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

Arthur A. Sas\textsuperscript{a,\textdagger}, Yalda Jamshidi\textsuperscript{b}, Dongling Zheng\textsuperscript{b}, Ting Wu\textsuperscript{a}, Jakob Korf\textsuperscript{c}, Behrooz Z. Alizadeh\textsuperscript{a}, Tim D. Spector\textsuperscript{d}, Harold Snieder\textsuperscript{a,\textdagger}\textsuperscript{d}

\textsuperscript{a}Unit of Genetic Epidemiology & Bioinformatics, Department of Epidemiology, University Medical Centre Groningen, University of Groningen, The Netherlands
\textsuperscript{b}Division of Biomedical Sciences, St. Georges Hospital Medical School, London, United Kingdom
\textsuperscript{c}University of Groningen, University Centre for Psychiatry, University Medical Centre Groningen, The Netherlands
\textsuperscript{d}Department of Twin Research & Genetic Epidemiology, King’s College, St. Thomas Campus, London, United Kingdom

\textbf{A R T I C L E   I N F O}

Article history:
Received 31 August 2011
Received in revised form 28 March 2012
Accepted 25 April 2012
Available online xxxx

Keywords:
Aging
Twins
Heritability
Cytokines
Interleukin

\textbf{ABSTRACT}

Previous epidemiologic studies have evaluated the use of immunological markers as possible tools for measuring ageing and predicting age-related pathology. The importance of both genetic and environmental influences in regulation of these markers has been emphasized. In order to further evaluate 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\textalpha, IL-6, IL-10 and Tumor Necrosis Factor (TNF)-\textalpha). In addition, the role of age as a possible moderator on these influences was evaluated.

\textbf{Methods:} The study was conducted in 1603 females from the Twins UK registry, with mean age ±SD of 60.4 ± 12.2 years, including 863 monozygotic twins (385 pairs and 93 singletons) and 740 dizygotic twins (321 pairs and 98 singletons). Heritability was estimated using structural equation modeling. The role of age as a moderator was evaluated using gene-age interaction models.

\textbf{Results:} Heritabilities were moderate for IL-1\textalpha (range: 0.27–0.32) and IL-10 (0.30) and low for IL-6 (range: 0.15–0.16) and TNF-\textalpha (range: 0.17–0.23). For IL-1\textalpha, heritability declines with age due to an increase in unique environmental factors. For TNF-\textalpha, heritability increases with age due to a decrease in unique environmental factors.

\textbf{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 unique to the individual.

\textcopyright 2012 Elsevier Ltd. All rights reserved.

1. Introduction

With a growing number of elderly in modern society, understanding ageing and age-related pathology is an increasingly important issue for current and future healthcare initiatives. Although the role of genetic [1] and environmental factors [2] influencing ageing are appreciated, the precise molecular and cellular mechanisms involved are still unclear [3].

15 The use of biochemical biomarkers for measuring ageing 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 ageing is known to be associated with a low grade elevation of inflammatory factors, attributed to the dysregulation of immune and inflammatory pathways with ageing [4–7]. In accordance with these findings, it has been demonstrated that chronic inflammation predisposes to long-term morbidity and mortality from many chronic, age-related diseases (such as chronic pulmonary cardiovascular disease [8–11]).

The synthesis and regulation of serum levels of inflammatory factors has been extensively studied, showing the significance of both environmental and genetic influences in this process [12–15]. The findings on the magnitude of genetic influences (heritability) are very inconsistent however, possibly due to relatively small sample sizes used. Furthermore, there are no studies which have evaluated the changes in environmental and genetic processes with age, in order to investigate the origin of the dysregulation of immune and inflammatory status with advancing age.

The present study aims to assess the genetic and environmental influences on baseline serum levels of four key cytokines involved
2.3. Measurements and covariates

Intra-class correlations (104 repeats for IL-1 samples in order to calculate intra-assay reliabilities expressed as 0.89, 0.88 and 0.76 respectively. All cytokine levels were adjusted for potential batch effects.

Log-transformation was necessary for all cytokines to obtain a better approximation of the normal distribution. After log transformation, all data fell well within 4 SDs of the mean. Also, visual inspection of scatter plots yielded no potential outliers deflating or inflating the correlation. Therefore, no values needed to be excluded and all were included in the analysis. Next, the effect of various known covariates (risk factors for cardiovascular disease) was tested using linear regression analysis. Covariates included in the analysis were HDL, LDL, triglycerides and fasting blood glucose. 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 to test for difference in baseline characteristics between MZ and DZ twins.

