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Fatma Mokhtar

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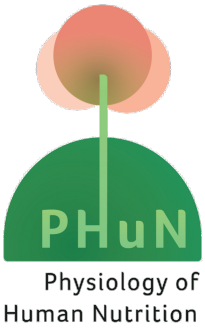
Fatma Bashir Abulgasem Mokhtar



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DISSERTATION

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Prof. dr. Pamela Habibović

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by

Fatma Bashir Abulgasem Mokhtar

Supervisors

Prof. dr. R. P. Mensink

Prof. dr. J. Plat

Assessment Committee

Prof. dr. R. Shiri-Sverdlov (chair)

Dr. C.J.H. van der Kallen

Prof. dr. K. Laitinen (University of Turku, Finland)

Dr. D.S. MacKay (University of Manitoba, Canada)

Prof. dr. E.C.M. Mariman

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CHAPTER 1

General introduction

General introduction

Background

According to the World Health Organization (WHO), 17.9 million people died in 2019 from cardiovascular diseases (CVDs) (1). Many different risk factors for CVD exist. Some of these risk factors are non-modifiable such as age, family history of CVD, and genetic background. The negative impact of many other risk factors, however, can be reduced through changes in lifestyle. These so-called modifiable risk factors include smoking, physical inactivity, diabetes, being overweight or obese, and high blood pressure. Another well-known modifiable risk factor is hypercholesterolemia, which is characterized by high serum cholesterol concentrations in the atherogenic low-density lipoprotein (LDL-C) fraction. Serum LDL-C is controlled by many different factors amongst others genetic background, endocrine factors, body weight, circadian rhythms, drugs, physical activity, and diet (2, 3). Cholesterol in the body can be obtained from the diet via intestinal absorption or by endogenous synthesis, mainly by the liver. Serum cholesterol concentrations vary widely between populations that have different dietary habits. Indeed, a change in dietary habits resulting in a reduced intake of cholesterol reduces serum cholesterol concentrations (4), though relatively modest (5). However, serum cholesterol concentrations within populations consuming comparable diets can also be very different. This suggests that wide inter-individual variation in cholesterol metabolism exists (6). Indeed, fractional cholesterol absorption between individuals consuming standardized diets varied between 29 – 81% (7). In general, however, the higher the absolute and fractional dietary cholesterol absorption rates, the lower the rates of cholesterol synthesis, biliary secretion, and fecal elimination are (8). People who absorb more cholesterol, deliver more cholesterol to the liver, which suppresses endogenous cholesterol synthesis and downregulates receptor-mediated uptake of LDL by the liver. Consequently, serum total cholesterol and LDL-C concentrations will increase, whereas the opposite occurs when cholesterol absorption is inhibited (9). Although the inter-individual variation is high, the intra-individual variation is low (10) suggesting that genetic background is an important determinant of cholesterol absorption.

Intestinal cholesterol absorption

Intestinal cholesterol absorption is a multistep process. Briefly, the intestinal micelle, that contains free cholesterol, interacts with the brush border membrane of the enterocyte. Here, the Niemann-Pick C1 like 1 protein (NPC1L1), which plays a critical role in cholesterol absorption (11), is highly expressed. Within the enterocyte, the cholesterol molecules first move to the endoplasmic reticulum, where they become esterified by acyl-CoA cholesterol acyltransferase (ACAT). In the endoplasmic reticulum, the esterified cholesterol is packed with triacylglycerol, phospholipids, and apolipoprotein-B48 to form chylomicrons (12). In this process, microsomal triglyceride transfer protein (MTP) plays an important role, as this enzyme transfers neutral lipids (triacylglycerol and cholesterol ester) into the newly formed chylomicrons (13). Via the basolateral membrane of the enterocyte, the chylomicrons are secreted into the lymph and subsequently travel to the blood. However, other essential proteins also determine the net flux of cholesterol into the enterocyte and as such the amount of cholesterol that is available for incorporation into mixed micelles. Two important proteins are the adenosine triphosphate (ATP) binding cassette (ABC) transporters ABCG5 and ABCG8. These proteins promote the efflux of cholesterol from the enterocyte back into the intestinal lumen for excretion (14). Thus, multiple genes are involved in this complex process. In fact, the precise mechanism of intestinal cholesterol absorption is still unsolved, and it is very likely that many more proteins play an important role. **Figure 1** presents a schematic overview of the intestinal cholesterol absorption pathway.

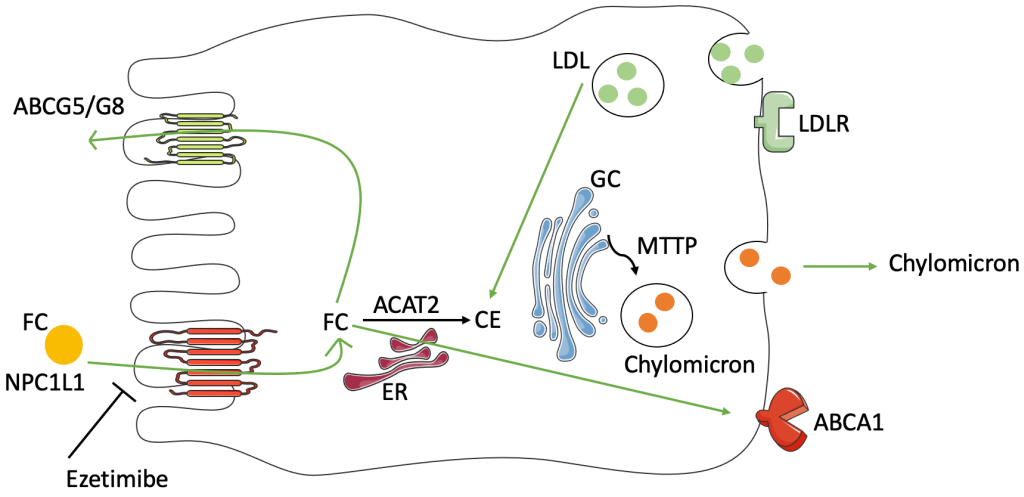

Figure 1. Schematic overview of the intestinal cholesterol absorption pathway. Free cholesterol (FC) enters the enterocyte via Niemann-Pick C1-like 1 (NPC1L1) and is esterified by Acetyl-CoA Acetyltransferase 2 (ACAT2) in the endoplasmic reticulum (ER). FC can also be transported back into the intestinal lumen via ATP-binding cassette members 5 and 8 (ABCG5/G8). Low-density lipoprotein (LDL) is taken up from the basolateral side via LDL receptor-mediated endocytosis. FC is also used by ATP-binding cassette A1 (ABCA1) to form high-density lipoprotein. Cholesterol ester (CE) is further processed in the Golgi complex (GC) with other components to form chylomicrons which are further transported to the lymphatic system. The drug ezetimibe is an NPC1L1 blocker and thus inhibits intestinal cholesterol absorption.

Biomarkers of intestinal cholesterol absorption

Phytosterols, also called plant sterols (e.g., campesterol and sitosterol), can be found in plant cell membranes and are therefore present in plant products such as vegetable oils, seeds, nuts, fruits, vegetables, and grain products (15, 16). Except for the plant sterols, also minute quantities of plant stanols (e.g. campestanol and sitostanol) can be found in plant-based products (17). Higher amounts are present in food products enriched with plant stanols derived from hydrogenated plant sterols. Saturation of the double bond at the C5-C6 position of the second ring in plant sterols results in the conversion of, for example, campesterol into campestanol and of sitosterol into sitostanol (18). Plant sterols and plant stanols, frequently esterified with fatty acids, are incorporated into certain so-called functional foods, as they effectively lower serum LDL-C concentrations (19).

Dietary intake of phytosterols ranges between 250-400 mg/day in European countries but varies widely between subjects (20). The intake of campesterol plus sitosterol is almost equal to that of cholesterol, but their intestinal absorption rates are markedly lower (21, 22). This lower intestinal absorption might relate to their chemical structure, differing in the side chain (**Figure 2**). The intestinal absorption of plant sterols is about 5%, while that of plant stanols is even lower (0.02-0.3%). For comparison, cholesterol absorption is 30-80% (7, 22-25). Another steroid, which is a metabolite of cholesterol, is cholestanol, which is present in small amounts in most mammalian tissues (26). It differs from cholesterol by the absence of the double bond in the second ring at the C5-C6 position (27). Intestinal absorption of cholestanol is also low and is estimated to be 3-4% (28).

Isotopic tracers can be used to measure intestinal cholesterol absorption. Though very precise, it is a labor-intensive and costly technique, thereby limiting its use in large-scale studies (29). However, fractional cholesterol absorption can also be estimated using a method developed by Miettinen and co-workers (30) by using serum total cholesterol-standardized levels of campesterol, sitosterol, or cholestanol. For that reason, non-cholesterol sterols are frequently used markers of cholesterol absorption, as plasma samples are easily accessible, and the non-cholesterol sterols are less laborious to measure. These non-cholesterol sterol markers have been validated in a randomly selected healthy population (30). In that study, ratios of plasma non-cholesterol sterols markers (campesterol, sitosterol, and cholestanol) to cholesterol were measured and compared with the quantification of intestinal cholesterol absorption as measured by the dual-isotope continuous feeding technique. It was shown that the plasma non-cholesterol to cholesterol ratios highly correlated with the absorption values determined by using the dual stable isotope technique.

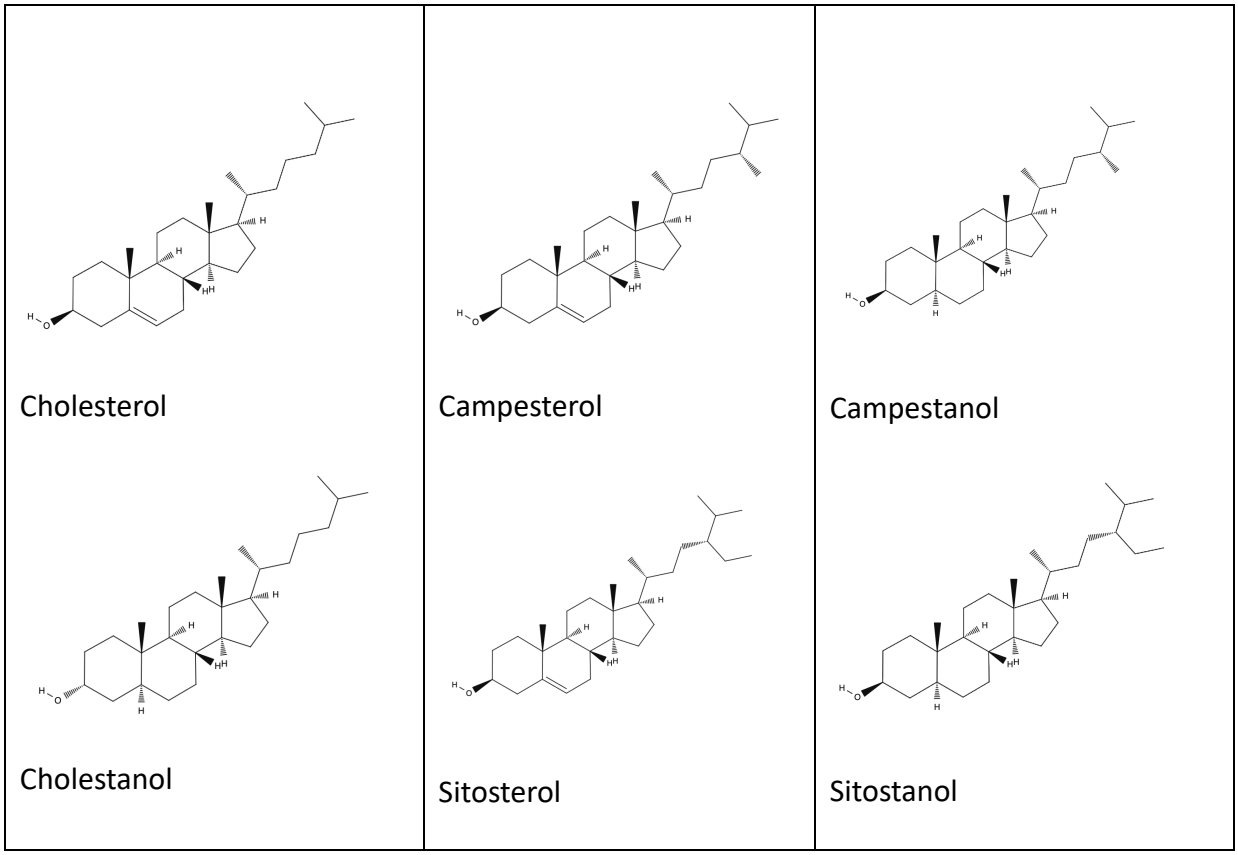

Figure 2. Chemical structure of cholesterol and a cholesterol absorption marker: cholestanol in the left part of the figure. In the middle, the chemical structures of two plant sterols are shown, that are also used to estimate cholesterol absorption: campesterol and sitosterol. In the right part of the figure, the chemical structures of two plant stanols are presented: campestanol and sitostanol. Structure source: MolView (31).

Genetic variation and intestinal cholesterol absorption

As already mentioned, cholesterol absorption varies widely between individuals, but is rather constant within an individual (10). This strongly suggests that this interindividual variation relates to genetic variations between individuals, as the diet was controlled for in that study.

