University of Groningen

(Genetic) Epidemiology of Inflammation, Age-related Pathology and Longevity
Sas, Arthur Alexander

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Chapter 2

The age-dependency of genetic and environmental influences on serum cytokine levels: a twin study

Scan this QR code to read the published article online.
The age-dependency of genetic and environmental influences on serum cytokine levels: A twin study

Arthur A. Sas a,1, Valla Jamshidi b, Dongling Zheng b, Ting Wu a, Jakob Korf b, Behrouz Z. Alizadeh b, Tim D. Spector a,*, Harald Snieder b,∗

a Department of Epidemiology and Biostatistics, University Medical Center Groningen, University of Groningen, The Netherlands
b Division of Biomedical Sciences, St. George’s Hospital Medical School, London, United Kingdom

Received 31 August 2011; revised 28 March 2012; accepted 20 April 2012

A R T I C L E  I N F O

Keywords:

ageing

twins

heritability

serum cytokine

A B S T R A C T

Previous epidemiological studies have evaluated the role of immunological markers as possible tools for the early detection of age-related changes in the human immune system. The importance of both genetic and environmental influences in regulation of these processes has been emphasized in order to further explore this relationship, the present study aims to investigate the relative influence of genetic and environmental factors on four key cytokines involved in the human immune response (Interleukin (IL)-1, IL-6, IL-10 and Tumor Necrosis Factor (TNF)-α). In addition, the role of age as a possible moderator in these processes was evaluated. Methods: The study was conducted in 1003 females from the Twins UK registry, with mean age 60 ± 12.2 years, including 863 MB twin pairs (320 pairs and 517 singletons) and 740 dizygotic twins (321 pairs and 568 singletons). Heritability was estimated using structural equation modeling. The role of age as a moderator was evaluated using age×age interaction models. Results: Heritability was moderate for IL-1α (range: 0.27–0.31) and IL-10 (0.20–0.22) and low for IL-6 (range: 0.15–0.18) and TNF-α (range: 0.17–0.21). For IL-10, heritability declines with age due to an increase in unique environmental factors. Conclusion: The current findings illustrate the importance of genetic and environmental influences on four cytokines involved in the human immune response. For two of these there is evidence that heritability changes with age owing to changes in environmental factors underlying the familiality. © 2012 Elsevier Ltd. All rights reserved.

1. Introduction

With a growing number of elderly in modern society, understanding age-related disorders is an increasingly important issue for current and future healthcare initiatives. Although the role of genetic [1] and environmental factors [2] influencing aging are appreciated, the precise molecular and cellular mechanisms involved are still unclear [3]. The use of biochemical biomarkers for measuring aging has been evaluated, in an attempt to identify individuals who are more prone to age-related pathology. In this context, it has been demonstrated that aging is known to be associated with a low grade inflammatory state and predicting age-related pathology. The importance of both genetic and environmental influences in regulation of these processes has been emphasized in order to further explore this relationship, the present study aims to investigate the relative influence of genetic and environmental factors on four key cytokines involved in the human immune response (Interleukin (IL)-1, IL-6, IL-10 and Tumor Necrosis Factor (TNF)-α). In addition, the role of age as a possible moderator in these processes was evaluated.

2. Methods

2.1. Subjects

The study was conducted in 1003 females from the Twins UK registry, with mean age 60 ± 12.2 years, including 863 MB twin pairs (320 pairs and 517 singletons) and 740 dizygotic twins (321 pairs and 568 singletons). Details of the Twins UK registry (including details on recruitment) were published elsewhere [22]. Zygosity was determined by questionnaire supplemented by DNA-fingerprinting in cases with disputed or uncertain zygosity.

2.2. Sample analysis

Serum IL-1α, IL-10, IL-6 and TNF-α were measured simultaneously using the bead-based high sensitivity human cytokine kit (INNO-SONIC, Inno-Mikki) according to the manufacturer’s protocol. Briefly, on the day of analysis, each blood sample was incubated with antibody-coated capture beads overnight at 4°C. After washing the beads, species-specific biotinylated detector antibodies are added and incubated with the beads for one hour. After removal of excess biotinylated antibodies, streptavidin–Phycoerythrin (Streptavidin-PE) is added and incubated for 30 minutes. Streptavidin-PE, the beads are analyzed on the Luminex-100 system (LiquiChip, Queens). Concentrations of the four cytokines were calculated from the standard curve provided by the manufacturer. The intra-assay variability for each cytokine. Sensitivity values for the kit are: IL-1α 0.06 pg/mL, IL-10 0.15 pg/mL, IL-6 0.10 pg/mL, TNF-α 0.09 pg/mL.

