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

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Chapter 7
General Discussion

The main focus of this thesis was to investigate the genetic and environmental influences underlying baseline serum or plasma levels of various inflammatory markers and the role of age as a possible moderator of these influences. We further examined the genetic overlap between inflammatory markers and neuroticism, a personality trait that strongly predicts both mental and somatic disorders (Ormel et al. 2013; Terracciano et al. 2008). The inflammatory markers studied are C-reactive protein (CRP), Interleukin-1 β (IL-1 β), Tumor Necrosis Factor- α (TNF- α), IL-10, IL-6, Immunoglobulin G (IgG) and fibrinogen. These markers were chosen because of their established role in the pathophysiology of complex diseases related to ageing (Jylhä et al. 2007, Prins et al. 2016). Finally, I introduced the hypothesis that mathematical models describing survival data represent a general biological “law of ageing” as illustrated by analyses of human survivorship data of different types of cancer patients and the entire Dutch population as well as in a variety of other organisms.

Summary of main results of the thesis

Step by step I explored the role of age by using increasingly more complex study designs. After adjustment for age and other covariates on the mean levels, I tested the extent to which genetic and/or environmental influences accounted for individual differences in blood levels of inflammatory markers IL-1 β , TNF- α , IL-10 and IL-6 (chapter 2). Heritabilities for the different covariate models are given in Table 1 and were moderate for IL-1 β and IL-10 and low for IL-6 and TNF- α .

Table 1: Summary of the heritabilities of various inflammatory markers studied in the thesis.

Chapter 1	Inflammation marker	Heritabilities (95% CI)
Chapter 2 (Sas et al., 2012b)	IL-1 β IL-6 IL-10 TNF- α	Range: 0.27 (0.17-0.36) – 0.33 (0.24-0.41) 0.15 (0.00-0.38) 0.30 (0.20-0.38) Range: 0.17 (0.05-0.28) – 0.23 (0.12-0.33)
Chapter 3 (Sas et al., 2014)	CRP Fibrinogen IgG	0.52 (0.01-0.78) 0.67 (0.35-0.79) 0.43 (0.01-0.79)
Chapter 4 (Sas et al., 2017)	CRP	Range: 0.46 (0.40-0.52) – 0.52 (0.46-0.58)

Abbreviations: CI, Confidence Interval; CRP, C-reactive Protein; IL – Interleukin; TNF, Tumor Necrosis Factor; Ig, Immunoglobulin.

In chapter 2, I modeled the variable age as a continuous moderator variable of the genetic and environmental influences on these inflammation markers. Cross-sectional twin data of 1,603 middle aged women from the Twins-UK registry were used (Spector & Williams. 2006). For IL-1 β , heritability declined with age due to an increase in unique environmental influences. For TNF- α , heritability increased with age due to a decrease in unique environmental factors.

Although longitudinal studies are the gold standard for studying changes in serum cytokine levels and variance components over time, such studies are difficult to conduct as they are time consuming and expensive to establish. By using age as a moderator of genetic and environmental influences, the statistical models allowed us to mimic longitudinal outcomes in a cross-sectional dataset.

In chapter 3, I then further tested the hypothesis of changing genetic and environmental influences with age on the inflammation marker CRP in a longitudinal twin study of over 6,000 female twins from the TwinsUK registry with up to three measurements over a 10-year follow-up period (Sas et al. 2012b).

CRP levels significantly increased from visit 1 to visit 2 and between visit 1 and visit 3, but not between visit 2 and visit 3. Heritability estimates for CRP were around 50% at all three time points, and thus very stable over time (0.46–0.52; Table 1). Adjustment for BMI only slightly reduced heritability estimates (0.49–0.51). The genetic correlations between visits were significantly smaller than one, ranging from 0.66 (visit 1 – visit 3) to 0.85 (visit 2–visit 3). These findings indicated, that a large part of the genetic influences over time could be attributed to the same genes. However, partly different genes regulating CRP levels also impact at different timepoints in an individual’s life course. Although significant, the environmental correlations between visits were consistently low (0.16 – 0.27) indicating that the environmental influences on CRP were mostly different at the three time points.