The genetic information of an organism is found in deoxyribonucleic acid (DNA), that is composed of two polynucleotide chains that wind around each other forming a double helix. The two DNA strands are made of nucleotides, and each nucleotide consists of a sugar molecule, a phosphate group, and one of the four nucleobases: adenine (A), thymine (T), guanine (G), or cytosine (C) (32). The genetic information that is necessary to produce a protein is called a gene. These genes are composed of different parts. The first part is the promoter. Other essential parts of a gene are the introns and exons. Different steps are

required for a gene to be expressed. First, information from a gene is transferred to messenger ribonucleic acid (mRNA) by a process known as transcription, which can be initiated by the binding of transcription factors to the promoter region. In this process, an enzyme called RNA polymerase II plays an important role and a pre-mRNA molecule is formed. For the synthesis of the pre-mRNA, both the introns and exons of DNA are transcribed. The information from the introns is then removed by a process called RNA splicing and mature mRNA is formed. Secondly, the mature mRNA will leave the nucleus and travels through the cytoplasm to the ribosomes. Ribosomes can now attach to the mRNA and translation will start, which refers to the process that the information of an mRNA molecule is used to build a polypeptide composed of many different amino acids. Finally, the polypeptide is converted into a functional protein (33) (**Figure 3**).

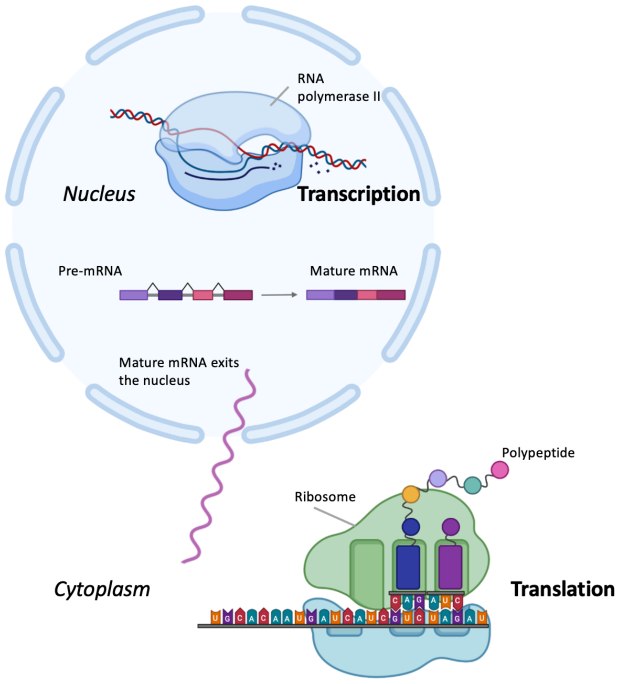

Figure 3. Gene expression through transcription and translation. Exons are the colored boxes, while the introns are the gray lines in the pre-mRNA in the nucleus. During transcription, mature mRNA is formed. During translation, the mature mRNA is used for the synthesis of a specific protein. This figure was created using BioRender (https://biorender.com/).

The genetic information in our DNA varies largely between individuals. Single nucleotide polymorphisms (SNPs) are single base-pair changes in the genomic DNA strains that are present with a frequency of more than 1% in an unrelated population (34). In **Figure 4**, for example, DNA variation between two individuals exists due to the substitution of the thymine nucleobase for the guanine nucleobase.

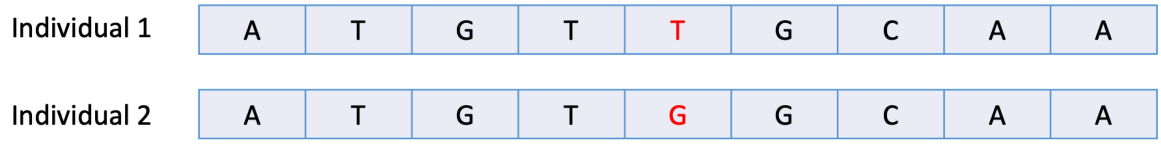

Figure 4. Single nucleotide polymorphisms (SNPs) are single base variations among different people. The figure shows a part of DNA in which one nucleobase of individual 1 (thymine (T)) is replaced by guanine (G) in individual 2.

SNPs can be categorized based on their effects on the amino-acid sequence of the coded protein. Synonymous SNPs do not cause a change in the encoded amino acids. However, nonsynonymous (missense) SNPs cause a change in the amino-acid sequence of the protein (35). Also, other polymorphisms exist. Indel polymorphisms, for example, are insertions or deletions of a nucleotide sequence. Indel is a type of genetic variation, in which a specific nucleotide is present (insertion) or absent (deletion), which can also alter protein structure or function depending on the site of the indel in the gene (36). Variant annotation can be used to find out the functional consequences of the DNA variants, which can further help in predicting the variant effects on the protein function and structure (37). Different genetic variants are shown in **Figure 5**. There is a great potential of using SNPs as markers to detect associations with phenotypic variations in a population. Some SNPs can have functional consequences if they are present within transcriptional regulatory elements, which can impact mRNA transcription stability or affect transcription factor binding affinity. These variations are associated with diversity in the population, individual responses to diet and medication, and susceptibility to disease (38, 39). For this reason, SNPs are a great tool to identify and map complex diseases and are a promising approach for medical decisions for healthcare providers to customize treatment tailored to individual patients (precision medicine) (40-42).

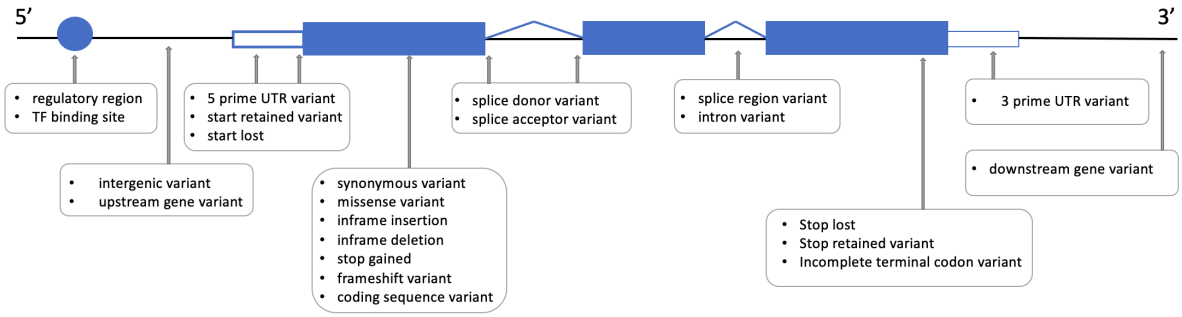

Figure 5. Possible locations of genetic variants and its consequence for the transcript. Consequences of variants on, for example transcripts can be obtained from the Ensembl variant effect predictor (43).

Another essential aspect of human genomics is linkage disequilibrium (LD), which refers to the nonrandom pattern of associations between alleles in a given population. An allele refers to a gene variant, and a haplotype to a set of alleles on the same chromosome (44). These associations between alleles are mainly due to physical proximity (physically close to each other on a chromosome) and are, therefore, more likely to be inherited together during meiosis (45). Thus, LD can impact the interpretation of genetic association studies. To reduce the number of outcomes when examining, for example, the association between SNPs and the likelihood of developing a disease, it is possible to select a SNP (tag SNP) that is representative for group of SNPs (e.g. haplotype) instead of testing all the variants (46).

Cholesterol absorbers and synthesizers

Based on cholesterol absorption and endogenous cholesterol synthesis markers, humans can be classified as high-cholesterol and low-cholesterol absorbers or as high-cholesterol and low-cholesterol synthesizers (47). These characteristics have been shown to relate to the cholesterol-lowering efficacy of an intervention (48, 49). Statin therapy, for example, is most effective in high-cholesterol synthesizers, while individuals classified as high-cholesterol absorbers will benefit more if they use cholesterol-absorption inhibitors such as products enriched with plant stanol and sterol esters or the drug ezetimibe (47, 50, 51). By defining

whether a patient is a cholesterol-synthesizer or cholesterol-absorber, a tailored regimen can be followed to achieve the best outcome and to reduce the cost of treatment. SNPs in genes involved in intestinal cholesterol absorption can relate to the efficacy of dietary interventions aimed to lower serum LDL-C concentrations. Weinberg *et al.* (52), for example, found that by consuming a high-cholesterol, high-polyunsaturated fat diet, individuals that were heterozygous for the A-IV-2 allele have a lower fractional cholesterol absorption than A-IV-1/1 homozygous individuals. Moreover, Plat *et al.* (53) observed that after consuming plant stanols, participants homozygous for the ABCG8 T400K genotype TT had a greater reduction in cholesterol absorption compared to the TK/KK genotype group.

Since different laboratories use different methods to monitor cholesterol absorption and synthesis (54), there are no generally accepted cut-off values to define individuals as high-cholesterol or low-cholesterol absorbers or as high-cholesterol or low-cholesterol synthesizers. Therefore, the need exists to better standardize analytical procedures to define individuals as high-cholesterol or low-cholesterol absorbers or synthesizers. In addition, mapping SNPs associated with cholesterol absorption and synthesis can help to identify cholesterol-absorbers or synthesizers and optimize personalized treatments.

The significance of biological networks

Cellular processes are controlled at multiple levels by regulating gene transcription and protein synthesis within complex networks (55). To obtain a deeper understanding of gene functions and interactions, several tools are available to visualize complex biological networks graphically and to predict gene functions by using available genomic and proteomics data (56). A frequently used software program is GeneMania (57). Based on several lines of evidence indicating the importance of SNPs in genes associated with intestinal cholesterol absorption, the focus of this thesis was directed toward a better understanding of the complex intestinal cholesterol absorption network.

Outline of the thesis

As mentioned before, intestinal cholesterol absorption varies widely between individuals. This thesis now aims to understand reasons for this interindividual variability. Not only SNPs in genes related to intestinal cholesterol absorption were examined, but also investigated the

interplay between the SNPs and endogenous cholesterol synthesis markers and LDL-C levels. Furthermore, genes that are affected by plant stanol ester intake were investigated, and genes related to intestinal cholesterol absorption were identified using different approaches. Moreover, enriched pathways were revealed. In the end, this knowledge should lead to a better understanding of the determinants of the complex intestinal cholesterol absorption network. **Figure 6** represents the outline of this thesis**.**

CHAPTER 2 first provides a systematic overview of the literature to summarize genetic variants associated with intestinal cholesterol absorption. Secondly, the genes that appeared from the systematic review were used to build an intestinal cholesterol absorption network using the GeneMANIA Cytoscape plugin**. CHAPTER 3** presents the association of pre-selected genes with intestinal cholesterol absorption and endogenous cholesterol synthesis rates, and LDL-C concentrations. In **CHAPTER 4**, we have genotyped participants' from five human intervention studies to investigate the association of all SNPs of the Axiom^{TM} Precision Medicine Research Array (PMRA) Kit (Thermo Fisher Scientific, Waltham, MA, USA) (58) with different non-cholesterol sterol absorption markers. To better understand the molecular mechanism of intestinal cholesterol absorption, we tried to identify in **CHAPTER 5** genes that are differentially regulated after the intake of plant stanol esters in high-cholesterol and low-cholesterol absorbers. The effects of acute plant stanol ester consumption on mRNA gene expression profiles in the duodenum and jejunum biopsies in two groups pre-classified as high-cholesterol or low-cholesterol absorbers were studied. Additionally, the mRNA expression for each group (high-cholesterol and low-cholesterol absorbers) was visualized in enterocyte cholesterol metabolism wikipathways, which was an output of the enrichment analysis. All data for this thesis were obtained from samples collected in five previous human intervention studies performed between 1997 and 2012 at Maastricht University, the Netherlands. All individuals included in these studies were from Maastricht and the surrounding area, a European population. The **General Discussion** discusses the significance of the results obtained, while possible implications and further studies are mentioned.

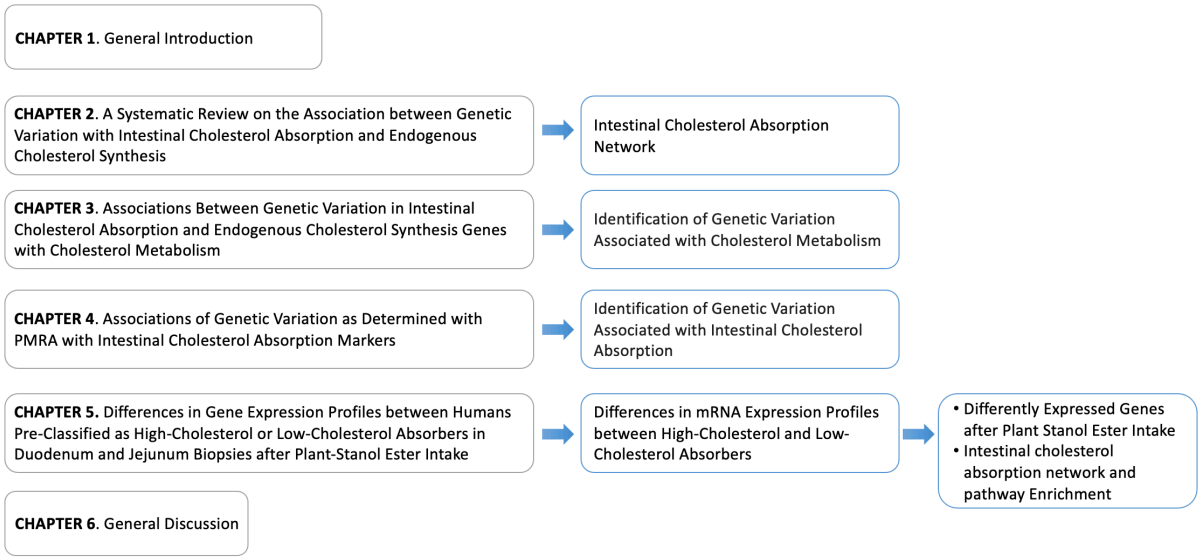

Figure 6. Outline of the thesis. Abbreviations: PMRA = Axiom™ Precision Medicine Research Array.