2.3. Measurements and covariates

Accuracy of the measurements was confirmed by replicating samples in order to calculate intra-assay reliabilities expressed as intra-class correlation (104 replicates for IL-1, IL-10, 117 replicates for IL-6, 108 replicates for IL-10 and 117 replicates for TNF-α). Intra-assay reliability estimates for IL-1, IL-6, IL-10 and TNF-α were 0.95, 0.96, 0.90 and 0.76 respectively. All cytokine levels were adjusted for potential batch-effects.

In order to allow adequate statistical power, it was necessary for all cytokines to obtain a reasonable approximation of the normal distribution. After log transformation a normal distribution of all cytokines was achieved. The relationship between any two cytokines was then tested using linear regression analysis. Covariates included in the analysis were BMI, sex, smoking status and BMI supplementation.

For the regression analysis, three models were used for each cytokine: (1) Age, (2) Age and BMI, (3) Age, BMI and any other significant covariates. The influence of smoking behavior, alcohol consumption and history of cardiovascular disease (CVD) as possible covariates were tested in a small subset of the data (n = 452). These factors did not significantly contribute as covariates (data not shown). The residuals were used in the model fitting. General Estimating Equations (GEEs) were used for test in between baseline characteristics between MB and DZ twins.

2.4. Analytical approach

The aims of our analyses were to estimate the relative influence of genetic and environmental factors on IL-1α, IL-10, IL-6 and TNF-α levels and the influence of age on these factors. Structural equation modeling (SEM) was the primary method of analysis. SEM is based on the comparison of the covariance–covariance matrices in MB and DZ twin pairs and allows separation of the observed phenotypic variance into genetic and environmental components: additive (A) or dominant (D) genetic components and common (C) and unique (E) environmental components. E also contains measurement error. Dividing each of these components by the total variance yields the different standardized components of the variance. We focused on additive genetic effects and common and unique environmental effects (by using ACE as the full model) for IL-6, IL-10 and TNF-α. For IL-10, we focused on additive genetic effects, genetic dominance and unique environmental effects (by using an ADE model, since its correlations among MB twins substantially exceeded twice that among DZ twins, which indicates dominance variance [23]). In addition, we calculated (A + E) models as all ACE models could not be distinguished, calculating the proportion (and magnitude) of a familial component. All available data was taken into account. Models were fitted to the raw data using maximum likelihood estimation. All available data was taken into account. Models were fitted to the raw data using maximum likelihood estimation. All available data was taken into account. Models were fitted to the raw data using maximum likelihood estimation. All available data was taken into account. Models were fitted to the raw data using maximum likelihood estimation.

Since regression analysis of age × cytokines (as described above) merely reflects the effect of the effect of age on the mean cytokine levels, gene–age interaction models were applied testing whether age mediated the effect on genetic and environmental variance components underlying different influences of cytokine levels. We fitted the basic gene–environment interaction models as described by Purcell [24], using age as a continuous moderator incorporating all the available (complete) twin pairs. All twins were grouped between 18 and 70 years of age in each group. Sensitivity values for the kit are: IL-1α 0.06 pg/mL, IL-10 0.15 pg/mL, IL-6 0.10 pg/mL, TNF-α 0.09 pg/mL.

2.3. Measurements and covariates

Accuracy of the measurements was confirmed by replicating samples in order to calculate intra-assay reliabilities expressed as intra-class correlation (104 replicates for IL-1, IL-10, 117 replicates for IL-6, 108 replicates for IL-10 and 117 replicates for TNF-α). Intra-assay reliability estimates for IL-1, IL-6, IL-10 and TNF-α were 0.95, 0.96, 0.90 and 0.76 respectively. All cytokine levels were adjusted for potential batch-effects.

In order to allow adequate statistical power, it was necessary for all cytokines to obtain a reasonable approximation of the normal distribution. After log transformation a normal distribution of all cytokines was achieved. The relationship between any two cytokines was then tested using linear regression analysis. Covariates included in the analysis were BMI, sex, smoking status and BMI supplementation.

For the regression analysis, three models were used for each cytokine: (1) Age, (2) Age and BMI, (3) Age, BMI and any other significant covariates. The influence of smoking behavior, alcohol consumption and history of cardiovascular disease (CVD) as possible covariates were tested in a small subset of the data (n = 452). These factors did not significantly contribute as covariates (data not shown). The residuals were used in the model fitting. General Estimating Equations (GEEs) were used for test in between baseline characteristics between MB and DZ twins.