In chapter 4, I tested whether neuroticism may have genetic or environmental overlap with plasma levels of the inflammatory markers CRP, IgG and fibrinogen, to gain further insight into the pathophysiology for both somatic and mental disorders. I focused on the personality trait neuroticism, as high neuroticism scores are highly predictive for developing mental and somatic health problems (Lahey, 2006). Also, high neuroticism scores have been associated with raised plasma levels of various inflammatory markers (McManus et al., 2013, Turiano et al. 2013).

Heritabilities were fair for neuroticism (0.55), CRP (0.52) and fibrinogen (0.67) and moderate for IgG (0.43) (Table 1). However, no significant phenotypic or genetic correlations were found between neuroticism and the inflammatory markers in a population cohort of healthy female twins (Sas et al. 2014).

As no phenotypic or genetic overlap between neuroticism scores and serum cytokine levels were found, higher neuroticism levels are not associated with higher inflammation.

One explanation of the observed relationship between neuroticism and inflammation markers as reported in the literature could be that confounding factors such as stressors and risk behavior are associated with higher neuroticism scores as well as higher serum levels of inflammatory markers (Sas et al. (2014)). This illustrates the importance of testing the influence of potential confounders influencing baseline levels of inflammatory markers. In accordance, in all of the studies in the present thesis the effects of established covariates on the data were assessed and statistically corrected for.

In chapters 5 and 6, I further worked out the idea that modelling cross-sectional data can sometimes be interpreted as if they were longitudinal. I looked at survivorship data which is based on cross-sectional measurements, because death is irreversible and can only be ascertained once per subject and proposed the hypothesis that cross-sectional survival data of a cohort may be useful as an estimate of a longitudinal property of an average prototypical individual.

My working hypothesis is that survivorship data describe a basic, intrinsic principle of aging of all individuals. We demonstrated in these chapters that mathematical formulas describing these data accurately describe survival curves (both human and animal), as well as common age-related pathologies such as colon and prostate cancers. An interesting feature however, is that with these curves we were not able to describe incidence data of external causes of pathology and death as illustrated by cervix cancer data caused by an infection of the human papilloma virus (HPV). This would imply that a cross-sectional population survivorship curve reflects alongitudinal biological ageing process in a prototypical individual.

Inflammation, ageing and chronic diseases: theoretical model

Recent work indicates, that inflammation is more predictive for reaching extreme old age in humans (Arai et al. 2015) than other biomarkers of ageing such as telomere length or renal function. In accordance, and mentioned in Chapter 2 and 3, chronic low-grade inflammation has been linked to less successful ageing as reflected in a higher risk of cardiovascular disease (CVD), cancer and ultimately death (Franceschi et al. 2007, Bruunsgaard et al. 2001). Much remains unclear on this issue however, as illustrated by recent work of Prins et al. (2016). In their large Mendelian randomization study no apparent direct causal influence of higher baseline CRP levels on 32 somatic and psychiatric disorders were observed. This illustrates that the underlying mechanisms that may explain the relationship between inflammation and healthy ageing are still unknown and need to be investigated.

Inflammation is tightly controlled by the immune system and is considered to be part of the acute physiological immune response to pathogens. Inflammation responses are adaptive, illustrated by more rapid reactivity and faster self-limitation by frequent exposure to stimuli (McDade, 2012a, Yattim&Lakkis. 2015). This means that when the infection is cured, the immune response normalizes. CRP-levels can rise 10-fold in the response to acute inflammation such as the flu. This means, that CRP levels of >10 mg/L are not relevant for research on chronic inflammation as reflected by baseline serum levels (McDade et al. 2012a). In contrast, chronic low-grade inflammation is a hallmark of systemic inflammatory activity and may, for example, indicate the presence of autoimmune disease, but is also found to be predictive for development of cancer, CVD and even psychiatric disorders (Singh et al., 2011). As a result, throughout my thesis, cases of “acute inflammation” are therefore excluded or controlled for throughout statistical model testing, as is common praxis in studies on baseline levels of inflammatory markers (Sas et al. (2012b, 2014)). What remains in our studies, are large groups of “healthy” individuals with CRP-levels in the normal range, allowing the study of the genetic and environmental origins of differences in baseline serum levels between individuals with potential impact on health and disease.

