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

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Chapter 1

Introduction

Life is irrevocably connected to death. Every living organism eventually dies; whether they are small like bacteria or large like elephants. In general, we all follow our own "walk of life", from birth to our death. This "walk of life" is better known as "ageing", a continuous process in which our bodies become "older".

The life course of most organisms is highly comparable. This means birth, youth, the reproductive and post-reproductive phase and –ultimately- death. How this endpoint is reached however (e.g., length and timing of the aforementioned phases, diseases organisms encounter and –ultimately- cause of death) is more individually defined. For humans, these "moderators of lifespan" can be divided into genetic influences (or genetic predisposition) on the one hand, and non-genetic influences (e.g., environmental influences and (risk)behaviours) on the other hand (Franceschi et al., 2000 and 2007, Vasto et al. 2007).

Table 1. Illustrative examples of genetic and non-genetic moderators of lifespan.

Genetic	Non-genetic
Telomere length has been associated with age. Telomeres are the "end-cap" of chromosomes, protecting the genetic information (or DNA). The telomere length however, declines with every cell cycle. This ultimately leads to loss of telomere functionality, cellular dysfunction (due to loss of genetic information) and subsequent cell death.	**Oxidative stress has been related to rate of ageing and development of disease.** Oxidative (metabolic) stress and (for example) cosmic radiation, are among the burdens organisms have to deal with on our planet. This radiation triggers production of free radicals in our cells, which damages DNA and also leads to cellular dysfunction and, ultimately, cell death.
Specific genetic mutations have been associated with development of (age-related) pathology. Genetic mutations are responsible for Progeria, a disease in which patients age at a rapid rate and die prematurely. Also, genetic mutations have been related to particular cancer types (BRCA1 à breast cancer).	**Unhealthy behaviour may increase the rate of ageing and the risk of an untimely death.** Smoking in humans make them more prone to develop cardiovascular diseases, lung- and bladder cancer and death from these causes.
Genetic ← → **Non-genetic**	
Ageing is associated with a low-grade elevation of inflammatory markers, attributed to the dysregulation of immune and inflammatory pathways with ageing. 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.	

In Table 1, some examples of these influences are explained in detail, illustrating how these mechanisms impact the ageing process. The precise contribution and interplay between these influences remain unclear however.

In order to further clarify these interactions, the use of biochemical biomarkers for quantifying the status or rate of 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 associated with a low-grade elevation of inflammatory markers, attributed to the dysregulation of immune and inflammatory pathways with ageing (Franceschi et al., 2000 and 2007, Vasto et al., 2007, Pawelec et al., 1999). In accordance with these findings, it has been shown that chronic inflammation predisposes to morbidity and mortality from many chronic, age-related diseases (such as chronic pulmonary and cardiovascular disease) (Bruunsgaard et al., 2001, Schnabel et al., 2009, Danesh et al., 1998, Luc et al., 2003).

The synthesis and regulation of serum levels of inflammatory markers has been extensively studied, showing the significance of both environmental and genetic influences in this process (Pantsulaia et al., 2001, Rahman et al., 2009, Su et al., 2009, Maat et al., 2004). The ideal study design to assess these influences as well as possible interactions and "moderators" are classical twin studies. These types of studies are typically analyzed using variance component models, also known as structural equation modeling (Neale & Cardon, 1992, Purcell, 2002, Snieder et al., 2010, Kyvik, 2000).

Variance component analysis of classic twin studies

Twin methodology allows estimation and quantification of the relative contribution of genes and environment to the disease or trait of interest. The classic twin study design is based on the idea that two kinds of twins exist. Monozygotic (MZ) twins are genetically "equal"; any phenotypic differences between them are due to their differential environments. Dizygotic (DZ) twins are genetically no more comparable then siblings (which share on average 50% of their segregating genes). The actual variance component analysis 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 includes measurement error). In general, any greater phenotypic similarity among MZ twins compared with DZ twins reflects the importance of genetic influences (assuming that both types of twins share environmental influences to the same extent) (Neale & Cardon, 1992, Purcell, 2002). The assumption of equal environmental sharing in MZ and DZ twins has been frequently criticized as a potential weakness of the twin design. However, studies specifically carried out to test it (e.g., studies conducted among twins where zygosities had been misassigned) have

shown no instances where violation of this assumption leads to important bias in interpretation of the results of classic twin studies (Snieder et al., 2010, Kyvik, 2000).