2.4. Analytical approach

The aims of our analyses were to estimate the relative influence of genetic and environmental factors on IL-1α, IL-10, IL-6 and TNF-α levels and the influence of age on these factors. Structural equation modeling (SEM) was the primary method of analysis. SEM is based on the comparison of the covariance–covariance matrices in MB and DZ twin pairs and allows separation of the observed phenotypic variance into genetic and environmental components: additive (A) or dominant (D) genetic components and common (C) and unique (E) environmental components. E also contains measurement error. Dividing each of these components by the total variance yields the different standardized components of the variance. We focused on additive genetic effects and common and unique environmental effects (by using ACE as the full model) for IL-6, IL-10 and TNF-α. For IL-10, we focused on additive genetic effects, genetic dominance and unique environmental effects (by using an ADE model, since its correlations among MB twins substantially exceeded twice that among DZ twins, which indicates dominance variance [23]). In addition, we calculated (A + E) models as all ACE models could not be distinguished, calculating the proportion (and magnitude) of a familial component. All available data was taken into account. Models were fitted to the raw data using maximum likelihood estimation. All available data was taken into account. Models were fitted to the raw data using maximum likelihood estimation. All available data was taken into account. Models were fitted to the raw data using maximum likelihood estimation. All available data was taken into account. Models were fitted to the raw data using maximum likelihood estimation. All available data was taken into account. Models were fitted to the raw data using maximum likelihood estimation. All available data was taken into account. Models were fitted to the raw data using maximum likelihood estimation.
variance by age, respectively. For example, a significant moderation
of additive genetic variance alone would suggest that the mag-
nitude of heritability of serum inflammatory factor levels changes
as the moderate increases or decreases. Variance components were
only tested for significance if the respective interaction terms
had been dropped from the model, e.g. A was not tested unless T
was not significant, to avoid modeling interactions in the absence
of main effects. In the final model, each parameter contributes sig-
ificantly to model fit (p < 0.05).

All data handling and preliminary analyses were done with
STATA version 10.1, Statacorp, TX, USA. Quantitative genetic
modeling was carried out using Mx software [25].

3. Results

Baseline characteristics of monozygotic (MZ) and dizygotic (DZ)
twins are summarized in Table 1. Except for age, no significant dif-
fierences were observed between MZ and DZ twins. Mean age ±
standard deviation (SD) was 50.4 ± 11.1 years for MZ twins and
53.1 ± 12.2 years for DZ twins (p = 0.01) and was adjusted for in
all models. Mean Body Mass Index (BMI), Low-Density Lipoprotein
(LDL), High-Density Lipoprotein (HDL), triglycerides, mean glucose
levels, IL-6, IL-10, IL-1β, TNF-α and TNF-β levels did not significantly
differ between MZ and DZ twins.

Table 1: General characteristics and baseline levels of studied variables by age group.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>HDL (mmol/L)</th>
<th>LDL (mmol/L)</th>
<th>Triglycerides (mmol/L)</th>
<th>Fasting glucose (mmol/L)</th>
<th>IL-10 (pg/L)</th>
<th>IL-6 (pg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–29</td>
<td>25.8 (4.7)</td>
<td>1.5 (0.5)</td>
<td>3.5 (1.0)</td>
<td>0.8 (0.4)</td>
<td>4.8 (1.0)</td>
<td>51.8 (82.7)</td>
<td>29.5 (29.3)</td>
</tr>
<tr>
<td>30–39</td>
<td>26.3 (5.4)</td>
<td>1.6 (0.5)</td>
<td>3.3 (1.0)</td>
<td>0.9 (0.4)</td>
<td>4.8 (1.2)</td>
<td>53.8 (102.0)</td>
<td>29.9 (44.9)</td>
</tr>
<tr>
<td>40–49</td>
<td>26.4 (5.1)</td>
<td>1.6 (0.5)</td>
<td>3.3 (1.0)</td>
<td>0.8 (0.4)</td>
<td>5.0 (0.9)</td>
<td>55.0 (100.2)</td>
<td>30.2 (57.2)</td>
</tr>
<tr>
<td>50–59</td>
<td>26.4 (5.1)</td>
<td>1.6 (0.5)</td>
<td>3.3 (1.0)</td>
<td>0.8 (0.4)</td>
<td>4.8 (1.2)</td>
<td>58.0 (102.0)</td>
<td>30.2 (57.2)</td>
</tr>
<tr>
<td>60–69</td>
<td>26.4 (5.1)</td>
<td>1.6 (0.5)</td>
<td>3.3 (1.0)</td>
<td>0.8 (0.4)</td>
<td>5.0 (0.9)</td>
<td>55.0 (100.2)</td>
<td>30.2 (57.2)</td>
</tr>
</tbody>
</table>