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CHAPTER 2

Genetic variation and intestinal cholesterol absorption in humans: A systematic review and a gene network analysis

Fatma B.A. Mokhtar, Jogchum Plat, Ronald P. Mensink

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Abstract

Intestinal cholesterol absorption varies widely between individuals, which may translate into differences in responsiveness to cholesterol-lowering drugs or diets. Therefore, understanding the importance of genetic variation on cholesterol absorption rates and the complex intestinal cholesterol network is important. Based on a systematic review, genetic variants in seven genes (*ABCG5, ABCG8*, *ABO, APOE*, *MTTP, NPC1L1,* and *LDLR*) were identified that were associated with intestinal cholesterol absorption. No clear associations were found for variants in *APOA4*, *APOB*, *CETP, CYP7A1*, *HMGCR*, *SCARB1*, *SLCO1B1*, and *SREBF1.* The seven genes were used to construct an intestinal cholesterol absorption network. Finally, a network with fifteen additional genes (*APOA1*, *APOA4*, *APOB*, *APOC2*, *APOC3*, *CETP*, *HSPG2*, *LCAT, LDLRAP1*, *LIPC*, *LRP1*, *OLR1*, *P4HB*, *SAR1B*, and *SDC1*) was generated. The constructed network shows that cholesterol absorption is complex. Further studies are needed to validate and improve this network, which may ultimately lead to a better understanding of the wide inter-individual variability in intestinal cholesterol absorption and the development of personalized interventions.

1. Aim and rationale of the review

Plasma cholesterol concentrations are the result of many interacting pathways including intestinal cholesterol absorption. To prevent hypercholesterolemia, a major risk factor for coronary heart disease (CHD), tight regulation of intestinal cholesterol absorption is therefore essential (1,2). However, fractional intestinal cholesterol absorption rates vary widely between individuals and ranged between 29% and 80%, when participants consumed a breakfast providing 64 mg of cholesterol. Within subject-variability, however, was small (3). These findings therefore suggest that genetic background is an important determinant of intestinal cholesterol absorption rates.

Intestinal cholesterol absorption can be lowered by drugs specifically targeting NPC1 like intracellular cholesterol transporter 1 (NPC1L1), a key transmembrane protein that transports cholesterol from the intestinal lumen into the enterocyte (4). Besides NPC1L1, many other proteins such as the ATP-binding cassette subfamily G member 5 and member 8 (ABCG5 and ABCG8, respectively) heterodimers (ABCG5/8) affect intestinal cholesterol absorption rates (5). This heterodimer is involved in the efflux of cholesterol over the apical membrane of the enterocyte back into the intestinal lumen (6), thereby decreasing overall intestinal cholesterol absorption efficiency. These proteins are not only associated with the transport of cholesterol but also of plant sterols, like campesterol and sitosterol. Plant sterols are structurally related to cholesterol, but are not synthesized by humans. Therefore, all plant sterols in plasma are diet-derived and their cholesterol-standardized plasma levels can be used as markers for fractional intestinal cholesterol absorption (7). In addition to apical influx and efflux transporters, other proteins within the enterocyte are important for cholesterol absorption. Acyl-CoA: cholesterol acyltransferase isoform 2 (ACAT2), apolipoprotein B48 (apoB48), and microsomal triglyceride transfer (MTTP), for example, are involved in intracellular cholesterol trafficking and chylomicron assembly. The chylomicrons, secreted over the basolateral membrane of the enterocyte into the lymphatic system, transport cholesterol and dietary lipids from the intestine to the periphery. In all these genes, single nucleotide polymorphisms (SNPs) are present that may at least partly explain the large variability between individuals in intestinal cholesterol absorption rates, which may also translate in differences in responsiveness to drugs or diets that inhibit cholesterol absorption. Therefore, a systematic overview of genetic variants in relation to intestinal cholesterol absorption was carried out. Cholesterol absorption was quantified by measuring plasma plant sterol levels or by using

isotope protocols. Markers for cholesterol synthesis were also reported, when available. Based on the findings of genetic variants associated with intestinal cholesterol absorption, an intestinal cholesterol absorption network was built.

2. Approach

2.1. Literature search and data synthesis

An online literature search was performed in three databases (Medline, Embase, and Cochrane Central Register of Clinical Trials) on July 16, 2021. The keywords used were: (cholesterol absorption Or plant sterol* Or plant stanol* Or sitosterol* Or phytosterol* Or campesterol* Or cholestanol*) AND (single nucleotide polymorphism* Or SNP* or genetic association Or genetic polymorphism* Or genome-wide association study Or GWA Or GWAS or genetic variability). The search was limited to "human" and the "English language". Moreover, reference lists of selected articles were manually searched. PRISMA guidelines were followed (8). Data of 21 studies could be used to examine cross-sectional relationships between gene polymorphisms with plasma or serum non-cholesterol sterol levels as markers for intestinal cholesterol absorption (9-29). Characteristics of these 21 studies are summarized in **Supplementary Table 1**. In two additional studies, cholesterol absorption was estimated using isotopes (30-31), which are summarized in **Supplementary Table 2**.

For the markers, absolute concentrations and cholesterol-standardized concentrations were extracted. When needed, non-cholesterol sterol or total cholesterol concentrations were converted into respectively µmol/L and mmol/L. Data reported as median (interquartile range) values were transformed into means ± standard deviation (SD) based on the method of Wan *et al*. (32). For the study of Teupser *et al.* (17), however, geometric means were reported, while values were log-transformed for the study of Lupattelli *et al.* (20).

2.2 Creating an intestinal cholesterol absorption network

GeneMANIA prediction software (33) was used to construct an intestinal cholesterol absorption network. For this, the seven genes of which genetic variation was related to cholesterol absorption, based on our literature review, were entered into the query box of the GeneMANIA Cytoscape plugin. The settings used were: max resultant genes = 15, max resultant attributes = 0, with the criteria: pathway, co-expression, genetic interaction, and co-

localization. Pathway means that two gene products are part of the same reaction; co-expression indicates that expression of the two genes are comparable under different conditions; genetic interaction implies that expression levels of one gene is influenced by the other gene; finally, co-localization means that the genes are expressed in the same tissue or their protein products are found in the same part of a cell. The analysis was run as a plugin with the Cytoscape tool version (3.8.2) (34). For each new gene, expression in the small intestine was checked via the National Center for Biotechnology Information (NCBI) (35). If a gene was not expressed in the small intestine, it was deleted from the network in Cytoscape and a new gene proposal as part of the network was requested. The network was constructed on July 26, 2021.

3. Cross-sectional relationships between gene polymorphisms with cholesterol absorption

Cross-sectional associations between genetic variants (e.g., SNPs and variable number of tandem repeats (VNTR)) with plasma non-cholesterol sterol levels were presented in 21 studies. These studies were mainly carried out in mildly hypercholesterolemic, but otherwise healthy subjects (**Table 1**). The reported variants were located in fifteen different genes: *ABCG5, ABCG8,* alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase (*ABO*)*,* apolipoprotein A4 (*APOA4), apolipoprotein E* (*APOE*), cholesteryl ester transfer protein (*CEPT*), cytochrome P450 family 7 subfamily A member 1 (*CYP7A1*), 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*), *MTTP*, *NPC1L1*, scavenger receptor class B member 1 (*SCARB1*), solute carrier organic anion transporter family member 1B1 (*SLCO1B1*), and sterol regulatory element binding transcription factor 1 (*SREBF1*). In the two studies that used isotopes, the reported variants were located in the *ABCG5, ABCG8,* apolipoprotein B *(APOB), APOE*, and low-density lipoprotein receptor *(LDLR)* (**Table 2**). Genes and protein names and chromosomal location of the genes are shown in **Supplementary Table 3**. Results are summarized in **Figure 1**. We found evidence that SNPs in *ABCG5, ABCG8*, *ABO, APOE, MTTP NPC1L1,* and *LDLR* were associated with intestinal cholesterol absorption. Only those genetic variants for which mechanistic studies have been performed (*ABCG8, apoE,* and *NPC1L1*) are discussed in the next paragraphs.

Table 1. Relationships between gene polymorphisms with serum or plasma cholesterol absorption and synthesis markers.

First author, year	Gene*	Polymorphism	rs-number+	Subjects	Genotype	n	Cholesterol absorption markers (mmol/mmol cholesterol)			Cholesterol synthesis markers (mmol/mmol cholesterol)	
							Campesterol	Sitosterol	Cholestanol	Lathosterol	Desmosterol
Berge,	*ABCG5*	Q604E	rs6720173	Healthy	QQ	91	1.58±0.61	1.18±0.41	2.07±0.40	1.57±0.67	0.99±0.27
2002 [9]					QE+EE	51	1.55±0.57	1.15±0.45	2.03±0.42	1.58±0.63	1.03±0.27
Miwa,	*ABCG5*	Q604E	rs6720173	Primary hyper-cholesterolemic	QQ	78		0.96±0.28		1.19±0.55	
2005 [10]					QE	21		0.96±0.37		1.16±0.38	
					EE	1		0.56		1.57	
Plat,	*ABCG5*	Q604E	rs6720173	Healthy	QQ	81	3.11±0.99	1.18±0.44		0.95±0.35	
2005 [11]					QE+EE	31	2.85±0.95	1.06±0.46		1.03±0.30	
Gylling,	*ABCG5*	Q604E	rs6720173	Mildy to moderately hyper-cholesterolemic	QQ	200		1.45±0.57[a]			
2009 [12]					QE+EE	57		1.29±0.45[b]			
Berge,	*ABCG8*	D19H	rs11887534	Healthy	DD	128	1.61±0.61[e]	1.19±0.42[e]	2.08±0.40[e]	1.53±0.60	0.90±0.26
2002 [9]					DH+HH	14	1.17±0.35[f]	0.88±0.30[f]	1.75±0.31[f]	1.84±1.06	1.01±0.32
Gylling,	*ABCG8*	D19H	rs11887534	Mildy to moderately hyper-cholesterolemic	DD	216	3.06±1.18[e]	1.46±0.59[e]	1.51±0.29[e]	1.21±0.44[a]	
2009 [12]					DH+HH	41	2.53±0.96[f]	1.19±0.45[f]	1.37±0.32[f]	1.39±0.58[b]	

Table 1 (continued). Relationships between gene polymorphisms with serum or plasma cholesterol absorption and synthesis markers.

First author, year	Gene*	Polymorphism	rs-number+	Subjects	Genotype	n	Cholesterol absorption markers (µmol/mmol cholesterol)			Cholesterol synthesis markers (µmol/mmol cholesterol)	
							Campesterol	Sitosterol	Cholestanol	Lathosterol	Desmosterol
Renner,	*ABCG8*	D19H	rs11887534	Healthy	DD	75	1.49±0.58[a]	1.18±0.40[a]		1.07±0.43	
2013 [13]					DH	18	1.06±0.66[b]	0.96±0.44[b]		1.14±0.68	
Renner,	*ABCG8*	D19H	rs11887534	Gallstone carriers	DD	14	1.26±0.87[a]	0.90±0.42		1.28±0.45	
2013 [13]					DH	11	0.94±0.22[b]	0.81±0.22		1.30±0.76	
Nissinen,	*ABCG8*	D19H	rs11887534	Gallstone free	DD	102	1.84±0.65[c]	1.23±0.47[g]	1.51±0.29[c]	0.83±0.38	0.75±0.13
2018 [14]					DH+HH	24			1.29±0.22[d]	0.89±0.35	0.77±0.14
Nissinen,	*ABCG8*	D19H	rs11887534	Gallstone carriers	DD	51	1.64±0.71[c]	1.09±0.46	1.46±0.26[a]	0.88±0.28	0.74±0.11
2018 [14]					DH+HH	15	1.40±0.54[d]	0.92±0.36	1.31±0.22[b]	0.93±0.38	0.77±0.12
Berge,	*ABCG8*	T400K	rs4148217	Healthy	TT	95	1.62±0.61	1.21±0.45[a]	2.08±0.44	1.48±0.57[a]	0.97±0.25[a]
2002 [9]					TK+KK	48	1.49±0.58	1.07±0.34[b]	2.00±0.31	1.73±0.78[b]	1.07±0.30[b]
Chan,	*ABCG8*	T400K	rs4148217	Obese	TT	37	2.66±1.18[e]	1.33±0.89[c]		2.50±0.86	
2004 [15]					TK	10	2.00±0.33[f]	0.90±0.26[d]		2.33±1.00	
Miwa,	*ABCG8*	T400K	rs4148217	Primary hyper-cholesterolemic	TT	76		0.96±0.34		1.14±0.45	
2005 [10]					TK	24		0.96±0.41		1.31±0.66	
Plat,	*ABCG8*	T400K	rs4148217	Healthy	TT	77	3.24±0.99[g]	1.25±0.46[g]		0.93±0.35	
2005 [11]					TK+KK	35	2.58±0.81[h]	0.92±0.32[h]		1.06±0.28	

Table 1 (continued). Relationships between gene polymorphisms with serum or plasma cholesterol absorption and synthesis markers.