Twin studies not only provide estimates of the relative contribution of genetic and environmental influences. They also allow exploration of interaction models, for example a gene-age interaction, as in the interaction models introduced by Purcell (Purcell, 2002). This model directly incorporates age as a continuous moderator into the model and allows to estimate whether and to what extent the A (or D), C and E components on a trait of interest are modified by age. In this gene-age interaction model, the phenotypic variance of the outcome variables is portioned into A, C, and E components with the path coefficients associated with each variable expressed as linear functions of age (e.g., A+T×M1, C+U×M1, E+V×M1) where M1 represents the value of the moderator (age in years), T, U & V represent the relative influence of the moderator on A, C and E and B represents linear effects on the outcome (Figure 1) (Purcell, 2002, Sas et al., 2012a).

Furthermore, with multivariate variance component models, making use of the 'Cholesky decomposition', cross-trait, cross-twin correlations of the MZ and DZ pairs can provide additional information to partition the phenotypic correlation between variables within individuals into A, C and E components (Neale & Cardon, 1992). This also includes the possibility of applying multivariate variance component models to longitudinal data. In the example in figure 2, baseline C-reactive Protein (CRP) levels are assessed multiple (up to 3) times and the heritability as well as the genetic and environmental correlations between different time points of CRP measurements was calculated.

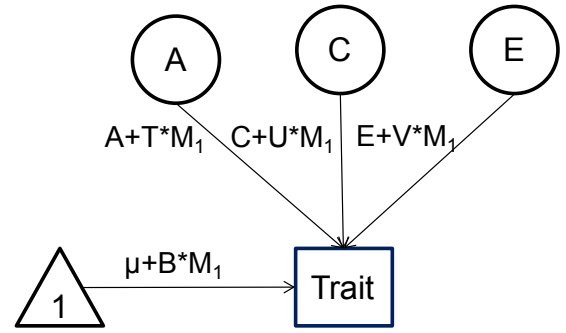

Figure 1. Partial path diagram for the basic gene-environment interaction model. A=additive genetic effects; C=common environmental effects; E=unique environmental effects; M=moderator (age); T=moderated component of A; U=moderated component of C; V=moderated component of E; B=linear effects of moderator on mean (forced entry). In path diagrams such as these unobserved (latent) factors are in circles, observed (measured) variables in squares and effects on the mean of the measured variable (trait) are indicated by the triangle.

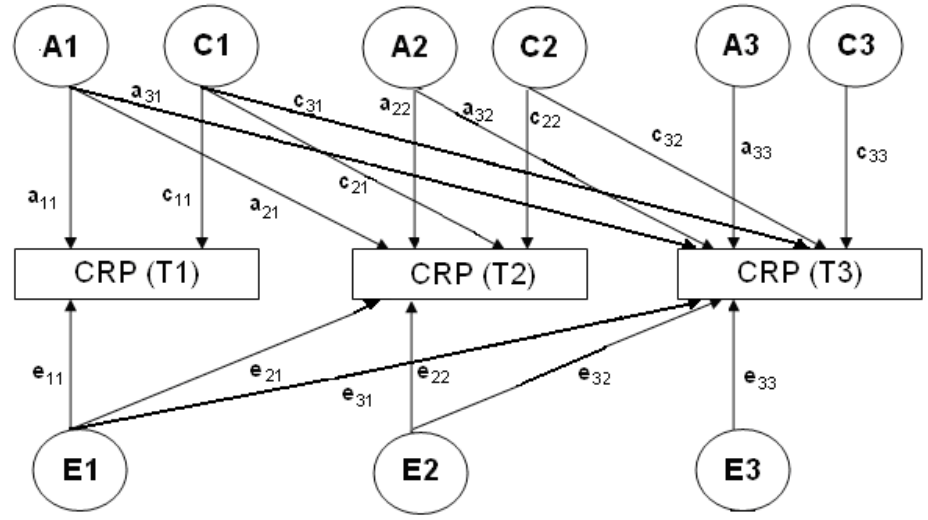

Figure 2. Path diagram for a multivariate model. For clarity, only one twin is depicted. A1, A2, A3 = Genetic variance components; C1, C2, C3 = common environmental variance components; E1, E2, E3 = unique environmental variance components; V1, V2, V3 = Visit 1, 2 and 3; a11 through a33 = genetic path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

This gives an estimation of genetic and environmental sources of the stability and change in CRP with increasing age. In this model it is possible to test whether the genes influencing baseline CRP-levels at e.g. visit 1 and 2 (and therefore different ages) are the same, partly the same or entirely different. If
they are partly the same, this multivariate model allows further determination of the amount of overlap between genes influencing CRP at different ages or visits (V1, V2, V3) by calculating the genetic correlation (rg) between (in this context) the different measurements of CRP-levels. Shared and unique environmental correlations can be calculated in a similar fashion (Sas et al., 2017).