Table 2: Power values and twinning correlations for IL-1β, IL-6, IL-10 andTNF-α.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Model</th>
<th>β</th>
<th>Correlation (r²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>(A + C)</td>
<td>0.05 (0.05–0.15)</td>
<td>0.02 (0.05–0.15)</td>
</tr>
<tr>
<td>IL-6</td>
<td>(A + C)</td>
<td>0.05 (0.05–0.15)</td>
<td>0.02 (0.05–0.15)</td>
</tr>
<tr>
<td>IL-10</td>
<td>(A + C)</td>
<td>0.05 (0.05–0.15)</td>
<td>0.02 (0.05–0.15)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>(A + C)</td>
<td>0.05 (0.05–0.15)</td>
<td>0.02 (0.05–0.15)</td>
</tr>
</tbody>
</table>

Fig. 1: Partial path diagram of the basic gene-environment interaction model. A = additive genetic effects; C = common environmental effects; E = unique environmental effects; N = moderated component of C; V = moderated component of E. R = linear effects of moderator on outcome. A = additive genetic effects; C = common environmental effects; E = unique environmental effects.

In Table 3, the contribution of the variance components on the
various cytokines are summarized. In virtually all cytokines, the
best fitting model was an AE-model, indicating the presence of
an additive genetic component in the variance of baseline serum
values. For IL-1β, AE was the best fitting model for all 3 covariate
models. Heritability is moderate, 0.32, 0.31 and 0.27 for model 1,
2 and 3 respectively. Heritability of IL-10 was also moderate;
0.31 in all three covariate models. Heritabilities for TNF-α were
low, ranging from 0.17–0.22.

Table 3: Genetic and environmental variance components (%MZ DZ) for IL-1β, IL-6, IL-10 and TNF-α.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Submodel 1</th>
<th>Submodel 2</th>
<th>Submodel 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>0.29 (0.00–0.57)</td>
<td>0.31 (0.00–0.57)</td>
<td>0.35 (0.00–0.60)</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.29 (0.00–0.57)</td>
<td>0.31 (0.00–0.57)</td>
<td>0.35 (0.00–0.60)</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.01 (0.00–0.04)</td>
<td>0.01 (0.00–0.04)</td>
<td>0.01 (0.00–0.04)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.00 (0.00–0.00)</td>
<td>0.00 (0.00–0.00)</td>
<td>0.00 (0.00–0.00)</td>
</tr>
</tbody>
</table>

In Table 4, the results of the gene-age interaction modeling is
presented, testing age as a continuous moderator of the variance
of various cytokines. In all cytokines, the best fitting model was
AE, indicating the presence of a significant contribution of the
A + C component, which indicates a significant
familial effect (A + C (95% CI) is 0.29 (0.20–0.38) for both models).

Table 4: Comparison of model fits. Submodels for IL-1β, IL-6, IL-10 and TNF-α.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Model</th>
<th>Submodel 1</th>
<th>Submodel 2</th>
<th>Submodel 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>0.13 (0.06–0.20)</td>
<td>0.13 (0.06–0.20)</td>
<td>0.13 (0.06–0.20)</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>0.13 (0.06–0.20)</td>
<td>0.13 (0.06–0.20)</td>
<td>0.13 (0.06–0.20)</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>0.01 (0.00–0.04)</td>
<td>0.01 (0.00–0.04)</td>
<td>0.01 (0.00–0.04)</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.00 (0.00–0.00)</td>
<td>0.00 (0.00–0.00)</td>
<td>0.00 (0.00–0.00)</td>
<td></td>
</tr>
</tbody>
</table>

We were able to demonstrate the presence of a significant addi-
tive genetic component in the regulation of baseline serum levels
of IL-1β, IL-6, IL-10 and TNF-α in female twins. We also showed that
age acts as a moderator on the additive genetic component in
regulation of baseline IL-1β and TNF-α serum levels (Figs. 2 and 3). Heritability humps due to a change in unique environ-
mental factors. This indicates changes in immune status or modera-
tion of inflammatory pathways with age.

The present study is one of the most extensive studies of its
kind in terms of sample size, therefore providing superior power
to most previous studies. Still, some power issues arise. For IL-6 and TNF-α, no evident genetic compo-
nents in the regulation of baseline serum levels of four key cyto-
kinemes involved in the human inflammatory response with poten-
tial relevance for ageing pathways. We also incorporated age in
the “fully adjusted” models as a potential moderator of genetic and
environmental factors.