First author, year	Gene*	Polymorphism	rs-number+	Subjects	Genotype	n	Cholesterol absorption markers (µmol/mmol cholesterol)			Cholesterol synthesis markers (µmol/mmol cholesterol)	
							Campesterol	Sitosterol	Cholestanol	Lathosterol	Desmosterol
Gylling,	*ABCG8*	T400K	rs4148217	Mildy to moderately hyper-cholesterolemic	TT	165	3.07±1.16[a]	1.48±0.51[e]			
2008 [12]					TK+KK	92	2.81±1.15[b]	1.31±0.58[f]			
Jiang,	*ABCG8*	T400K	rs4148217	Gallstone / gallstone-free	TT	39	2.45 ± 1.25[c]	3.74 ± 4.25			
2009 [16]					TK+KK	4	0.71 ± 0.20[d]	0.66 ± 0.66			
Berge,	*ABCG8*	Y54C	rs4148211	Healthy	YY	54	1.51±0.57	1.10±0.42	2.01±0.37	1.57±0.60	0.98±0.25
2002 [9]					YC+CC	85	1.60±0.61	1.20±0.42	2.06±0.39	1.57±0.70	1.00±0.28
Miwa,	*ABCG8*	Y54C	rs4148211	Primary hyper-cholesterolemic	CC	67		0.98±0.34		1.12±0.45	
2005 [10]					CY	30		0.95±0.40		1.27±0.57	
					YY	3		0.61±0.11		1.38±0.98	
Jiang,	*ABCG8*	Y54C	rs4148211	Gallstone / gallstone-free	CC	37	2.47± 1.28[a]	3.97 ± 0.73			
2009 [16]					YC+YY	6	1.19 ± 0.73[b]	0.93 ± 0.64			
Berge,	*ABCG8*	A632V	rs6544718	Healthy	AA	94	1.52±0.57	1.16±0.39	2.05±0.41	1.57±0.70	1.01±0.29
2002 [9]					VA+VV	49	1.68±0.63	1.20±0.48	2.04±0.39	1.56±0.57	0.99±0.23
Plat,	*ABCG8*	A632V	rs6544718	Healthy	AA	70	3.06±0.95	1.17±0.47		0.95±0.32	
2005 [11]					VA+VV	42	2.99±1.03	1.11±0.40		1.01±0.36	
Miwa,	*ABCG8*	M429V	rs147194762	Primary hyper-cholesterolemic	MM	92		0.93±0.34a		1.19±0.51	
2005 [10]					MV	8	1.35±0.52b			0.84±0.36	

Table 1 (continued). Relationships between gene polymorphisms with serum or plasma cholesterol absorption and synthesis markers.

First author, year	Gene*	Polymorphism	rs-number+	Subjects	Genotype	n	Cholesterol absorption markers (µmol/mmol cholesterol)			Cholesterol synthesis markers (µmol/mmol cholesterol)	
							Campesterol	Sitosterol	Cholestanol	Lathosterol	Desmosterol
Teupser, 2010 [17]	*ABCG8*		rs41360247	Healthy and	TT		2.70±1.01[g]	1.10±1.01[g]			
					TC		2.40±1.01[h]	0.85±1.02[h]			
					CC		2.30±1.05[i]	0.68±1.11[i]			
Teupser, 2010 [17]	*ABCG8*		rs4245791	Healthy and	TT		2.50±1.01[g]	0.92±1.01[g]			
					TC		2.80±1.01[h]	1.11±1.01[h]			
					CC		3.10±1.01[i]	1.31±1.02[i]			
Teupser, 2010 [17]	*ABO*	rs657152		Healthy and	GG		2.60±1.01[g]	0.99±1.01[g]			
					GT		2.80±1.01[h]	1.06±1.01[h]			
					TT		2.70±1.01[i]	1.04±1.02[i]			
Teupser, 2010 [17]	*ABO*§			Healthy and	O	623	2.71±1.01[e]	0.95±1.02[a]			
					A	777	2.85±1.01[f]	1.00±1.02[b]			
					B	237	2.84±1.02[f]	0.98±1.03[b]			
					AB	102	2.83±1.03[f]	0.97±1.05[b]			
Teupser, 2010 [17]	*ABO*§			Healthy blood	O	301	2.84±1.02[c]	1.25±1.02[c]			
					A	296		1.33±1.02[d]			
					B	111	3.04±1.02[d]	1.32±1.04[d]			
					AB	52		1.32±1.05[d]			
							3.02±1.03[d]				
							3.01±1.04[d]				
Plat, 2002 [18]	*APOA4*	Gln360→His		Healthy	A-IV-1/1	97	3.08±1.01			0.97±0.33	
					A-IV-1/2	15	2.75±0.69			0.99±0.41	
Plat, 2002 [18]	*APOA4*	3.04±1.00		Healthy	A-IV-T/T	72	Thr347→Ser			0.99±0.38	
		3.09±1.02			A-IV-S/T	34				0.96±0.25	
		2.59±0.40			A-IV-S/S	6				0.84±0.23	

Table 1 (continued). Relationships between gene polymorphisms with serum or plasma cholesterol absorption and synthesis markers.

First author, year	Gene*	Polymorphism	rs-number+	Subjects	Genotype	n	Cholesterol absorption markers (µmol/mmol cholesterol)			Cholesterol synthesis markers (µmol/mmol cholesterol)	
							Campesterol	Sitosterol	Cholestanol	Lathosterol	Desmosterol
Uusitupa,	*APOE*§		rs429358	Hyper-cholesterolemic	E3: E3E3	19	2.13±0.65[c]	1.36±0.39[e]	1.22±0.24[a]	1.61±0.55	0.70±0.14[a]
1997 [19]			rs7412		E4: E4E4 + E3E4	16	3.45±1.49[d]	2.02±0.72[f]	1.40±0.25[b]	1.40±0.46	0.62±0.10[b]
Plat,	*APOE*§		rs429358	Healthy	E2: E2E2+E2E3+E2E4	12	2.99±1.27			0.92±0.24	
2002 [18]			rs7412		E3: E3E3	72	3.05±0.94			1.00±0.34	
					E4: E3E4+E4E4	28	3.01±0.97			0.94±0.36	
Lupattelli,	*APOE*§		rs429358	Primary hyperlipidemic	E3: E2E3+E3E3	9+55	1.70±0.30	1.95±0.20[a]		1.90±0.20	
2013 [20]			rs7412		E4: E3E4+E4E4	22+1	1.80±0.30	2.05±0.20[b]		2.01±0.22	
MacKay,	*APOE*§		rs429358	Mildly	E3: E2E3+E3E3	40	2.33±0.99	1.21±0.52	1.63±0.31	1.38±0.55	0.82±0.19
2015 [21]			rs7412	hyper-cholesterolemic	E4: E3E4+E4E4	23	2.05±0.72	1.09±0.43	1.69±0.30	1.40±0.70	0.79±0.19
Plat,	*CETP*	Taq IB		Healthy	01/1	42	2.85±0.86			0.92±0.23	
2002 [18]					01/2	52	3.18±1.10			1.05±0.41	
					02/2	18	3.02±0.85			0.88±0.28	
De Castro-Orós,	*CYP7A1*	_204A > C	rs3808607	No systemic disease	TT§	31	1.91±0.83	1.88±0.83		2.03±0.85	
2011 [22]					GT+GG	36	2.04±0.71	1.85±0.73		1.94±1.36	
MacKay,	*CYP7A1*	_204A > C	rs3808607	Mildly	TT§	20	2.12±0.70	1.14±0.41	1.59±0.28	1.72±0.66[a]	0.86±0.17
2015 [21]				hyper-cholesterolemic	GT	35	2.25±1.06	1.16±0.56	1.69±0.32	1.22±0.54 [b]	0.77±0.16
					GG	8	2.40±0.62	1.23±0.38	1.64±0.31	1.30±0.41[a,b]	0.88±0.29

Table 1 (continued). Relationships between gene polymorphisms with serum or plasma cholesterol absorption and synthesis markers.

First author, year	Gene*	Polymorphism	rs-number+	Subjects	Genotype	n	Cholesterol absorption markers (µmol/mmol cholesterol)			Cholesterol synthesis markers (µmol/mmol cholesterol)	
							Campesterol	Sitosterol	Cholestanol	Lathosterol	Desmosterol
Plat,	*HMGCR*	VNTR		Healthy	10/Oct	35	3.17±0.98			1.01±0.37	
2002 [18]					10/> 10	53	3.11±1.06			0.94±0.30	
					> 10/> 10	24	2.67±0.71			0.99±0.37	
Wolff,	*MTTP*	-493G/T	rs1800591	Moderate and untreated cardiovascular risk factors	GG§	30		1.04±0.68[b]	1.30±0.54[b]	1.53±0.83	1.14±0.38
2011 [23]					GT	30		1.14±0.85[b]	1.36±0.62[b]	1.42±0.56	1.06±0.31
					TT	9		2.61±3.14[a]	1.99±0.90[a]	1.89±0.65	1.09±0.13
Lupattelli,	*NPC1L1*	c.816C. G (L272L)	rs2072183	Primary	CC§	49	1.61±0.30[c]	1.94±0.20[a]		1.97±0.20	
2013 [20]				hyperlipidemic	CG+GG	36+2	1.86±0.30[d]	2.03±0.20[b]		2.00±0.02	
Martin,	*NPC1L1*§	_133A>G	rs17655652 rs41279633	Treatment-naive ADH	AC/AC	20	1.99±0.41	2.04±0.96		1.61±0.77[a]	
2010 [24]		_18C>A			All others	59	2.12±0.27	2.08±1.00		1.30±0.46[b]	
Chan,	*NPC1L1*	1735C-25432A-	rs2072183	Obese	02/Feb	8	1·57±2·60	1.09±0.67		2.63±0.93	
2008 [25]			rs545917974		Non-2/2	8	2.69±1.02	1.11±0.62		2.99±0.90	
Plat,	*SCARB1*	HaeIII		Healthy	01/Jan	25	2.90±0.72			1.15±0.44[a]	
2002 [18]					1/2 + 2/2	87	1.04±3·07			0.92±0.28[b]	
Gerloff,	*SLCO1B1*§	*1a, *1b, *5	rs2306283	Healthy	*1a/*1a	10	2.45±1.15			1.59±0.50	
2006 [26]			rs2306238		*1a/*1b*1b/*1b	10	2.47±0.97			1.74±0.57	
					*1a/*5	8	2.12±0.77			1.33±0.21	
Pasanen,	*SLCO1B1*	c.521T>C	rs4149056	Healthy	c.521TT	16	3.25±1.03	1.66±0.54	1.66±0.19	1.27±0.42	0.94±0.27[a]
2008 [27]					c.521TC	12	3.28±0.99	1.60±0.52	1.66±0.19	1.31±0.41	0.92±0.27[a,b]
					c.521CC	4	2.84±0.69	1.33±0.36	1.57±0.13	1.67±0.28	1.34±0.19[b]

Table 1 (continued). Relationships between gene polymorphisms with serum or plasma cholesterol absorption and synthesis markers.

First author, year	Gene*	Polymorphism	rs-number+	Subjects	Genotype	n	Cholesterol absorption markers (µmol/mmol cholesterol)			Cholesterol synthesis markers (µmol/mmol cholesterol)	
							Campesterol	Sitosterol	Cholestanol	Lathosterol	Desmosterol
Laaksonen,	*SREBF1*	G952G	rs2297508	Moderate hyper-cholesterolemic	GG+GC	81	2.55±1.79			1.05±0.42[a]	
2006 [28]					CC	14	2.09±1.02			1.42±0.79[b]	
Berthold,	*SREBF1*	G952G	rs2297508	Healthy	GG+GC	61	2.04±0.81	1.19±0.43	1.43±0.26	1.37±0.40	0.71±0.14
2008 [29]					CC	11	1.85±0.69	1.40±0.44	1.49±0.37	1.42±0.45	0.73±0.15

Abbreviations: ADH: autosomal dominant hypercholesterolemia, CVD: cardiovascular disease, VNTR: variable number tandem repeat
Values are means ± SD. However, Teupser *et al.* [17] have used geometric means, while values were log-transformed for the study of Lupattelli *et al.* [20].

* Full gene names are given in **Supplementary Table 3**.

+ The rs-number is a unique label to identify a specific single nucleotide polymorphism (SNP).

§ Polymorphism refers to a haplotype.

Significantly different between genotype groups: [a,b] ($P \leq 0.05$), [c,d] ($P \leq 0.01$), [e,f] ($P \leq 0.005$), [g,h,i] ($P \leq 0.001$).

Table 2. Relationships between gene polymorphisms with cholesterol absorption and synthesis as measured with isotopes.

First author, year	Gene*	Polymorphism	rs-number+	Subjects	Genotype	n	Cholesterol absorption	Cholesterol synthesis	
							% cholesterol aborption per day	% of the cholesterol pool synthesized per day	mg/kg per day
Santosa,	*ABCG5*	Q604E	rs6720173	Obese	QQ	19	59.7±18.5[a]	8.5±6.8	
2007 [30]					QE	13	57.2±11.8[b]	13.9±9.1	
					EE	3	86.5±13.3[a,b]	7.9±4.9	
					QE+EE	16	62.6±16.6	12.8±8.6	
	ABCG8	D19H	rs11887534		DD	26	58.8±15.9	10.1±6.7	
					DH	9	67.5±21.2	11.4±11.0	
	ABCG8	T400K	rs4148217		TT	22	64.4±19.4	10.4±8.9	
					TK+KK	13	55.4±12.2	10.4±6.0	
	ABCG8	Y54C	rs4148211		CC	16	64.9±15.4	10.0±8.8	
					CY	12	54.2±13.5	9.0±5.3	
					YY	7	64.1±25.6	14.1±9.1	
					CY+YY	19	57.9±18.8	10.8±7.2	

Table 2 (continued). Relationships between gene polymorphisms with cholesterol absorption and synthesis as measured with isotopes.

First author, year	Gene*	Polymorphism	rs-number+	Subjects	Genotype	n	Cholesterol absorption	Cholesterol synthesis	
							% cholesterol aborption per day	% of the cholesterol pool synthesized per day	mg/kg per day
Gylling,	*APOB*			Healthy	X1X1	13	48.9±10.8		7.5±4.7
1995 [31]					X1X2	31	47.0±9.5		9.7±3.3
					X2X2	8	48.9±15.6		8.8±5.9
	APOE§				E2: E2E2+E2E3+E2E4	8	40.2±7.9[c]		11.1±2.5[a]
					E3: E3E3	28	46.3±7.4[d]		9.0±3.7[b]
					E4: E3E4+E4E4	16	52.7±11.2[d]		8.3±4.0[b]
	LDLR	Pvu II			P-P-	25	46.7±10.0[a]		8.6±4.0[a]
					P-P+	15	50.4±12.8[b]		9.2±4.3[b]
					P+P+	3	57.4±6.1[b]		6.3±0.9[b]

Values are means ± SD.