2.4. Analytical approach

The aims of our analysis were to estimate the relative influence of genetic and environmental factors on IL-1β, IL-6, IL-10 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 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) 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-1β, we focused on additive genetic effects, genetic dominance and unique environmental effects (by using an ADE model), since its correlations among MZ twins substantially exceeded twice that among DZ twins, which indicates dominance variance [23]. In addition, we calculated (A + C) in models were AE or CE models could not be distinguished, calculating the presence (and magnitude) of a familial component. All available data was taken into account. Models were fitted to the raw data using normal maximum likelihood theory, allowing the use of information for the estimation of variance (but not covariance) provided by unpaired twin observations.

Since regression analysis of age on cytokines (as described above) merely reflects the estimation 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 individual differences 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 cytokines were adjusted for BMI and any other covariates if significant (model 3) and their residuals used in model fitting. The effect of age on the mean cytokine levels was incorporated in the interaction model itself (see below). In this gene-environment interaction model (Fig. 1), the phenotypic variance of the outcome variables (i.e., serum IL-1β, IL-6, IL-10 and TNF-α) is partitioned into A, C or D, and E components with the path coefficients associated with each variable expressed as linear functions of the moderator (e.g., A + T × M1, C/D + U × M1, E + V × M1), where T represents the effects of the moderator on additive genetic variance and U and V represents the effects of the moderator on common environmental/dominant genetic and unique environmental variance, respectively. In addition, the effect of the moderator on the mean is also modeled (e.g., μ + B × M1), where M1 represents the value of the moderator and B represents linear effects on the outcome. The mean structure encompasses any phenotypic correlation between age and the outcome variables. A significant compromise of model fit when parameters T, U or V are fixed to zero reflects evidence of significant moderation of additive genetic, common environmental/dominant genetic or unique environmental
variance by age, respectively. For example, a significant moderation of additive genetic variance alone would suggest that the magnitude of heritability of serum inflammatory factor levels changes as the moderator 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 significantly 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 differences were observed between MZ and DZ twins. Mean age ± standard deviation (SD) is 60.4 ± 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, blood glucose levels, IL-1β, IL-6, IL-10 and TNF-α levels did not significantly differ between MZ and DZ twins.

Twin correlations and percentage of variance explained by covariates (R²-values) are summarized in Table 2. IL-1β was only significantly influenced by LDL (p < 0.01). IL-6 and IL-10 were significantly influenced by BMI (p < 0.01). TNF-α was significantly influenced by BMI (p < 0.01) and HDL (p < 0.01).

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, 0.32 and 0.27 for model 1, 2 and 3 respectively. Heritability of IL-10 was also moderate; 0.30 in both covariate models. Heritabilities for TNF-α were low, ranging from 0.17–0.23 depending on the covariate model used. Structural equation modeling could not distinguish between an AE and a CE-model in model 2 and 3. However, we found a significant contribution of the A + C component, which indicates a significant familial effect (A + C (95% CI) is 0.22 (0.10–0.33), 0.20 (0.09–0.31) and 0.15 (0.04–0.28) for covariate model 1, 2 and 3 respectively). For IL-6, no distinction could be made between AE or CE models based on P-value or AIC also. Again, we found 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). Heritability is low, ranging from 0.15–0.16 depending on the model used. For IL-1β and IL-10, the full model indicated the presence of a Dominant genetic factor. However, this contribution of D did not reach significance.

In Table 4, the results of the gene-age interaction modeling is presented, testing age as a continuous moderator of the variance components for all cytokines. No evidence of gene-age interaction was found for IL-6 and IL-10. For IL-1β, a decline in heritability with age was observed (Fig. 2a). Heritability is approximately 0.43 at age 20 and 0.22 at age 70, caused by an increase in the unique environmental influences with age (p < 0.01), total variance increased with age (Fig. 2b). For TNF-α, an increase in heritability with advancing age was observed (Fig. 3a). Heritability is below 0.10 at age 20 and approximately 0.17 at age 70, caused by a decrease in unique environmental influences with age (p < 0.01), total variance decreased with age (Fig. 3b). SEM-analysis after stratification of the data (individuals below and above the median of age) provided similar (but non-significant) results regarding the trend of heritabilities displayed in Figs. 2b and 3b. Heritabilities (95% CI) in younger individuals (<62 years of age) were 0.31
Comparative model fits for age as a continuous moderator on IL-1

