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

Ibi, D.

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General Discussion and Future Perspectives

In this thesis, we aimed to gain new insights into the underlying pathophysiology of cardiometabolic disease and the long-term and cumulative exposure of its risk factors over the life course, thereby facilitating the search for preventive and curative strategies of cardiometabolic disease. In the first part of this thesis, we focused on the genetic determinants of lipid metabolism during both fasting and postprandial states. In the second part, we studied the age-related changes of cardiometabolic risk factors over the life course across four generations. In this chapter, I review and interpret the main findings, and describe implications for public health and future preventive and curative strategies to reduce the burden of cardiometabolic disease.

PART I –GENETIC DETERMINANTS OF LIPID METABOLISM

Genetics of postprandial TG metabolism

In **Chapter 2** we first performed a GWAS on postprandial TG concentrations at 150 min after a mixed-meal intake as well as on fasting triglyceride (TG) concentrations to use as a basis for comparison. These analyses showed that the major loci/genes that affect fasting TG concentrations also play a major role in determining postprandial TG concentrations, such as *LPL, APOA1, APOE, CLIP2* and *GCKR*. In addition, the effects of these genes on postprandial and fasting TG concentrations appear to be similar. These results suggest that fasting and postprandial TG metabolism involve for the most part the same genes/pathways. From a clinical perspective, this suggests that postprandial TG do not provide major additional information to improve cardiovascular disease (CVD) risk prediction. Given that the same genes have similar effects on fasting and postprandial TG concentrations, a single TG genetic score might be sufficient for CVD risk prediction. As a consequence, pharmacological modulation of the same genes would suffice to lower both fasting and postprandial TG concentrations to lower cardiovascular risk, given that fasting and postprandial TG concentrations are both strong risk factors for CVD.

Since postprandial TG concentrations are highly correlated with fasting TG concentrations, the genes affecting postprandial TG concentrations 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 of the postprandial TG response independent of fasting TG concentrations. We identified rs7350789, mapping to the *hepatic lipase* (*LIPC)* gene that encodes for hepatic lipase (HL), to be associated with the TG response. *LIPC* has been previously associated with fasting TG levels in large-scale meta-analyses (1), but the effect size was quite small, which explains the lack of an association with fasting or postprandial TG lev-

els in our study. In addition to having a stronger effect on postprandial TG response independent of fasting TG concentrations, association analyses of the *LIPC* SNP with lipoprotein profiles indicated that the hepatic lipase mechanism of action in postprandial TG metabolism is different from that in the fasting state. However, the small absolute effect on postprandial TG response suggests that this *LIPC* variation is quantitatively not a major contributor of postprandial TG metabolism, and thus might have a negligible effect on CVD risk.

Altogether, our results indicate that postprandial TG concentrations are determined by the same major genes that determine fasting TG concentrations. *LIPC* contributes to postprandial TG metabolism independent of fasting TG concentrations, but common variation in *LIPC* is not a major determinant of postprandial TG concentrations.

Hepatic lipase: A pro- or anti-atherogenic protein?

In **Chapter 2**, we did not specifically study the effects of genetically decreased HL via rs7350789-A and CVD outcomes. However, we performed association analyses between rs7350789-A and nuclear magnetic resonance (NMR)-based lipoproteins and metabolites, which are well described intermediate phenotypes between genetic variation and CVD outcomes. Overall, our results showed that genetically decreased HL has pro- and anti-atherogenic effects on fasting and postprandial lipoprotein metabolism. Given these opposite effects, it is thus difficult to predict the HL effect on CVD risk. Publicly available data from Cardiogram show that rs7350789-A has no effect on CVD risk (2), which suggests the absence of an HL effect on CVD risk in the general population. This is in concordance with most population-based studies, which have shown minimal to no effect .

However, in some cohorts/studies, *LIPC* variation has been controversially correlated with CVD risk. For example, in the Copenhagen Heart Study carriers of the rs1800588-T allele, which leads to a 15–30% lowering of HL activity, had higher HDL-C levels and paradoxically a 1.7 fold higher risk of ischemic CVD compared to the C-allele carriers (3). On the other hand, a later study using subjects from the same cohort reported that the cumulative incidence of ischemic CVD over 28 years of follow-up did not differ among the rs1800588 genotypes (4). A reason for these differences in conclusions may be the different effects/roles of HL in lipoprotein metabolism. Our data showed that associations of the *LIPC* variant with fasting and postprandial lipoprotein levels versus postprandial lipoprotein responses revealed different effects on pro- and anti-athterogenic lipoproteins. This shows that the context studied should be taken into consideration when assessing disease risk of the *LIPC* variant. Previous data suggested that high HL activity is anti-atherogenic in