Individual differences in baseline levels of inflammatory markers have been studied extensively before, and provide clues on both functioning of the immune system (Sas et al. 2012b, 2014, 2017), as well as on its potential adverse outcomes (Franceschi et al. 2007, Bruunsgaard et al. 2001). It is clear, also from this thesis, that genetic influences play an important role (Table 1). However, these effects only partially account for the individual differences in these baseline levels: much is also determined by unique environmental (non-genetic) effects (Sas et al. (2012b, 2014, 2017)). What exactly are these effects and how do they account for variance in baseline levels of inflammatory markers? One example is the amount of adipose tissue and BMI, which is a known source of pro-inflammatory cytokines like IL-6 and of CRP (Schäffler et al. (2006)). In the studies presented in my thesis, BMI accounts for about 5% of the variance. Furthermore, according to other studies, smoking behavior may also account for some of the variance (Dietrich et al. 2007). But even when controlling for these effects through covariate analysis, a substantial unique environmental influence remains (Sas et al. (2012b, 2017)). Most likely, not all environmental covariates are known or at least not accounted for. In my opinion, an alternative source of environmental variance could originate from the development of our immune system throughout life. This idea is supported by the work of McDade (2012a), emphasizing the influence of environment on individual characteristics of our immune system. His work describes the relationship between the frequency of exposure to immune system triggering pathogens in childhood and serum cytokine and CRP levels in adulthood. More specifically, he provided evidence that more frequent stimuli of the immune system in childhood assessed

as more episodes of diarrhea, more frequent exposition to animal feces or pathogens in standing water leads to significantly lower levels of serum cytokines and CRP in adulthood. Significantly lower baseline serum CRP levels are found in populations with high frequencies of exposure to these stimuli such as those from the Philippines in comparison with the United States of America, even when differences in adult adiposity are statistically taken into account (McDade et al. 2012b). Apparently, the immune system learns from repeated stimulation in that frequent exposures help to optimize the process of “turning on” the immune cascade when necessary and to “switch off” as soon as possible. This more effective immune system helps to prevent damage to the body through unnecessary exposure to inflammation and leads to significantly lower levels of serum cytokine levels and CRP throughout life. As mentioned, such lower levels of low-grade inflammation could favor positive health outcomes in terms of lower incidences of aging-related disorders. Possible clinical implications of this theory in terms of disease outcome are provided by Prins et al. (2016). Here, a Mendelian randomization study was performed that uses multi-SNP genetic risk scores of CRP as instrumental variables to study the causal role of CRP in a wide range of chronic disease outcomes. No apparent causally increased risk of CRP levels on these outcomes was demonstrated, but, interestingly, a higher lifelong exposure to CRP as indicated by a higher genetic risk score provides a potentially causal protective effect on the development of schizophrenia. The explanation of this unexpected protective effect may be analogous to the model from McDade explained above in that a higher cumulative CRP exposure” in youth “trains” the immune system and offers protection against the onset of schizophrenia in late adolescence or early adulthood. Similar results and interpretations are described in a recent paper of Hartwig et al. (2017).

A study by Arai and colleagues (Arai et al. (2015)) confirm the role of low-level inflammation as a strong predictor of survival, cognition and capability in old age. Even though centenarians themselves have high levels of systemic inflammation markers, these individuals apparently age slower, hypothetically due to lower levels of inflammation in child- and adulthood. This is supported by the observation that offspring of centenarians have significantly lower levels of inflammatory markers compared to non-centenarian offspring. Thus, there seems to be a significant genetic component in this mechanism, but genetics can probably only account for part of this relationship. In terms of “environmental components”, again McDade’s model may well be applicable here, since early (childhood) exposure to pathogens is likely to be part of the shared family environment. In terms of McDade’s model this hypothetically means that pathogen exposure could lead to lower serum cytokine and CRP levels in adulthood, offering a protective mechanism against ageing and increasing the probability to become “centenarian” (figure 1, left side). Still high levels of inflammation are observed at old age (Arai et al., 2015), which increases the risk of sickness and death, but the

onset seems to be delayed in these individuals. The potential protective effect as proposed by McDade, therefore, may be postponing the negative effects of higher chronic inflammation levels, rather than guaranteeing a permanent protective effect.