Aims and scope of the thesis

The present thesis aims to apply several aspects of twin modeling on two cohorts of twin data, using data from the TwinsUK Registry (Spector & Williams, 2006) and Twin Interdisciplinary Neuroticism Study (TWINS) (Riese et al., 2013), in order to investigate the genetical and environmental influences underlying baseline serum levels of various well established inflammatory markers (C-Reactive Protein [CRP], Fibrinogen, Immunoglobulin-G [IgG], Interleukin[IL]-1β, IL-6, IL-10 and Tumor Necrosis Factor [TNF]-α) (Sas et al., 2012a). The role of age as a possible moderator of these influences is investigated, using a cross-sectional study design with gene-age interaction modeling in chapter 2 (IL-1β, IL-6, IL-10 and TNF-α) (Sas et al., 2012a) and a longitudinal study design using a trivariate Cholesky decomposition model in chapter 3 (CRP) (Sas et al., 2017). In chapter 4, the shared genetic background of neuroticism (a well established personality trait that is predictive for both mental and somatic disorders) and baseline levels of inflammatory markers (CRP, fibrinogen, IgG) is investigated (Sas et al., 2014). In chapters 5 and 6 we change to a more general perspective; instead of investigating underlying mechanisms through inflammatory biomarkers of ageing, we introduce the hypothesis that mathematical models describing survivorship (or mortality) data (in this context Gompertz' law was used) represent a biological "law of ageing" (Sas et al., 2012b, Sas & Korf, 2018 (to be published). In chapter 7 we conclude with a general discussion and some implications of our findings.

Detailed content of the thesis

In chapter 2, the relative influence of genetic and environmental factors on four key cytokines involved in the human immune response (IL-1β, IL-6, IL-10 and TNF-α) is assessed. In addition, the role of age as a possible moderator on these influences was evaluated. The study was conducted in 1,603 females from the Twins UK registry, 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 (Sas et al., 2012a). We tested the hypotheses that (1) genetic (rather then environmental) factors are more important in the regulation of baseline cytokine levels and that (2) these factors are influenced (moderated) by age.

In chapter 3 we investigated the stability of genetic and environmental factors influencing serum CRP levels with advancing age. A maximum of 6,201 female twins from the TwinsUK registry with up to three CRP measurements over a 10 year follow up period were included in this study. A trivariate path model (Cholesky decomposition) was used, estimating the heritability of CRP at different times of measurement as well as the genetic correlation between different time points, giving an estimation of the stability of genetic and environmental influences with advancing age (Sas et al., 2017). We will defend the hypothesis that the genetic factors remain largely stable over time (with advancing age).

Neuroticism is an important marker of vulnerability for both mental and physical disorders, e.g. anxiety, depression, atopic eczema, cardiovascular disease and (ultimately) mortality, which in general are the same mental and physical disorders as related to inflammatory markers. In chapter 4, the phenotypic and genetic relationship between neuroticism and three commonly used inflammatory markers (CRP, fibrinogen and IgG) is determined. The study was conducted in 125 Dutch female twin pairs. For each participant, four different neuroticism scores and serum levels of the abovementioned inflammatory markers were available. Heritabilities, phenotypic and genetic correlations were estimated using bivariate structural equation modeling (Sas et al., 2014). We tested the hypothesis that, considering their similar effect on health and apparently ageing, phenotypic and genetic correlations must be significant between neuroticism and the aforementioned inflammatory markers.

In chapters 5 and 6, we argue that Gompertz' demographic law on survivorship can be used as a simple and generally applicable "law of ageing", by applying the principle of ergodicity. In this context, a (cross-sectional) population survival curve hypothetically reflects the longitudinal ageing process in a single, average organism of that population. This hypothesis was illustrated with quantitative analyses of human survivorship data of different types of cancer patients and the entire Dutch population and of a variety of other organisms: mice under caloric restriction, male and female houseflies *(Musca domestica)* fed with a variety of diets, male and female West-Indian fruit flies *(Anastrepha obliqua)* and male and female wasps *(Diachasmimorpha longicaudata)* (Sas et al., 2012b, Sas & Korf, 2018 (to be published)).

Finally, I will integrate and discuss all research findings, strengths and shortcomings of the studies as well as (clinical) implications and recommendations for further research in the general discussion.

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