* Full gene names are given in **Supplementary Table 3**.

+ The rs-number is a unique label to identify a specific single nucleotide polymorphism (SNP).

§ Polymorphism refers to a haplotype.

Significantly different between genotype groups : [a,b] ($P \leq 0.05$), [c,d] ($P \leq 0.01$).

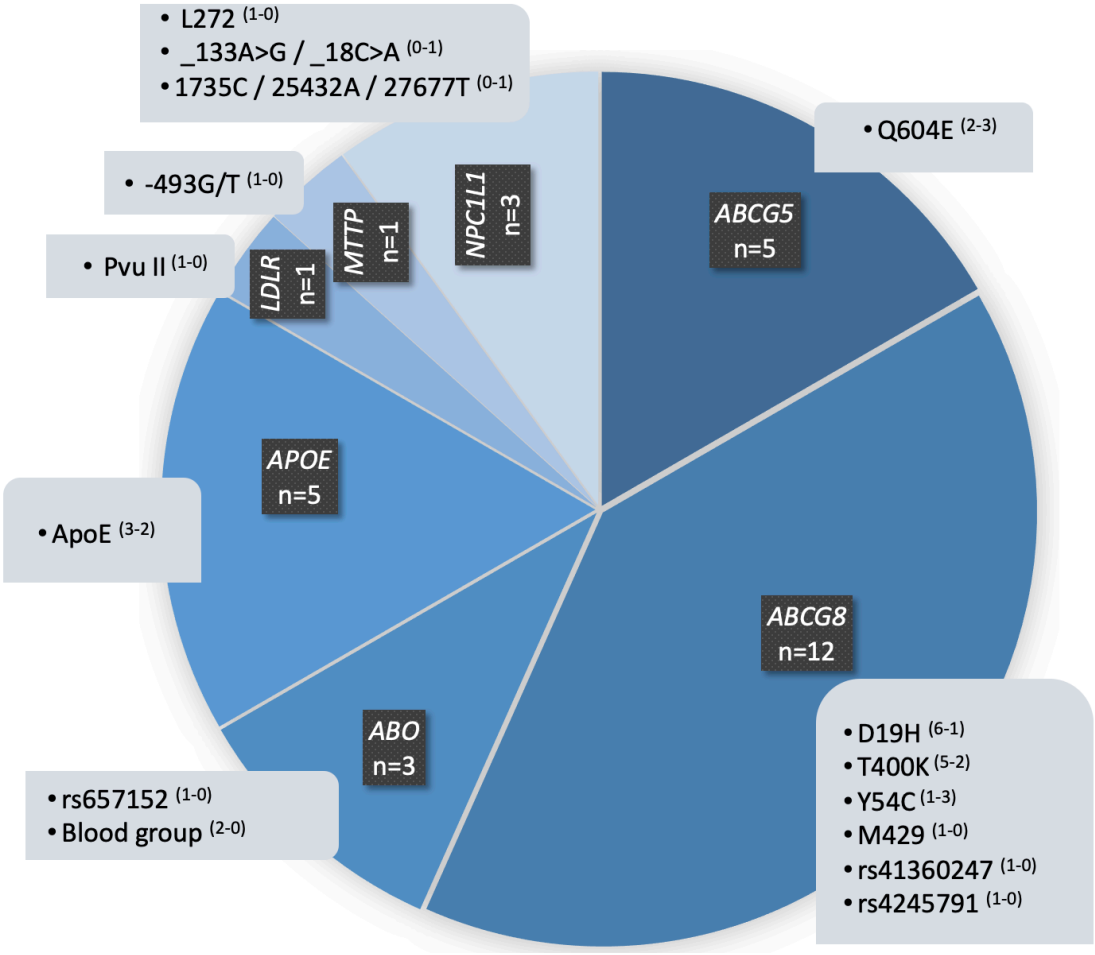

Figure 1. Number of cross-sectional studies that found statistically significant relationships between genetic variation in a particular gene with intestinal cholesterol absorption.
n: indicates the total number of data points included in the review. On the outside of the circle, the polymorphism of the gene is shown. The numbers in superscript refer respectively to the number of studies that observed or did not observe a statistically significant relationship with intestinal cholesterol absorption for a certain single nucleotide polymorphism (SNP) or haplotype. Full gene names are given in **Supplementary Table 3**.

3.1. ABCG8

Important regulators that act as a cholesterol efflux pump are ABCG5 and ABCG8. In transgenic mice, overexpression of the human *ABCG8* gene decreased fractional cholesterol absorption by about 50% and increased biliary cholesterol levels more than five-fold. These effects were associated with a compensatory two to four-fold increase in hepatic cholesterol synthesis and a three- to sixth fold increase in fecal neutral sterol excretion (36). In contrast, deletion of *ABCG8* in a murine model resulted in a significant increase in intestinal sterol

absorption and a decrease in the hepatic secretion of cholesterol (37). This data clearly illustrates the reciprocal character of intestinal cholesterol absorption and endogenous cholesterol synthesis.

3.1.1. D19H (rs11887534)

All four studies yielding 6 data points included in our review (9, 12, 13, 14) showed that carriers of the H-allele (DH or HH) of the *ABCG8* D19H polymorphism had a lower cholesterol absorption as compared with carriers of the wild-type allele (DD), which was not always compensated for by a higher cholesterol synthesis. In the study that measured cholesterol absorption with isotopes, no significant differences between the genotype groups were found (30). No significant differences in serum lipoprotein profiles were evident (9, 12, 13, 14). However, in another study (38), which was not included in this review since cholesterol absorption markers were not measured, concentrations of total cholesterol and triacylglycerol were lower in carriers of the H-allele (DH or HH). To examine underlying mechanisms, Renner *et al.* (13) investigated whether the D19H polymorphism in humans affected the intestinal expression of *ABCG8* in ileal mucosal biopsies. However, no differences in mRNA levels were found. Moreover, ABCG8 protein concentrations were comparable, while in silico modelling suggested that the D19H polymorphism did not affect the 3-dimensional structure of the protein (39). It has, however, been suggested that the reduction in intestinal cholesterol absorption associated with this polymorphism is due to the substitution of histidine (negatively charged) with aspartic acid (positively charged) at amino acid 19, which may alter the transport efficiency of ABCG8 (39).

3.1.2. T400K (rs4148217)

The T400K polymorphism in *ABCG8* was clearly related with intestinal cholesterol absorption. In five studies, absorption was lower in carriers of the minor allele (TK or KK) as compared to wild-type carriers (TT) (9, 11, 12, 15, 16). Two studies found no associations (10, 30), while cholesterol synthesis was only increased in carriers of the minor allele (TK or KK) in the study of Berg *et al.* (9). For serum lipoproteins, plasma triacylglycerol concentrations were significantly lower in carriers of the minor allele (TK) compared with carriers of the major allele (TT) in one study (15), while the opposite was found in another study (11). No associations were found with LDL-C and HDL-C (9, 11, 16, 21). A possible explanation for the lower

cholesterol absorption in carriers of the minor allele (TK or KK) could be due to differences at the post-transcriptional and translational levels. The function of ABCG8 as a sterol transporter depends on the formation of ABCG8/ABCG5 heterodimers and translocation from the Golgi complex to the apical membrane. The change of threonine for lysine at amino acid 400 in exon 8 may enhance the heterodimer functionality, thereby increasing the transport of cholesterol and plant sterols for the (TK+KK) polymorphism from the enterocyte back into the intestinal lumen (11, 40).

3.2. APOE

ApoE plays a central role in fat and cholesterol metabolism and is part of all lipoproteins. Polymorphisms in *APOE* result in three common alleles (E2, E3, and E4), which can result in six different genotypes (E2E2, E2E3, E2E4, E3E3, E3E4, and E4E4). The most frequent isoform is ApoE3 (41). We found two studies in which carriers of at least one E4 allele had a higher intestinal cholesterol absorption as compared to those carrying an E3 allele (19, 20). These subjects also had a lower cholesterol synthesis but higher serum total serum cholesterol concentrations (19). In the study of Plat *et al.* (18), carriers of ApoE2 (E2E2 + E2E3 + E2E4) had lower LDL-C concentrations at baseline as compared with ApoE3 (E3E3) and ApoE4 subjects (E3E4+ E4E4). A possible mechanistic explanation for the cross-sectional associations between ApoE genotype and serum total and LDL-C concentrations is not entirely clear. It can be speculated that the different ApoE isoforms regulate the transport of cholesterol into cells differently. It has been found that the affinity of ApoE2 for lipoprotein receptors is lower than that of E3 and E4, due to the amino acid substitutions of the different ApoE isoforms (42). Furthermore, the clearance of chylomicron remnants is faster in ApoE4 than in ApoE3 individuals. Combined with the increased cholesterol absorption in ApoE4 subjects, these effects may increase the intrahepatic pool of free cholesterol, which downregulates cholesterol synthesis (43). How this translates to differences in intestinal cholesterol absorption is not clear. However, it is most likely that it is not a direct effect of the ApoE polymorphism, but more a consequence of other differences in cholesterol metabolism between the various ApoE genotypes.

3.3. NPC1L1

A second important transporter protein involved in cholesterol absorption is the NPC1L1 protein. NPC1L1 transports sterols from the intestinal lumen into the enterocytes. This protein is the primary target for the drug ezetimibe, which potently inhibits intestinal absorption of biliary and dietary cholesterol without affecting the absorption of triacylglycerols, bile acids, or fat-soluble vitamins (44). Treatment with ezetimibe also reduces the absorption of plant sterols (45), which illustrates that just like for ABCG5/G8 mediated efflux, plant sterols, and cholesterol also share the same influx transporter.

3.3.1. L272L (rs2072183)

Lupatelli and colleagues found an increased cholesterol absorption in patients with primary hyperlipidemia that carried the CG or GG genotype of the L272L polymorphism of *NPC1L1* (20). How the *NPC1L1* L272L polymorphism is functionally related to cholesterol absorption is unknown (20). It is, however, known that this polymorphism results in a synonymous substitution which does not alter the amino acid sequence of the protein but may affect translation rates and protein folding (46).

4. Intestinal cholesterol absorption network

Since one of our aims was to better understand the physiology of intestinal cholesterol absorption, the seven genes of which genetic variation was related to intestinal cholesterol rates (*ABCG5*, *ABCG8*, *ABO, APOE, LDLR, MTPP,* and *NPC1L1*) were used to construct a network for intestinal cholesterol absorption. The network was generated by entering the seven genes of which genetic variation was associated with intestinal cholesterol absorption into GeneMania (33). Fifteen new genes, that were related to these seven identified genes and expressed in the small intestine, were requested to create a network of in total 22 genes**.** These fifteen new genes were: apolipoprotein A1 *(APOA1), APOA4, APOB,* apolipoprotein C2 (*APOC2*), apolipoprotein C3 (*APOC3*), *CETP*, heparan sulphate proteoglycan 2 (*HSPG2*), lecithin-cholesterol acyltransferase *(LCAT*), low density lipoprotein receptor adaptor protein 1 (LDLRAP1), lipase C, hepatic type (LIPC), LDL receptor related protein 1 (LRP1), oxidized low density lipoprotein receptor 1 (*OLR1*), prolyl 4-hydroxylase subunit beta (*P4HB*), secretion associated Ras related GTPase 1B (*SAR1B*), and syndecan 1 (*SDC1*) (**Supplementary Table 4**).

Pathway and co-expression interaction between the genes added the most to the network, while genetic interaction and co-localization contributed the least (**Figure 2**).

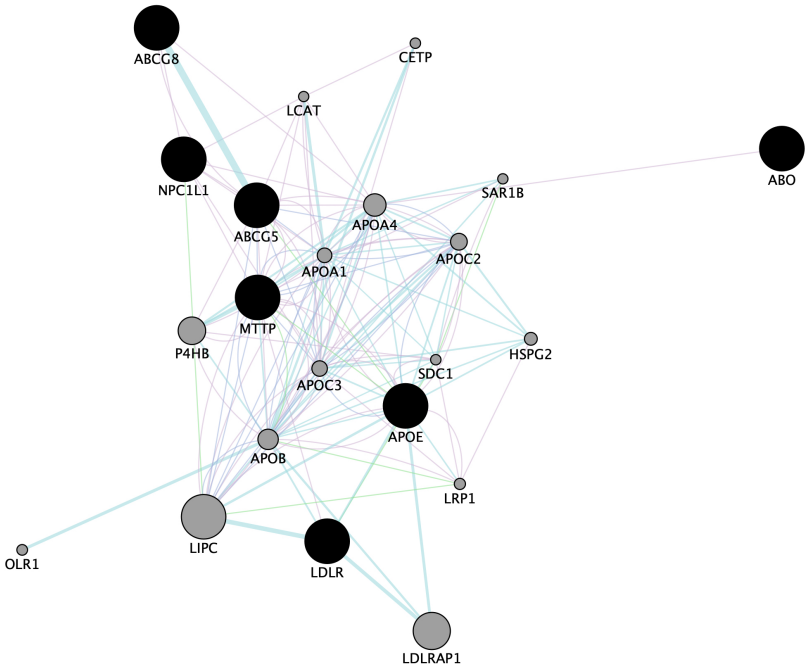

Figure 2. An intestinal cholesterol absorption network.
Seven genes (black circles) were identified by our systematic literature review and fifteen new genes (gray circles) were predicted with GeneMANIA Cytoscape plugin.
The larger the circle size of the new genes, the more likely that the gene is functionally related to the genes in the network. The type of interaction (network category) between genes is illustrated by different colours. Pathway means that two gene products are part of the same reaction; co-expression indicates that expression of the two genes are comparable under different conditions; genetic interaction implies that expression levels of one gene is influenced by the other gene; finally, co-localization means that the genes are expressed in the same tissue or their protein products are found in the same part of a cell. Full gene names are given in **Supplementary Table 4**.