The Gompertz-model I discussed in **chapter 5 and 6** in my opinion fits this hypothesis. It is a model for describing survival curves (as discussed in section 1 of the discussion) but may be well suitable for describing an underlying universal biological mechanism of ageing. With the model an aging effect is described with an exponential function, which reveals the time point at which this underlying mechanism becomes most relevant in this model. This is illustrated in Figure 1 (right side), where at age +/-60 the “bending point” precedes the steepest descent of the survivorship curve. The exact bending point may be different between cohorts and between species. Hypothetically, this means that even though a certain exponential increase in systemic inflammation during life is fixed (“programmed”) for a particular population, the time point at which this becomes clinically relevant is different for each individual. This, in my opinion, may depend both on genetics, as well as on “environmental training” of the immune system as proposed by McDade (McDade et al. 2012a).

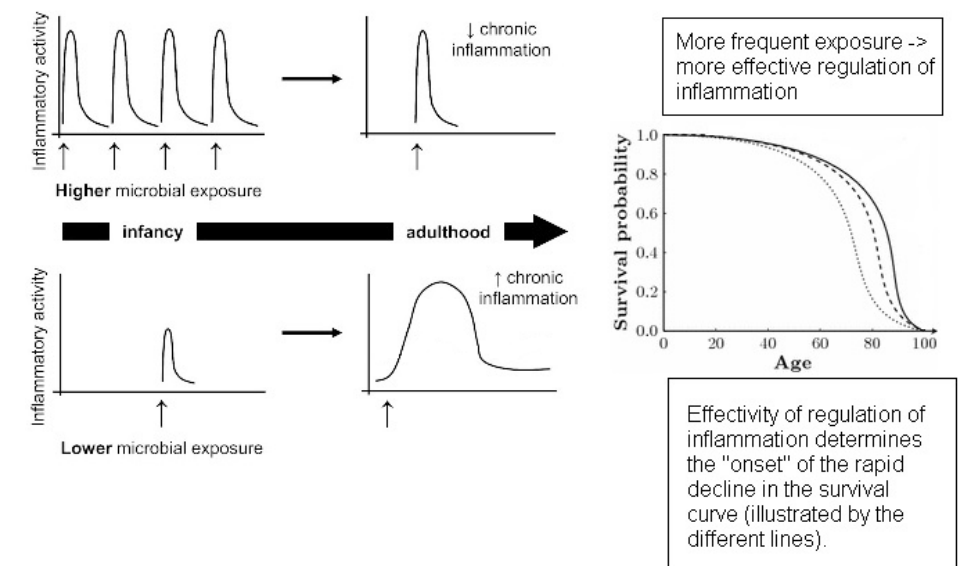


Figure 1: a theoretical model on inflammation and ageing. The left side of the figure (figure based on Fig. 3 proposed by McDade (2012a), with slight modification) represents the development of the immune system during infancy and adulthood. Note that more frequent stimuli and higher microbial exposure lead to more powerful but shorter responses in adulthood, whereas lower exposure and less stimuli lead to less effective responses with less effective regulatory mechanisms, enhancing chronic (low-grade) inflammation. The right side represents my hypothesis (for details see text) of these effects in later life, illustrated by earlier / later onset of the “bending point” thus influencing the point at which chronic inflammation becomes clinically relevant.

In chapter 5, the term “frailty” has also been introduced as a way to understand the ageing process underlying the Gompertz model (Gompertz, 1825). Frailty is defined as the lack of “reserve capacity” of our body; the ability to counter disturbances of our internal environment like infection, disease and (ultimately) death (Fried et al. 2001; Schuurmans et al. 2004; Strawbridge et al. 1998). Measurements of frailty include decline in muscular strength, unintentional weight loss, fatigue, low physical activity level, and slow walking speed (Fried et al. (2009). Frailty is considered to be the result of a complex interplay of genetic and environmental factors in an individual, all adding up to risk of developing disease and death. In the context of the Gompertz model, a population survival curve may reflect the lifelong decrease in “reserve capacity”, and thus increasing frailty.