familial hypercholesterolemia and pro-atherogenic in hypertriglyceridemia. Thus, theoretically from a therapeutical perspective, pharmaceutical HL lowering would be beneficial in hypertriglyceridemic patients, whereas, HL enhancement would benefit those with familial hypercholesterolemia. Furthermore, gene-diet interaction has also been shown to be an important factor that modifies the effects of HL on lipoprotein metabolism (5-7). The T allele of the *LIPC* promoter variant rs1800588 was correlated with higher HDL-C levels in individuals who usually consume a low-fat diet, and with lower high-density lipoprotein cholesterol (HDL-C) levels in those consuming a high-fat diet (7). This suggests that dietary intervention could consider, among others factors, the *LIPC* genotype. Finally, *LIPC* variation has been shown to interact with lipid-lowering medication in determining LDL particle size and CVD regression (8). More specifically, homozygous CC patients for the promoter polymorphism -514C/T (rs1800588) had a greater decrease in HL activity and a greater increase in low-density lipoprotein (LDL) buoyancy with lipid-lowering therapy compared to both homozygous and heterozygous carriers of the T allele.

Taken together, it is clear that HL plays a complex role in lipid metabolism. In order to answer whether HL is a pro- or anti-atherogenic protein, the context in which it is asked should be taken into account. Should HL be used as target for CVD? The answer would be no for the general population, and perhaps yes for specific subgroups of patients, such as those with specific underlying lipid conditions. In addition to pharmacological intervention based on HL activity, *LIPC* genotype could be considered for personalized lifestyle interventions and other drug interventions such as statins.

Genetics of TG levels

The potential of LPL enhancement for CVD risk reduction

Elevated levels of circulating TG and TG-rich lipoproteins are major risk factors for CVD, independent of elevated LDL-C. Thus, in addition to drugs that reduce LDL-C, such as statins, TG-lowering therapies have been suggested for CVD risk prevention. As we have shown in this thesis, LPL is a key player in TG metabolism, both in the fasting and postprandial state. Therefore, in **Chapter 3**, we ''mimicked'' an RCT study using genetic scores for plasma TG instead of drugs. More specifically, we explored the effects of genetically-decreased TG levels via the *LPL* gene on lipoprotein metabolism (as a proxy for LPL targeting drugs), with or without the background of genetically-decreased LDL-C (a proxy for LDL-C lowering drugs, such as statins). Our results show 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.

This can explain the previously reported additive genetic effects of TG-lowering via *LPL* on CVD risk reduction (9,10). Lotta *et al.* has shown that both genetically-influenced lower TG levels via *LPL* alleles and genetically-influenced lower LDL-C levels have an additional 10% lower CVD risk compared to those with genetically-influenced lower LDL-C levels only. From a clinical perspective, this suggests that 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.

While commonly available TG-lowering drugs such as fibrates (11-13), omega-3-fatty-acids (14,15) and niacin (16) have shown a profound TG reduction (25–45%), their contribution to CVD reduction when combined with statins has remained inconclusive. A new fibrate called prefibrate (K-877, Kowa) (17,18), and a high dose of omega-3 fatty acid (Epanova, AstraZeneca)(19,20), which have shown better efficacy, are currently in Phase 3 clinical trials. Whether these medications will provide additional CVD risk benefit to statins remains to be seen. Other novel and emerging treatments for TG have focused on modulation of LPL inhibitors, including apolipoprotein C-III (apo C-III) and angiopoietin-likeprotein 3 (ANGPTL3). Currently, there are two antisense apo C-III inhibitors (volanesorsen and AKCEA-APOCIII-LRx) (21-24) and two anti-ANGPTL3 therapies (the monoclonal antibody evinacumab and the antisense IONIS-ANGPTL3-LRx inhibitor)(25-28), which have shown favourable TG and other lipid changes in patients with high TG and atherosclerotic CVD. While these anti-apo C-III and anti-ANGPTL3 drugs seem promising so far, more outcome clinical trials are needed to firmly establish their efficacy and safety.

Another important modulator of LPL activity is apolipoprotein A-V (apo A-V), which in contrast to apo C-III and ANGPTL3 promotes LPL activity (29). Genetic variation in the *APOA5* gene encoding apo AV strongly affects TG levels (30,31), but the potential clinical impact and underlying mechanisms relative to LPL are incompletely elucidated. Therefore, in **Chapter 4**, we aimed to further investigate the role of effects of *APOA5* genetic variation on plasma lipoproteins and CAD risk, separately and in combination with variation in *LPL* and LDL-C through MR approaches. Our results showed that TG-lowering via *APOA5* provided additional beneficial effects on CAD risk and the lipoprotein profile on top of TG-lowering via *LPL* and LDL-C-lowering. In addition, the associations with NMR-based lipoproteins and other metabolites suggested 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. Regardless of the precise mechanism(s)of action, our results

genetically validate apo A-V as potential target for TG reduction and primary CAD prevention. While suppressing (downregulating) is easier than enhancement in drug development, there are options for introducing apo AV into the blood. However, major work is needed in the future to bring these options into practice.