Network category:

Pathway

Co-expression

Genetic Interaction

Co-localization

Some of the suggested fifteen genes were certainly logical candidates, while the role for others in the network is less clear and ambiguous. Lipids and cholesterol that are absorbed by the enterocytes of the small intestine are transported to the endoplasmic reticulum and incorporated into chylomicrons. In this step *ApoB*, *ApoC2*, and *ApoC3* are clearly involved (47, 48). Furthermore, *ApoA4* is also linked to intestinal lipid metabolism, since its expression is upregulated upon intestinal lipid absorption. The role of *LIPC,* which encodes for hepatic lipase (HL) is less evident. It is well known that HL is secreted by the hepatocytes (49) and catalyses the hydrolysis of triacylglycerols and phospholipids present in circulating plasma lipoproteins, including chylomicron remnants (50). Though expressed in the intestine, no role for HL in enterocytes has been described so far. LDLRAP1 interacts with the LDL receptor as it is required for LDLR to be functional, as has been described for hepatocytes (51). Genome-wide association studies (GWAS) studies have shown that a SNP in the *LDLRAP1* gene (rs12027135) is associated with plasma total cholesterol and LDL-cholesterol concentrations (52). Finally, both CETP, which plays a central role in cholesterol and triacylglycerol metabolism in the circulation (53), as well as LCAT, which is crucial for the esterification of cholesterol in plasma (54) are unexpected candidates in the intestinal cholesterol absorption network. Further studies are needed to validate and improve this network.

5. Conclusion

Genetic variants in *ABCG5, ABCG8*, *ABO, APOE*, *LDLR, MTTP,* and *NPC1L1* were identified that were associated with intestinal cholesterol absorption. When interpreting the results, however, it should be realized that the relationship between SNPs with cholesterol metabolism may depend on subject characteristics, such as health status, gender, and ethnicity (55). Therefore, further studies are needed with different populations to better understand the wide inter-individual variability in intestinal cholesterol absorption. Also, GWAS studies may help identifying new genes and genetic variations contributing to the complex intestinal cholesterol absorption network. Also, only a limited number of studies have examined relations of combinations of multiple SNP with cholesterol absorption. Third, intervention studies especially designed to answer the question whether a genetic variant or a combination of variants modifies the response towards an intervention are needed.

The constructed intestinal cholesterol absorption network is complex, and our knowledge of genes involved in this process is not complete. All genes of the created intestinal cholesterol

absorption network should be further explored to find if specific SNPs in these genes that may affect the level of absorbed cholesterol via the small intestine, while also interaction between the genes in the network should be evaluated. Further studies are needed to validate and improve this network, which may ultimately lead to a better understanding of the wide inter-individual variability in intestinal cholesterol absorption and the development of personalized interventions.

Supplemental data

Supplementary Table 1. Characteristics of the studies that measured cholesterol absorption and synthesis using non-cholesterol sterol markers in serum or plasma.

First author, year	Country	Sample size (n)	Male/ Female (%)	Gene*	Polymorphism	Subjects	Genotype	n	Male/ Female (%)	Age (years)+	BMI (kg/m²)+	Cholesterol absorption markers	Cholesterol synthesis markers
Berge,	USA	148	50/50	*ABCG5*	Q604E	Healthy	QQ	91		55±11§		Campesterol Sitosterol Cholestanol	Lathosterol Desmosterol
2002 [9]							QE+EE	51					
				ABCG8	D19H		DD	128					
							DH+HH	14					
				ABCG8	Y54C		YY	54					
							YC+CC	85					
				ABCG8	T400K		TT	95					
							TK+KK	48					
				ABCG8	A632V		AA	94					
							VA+VV	49					
Miwa,	Japan	100	48/52	*ABCG5*	Q604E	Primary hyper-	QQ	78		62.4±12.1§	23.0±3.5§	Sitosterol	Lathosterol
2005 [10]							QE	21					
							EE	1					
				ABCG8	Y54C		CC	67					
							CY	30					
							YY	3					
				ABCG8	T400K		TT	76					
							TK	24					
				ABCG8	M429V		MM	92					
							MV	8					

Supplementary Table 1 (continued). Characteristics of the studies that measured cholesterol absorption and synthesis using non-cholesterol sterol markers in serum or plasma.

First author, year	Country	Sample size (n)	Male/ Female (%)	Gene*	Polymorphism	Subjects	Genotype	n	Male/ Female (%)	Age (years)+	BMI (kg/m²)+	Cholesterol absorption markers	Cholesterol synthesis markers
Plat,	The Netherlands	112	37/63	*ABCG5*	Q604E	Healthy	QQ	81		33±16§	22.9±3.6§	Campesterol Sitostero	Lathosterol
2005 [11]							QE+EE	31					
				ABCG8	T400K		TT	77					
							TK+KK	35					
				ABCG8	A632V		AA	70					
							VV+VA	42					
Gylling,	Finland	282	46/54	*ABCG5*	Q604E	Mildy to moderately hyper-cholesterolemic	QQ	200	46/54§	54.1±8.4§	25.8±3.4§	Campesterol Sitosterol Cholestanol	Lathosterol
2009 [12]							QE+EE	57					
				ABCG8	D19H		DD	216					
							DH+HH	41					
				ABCG8	T400K		TT	165					
							TK+KK	92					

Supplementary Table 1 (continued). Characteristics of the studies that measured cholesterol absorption and synthesis using non-cholesterol sterol markers in serum or plasma.

First author, year	Country	Sample size (n)	Male/ Female (%)	Gene*	Polymorphism	Subjects	Genotype	n	Male/ Female (%)	Age (years)+	BMI (kg/m²)+	Cholesterol absorption markers	Cholesterol synthesis markers
Renner,	Germany	134		*ABCG8*	D19H	Healthy	DD	75		57±12.7§	25.2±3.5§	Campesterol Sitosterol	Lathosterol
2013 [13]							DH	18					
		45				Gallstone carriers	DD	14		61±12.7§	26.6±4.0§		
							DH	11					
Nissinen,	Finland	126	17/83	*ABCG8*	D19H	Gallstone free	DD	102		11.4±4.5§	19.0±3.4§	Campesterol Sitosterol Cholestanol	Lathosterol Desmosterol
2018 [14]							DH+HH	24					
		66	24/76			Gallstone carriers	DD	51		11.8±4.9§	19.2±3.2§		
							DH+HH	15					
Chan,	Australia	47	100/0	ABCG8	T400K	Obese	TT	37	100/0	54±7	32±3	Campesterol Sitosterol	Lathosterol
2004 [15]							TK	10	100/0	55±12.6	32±3.6		

Supplementary Table 1 (continued). Characteristics of the studies that measured cholesterol absorption and synthesis using non-cholesterol sterol markers in serum or plasma.

First author, year	Country	Sample size (n)	Male/ Female (%)	Gene*	Polymorphism	Subjects	Genotype	n	Male/ Female (%)	Age (years)+	BMI (kg/m²)+	Cholesterol absorption markers	Cholesterol synthesis markers
Jiang,	China	43	65/35	*ABCG8*	T400K	Gallstone / gallstone-free	TT	39		Gallstone:	Gallstone:	Campesterol Sitosterol	
2009 [16]							TK+KK	4		54 ± 11.9	21.4 ± 3.1		
										Gall-stone free:	Gall-stone free:		
					Y54C		CC	37		51 ± 8.4	22.3 ± 1.4		
							YC+YY	6					
Teupser,	Germany	4412		*ABCG8*	rs41360247	Healthy and CVD patients	TT			18-74§		Campesterol Sitosterol	
2010 [17]							TC						
							CC						
				ABCG8	rs4245791		TT						
							TC						
							CC						
				ABO	rs657152		GG						
							GT						
							TT						

Supplementary Table 1 (continued). Characteristics of the studies that measured cholesterol absorption and synthesis using non-cholesterol sterol markers in serum or plasma.

First author, year	Country	Sample size (n)	Male/ Female (%)	Gene*	Polymorphism	Subjects	Genotype	n	Male/ Female (%)	Age (years)+	BMI (kg/m²)+	Cholesterol absorption markers	Cholesterol synthesis markers
Teupser, 2010 [17]				*ABO*¥	Blood group (CARLA cohort)		O	623					
							A	777					
							B	237					
							AB	102					
				ABO¥	Blood group (Blood donors)		O	301		18-68§			
							A	296					
							B	111					
							AB	52					
Plat, 2002 [18]	The Netherlands	112	37/63	*APOA4*	Gln360→His	Healthy	A-IV-1/1	97		33±16§	23±2.8§	Campesterol	Lathosterol
							A-IV-1/2	15					
				APOA4	Thr347→Ser		A-IV-T/T	72					
							A-IV-S/T	34					
							A-IV-S/S	6					

Supplementary Table 1 (continued). Characteristics of the studies that measured cholesterol absorption and synthesis using non-cholesterol sterol markers in serum or plasma.

First author, year	Country	Sample size (n)	Male/ Female (%)	Gene*	Polymorphism	Subjects	Genotype	n	Male/ Female (%)	Age (years)+	BMI (kg/m²)+	Cholesterol absorption markers	Cholesterol synthesis markers
Plat, 2002 [18]				*APOE*¥			E2: E2E2+E2E3+E2E4	12					
							E3: E3E3	72					
							E4: E3E4+E4E4	28					
				CETP	Taq IB		1/1	42					
							1/2	52					
							2/2	18					
				HMGCR	VNTR								
							10/10	35					
							10/> 10	53					
							> 10/> 10	24					
				SCARB1	HaeIII		1/1	25					
							1/2 + 2/2	87					

Supplementary Table 1 (continued). Characteristics of the studies that measured cholesterol absorption and synthesis using non-cholesterol sterol markers in serum or plasma.

First author, year	Country	Sample size (n)	Male/ Female (%)	Gene*	Polymorphism	Subjects	Genotype	n	Male/ Female (%)	Age (years)+	BMI (kg/m²)+	Cholesterol absorption markers	Cholesterol synthesis markers
Uusitupa, 1997 [19]	Finland	35		*APOE*¥		Hyper-cholesterolemic	E3: E3E3	19				Campesterol Sitosterol Cholestanol	Lathosterol Desmosterol
							E4: E4E4+E3E4	16					
Lupattelli, 2013 [20]	Italy	87	51/49	*APOE*¥		Primary hyperlipidemic	E3: E2E3+E3E3	9+55		53±12	27±3.7	Campesterol Sitosterol	Lathosterol
							E4: E3E4+E4E4	22+1		41±12.0	25±2.6		
				NPC1L1	c.816C.G (L272)		CC	49		52±14	26±3		
							CG+GG	36+2		48±11	25±4		
MacKay, 2015 [21]	Canada	63		*APOE*¥		Mildly hyper-cholesterolemic	E3: E2E3+E3E3	40	45/55	56.1±7.9	29.1±6.6	Campesterol Sitosterol Cholestanol	Lathosterol Desmosterol
							E4: E3E4+E4E4	23	26/74	54.4±9.6	28.3±5.0		
				CYP7A1			TT	20	40/60	55.0±8.8	31.7±7.8		
							GT	35	34/66	56.1±8.7	27.7±4.8		
							GG	8	50/50	53.8±7.6	26.7±4.3		

Supplementary Table 1 (continued). Characteristics of the studies that measured cholesterol absorption and synthesis using non-cholesterol sterol markers in serum or plasma.

First author, year	Country	Sample size (n)	Male/ Female (%)	Gene*	Polymorphism	Subjects	Genotype	n	Male/ Female (%)	Age (years)+	BMI (kg/m²)+	Cholesterol absorption markers	Cholesterol synthesis markers
De Castro-Orós,	Spain	67	51/49	*CYP7A1*	_204A > C	No systemic disease	TT	31	48/52	45.0±15.2	26.1±3.2	Campesterol Sitosterol	Lathosterol
2011 [22]							GT+GG	36	53/47	39.6±13.5	25.0±3.7		
Wolff,	France	69	0/100	*MTTP*	-493G/T	Moderate and untreated cardiovascular risk	GG	30	0/100	53.3±8.3§	28.1±5.0§	Sitosterol Cholestanol	Lathosterol Desmosterol
2011 [23]							GT	30	0/100				
							TT	9	0/100				
Martin,	Spain	79		*NPC1L1*¥	_133A>G	Treatment-naive ADH	AC/AC	20				Campesterol Sitosterol	Lathosterol
2010 [24]					_18C>A		All others	59					
Chan,	Australia	16	100/0	*NPC1L1*¥	1735C	Obese	2/2	8	100/0	53±7	32±3	Campesterol Sitosterol	Lathosterol
2008 [25]					25432A		Non-2/2	8	100/0	55±7	32±4		
					27677T								

Supplementary Table 1 (continued). Characteristics of the studies that measured cholesterol absorption and synthesis using non-cholesterol sterol markers in serum or plasma.

First author, year	Country	Sample size (n)	Male/ Female (%)	Gene*	Polymorphism	Subjects	Genotype	n	Male/ Female (%)	Age (years)+	BMI (kg/m²)+	Cholesterol absorption markers	Cholesterol synthesis markers
Gerloff	Germany	28	100/0	*SLCO1B1*¥	*1a	Healthy	*1a/*1a	10	100/0	28.3±4.3		Campesterol	Lathosterol
2006 [26]					*1b		*1a/*1b*1b/*1b	10	100/0	32.5±5.5			
					*5		*1a/*5	8	100/0	33.0±5.5			
Pasanen,	Finland	32	56/44	*SLCO1B1*	c.521	Healthy	c.521TT	16	50/50	19-28	25.3-26.9	Campesterol Sitosterol Cholestanol	Lathosterol Desmosterol
2008 [27]							c.521TC	12	58/42	20-34	20.3-27.4		
							c.521CC	4	75/25	21-25	19.3-27.1		
Laaksonen,	Finland	95	84/16	*SREBF1*	G952G	Moderate hyper-cholesterolemic	GG+GC	81		26-69		Campesterol	Lathosterol
2006 [28]							CC	14					
Berthold,	Germany	72	100/0	*SREBF1*	G952G	Healthy	GG+GC	61	100/0	31.7±9.3	25.8±3.3	Campesterol Sitosterol Cholestanol	Lathosterol Desmosterol
2008 [29]							CC	11	100/0	30.7±9.4	25.6±6.0		

Abbreviations: ADH: autosomal dominant hypercholesterolemia, BMI: body mass index, CVD: cardiovascular disease, VNTR: variable number tandem repeat.