An interesting feature of this approach, is that the rapid development of frailty during midlife apparently is followed by a deceleration at old age. This latter effect is reflected in the “tail” of the survival curve in Figure 2. In the context of my thesis, this could mean that over life, an increase in low-grade inflammation is associated with accelerated ageing and enhanced mortality risk. The tail of the curve may represent individuals with a delayed onset of the increase of inflammatory marker levels with age, as discussed above. This idea is supported by the findings of Arai et al. (2015), where suppression of chronic inflammation was predictive of (extreme) old age, cognition and capability in centenarians.

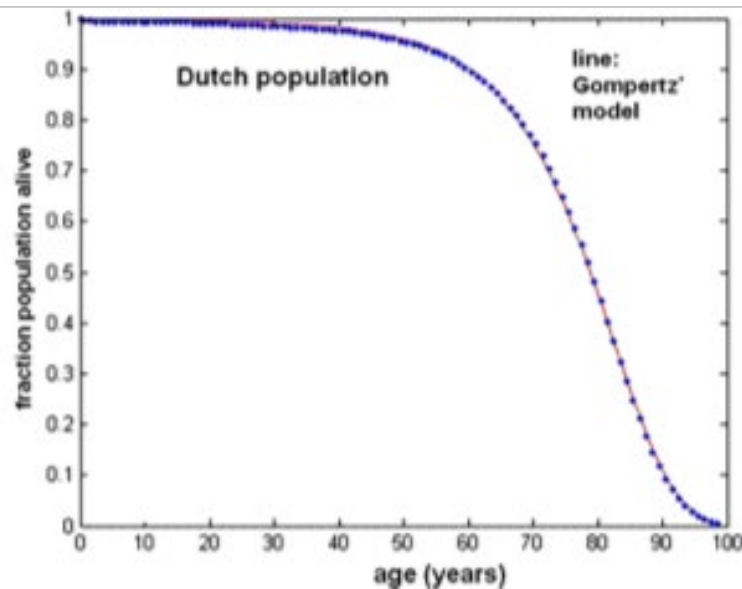


Figure 2: Survival curve of the total Dutch population (CBS, 2003. Sas et al. (2012a)

In his paper, McDade legitimately asked the question: “Is inflammation a silent killer?” (McDade (2012a)). This question has not been fully answered to date. As pointed out in the current thesis, there is a well-established relationship between inflammation and disease, in which both genetic and environmental factors play their part. But the main question remains: Should non-clinical inflammation, defined as chronic low-grade inflammation, be regarded as causal for the development of age related diseases? Or is it merely an innocent bystander of the (sub)clinical disease process, which is accompanied by systemic low-grade inflammation and, therefore, predictive for the development of disease? What data and study designs do we need to further investigate this issue? And what could be the possible (clinical) implications of the findings in my thesis? I will discuss these questions in the next sections.

Relationship between inflammation markers and age-related chronic disease: Predictive or causal?

Among the possible causes of unhealthy ageing, the role of inflammation has been discussed extensively before in the literature as well as in this thesis. It is clear that inflammatory processes play an important role in ageing and the development of disease. But the most important and intriguing question largely remained unanswered. There are two alternatives:

- 1) Is there a causal relationship between serum levels of inflammation markers and ageing and, consequently, age-related diseases? Or
- 2) Is their potential predictive value merely a reflection of the underlying disease process?

In order to analyze and discuss this matter in more detail, I will use illustrative literature on the relationship between inflammation and cancer, psychiatric disorders and CVD.