In **Chapter 3** and **Chapter 4**, we used genetic risk scores for plasma TG levels as proxies for the potential effect of drugs on plasma TG and CVD risk. However, when interpreting the results of these studies, several assumptions and limitations of the MR approach should be taken into consideration. First, when translating genetic findings into pharmacological strategies, it should be noted that the consequences of lifelong exposure to genetic effects may differ from the relatively short-term pharmacological effects of drugs. Second, MR assumes that genetic variants are associated with the end point of interest only via the pathway of the exposure of interest, and thus pleiotropic effects could invalidate the results. Even though in **Chapter 3** and **Chapter 4** we chose genetics variants so that we could minimize possible pleiotropic effect, any remaining pleiotropy cannot be excluded.

PART II – CARDIOMETABOLIC AND GENETIC RISK PROFILES OVER THE LIFE COURSE IN DIFFERENT GENERATIONS OF MEN AND WOMEN

Current and future trends of obesity and related cardiometabolic risk factors in the Netherlands

The findings of **Chapter 5** showed that the younger generations in the Doetinchem cohort study have a higher prevalence of overweight and obesity, and from an earlier age onwards compared to the older generations. This is similar to the increasing prevalence of overweight and obesity in the general Dutch adult population over the last decades. Based on data from the Dutch Central Bureau for Statistics (CBS), obesity rates in adults of 20 years and older have more than doubled from 1990 (6.3%) to 2017 (14.2%), a time window from our baseline (round 1) to the last follow-up measurements (round 6) (32). Some 36 % of all adults were overweight in 2017, which means that at that time point half of the Dutch population aged 20 years or older was overweight or obese. In addition, a prediction model by the World Health Organization (WHO) (Adulthood obesity prevalence forecasts) in 2013 indicated that by 2030, 17 % of Dutch adults will be obese, out of which 8% will be men and 9% women (33). The earlier age of obesity in the younger generations results in an increased lifelong exposure to obesity. This indicates that the burden of obesity-related chronic diseases such as type 2 diabetes (T2D) and CVD may further

increase in the coming years. In addition, in our study, we observed that obesity increased and cardiometabolic risk factors deteriorated with age in all generations, which likely adds to the burden of cardiometabolic disease in ageing populations.

In the recent decades, the number of people diagnosed with T2D has more than doubled globally, mainly due to increased obesity prevalence (34-36). Nevertheless, we did not see an obvious increase in T2D in the younger generations of the Doetinchem study (**Chapter 5**). Since the youngest generations had merely reached 50 years in the last included measurement round, it remains to be assessed whether the relative prevalence of T2D will increase or not in the future. Another study in the Netherlands predicted a worsening of T2D prevalence in the years to come (37). More specifically, based on these projections, in the next 20 years, some 300,000 additional cases of T2D will occur, with about 100,000 extra cases if the increase in the prevalence of obesity continues at the present rate. This emphasizes the need for primary prevention of obesity.

However, it should be noted that while overweight and obesity have increased over the recent years, prevention and improved treatment of CVD events (bypass, atherectomy, stents, etc) have also vastly improved in the same time period (38-40). The preventive measures include, amongst others, more public awareness and legislation with regard to smoking, but also the widespread use of CVD preventive medication such as statins. This may have partly counteracted the adverse effects of obesity on cardiometabolic disease, and likely contributed to the actual decrease in CVD mortality over the last decades. In the Netherlands, the number of CVD deaths has decreased from approximately 50,000 in 1970 to 37,000 in 2019, a reduction of 25% (41). Given that obesity is a major contributor to CVD cases, further work on successfully reducing obesity in the future will help to maintain and even increase the progress made so far in the CVD trends.

Life course genetics of cardiometabolic risk factors

While the environment we live in plays a major role in the development of obesity and associated cardiometabolic risk factors, not everyone who is exposed to the same environmental exposures will have similar risk of cardiometabolic disease. The inter-individual variability is also clearly affected by the genetic makeup of an individual, which may increase or decrease risk of cardiometabolic disease in combination with non-genetic/environmental and lifestyle factors. In addition, these non-genetic effects change over the life course, since over the long term our environmental exposure is not constant and dynamic gene-age interactions thus likely affect disease risk. In **Chapter 6** we studied the effects of specific genetic loci

on adult body weight over life course and found that some genetic loci have a stable effect over the life course, whereas others may be age-dependent. More specifically, we found that the *TOMM40-APOE* locus is associated with weight gain in young and middle adulthood individuals and with progressive weight loss after the age of 50. The *TOMM40-APOE* locus has been shown to increase the risk of Alzheimer's disease (42,43), 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.