We present the genetic and environmental sources of
individual differences in baseline levels of four key cyto-
kinemes involved in the human inflammatory response with potential relevance for ageing pathways. In addition, we also
incorporated age in the “fully adjusted” models as a potential moderator of genetic and
environmental factors.

We were able to demonstrate the presence of a significant addi-
tive genetic component in the regulation of baseline serum levels
of IL-1β, IL-6, IL-10 and TNF-α in female twins. We also showed that
age acts as a moderator on the additive genetic component in
regulation of baseline IL-1β and TNF-α serum levels (Figs. 2 and 3). Heritability humps due to a change in unique environ-
mental factors. This indicates changes in immune status or modera-
tion of inflammatory pathways with age.

The present study is one of the most extensive studies of its
kind in terms of sample size, therefore providing superior power
to most previous studies. Still, some power issues arise. For IL-6 and TNF-α, no evident genetic compo-
nents in the regulation of baseline serum levels of four key cyto-
kinemes involved in the human inflammatory response with potential relevance for ageing pathways. We also incorporated age in
the “fully adjusted” models as a potential moderator of genetic and
environmental factors.
were observed as SEM could not distinguish between a CE and AE model. However, analysis of the A+E component in these models suggest the presence of a significant contribution of a familial component. The full (ACE) model, which was applied, revealed lower heritability as was observed in none (but all of the) previous studies on IL-1 [14, 25-28].

A limitation of the present study is that it cannot distinguish between age, birth cohort effects and calendar time effects. This is a known limitation for these kind of studies (due to their cross-sectional design). Longitudinal studies would be necessary to address these issues.

In the present study, behavioral covariates like smoking behavior, alcohol consumption and physical exercise were not taken into account as potential covariates. Though significant associations between these covariates and immunological traits have been demonstrated in the past, no significant contributions of these covariates to baseline serum levels of the studied cytokines were observed. Regression analyses in a subset of the individuals where data on smoking behavior, alcohol consumption and history of Cardiovascular disease (CVD) (including coronary-vascular accidents) was available (n=425) yielded no significance of these covariates.

We did not observe a significant relationship (or R²-values) between age and cytokine levels in the current study, in contrast to other studies. A possible explanation for this is that strict adjustment for batch effect as applied in may have removed some of the association between serum cytokine levels and age as a result of some imbalance of the age distribution across batches. This has no impact on the results and conclusions drawn however, since a lack of effect of age on the mean values does not imply a lack of effect on the variance components.

An important difference with previous studies is the inclusion of IL-1β and IL-10 in the analysis. No heritabilities on baseline serum levels of these cytokines have been published to date, we are the first to demonstrate genetic influences in the regulation of these baseline serum levels. The role of IL-1β in particular, is the association of anti-inflammatory cytokines with healthy ageing and longevity. The principal route function of IL-1β appears to be the limited and ultimately terminating inflammatory response, which hypothetically offers protection against various age-related pathologies [20,21].

An interesting feature of our study is that we are the first to show modulation of unique environmental influences in the regulation of baseline IL-1β and TNF-α by age. For IL-1β this may be a direct effect of an increasing importance of unique environment during life (e.g. habits, social network, and environment), leading to a decrease in heritability over life. On the other hand, it may also represent increasing homocysteine discordance (in terms of ‘internal environment’) between twins. The latter seems more plausible as it is difficult to envision a lifelong increase in discordance in the ‘physical’ unique environment (e.g. lifestyle) causing a lifelong decrease in heritability over this cytokine.

For TNF-α heritability increases with age due to a decreasing discordance in unique environmental factors. No solid explanation can be given for this phenomenon, but it is clear that genetic factors become more important in regulation of TNF-α during life.

Analyzing heritabilities calculated with SEM in a stratified (younger and older individuals) analysis provided a similar trend but no significant differences in heritabilities for IL-1β and TNF-α alpha as observed in Figs. 2b and 3b. We conclude from these results that a stratified SEM-analysis is probably less powerful than the CoT model used in the present paper, which is applied for the entire age range.

The present study shows evidence of a substantial role for genetics in the regulation of baseline cytokine levels. Moreover, we emphasize the importance of (changing) environmental factors (i.e. ‘internal environment’) during life, hypothetically causing a lifelong decrease in heritability of this inflammatory cytokine. We conclude from these results that a stratified SEM-analysis is probably less powerful than the CoT model used in the present paper, which is applied for the entire age range.