Cancer and CVD constitute the most important causes of death in Western societies (Luc et al. (2003)). For cancer, there is a strong relationship between inflammation marker levels and a variety of immunological cancers such as lymphoma, multiple myeloma (i.e. Kahlers Disease) and leukemia, as well as non-immunological cancers such as colon carcinoma (Aggawar et al. 2006, Lu et al. 2006). In other types of cancer, a positive association with inflammatory markers such as IL-6 and CRP has also been found. However, it remains unknown whether this points to a causal relationship (Heikkila et al. 2008). Two possible explanations for this lack of knowledge on causal relationships are mentioned in the literature. First, it may be due to insufficiently powered prospective studies (Singh et al. 2011) or second, these causal relationships may simply not exist. This would imply, that elevated cytokine and CRP levels are merely a reflection of low-grade inflammation seen when someone is diagnosed with a disease and, therefore, can be considered a consequence rather than cause of the disease.

In the literature, the relationship between inflammation markers and psychiatric disorders has been studied extensively as well (Turiano et al. 2013). In chapter 4, I investigated the shared genetic and environmental factors between neuroticism, a risk factor for psychiatric disorders, and CRP, fibrinogen and IgG (Sas et al. (2014)). Neither evidence of a correlation between neuroticism and immunological markers nor shared genetic and environmental influences on this relationship were observed. If neuroticism would have had a causal role at least some degree of overlap would have been expected. However, the study sample to address this question was relatively small and therefore possibly lacked power to pick up effects. Prins et al. (2016) used Mendelian randomization to study causal relationships between CRP and a variety of somatic and psychiatric outcomes. Here, again, no significant causal relationships were found, with the notable exception of a putatively causal protective effect of CRP on the development of schizophrenia. This could indicate that (at least for some psychiatric disorders) a causal effect of prior inflammation may exist.

The role of inflammation markers as potential predictors of CVD has been well established (Golia et al., 2014, Singh et al., 2011, McManus et al., 2013). The development of CVD is mainly driven by atherosclerosis. A deterioration process caused by fatty depositions (i.e. cholesterol) sticking to the blood vessel wall, which in turn cause chronic low-grade inflammation (Libby et al. 2002). That is, the atherosclerotic process promotes the attraction of macrophages and an increase CRP and fibrinogen-levels through increases in IL-6 levels and the soluble IL-6 receptor (IL6R). This mechanism has been proven to be a causal mechanism, even after ruling out CRP and fibrinogen as possible mediators of IL6 levels (Swerdlow et al. 2012). Moreover, increased IL-6 levels have been associated with an increased risk of CVD (Ridker et al. (2000), Danesh et al. (2008), Sattar et al. (2009)). In a Mendelian randomization analysis, Swerdlow et al. (2012) reported that carriers of a genetic marker for IL6R had lower CRP and fibrinogen levels and lower odds for developing CVD. In my opinion, this mechanism provides a clue regarding causal mechanisms in the development of CVD and even provides room for a potential therapeutic intervention. This means, although highly speculative, that IL6R is a potential clinical target for future medication in order to keep CRP and fibrinogen levels low and in this way reduce risk for developing CVD.

In chapter 2 and 3, the genetic and environmental influences on serum cytokine and CRP levels were studied in twins. In Chapter 2, I reported on different genetic and environmental influences at different ages for IL-1 β and TNF- α . Furthermore, in chapter 3, I reported on changes in environmental influences on CRP over time. Although I did not look at the use of these markers as predictors of healthy ageing or the development of disease, it could be hypothesized that changes in unique environmental influences are due to aging related increases in discordance between twins

of a pair. This means that a strict genetic regulation of serum cytokine and CRP levels as markers of accelerated ageing and adverse disease outcomes is less plausible. However, the importance of genetic influences transpired in chapter 3 as well, since relatively high and stable genetic correlations were observed with age. It seems that the regulation of CRP levels results from partly stable and partly varying genetic factors while the influence of the environment is mostly due to different factors over time.

Could serum inflammation marker levels be predictive, or even causal, for CVD? Based on the arguments described above, I think they do. As pointed out, a clear connection between IL6 and IL6R levels and CVD is established (Swerdlow et al. (2012)). Regarding its clinical implications, it is a matter of figuring out how to implement this knowledge in effective interventions. Could the use of “simple” non-steroidal anti-inflammatory drugs (NSAIDs) be sufficient to provide a beneficial effect (“one Ibuprofen a day keeps the doctor away”), or should a more aggressive therapy (in terms of immune suppression) be initiated to achieve a significant result in terms of decreasing CVD development? If so, the risk of possible unfavorable side effects (immune deficiency, opportunistic infections) should definitely be taken into account.