In addition to body weight, age-dependent genetic effects have been observed for other cardiometabolic risk factors including total cholesterol (44), LDL-C (45) and blood pressure (46). For example, some genes have been shown to increase cholesterol levels and blood pressure more at a later age compared to younger ages. These findings are clinically highly relevant because different treatment strategies might be warranted at different ages if the mechanisms involved in regulating cardiometabolic risk factors vary across the life course. In addition, these gene-environment-age interactions suggest that risk prediction scores used for prevention of cardiometabolic disease may need to include different genes or risk factors depending on the age of the individual. In addition to body weight, cholesterol and blood pressure, further work on exploring whether similar age-related genetic effects are significant for other cardiovascular risk factors is needed to better capture cardiometabolic risk over the life course.

Another important implication of our findings in **Chapter 6** is that age-dependent genetic effects might introduce bias in Mendelian Randomization (MR) studies. Conventional MR analyses are conducted on the basis of a constant effect of the genetic variant on the exposure over time, even though this has not yet been established as an assumption of the MR. Usually, the larger the degree to which the associations between genetic variants and exposure vary with age, the larger the potential bias (47). Therefore, before conducting MR studies, is important to carefully examine the age-dependent relationships between genetic variants and the exposures of interest. Based on such information, the genetic variants that lead to potential bias could be excluded or alternative MR methods that consider repeated measures of the gene-exposure associations may be used.

FUTURE PERSPECTIVES

Nature and Nurture in Cardiometabolic Health

In this thesis, we show that cardiometabolic disease is a complex disease that is associated with a combination of multiple genes and environmental factors that, in addition, may have different interactions over the life course. Each of these factors is like a piece of a big cardiometabolic "puzzle", which need to be connected to fully capture the whole picture of cardiometabolic disease risk. The more we understand about these factors and their contribution to cardiometabolic disease risk, i.e. the more pieces we add together, the more we may be able to improve cardiometabolic health in a personalized way. The completion of the Human Genome has provided an extraordinary foundation for increasing knowledge on the role of genetics in disease. From BMI and lipids to many other phenotypes, large-scale genome-wide association studies have identified numerous genetic loci and related mechanisms. However, we are still lacking a comprehensive understanding of the non-genetic drivers and their interaction with genetics in health and disease. Most of the epidemiological studies have typically focused on 1 or few exposures/ risk factors at a time, while in real-life conditions we are exposed to multiple risk factors at once. These multiple exposures may not only have risks that add up, but they may also interact with each other and with genetic factors to multiply or attenuate their effects. Therefore, it is important that future research focuses on a comprehensive approach that captures the multiple co-occurring risk factors of cardiometabolic disease.

Major research initiatives (48-50), among which Exposome_NL(49), are now focused on investigating the Human Exposome, which can be considered the environmental analog and the complement to the Human Genome. The exposome concept, first introduced in 2005 (51), captures the totality of exposures we face throughout our lives including the air we breathe, our social interactions, the psychological stresses we face and lifestyle choices such as smoking, diet and physical. Since many aspects of our environment are modifiable, the Human Exposome offers a huge potential for disease prevention. Hopefully in the future, the Human Genome and Human Exposome will be synchronized to complete the whole puzzle of human health and disease, enabling personalized medicine to become the future of the healthcare.

Prevention at a population level

Given the dominant role of non-genetic factors in cardiometabolic disease, interventions that decrease the risk associated with environmental exposures in the entire population should be considered as a first step in cardiometabolic disease prevention. Such population-level approaches may include policy changes that focus on

improving and maintaining a healthy lifestyle from young age onwards to minimize the burden of CVD. For example, food retail policies should encourage the availability of outlets selling healthy food and decrease the density of fast-food outlets. In addition, pricing strategies, such as lowering the cost of fruits and vegetables and increasing taxation of unhealthier products, as well as nudging strategies that promote more physical activity in daily life could efficiently shift risk factor levels of the entire population in a favourable direction. Finally, even though smoking has declined in the recent decades, more smoking bans should be implemented to further reduce smoking and improve cardiometabolic health.

Prevention at an individual-level/Personalized approaches

As the precision of risk prediction tools increases with the advances in the Human Genome and Human Exposome, it enhances our ability to identify individuals who are at higher risk for cardiometabolic disease. Once this has been achieved, individual-level preventive strategies adapted to age, gender and an individual's genetic profile may complement population-based strategies to prevent cardiometabolic disease. In addition to prevention at an individual-level, the genetic variation may also be useful for personalized intervention to treat cardiometabolic disease. Thus, both future preventive and curative strategies should include a personalized approach that aims to provide optimal cardiometabolic health to individuals.

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