Genetics and life course epidemiology of cardiometabolic disease: towards personalized medicine

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SUMMARY

The global rise in cardiometabolic disease, which is driving much of the global non-communicable disease burden, has substantially increased over the past decades. Despite advances in cardiometabolic disease research, endeavors to successfully address cardiometabolic disease remain challenging due it its complex nature. This emphasizes the need for more effective preventive and curative strategies for cardiometabolic disease. With the studies described in this thesis, we aimed to gain insights into the underlying mechanisms of cardiometabolic disease, and the long-term and cumulative exposure of its risk factors over the life course, thereby facilitating the search for better preventive and curative strategies. **Chapter 1** serves as a general introduction to cardiometabolic disease, particularly to lipid metabolism and obesity, and to the environmental and genetic factors that determine cardiometabolic risk throughout the life course.

The first part of this thesis focuses on the genetics and thus underlying mechanisms of lipid metabolism during both fasting and postprandial (non-fasting) states as atherogenic dyslipidemia is a major component of cardiometabolic disease. In addition to abnormally elevated levels of low-density lipoprotein-cholesterol (LDL-C) and low levels of high-density-lipoprotein-cholesterol (HDL-C), atherogenic dyslipidemia is characterized by elevations in triglycerides (TG) and triglyceride-rich lipoproteins (TRLs), which are major risk factors for cardiovascular disease, independent of LDL-C. Thus, in addition to drugs that reduce LDL-C, such as statins, therapies that lower TRL and TG levels are needed for CVD prevention. Several therapies, which in particular have been focused on LPL as a key player in TG metabolism, have been in development. However, their contribution to CVD reduction when combined with statins has remained inconclusive. Furthermore, while most of the current research has been focused on the fasting states, it is important to also gain insights into the mechanisms involved in postprandial lipoprotein metabolism as we spend most of the day in a postprandial state due to frequent food intake.

In **chapter 2** we first performed a GWAS on postprandial TG levels at 150 min after a mixed-meal intake as well as on fasting TG levels to use as a basis for comparison. These analyses showed that the major loci/genes that affect fasting TG levels (as *LPL, APOA1, APOE, CLIP2 and GCKR*) also play a major role in determining postprandial TG levels, and the effects of these genes appear to be similar on fasting and postprandial levels. Since postprandial TG levels are highly correlated with fasting TG levels, the genes affecting postprandial TG levels do not give clear insights into the mechanisms underlying the metabolic capacity to deal with dietary stimuli. Therefore, in

chapter 2 we aimed to identify genetic variants that determine the postprandial TG response to a mixed meal, independently from fasting TG levels. To this end, we performed a genome-wide association study (GWAS) on postprandial TG response at 150 min after the meal consumption in 5,630 participants of the Netherlands Epidemiology of Obesity (NEO) study. We identified rs7350789-A, mapping to the hepatic lipase (*LIPC*) gene that encodes for hepatic lipase, to be associated with a smaller increase in TG levels after a meal. Next, we performed association analyses of rs7350789-A with lipoprotein profiles, which indicated that the hepatic lipase mechanism of action in postprandial TG metabolism is different from that in the fasting state. Altogether, we concluded that, in this population-based study, postprandial TG levels are determined by the same major genes that determine fasting TG levels. *LIPC* contributes to postprandial TG metabolism independent of fasting TG levels, but common variation in *LIPC* is not a major determinant of postprandial TG levels.

Using Mendelian Randomization approaches, recent studies have suggested that drugs that enhance LPL-mediated lipolysis are likely to provide additional cardiovascular benefits in addition to existing LDL-C–lowering agents. Using a similar approach, in **chapter 3**, we aimed to gain insights into the underlying mechanisms behind these observed effects, by assessing the causal associations between genetically-decreased TG levels via the *LPL* gene (as a proxy for LPL targeting drugs) on lipoprotein metabolism, with or without the background of genetically-decreased LDL-C (a proxy for LDL-C-lowering drugs, such as statins) in 4,838 participants of the NEO study and 6,999 participants the Oxford Biobank (OBB). We found that TG-lowering on top of LDL-C-lowering has additive beneficial effects on lipids and lipoproteins compared to TG-lowering or LDL-C-lowering only, which explains the previously reported additional cardiovascular benefits of *LPL* genetic variants on top of LDL-C-lowering. We concluded that, from a clinical perspective, the pharmacological modification of LPL may be useful as an additional target for CVD prevention. This is of great importance as in some patients, despite optimal LDL-C-lowering, a substantial residual CVD risk remains, which may be due to increased TG and TG-rich lipoproteins.