Implications and future research

So, where to go next? What can be learned from these findings and in which direction should research continue in order to understand and prevent the premature development of age-related diseases? It is necessary to unravel the genetic and environmental influences underlying baseline serum inflammatory markers further, also looking at its relationship with the development of, or protection against, age-related disease. In this section, I would like to point out three topics, that provide some directions for further research and potential clinical implications.

First, McDade’s point of view on development of the immune system is an interesting starting point. What happened during aging with the young individuals he studied in later life? Could his theory be the basis for people to remain more healthy and possibly become centenarians? Centenarians seem to benefit from a better regulation of their immune system, leading to a later onset of aging related deregulation of the immune system. Microbial exposures in early life lead to a “catch-up event” in later life, which still implies the same health risks as in average individuals, but this simply happens at a later time point (McDade et al. 2012a, Arai et al.2015). Two interesting questions arise from this topic, which should be looked at in future research:

- 1) Is this later-onset of aging related immune deregulation compatible with McDade’s theory? Do centenarians benefit from an intensive “training” of their immune system at an early age?

- 2) Is this “catch-up event” compatible with my hypothesis on describing biological ageing principles by survival curves? And, even more interesting, does an exponential function which kicks in in later life (i.e. predicted by Gompertz law), account for the later or earlier onset of ageing related deregulation of the immune system?

In order to answer these questions, a retrospective (case-control like) study among centenarians and non-centenarians could be helpful to assess predictors of healthy ageing. In the present context, the study in particular should focus on sickness as a child suffered from and (lack of) hygienic circumstances when growing up (question 1). This could be done through questionnaires in which demographic data on place of birth (city? farm?) and socio-economic status of the family (predictor of hygienic standards / clean environment) could easily be questioned through the offspring of centenarians, even if their parents are no longer alive.

Question two is also complicated to answer. First of all, the effect of hygiene / sickness on the survival curve should be tested (starting of the “bending point, see Figure 1) as described in the section regarding question 1 above. After that, additional analyses through modeling of survival curve data (as been discussed in chapter 5 and 6) could be done by using cross-sectional data (age - death) with stratification through (for example) hygienic standards, sickness as a child (frequency) and test whether these survival curves can adequately be modelled by a mathematical (exponential) function (in the present context Gompertz Law). An alternative approach is through in vivo models (animal studies), where the young animals are raised in different hygienic regimes (microbial expositions). Through further observations in adulthood and again through survival data one can assess the plausibility of McDades theory as well as the capability of describing the survivorship data through Gompertz Law.

The genetic and environmental influences on the regulation of levels of inflammatory markers during aging is the second topic. As pointed out, I demonstrated moderate to high heritability's of IL-1 β , IL-6, IL-10, TNF- α , CRP, IgG and Fibrinogen (Table 1). But how do genetics help us fight disease? In my opinion, the final perhaps utopian result of research on this topic should lead to a clinical lab test which reveals a genetic risk score for an age-related disease using only a single drop of blood. Following the presented results and discussion in my thesis, this could work through (genetic risk scores of) serum cytokine levels (higher levels, higher risk). Persons who are more “at risk” could then be offered to prophylactically take anti-inflammatory drugs to lower serum cytokine levels, thus reducing risk of developing aging related disorders. One could argue that for most diseases (cancer, CVD) a lot is already known on genetic factors and a lot of predictive testing can be done, including assessment of genetic risk scores (Knowles & Ashley, 2018), but the addition of a genetic inflammation risk score may be cost effective and predictive of multiple diseases. Ultimately a single test could give information of the genetic propensity for high levels of chronic

inflammation and may aid clinical decision making in combination with comprehensive assessment of risk profiles and environmental exposures, i.e., measures on lifestyle.

