, F. Deidda, M.B. Whalen, L. Crisponi, A. Concas, C. Podda, S. Uzzau, P. Scheet, D.L. Longo, E. Lakatta, G.R. Abecasis, A. Cao, D. Schlessinger, M. Uda, S. Sanna, F. Cucca, A genome-wide association scan on the levels of markers of inflammation in Sardinians reveals associations that underpin its complex regulation, PLoS Genet. 8 (2012) e1002480, http://dx.doi.org/10.1371/journal.pgen.1002480.
[53] A. Vaez, R. Jansen, B.P. Prins, J.-J. Hottenga, E.J.C. de Geus, D.I. Boomsma, B.W.J.H. Penninx, I.M. Nolte, H. Snieder, B.Z. Alizadeh, In silico post genome-wide association studies analysis of C-reactive protein loci suggests an important role for interferons, Circ. Cardiovasc. Genet. 8 (2015) 487–497, http://dx.doi.org/10.1161/CIRCGENETICS.114.000714.