Intestinal cholesterol absorption in humans

Summary

Elevated concentrations of cholesterol in the blood, also known as hypercholesterolemia, increase the risk of developing cardiovascular disease (CVD). Amongst others, intestinal cholesterol absorption plays a crucial role in determining blood cholesterol concentrations. The amount of absorbed cholesterol via the small intestine varies between - but less within - individuals. This suggests that variations in cholesterol absorption are related to differences in genetic background. Consequently, individuals can be categorized as high-cholesterol or low-cholesterol absorbers. This is an important concept, as variation in intestinal cholesterol absorption has been associated with the presence of different metabolic disorders. Also, it can be envisaged that low-cholesterol and high-cholesterol absorbers respond differently to cholesterol-lowering treatments. High-cholesterol absorbers, for example, may respond better to interventions that inhibit intestinal cholesterol uptake than low-cholesterol absorbers do. The main aim of this thesis was therefore to better understand (i) reasons for this interindividual variability in intestinal cholesterol absorption and (ii) the complex intestinal cholesterol network.

In **Chapter 2,** associations of genetic variants with intestinal cholesterol absorption were systematically reviewed. Genetic variants in seven genes were associated with intestinal cholesterol absorption: *ABCG5*, *ABCG8*, *ABO*, *APOE*, *MTTP*, *NPC1L1*, and *LDLR*. In that chapter, an intestinal cholesterol absorption network was also constructed using these seven genes with the help of GeneMANIA Cytoscape plugin. The constructed network revealed the complex nature of intestinal cholesterol absorption. It was concluded that further research is needed to validate and improve this network, which could eventually result in a better understanding of the differences in cholesterol absorption rates and the formulation of personalized treatment interventions.

Single nucleotide polymorphisms (SNPs) in certain genes have been associated with cholesterol metabolism and may partly explain the large inter-individual variability in intestinal cholesterol absorption. In **Chapter 3** and in **Chapter 4,** associations of SNPs with

intestinal cholesterol absorption have been investigated in a cross-sectional study. First, in **Chapter 3**, SNPs were selected from genes that encoded proteins involved in intestinal cholesterol absorption. As cholesterol absorption and endogenous cholesterol synthesis are generally negatively related, also SNPs of genes involved in endogenous cholesterol synthesis were selected. For these selected SNPs, associations with intestinal cholesterol absorption markers, endogenous cholesterol synthesis markers, and serum low-density lipoprotein cholesterol (LDL-C) were calculated. A SNP in *ABCG5* (rs4245786) and the tag SNP *ABCG8* (rs4245791) were significantly associated with intestinal cholesterol absorption markers. In contrast, SNPs in *NPC1L1* (rs217429 and rs217416) were significantly associated with endogenous cholesterol synthesis. Finally, the tag SNP in *HMGCR* (rs12916) and a SNP in *LBR* (rs12141732) were significantly associated with serum LDL-C concentrations. Of note, the other SNPs in the cholesterol absorption or synthesis genes were not associated with serum LDL-C concentrations.

In **Chapter 4**, associations of a large data set of more than 160,00 SNPs with intestinal cholesterol absorption markers have been analyzed. For this total cholesterol-standardized (TC-standardized) campesterol and sitosterol levels, which are validated intestinal cholesterol absorption markers, were tested in 457 individuals of European descent. Only those SNPs that showed a consistent association with both markers, i.e. campesterol and sitosterol, were considered to be relevant. These SNPs were located in or between *ABCG8, EIF2B5, EPHB3, C4orf26, CDKL2, NR3C2, LOC285626, BMP6, HLA-G*, *HLA-H, WBSCR27, WBSCR28, TMTC4,* and *COL4A2*. These genes were used to construct a protein-protein interaction (PPI) network, which could be linked to 30 unique WikiPathways. This study highlighted the discovery of numerous unexplored genes and pathways potentially associated with intestinal cholesterol absorption that warrants further investigation.

Finally, in **Chapter 5**, gene expression profiles of a previous randomized, double-blind crossover study with participants of European descent were analyzed. The differently expressed genes (DEGs) in participants pre-classified as high-cholesterol and low-cholesterol absorbers were analyzed before and after the intake of plant stanol-esters, which are known to inhibit intestinal cholesterol absorption, in two parts of the small intestine: duodenum and jejunum. In the duodenum, 181 DEGs in the high-cholesterol absorbers and 482 DEGs in the

low-cholesterol absorbers were identified. In the jejunum, the corresponding numbers were 366 and 316 DEGs. Generally, changes in gene expression were in the opposite direction between the low-cholesterol absorbers and the high-cholesterol absorbers. The resulting DEGs were used for enrichment analysis using WikiPathways, KEGG, and Reactome. From this study, it is clear that responses in gene expression profiles differed between subjects that were a priori defined as low-cholesterol or high-cholesterol absorbers. These differences provide leads to better understand the molecular intestinal characteristics of low-cholesterol versus high-cholesterol absorbers, before and after exposure to an intervention that lowers intestinal cholesterol absorption. Whether these results can also be used to better understand the etiology of metabolic diseases possibly related to intestinal cholesterol absorption warrants further investigation.

In conclusion, the present thesis has deepened our understanding to explain the large interindividual variability in intestinal cholesterol absorption in apparently healthy individuals. To what extent findings can be extrapolated to other populations and can be confirmed in larger cohorts warrants further study. Also, majority of the identified SNPs or genes that were part of the created intestinal cholesterol absorption networks has not been associated with intestinal cholesterol absorption before. Thus, future studies should be carried out to gain a better understanding of the relationship between these genes and intestinal cholesterol absorption.