In addition to LPL, several LPL modulators have been shown to affect circulating TG levels the risk of CVD, but their effects on the detailed lipoprotein profile have never been studied in depth. Elucidation of these specific effects can provide insight in the therapeutic potential of LPL modulators in reducing CVD risk. Therefore, in **chapter 4**, we aimed to investigate the role of apolipoprotein A-V , a natural activator of LPL and potent TG regulator, on lipid metabolism. To this end, we performed

Mendelian Randomization analyses in 309,780 European-ancestry participants from the UK Biobank, evaluating the effects of genetically-decreased TG levels by apolipoprotein A5 (*APOA5*) gene alone and/or *LPL* with or without a background of genetically-decreased LDL-C levels on CAD risk. Next, we studied the effects of genetically-decreased TG levels via *APOA5* and *LPL* with over 100 lipoprotein measures in a combined sample from the Netherlands Epidemiology of Obesity study (N=4,838) and the Oxford BioBank (N=6,999). With the results from these analyses we concluded that TG-lowering via *APOA5* provides additional beneficial effects on CAD risk and the lipoprotein profile on top of TG-lowering via LPL and LDL-C-lowering, thus genetically validating apo A-V as potential target for TG reduction and primary CAD prevention. In addition, our results suggest that while LPL might be the main pathway via which apo A-V regulates TG levels, other possible mechanisms might be involved and should be further investigated in future studies.

Utilizing the long follow-up of nearly 30 years of the Doentichem cohort , on the second part of the thesis we take the opportunity to focus on the cardiometabolic and genetic risk profiles over the life course in different generations of men and women. As introduced in **chapter 1**, although cardiometabolic disease usually manifests itself at middle age or beyond, it is the result of a multifactorial disease process, where the effects of genetics and an unhealthy lifestyle accumulate with ageing. Thus, in order to fully understand the mechanisms that underpin this complex trait, insight into the dynamics of the cardiometabolic risk factors at different time points in life is fundamental.

The environment we live, which is a major contributor to the obesity pandemic and consequently to the cardiometabolic disease, has become more obesogenic (obesity-promoting) in the recent years. The exposure to such obesogenic environments has been reported to be higher for the younger generations compared to those born decades ago, which consequently could lead to higher rates of obesity and cardiometabolic disease. In **chapter 5**, we aimed to assess how the changing obesogenic environments are affecting different generations in the Netherlands by studying the development of anthropometric measures of obesity and related cardiometabolic risk factors (hypertension, hypercholesterolemia, low HDL-C, type 2 diabetes and inflammation) during 26 years of follow-up across four different generations (10-year age groups) of men and women of the Doetinchem cohort (n=6314 at baseline). The findings of **chapter 5** showed that the younger generations had obesity at an earlier age, but did not reach higher levels at midlife and beyond. This increased exposure to obesity was not (yet) associated with increased prevalence of cardiometabolic risk factors. However, the earlier age of obesity in the younger

generations results in an increased lifelong exposure to obesity, which indicates that the burden of cardiometabolic disease may further increase in the coming years in the Netherlands. In addition, in our study, we observed that obesity increased and cardiometabolic risk factors deteriorated with age in all generations, which may add to the burden of cardiometabolic disease in ageing populations.

In addition to the lifestyle and environmental factors, multiple genetic variants have been associated with obesity. Individual variations in body weight, however, are not constant throughout the life course, and might be attributed to the interaction of the genetic factors with the changing obesogenic environment. Thus, in **chapter 6** we investigated the temporal aspect of the genetic basis of obesity, by performing GWAS analyses on longitudinal measures of body weight at specific age groups from 30-70 years in 4,619 men and women of the Doetinchem cohort. In this first ever GWAS that was performed on longitudinal changes in body weight during adult life, we found that some genetic variants have a stable effect over the life course, whereas others are age-dependent. More specifically, we found that the TOMM40-APOE locus was associated with weight gain in young and middle adulthood individuals and with progressive weight loss after the age of 50. Interestingly, the TOMM40-APOE locus is an established risk factor for Alzheimer's disease, suggesting that the progressive weight loss that we observed after the age of 50 could be related to the pathological processes underlying Alzheimer's disease. From **chapter 6** we conclude that some genes may have a variable effect on body weight over the life course of an individual, and that future studies including other relevant environmental and lifestyle factors are needed to further dissect the complex trait of obesity.

To conclude this thesis, in **chapter 7** we discuss the main findings and their implications for future preventive and therapeutic strategies for cardiometabolic disease. Altogether, this thesis has provided us with a better understanding of the pathophysiology of cardiometabolic disease and the long-term and cumulative effects of its risk factors over the life course of an individual.