* Full gene names are given in **Supplementary Table 3**.

+ Values are means ± SD or ranges.

§ Values refer to all participants.

¥ Polymorphism refers to a haplotype.

Supplementary Table 2. Characteristics of the studies that measured cholesterol absorption and synthesis using isotopes.

First author, year	Country	Sample size (n)	Male/ Female (%)	Gene*	Polymorphism	Subjects	Genotype	n	Male/ Female (%)	Age (years)+	BMI (kg/m²)+	Cholesterol absorption (unit)	Cholesterol synthesis (unit)
Santosa, 2007 [30]	Canada	35	0/100	*ABCG5*	Q604E	Obese	QQ	19	0/100	49.4±6.7§	31.4±2.8§	% of cholesterol per day	The fraction of the cholesterol pool that is synthesized per day (Fractional Synthetic Rate, expressed as %)
							QE	13	0/100				
							EE	3	0/100				
							QE+EE	16	0/100				
				ABCG8	C54Y		CC	16	0/100				
							CY	12	0/100				
							YY	7	0/100				
							CY+YY	19	0/100				
				ABCG8	D19H		DD	26	0/100				
							DH	17	0/100				
				ABCG8	T400K		TT	22	0/100				
							TK+KK	13	0/100				

Supplementary Table 2 (continued). Characteristics of the studies that measured cholesterol absorption and synthesis using isotopes.

First author, year	Country	Sample size (n)	Male/ Female (%)	Gene*	Polymorphism	Subjects	Genotype	n	Male/ Female (%)	Age (years)+	BMI (kg/m²)+	Cholesterol absorption (unit)	Cholesterol synthesis (unit)
Gylling,	Finland	52	100/0	*APOB*		Healthy	X1X1	13	100/0	54.5±7.2§	27.6±1.1	% of cholesterol per dav	mg/kg per day
1995 [31]							X1X2	31	100/0		26.9±0.5		
							X2X2	8	100/0		23.5±1.3		
				APOE¥			E2: E2E2+E2E3+E2E4	8	100/0		27.1±1.2		
							E3: E3E3	28	100/0		27.0±0.6		
							E4: E3E4+E4E4	16	100/0		25.5±0.9		
				LDLR	Pvu II		P-P-	25	100/0		26.0±0.7		
							P-P+	15	100/0		27.8±0.8		
							P+P+	3	100/0		29.4±1.4		

Abbreviations: BMI: body mass index.
* Full gene names are given in **Supplementary Table 3**.
+ Values are means ± SD or ranges.
§ Values refer to all participants.
¥ Polymorphism refers to a haplotype.

Supplementary Table 3. List of genes included in the review with chromosomal location and protein name.

Gene symbol in HGNC	Gene name in HGNC	Chromosome location	Protein recommended name in UniProt
ABCG5	ATP binding cassette subfamily G member 5	2p21	ATP-binding cassette sub-family G member 5
ABCG8	ATP binding cassette subfamily G member 8	2p21	ATP-binding cassette sub-family G member 8
ABO	ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase	9q34.2	Histo-blood group ABO system transferase
APOA4	Apolipoprotein A4	11q23.3	Apolipoprotein A-IV
APOB	Apolipoprotein B	2p24.1	Apolipoprotein B-100
APOE	Apolipoprotein E	19q13.32	Apolipoprotein E
CETP	Cholesteryl ester transfer protein	16q13	Cholesteryl ester transfer protein
CYP7A1	Cytochrome P450 family 7 subfamily A member 1	8q12.1	Cytochrome P450 7A1
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase	5q13.3	3-hydroxy-3-methylglutaryl-coenzyme A reductase

Supplementary Table 3 (continued). List of genes included in the review with chromosomal location and protein name.

Gene symbol in HGNC	Gene name in HGNC	Chromosome location	Protein recommended name in UniProt
LDLR	Low density lipoprotein receptor	19p13.2	Low-density lipoprotein receptor
MTTP	Microsomal triglyceride transfer protein	4q23	Microsomal triglyceride transfer protein large subunit
NPC1L1	NPC1 like intracellular cholesterol transporter 1	7p13	NPC1-like intracellular cholesterol transporter 1
SLCO1B1	Solute carrier organic anion transporter family member 1B1	12p12.1	Solute carrier organic anion transporter family member 1B1
SCARB1	Scavenger receptor class B member 1	12q24.31	Scavenger receptor class B member 1
SREBF1	Sterol regulatory element binding transcription factor 1	17p11.2	Sterol regulatory element-binding protein 1

Abbreviations: HGNC: HUGO Gene Nomenclature Committee, which can be accessed at https://www.genenames.org], UniProt: The Universal Protein, which can be accessed at https://www.uniprot.org.

Supplementary Table 4. Gene symbols and gene names with chromosomal location and protein names of the cholesterol absorption network.

Gene symbol in HGNC	Gene name in HGNC	Chromosome location	Protein recommended name in UniProt
ABCG5	ATP binding cassette subfamily G member 5	2p21	ATP-binding cassette sub-family G member 5
ABCG8	ATP binding cassette subfamily G member 8	2p21	ATP-binding cassette sub-family G member 8
ABO	ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase	9q34.2	Histo-blood group ABO system transferase
APOE	Apolipoprotein E	19q13.32	Apolipoprotein E
LDLR	Low density lipoprotein receptor	19p13.2	Low-density lipoprotein receptor
MTTP	Microsomal triglyceride transfer protein	4q23	Microsomal triglyceride transfer protein large subunit
NPC1L1	NPC1 like intracellular cholesterol transporter 1	7p13	NPC1-like intracellular cholesterol transporter 1
APOA1	Apolipoprotein A1	11q23.3	Apolipoprotein A-I
APOA4	Apolipoprotein A4	11q23.3	Apolipoprotein A-IV
APOB	Apolipoprotein B	2p24.1	Apolipoprotein B-100
APOC2	Apolipoprotein C2	19q13.32	Apolipoprotein C-II
APOC3	Apolipoprotein C3	11q23.3	Apolipoprotein C-III
CETP	Cholesteryl ester transfer protein	16q13	Cholesteryl ester transfer protein
HSPG2	Heparan sulphate proteoglycan 2	1p36.12	Basement membrane-specific heparan sulfate proteoglycan core protein

Supplementary Table 4 (continued). Gene symbols and gene names with chromosomal location and protein names of the cholesterol absorption network.

Gene symbol in HGNC	Gene name in HGNC	Chromosome location	Protein recommended name in UniProt
LCAT	Lecithin-cholesterol acyltransferase	16q22.1	Phosphatidylcholine-sterol acyltransferase
LDLRAP1	Low density lipoprotein receptor adaptor protein 1	1p36.11	Low density lipoprotein receptor adapter protein 1
LIPC	Lipase C, hepatic type	15q21.3	Hepatic triacylglycerol lipase
LRP1	LDL receptor related protein 1	12q13.3	Prolow-density lipoprotein receptor-related protein 1
OLR1	Oxidized low density lipoprotein receptor 1	12p13.2	Oxidized low-density lipoprotein receptor 1
P4HB	Prolyl 4-hydroxylase subunit beta	17q25.3	Protein disulfide-isomerase

Seven genes (*ABCG5*, *ABCG8*, *ABO*, *APOE*, *LDLR*, *MTTP*, and *NPC1L1;* see **Supplementary Table 3**) were identified by the systematic review and were used to construct a network for intestinal cholesterol absorption. Using the GeneMANIA Cytoscape plugin, fifteen new genes were identified (*APOA1*, *APOA4*, *APOB*, *APOC2*, *APOC3*, *CETP*, *HSPG2*, *LCAT*, *LDLRAP1*, *LIPC*, *LRP1*, *OLR1*, *P4HB*, *SAR1B*, and *SDC1*).
Abbreviations: HGNC: HUGO Gene Nomenclature Committee, which can be accessed at https://www.genenames.org], UniProt: The Universal Protein, which can be accessed at https://www.uniprot.org.

List of abbreviations

ABCG5	ATP-binding cassette subfamily G member 5
ABCG8	ATP-binding cassette subfamily G member 8
ABO	ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase
ACAT2	acyl-CoA: cholesterol acyltransferase isoform 2
APOA1	apolipoprotein A1
APOA4	apolipoprotein A4
APOB	apolipoprotein B
ApoB48	apolipoprotein B48
APOC2	apolipoprotein C2
APOC3	apolipoprotein C3
APOE	apolipoprotein E
CETP	cholesteryl ester transfer protein
CHD	coronary heart disease
CYP7A1	cytochrome P450 family 7 subfamily A member 1
GWAS	Genome-wide association studies
HSPG2	heparan sulphate proteoglycan 2
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase
LCAT	lecithin-cholesterol acyltransferase
LDLR	low density lipoprotein receptor
LDLRAP1	low density lipoprotein receptor adaptor protein 1
LIPC	lipase C, hepatic type
LRP1	LDL receptor related protein 1
MTTP	microsomal triglyceride transfer
NCBI	National Center for Biotechnology Information
NPC1L1	NPC1 like intracellular cholesterol transporter 1
OLR1	oxidized low density lipoprotein receptor 1
P4HB	prolyl 4-hydroxylase subunit beta
RFLP	restriction fragment length polymorphism
SAR1B	secretion associated Ras related GTPase 1B
SCARB1	scavenger receptor class B member 1

SD	standard deviation
SDC1	syndecan 1
SLCO1B1	solute carrier organic anion transporter family member 1B1
SNP	single nucleotide polymorphism
SREBF1	sterol regulatory element binding transcription factor 1
VNTR	variable number of tandem repeats

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CHAPTER 3

Associations between SNPs in intestinal cholesterol absorption and endogenous cholesterol synthesis genes with cholesterol metabolism

Maite M. Schroor [a], Fatma B.A. Mokhtar [a], Jogchum Plat and Ronald P. Mensink

[a] These authors contributed equally to this work.

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Abstract

Single nucleotide polymorphisms (SNPs) have been associated with cholesterol metabolism and may partly explain large inter-individual variability in intestinal cholesterol absorption and endogenous cholesterol synthesis rates. This cross-sectional study therefore examined whether SNPs in genes encoding for proteins involved in intestinal cholesterol absorption (*ABCG5, ABCG8,* and *NPC1L1*) and endogenous cholesterol synthesis (*CYP51A1*, *DHCR7*, *DHCR24*, *HMGCR*, *HSD17B7*, *LBR,* and *MSMO1*) were associated with intestinal cholesterol absorption markers (total cholesterol (TC) standardized campesterol and sitosterol levels), an endogenous cholesterol synthesis marker (TC-standardized lathosterol levels), and serum low-density lipoprotein cholesterol (LDL-C) concentrations in a European cohort. *ABCG5* (rs4245786) and the tag SNP *ABCG8* (rs4245791) were significantly associated with serum campesterol and/or sitosterol levels. In contrast, *NPC1L1* (rs217429 and rs217416) were significantly associated with serum lathosterol levels. The tag SNP in *HMGCR* (rs12916) and a SNP in *LBR* (rs12141732) were significantly associated with serum LDL-C concentrations. SNPs in the cholesterol absorption genes were not associated with serum LDL-C concentrations. SNPs in *CYP51A1, DHCR24, HSD17B7,* and *MSMO1* were not associated with the serum non-cholesterol sterols and LDL-C concentrations. Given the variable efficiency of cholesterol-lowering interventions, the identification of SNPs associated with cholesterol metabolism could be a step forward towards personalized approaches.

1. Introduction

Cholesterol homeostasis is determined by the interaction between various complex processes, including intestinal dietary and biliary cholesterol absorption and endogenous cholesterol synthesis (1,2). For the uptake of sterols into the enterocyte, the apical transporter Niemann-Pick C1-Like 1 (NPC1L1) plays a key role (3). After absorption, the sterol efflux pump ATP-binding cassette (ABC) transporters G5 and G8 secrete a fraction of these sterols back into the intestinal lumen, while the remaining part is incorporated into chylomicrons and secreted into the circulation (**Figure S1**) (4). De novo cholesterol synthesis, which involves approximately 30 reactions and more than 20 different enzymes, mainly takes place in the liver (2). Other tissues, however, synthesize cholesterol as well (2). The endogenous cholesterol synthesis pathway starts with acetyl-CoA, which is converted into the intermediate lanosterol in a multistep process. Lanosterol is ultimately converted into cholesterol via either the Bloch or the Kandutsch–Russell pathway (**Figure S2**). The intermediates in these two pathways differ, but the same enzymes are involved (5–8). To estimate fractional intestinal cholesterol absorption, cholesterol-standardized campesterol and sitosterol levels can be used, while those of the Kandutsch–Russell pathway intermediate lathosterol reflect endogenous cholesterol synthesis rates. The use of these markers has been validated by correlating their plasma levels to stable isotope tracer measurements (9).