Genome Wide Association Studies (GWAS) are used to identify Single Nucleotide Polymorphisms (SNPs) contributing to the regulation of serum levels of inflammatory markers. These SNPs can be used in causal research on the relationship between serum cytokine levels and age related diseases as was done for CRP-levels and schizophrenia, discussed above (Prins et al. 2016)). In addition, causal relationships between inflammation markers and ageing (“inflammageing”) can be studied using genetic risk scores as instrumental variables. To date, GWAS are already performed on CRP, fibrinogen, IL-6 and TNF- α (Prins et al. 2016, Sabater-Lleal et al. 2013, Ridker et al. 2008, Dehghan et al. 2011). For IgG, IL-10 and IL-1 β currently no GWAS-results exist. In my thesis I focused on only a small number inflammation markers, whereas a lot more are known and can be studied. It is clear that more information is needed on the exact relationship between serum inflammatory marker levels and aging related development of disorders.

A final topic of interest that arises from my discussion, is on the potential protective effects of (anti-inflammatory) drugs on the risk of developing aging related disorders. In this context, the benefits of statins on the development of CVD (through lowering serum cholesterol levels) is well established (Bertrand & Tardif, 2017). Recent findings suggest however that, interestingly, statins have pleiotropic effects (including anti-inflammatory properties) which could lead to reduction of cardiovascular risk regardless of their hypolipidemic effects (Braunwald, 2012, Schönbeck & Libby, 2004). The role of “strictly” anti-inflammatory drugs remains controversial however. The benefits of, for example, Methotrexate (Micha et al. 2011) and Colchicine (Crittenden et al. 2012) have been studied, showing a substantial benefit in terms of reduction of cardiovascular risk in patients with rheumatoid arthritis, psoriasis, polyarthritis and gout. These results are not generalizable to healthy subjects however, since only patients with “abnormal” (chronic) inflammatory responses are included. Also, there have been trials on evaluating the effects of new drugs (including therapies targeting specific inflammation markers through monoclonal antibodies) on cardiovascular risk. In this context, the CANTOS-Trial evaluates the effectiveness of Canakinumab on reducing cardiovascular risk (Ridker et al. 2017). Canakinumab is a monoclonal IgG1 antibody targeting specifically IL1 β . Results are promising, showing a significant decrease in both incidence of myocardial infarction and CRP levels. Disadvantages however are its association with serious infections including sepsis, as well as potentially accelerated proliferation of existing cancers and metastasis through blockage of the natural (beneficial) properties of IL-1 β (Galis et al. 1994, Li et al. 2012).

The brief summary above makes clear that inflammation is a potential therapeutic target in terms of reducing cardiovascular risk (and thus ageing). The main challenge for the nearby future, in my opinion, is to identify anti-inflammatory agents, either known or yet to be developed, which cause minimal harm in terms of unwanted (side) effects as described above, but still have a maximal protective effect. Could this be by taking “good old” Aspirin and Colchicin, or do we absolutely need new drugs like Canakinumab? Probably, the truth will be somewhere in the middle. In my opinion, if a person is to receive these drugs it is a matter of setting clear indications and contra-indications for each drug. Which patient can receive which drug, with maximal benefits, but without the risk of harming them through side effects and complications of the treatment? Some (perhaps “healthier”) patients can be given more aggressive therapies if deemed beneficial (like Canakinumab) since they can cope with the potential side effects better as, for example, older (frail) patients.

I would like to end this discussion with a cautionary note. Even if we are able to develop an “anti-aging pill”, treating people properly will not be that easy in my opinion. We must realize, that in terms of disease prevention, the downsides of treating healthy individuals without any symptoms must be carefully considered. This means that apparently healthy people are forced to take drugs (after a certain age, or even lifelong), in order to achieve some benefit later in life, even though at the moment of starting the therapy there may not be any obvious signs of pathology yet. As a physician, I know that compliance in medication use is a big issue for most people. Two examples are blood sugar monitoring and insulin treatment in kids and (young) adults, or adults who refuse to take pain medication because “they are not fond of painkillers”. Even when we find out about the exact mechanisms and exact therapeutic targets, getting people to take these drugs could probably be an even bigger challenge than identifying our “miracle drug”.

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