Table 4

Parameter estimates of best fitting univariate structural equation models for IL-1, IL-6, IL-10 and TNF-α.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Model</th>
<th>Univariate model fitting:</th>
<th>A2/A + C2</th>
<th>C2/D2</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>1 ADE</td>
<td>0.12 (0.00–0.37)</td>
<td>0.22 (0.00–0.41)</td>
<td>0.65 (0.57–0.74)</td>
<td></td>
</tr>
<tr>
<td>IL-1α</td>
<td>1′ A</td>
<td>0.32 (0.24–0.41)</td>
<td>–</td>
<td>0.68 (0.59–0.76)</td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>2 ADE</td>
<td>0.13 (0.00–0.38)</td>
<td>0.22 (0.00–0.42)</td>
<td>0.65 (0.57–0.74)</td>
<td></td>
</tr>
<tr>
<td>IL-1α</td>
<td>2′ A</td>
<td>0.33 (0.24–0.41)</td>
<td>–</td>
<td>0.68 (0.59–0.76)</td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>3 ADE</td>
<td>0.19 (0.00–0.36)</td>
<td>0.09 (0.00–0.35)</td>
<td>0.72 (0.63–0.81)</td>
<td></td>
</tr>
<tr>
<td>IL-1α</td>
<td>3′ A</td>
<td>0.27 (0.17–0.36)</td>
<td>–</td>
<td>0.73 (0.64–0.83)</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>1 ACE</td>
<td>0.16 (0.00–0.38)</td>
<td>0.13 (0.00–0.31)</td>
<td>0.71 (0.62–0.80)</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>1 ADE</td>
<td>0.16 (0.00–0.37)</td>
<td>0.16 (0.00–0.38)</td>
<td>0.68 (0.59–0.78)</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>1 ACE</td>
<td>0.17 (0.00–0.33)</td>
<td>0.05 (0.00–0.24)</td>
<td>0.78 (0.67–0.90)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: A2, Additive genetic components; C2, Shared environmental components; D2, Dominant genetic components; E2, Unique environmental component.

Table 4

Comparative model fits for age as a continuous moderator on IL-1β, IL-6, IL-10 and TNF-alpha. Bold values indicate best fitting models.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Model</th>
<th>Univariate model fitting:</th>
<th>A2LL</th>
<th>A2LL</th>
<th>Adf</th>
<th>P</th>
<th>AIC</th>
<th>Model test</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>1 ADETUVB</td>
<td>2944.66</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IL-6</td>
<td>1 ACETVB</td>
<td>2946.89</td>
<td>2.22</td>
<td>2</td>
<td>0.33</td>
<td>–</td>
<td>1.78</td>
<td>4 vs. 1</td>
</tr>
<tr>
<td>IL-10</td>
<td>1 ACETVB</td>
<td>3858.36</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1 ACETVB</td>
<td>1563.17</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations: –2LL, –2-log likelihood; A, additive genetic variance; AIC, Akaike’s Information Criterion; B, linear effects of age on means of the outcome variables; BMI, Body Mass Index; C, common environmental variance; df, degrees of freedom; E, unique environmental variance; T, moderation of additive genetic variance by age; U, moderation of common environmental variance by age; V, moderation of unique environmental variance by age.

4. Discussion

The present study assessed the genetic and environmental sources of individual differences in baseline levels of four key cytokines 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.

We were able to demonstrate the presence of a significant additive 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 changes due to a change in unique environmental factors. This indicates changes in immune status or moderation 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 compared to most previous studies. Still, some power issues arise. For IL-6 and TNF-α submodel 2 and 3, no evident genetic compo-
nent was observed as SEM could not distinguish between a CE and AE model. However, analysis of the A + C component in these models suggests the presence of a significant contribution of a familial component. The full (ACE) model reported, indicates a substantial lower heritability as was observed in some (but not all) of the previous studies on IL-6 [14,15,26–28].

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 analysis in a subset of the individuals where data on smoking behavior, alcohol consumption and history of Cardiovascular disease (CVD) (including cerebro-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 by us may have removed some of the association between mean cytokines values 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 values. The reason for including IL-10 in particular, is the association of anti-inflammatory cytokines with healthy ageing and longevity. The principal routine function of IL-10 appears to be to limit and ultimately terminate inflammatory responses, 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 moderation of unique environmental influences on 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 homeostatic 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 of this inflammatory 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-alpha as observed in Figs. 2b and 3b. We conclude from these results that a stratified SEM-analysis is probably less powerful than the GxE model used in the present paper, which is applied over 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 dysregulation of inflammatory pathways during life. This implies that a strictly genetic relationship between cytokines, genes and pathology cannot be observed, even though there is an evident relationship between cytokine genes and levels on the one hand and cytokine levels and pathology on the other hand.

In conclusion, this study emphasizes the role of genetics and environmental factors in regulation of four potential ‘biomarkers of ageing’ that play a key role in the human immune and inflammatory responses. The present study supports the hypothesis that the variety in age-related phenotypes is a combination of both environmental factors and complex genetic pathways. Finally, our results indicate that the relative role of genetics and environment involving immune functioning may change over a lifetime, illustrating the potential of the studied cytokines as potential ‘biomarkers of ageing’.

References