A reciprocal relation exists between intestinal cholesterol absorption and endogenous cholesterol synthesis (10). For example, statin treatment decreases cholesterol synthesis but increases cholesterol absorption (11), while ezetimibe treatment results in the opposite effects (12). Furthermore, large inter-individual differences are present in relative intestinal cholesterol absorption and endogenous cholesterol synthesis rates. To illustrate, intestinal cholesterol absorption values ranged from approximately 29% to 80% in healthy adults. However, within subject-variability was small (13). For the cholesterol synthesis marker lathosterol, an intra-individual variation of around 23% and an inter-individual variation of more than 50% has been reported for healthy adults (14). Genetic variants, including single-nucleotide polymorphisms (SNPs), might at least partly explain these large inter-individual variations and the wide ranges between individuals in responses to lipid-lowering medications (15). In fact, some SNPs in intestinal cholesterol absorption genes have already

been associated with fractional cholesterol absorption rates (16–19). Additionally, several studies have reported associations between SNPs in genes related to intestinal cholesterol absorption and endogenous cholesterol synthesis with lipid-lowering effects of both pharmacological (20–23) and dietary interventions (24,25). However, whether these associations relate to differences in intestinal cholesterol absorption and endogenous cholesterol synthesis rates has unfortunately not been documented. Identification of SNPs associated with intestinal cholesterol absorption and endogenous cholesterol synthesis is important, as findings may contribute to the development of personalized interventions aimed at improving cholesterol metabolism. The present study therefore investigated in a European population the relation between a number of selected SNPs in genes essential in intestinal cholesterol absorption—*ABCG5*, *ABCG8*, and *NPC1L1*—and SNPs in genes involved in endogenous cholesterol synthesis—*CYP51A1*, *DHCR7*, *DHCR24*, *HMGCR*, *HSD17B7*, *LBR*, and *MSMO1*—with serum intestinal cholesterol absorption markers (total cholesterol (TC) standardized levels of campesterol and sitosterol), an endogenous cholesterol synthesis marker (TC-standardized levels of lathosterol), and LDL-C concentrations.

2. Materials and methods

2.1. Study Population

The present study included participants' baseline data from five human intervention studies (Study 1 to Study 5), performed between 1997 and 2012 at Maastricht University, the Netherlands. All participants were recruited from Maastricht and the surrounding area, and data from N = 456 were available for the present study. Overall, the study sample consisted of healthy adults aged ≥18 years old. The body mass index (BMI) was calculated for each participant by diving their body weight (kg) by the square of height (m). Most participants had a normal weight (N = 225; 49.3%) or were overweight (N = 179; 39.3%). BMI of few participants fell within the underweight (N = 7; 1.5%), obesity class I (N = 28, 6.1%) or obesity class II (N = 6; 1.3%) range (26). None of the participants used medication known to affect lipid metabolism. Details of the studies have been published (27–30), except for Study 4, which was a 6-week randomized, double-blinded, placebo-controlled parallel trial evaluating effects of plant-sterol ester supplementation as part of a combined lifestyle intervention. For the analysis of this project, we only used samples that were collected at baseline or at the end of a control period. All studies were approved by the Medical Ethics Committee of

Maastricht University and were conducted according to the principles laid down in the Declaration of Helsinki. Written informed consent was obtained from all participants.

2.2. Blood Sampling and Biochemical Measurements

Blood samples were drawn from participants after an overnight fast. At least one hour after venipuncture, serum was obtained by centrifugation at 2000× *g* for 15–30 min at 4 °C and aliquots were stored at −80 °C. The concentrations of TC (CHOD/PAP method; Roche Diagnostics Systems Hoffmann-La Roche Ltd., Basel, Switzerland), high-density lipoprotein cholesterol (HDL-C) (precipitation method by adding phosphotungstic acid and magnesium ions, and CHOD/PAP method; Roche Diagnostics Systems Hoffmann-La Roche Ltd., Basel, Switzerland) and *triacylglycerol* (TAG) corrected for free glycerol (GPO-Trinder; Sigma Diagnostics, St Louis, USA) were determined in serum by using enzyme-based methods. LDL-C concentrations were calculated using the Friedewald equation (31).

Serum concentrations of the intestinal cholesterol absorption markers campesterol and sitosterol, and the endogenous cholesterol synthesis marker lathosterol were analyzed using gas chromatography with flame-ionization detection (GC-FID) in Study 1 and Study 5, while GC-mass spectrometry (GC-MS) was used in the three other studies. Further details on the non-cholesterol sterol analysis have been presented in the article by Mackay et al. (32). Campesterol, sitosterol, and lathosterol concentrations are transported in plasma by cholesterol-rich lipoproteins, and therefore their concentrations were corrected for the differing number of lipoprotein particles by standardizing the concentrations of the markers to the TC concentrations (10^2 × µmol/mmol TC) as measured with the CHOD/PAP method.

2.3. DNA Extraction, Genotyping, and Quality Control

Genomic DNA was isolated from either full blood or buffy coats using the QIAamp genomic DNA isolation kit (Westburg BV, Leusden, the Netherlands) according to the instructions of the manufacturer. After isolation, the purity of the genomic DNA was checked by measuring the 260/280 nm and the 260/230 nm ratios (NanoDrop; ND-1000 spectrophotometer, Isogen Lifescience B.V., De Meern, The Netherlands). For all samples, ratios varied between 1.7 and 1.9 and around 2.0, respectively. DNA concentrations were calculated using the relationship that an A_{260} of 1.0 corresponds with 50 µg/mL DNA. All samples were stored at -80 °C after isolation. After thawing, the quality of about 5% of the samples was tested by evaluating the

degradation of DNA on agarose gels before further analysis. Results indicated that the quality of these samples was sufficient for genotyping. In the end, 471 DNA samples were genotyped by using the Axiom^{TM} Precision Medicine Research Array (PMRA) Kit (Thermo Fisher Scientific, Waltham, MA, USA) (33).

After running the arrays, the software package PLINK (version 1.90 beta; www.cog-genomics.org/plink/1.9/) (34) was used to exclude SNPs: (1) with >2% missing data, (2) located on sex chromosomes, (3) with a minor allele frequency (MAF) < 0.05, or (4) that deviated from Hardy–Weinberg Equilibrium (HWE) based on a p-value < 1×10^{-10}. Six subjects were removed because they had a heterozygosity rate ± 3 standard deviations (SDs) from the mean heterozygosity rate. Nine subjects were excluded because there was a sex discrepancy between DNA results with clinical records. Ultimately, 456 samples and 306,898 SNPs passed the quality-control criteria. Only SNPs in genes with a clear role in intestinal cholesterol absorption (*ABCG5, ABCG8,* and *NPC1L1*) or endogenous cholesterol synthesis (*CYP51A1, DHCR7, DHCR24, HMGCR, HSD17B7, LBR*, and *MSMO1*) that were present on the array and had passed the quality control steps were included in this study. An overview of the full gene names is provided in **Table S1**. The rs-numbers of the selected SNPs are presented, except for two SNPs in *ABCG8* for which the rs-numbers were unknown. For these SNPs, their Affymetrix SNP ID (AX-number), i.e., their unique probe set identifier, is given. **Table S2** presents information about these two SNPs that were provided by the PMRA array.

2.4. Statistics

Continuous values are reported as mean ± SD and categorical values as N (%). Visual inspection of histograms and Q-Q plots of the residuals showed a skewed distribution for TAG and concentrations were therefore log-transformed. Analysis of variance (ANOVA) was used to examine whether continuous variables differed significantly between the five studies. A chi-square test was used for categorical variables.

Possible deviations of the genotype frequencies from those expected under Hardy–Weinberg equilibrium (HWE) were assessed using chi-square tests in Microsoft Excel. Thereafter, SNPs with a genotype group with a frequency of <12 participants, which equals <2.5% of the sample size, were moved to the supplements. All SNPs in *DHCR7* were moved to the supplements due to this reason. Only for SNPs with a genotype group with a frequency of >12 participants, linkage disequilibrium (LD) was estimated and reported as r^2-values for pairs

of SNPs < 500 kB apart using the Haploview software package (version 4.1, Broad Institute of MIT and Harvard, Cambridge, MA, USA) (35). A threshold of $r^2 \geq 0.8$ was used to define SNPs in LD. Haplotype blocks were constructed in Haploview by using the default algorithm as defined by Gabriel et al. (36). In short, blocks were generated by this algorithm when at least 95% of the informative SNPs were in strong LD (36). Furthermore, the Tagger program in Haploview version 4.1 was used to select tag SNPs using the pairwise tagging approach (35). Selection criteria were a r^2 threshold ≥ 0.8 and a log of the likelihood odds ratio (LOD) threshold of 3.0. Results of the statistical analysis of the tag SNPs are presented in the main text, whereas results for the captured SNPs have been placed in the supplemental information.

Linear regression analyses, corrected for the factor study, were used to examine associations among the TC-standardized non-cholesterol sterols and LDL-C concentrations. Additionally, the general linear model (GLM) was used to examine associations between the SNPs with serum non-cholesterol sterol levels, and LDL-C and TC concentrations. The analyses were adjusted for the factor study. In case of a statistically significant effect of a SNP, the differences in TC-standardized non-cholesterol sterol levels, serum LDL-C concentrations, or serum TC concentrations between the genotype groups were compared with a Bonferroni post-hoc test. The Benjamini–Hochberg multiple testing correction with a false discovery rate of 0.2 was applied to the GLM results for each gene separately. Only SNPs with genotype groups consisting of at least 12 individuals were included in the Benjamini–Hochberg correction. If the original p-value obtained from the general linear model analysis was smaller than the Benjamini–Hochberg critical value, the p-value was considered statistically significant. Next, for SNPs that were significantly associated with TC-standardized non-cholesterol sterols or LDL-C concentrations, an additive, dominant, or recessive multiple linear regression model was built with adjustment for the factor study. The additive model was used when the Bonferroni post-hoc test indicated that all three genotypes were significantly different or when the post-hoc test did not show which genotypes differed significantly. A dominant or recessive model was used when the Bonferroni post-hoc indicated a significant difference between only two genotypes. A dominant model was used if the least frequent homozygous genotype (e.g., aa) and the heterozygous genotype (e.g., aA) had a comparable relation with the outcome (i.e., the non-cholesterol sterols or LDL-C). The dominant model used the major homozygous group as reference, hence, AA was

compared with aa + aA. Moreover, a recessive model was used if the least frequent homozygous genotype and the heterozygous genotype did not have a comparable relation with the outcome. The recessive model thus compared AA + aA with aa. All analyses were carried out using SPSS for Mac OS X (version 26.0, SPSS Inc., Chicago, IL, USA).

3. Results

Baseline characteristics for all participants and the five studies separately are shown in **Table S3**. Significant differences between the studies were reported for all characteristics of the participants (all $p < 0.05$), except for gender ($p = 0.064$).

3.1. Associations between Markers for Cholesterol Absorption and Cholesterol Synthesis, and Serum LDL-C Concentrations

Linear regression analyses showed that, after controlling for the factor study, sitosterol was positively associated with campesterol ($\beta = 1.39 \times 10^2$ µmol/mmol TC; $p < 0.001$) and inversely with lathosterol ($\beta = -0.09 \times 10^2$ µmol/mmol TC; p-value = 0.025). In addition, campesterol showed a significant inverse association with lathosterol ($\beta = -0.10 \times 10^2$ µmol/mmol TC; p-value < 0.001). Campesterol, sitosterol, and lathosterol were not significantly associated with serum LDL-C concentrations (all $p > 0.05$) (**Table S4**).

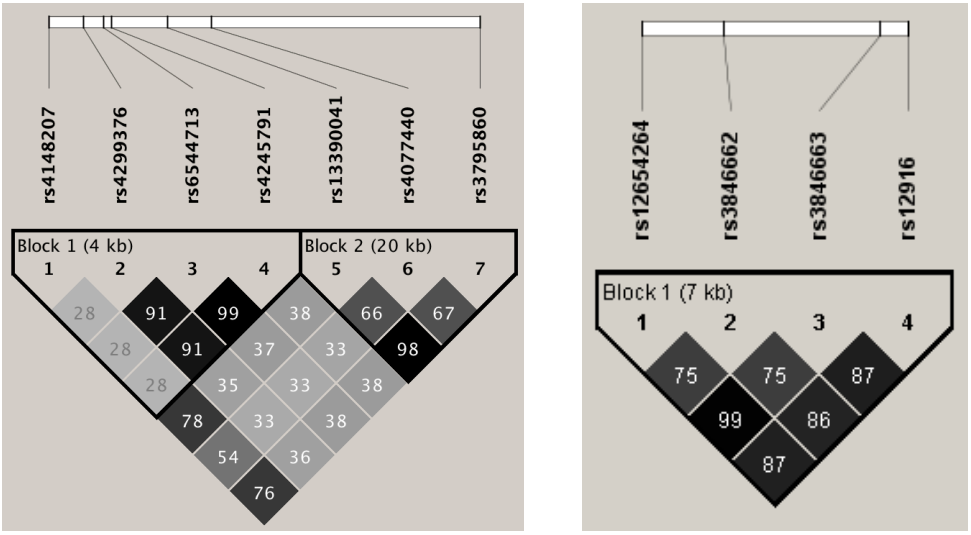
3.2. The Location and Allele Frequencies of the Selected SNPs

Table S5 shows the location and allele frequencies of the selected SNPs. The majority of SNPs were located in an intron and all SNPs had a call rate of ≥98.2%. The reference and alternative allele frequencies of the SNPs in our cohort were comparable to those of the European population, which were obtained from the National Center for Biotechnology Information (NCBI) (37). Five of the 12 selected SNPs in the *ABCG8* gene (AX_11180448, rs41360247, rs4245791, rs4299376, rs6544713) deviated significantly from HWE ($p < 0.05$). All other SNPs were in HWE (all $p > 0.05$).

3.3. Linkage Disequilibrium and Tagging for SNPs in Genes Related to Intestinal Cholesterol Absorption

SNPs in *ABCG8* (rs4299376, rs6544713, and rs4245791) were in high LD (all $r^2 > 0.90$) and consequently included in a haplotype block (**Figure 1a**). Haplotype block 2 included *ABCG8* (rs13390041, rs4077440, and rs3795860). Of these SNPs, rs13390041 and rs3795860 showed

a high LD (r^2 = 0.98). The tag SNP *ABCG8* (rs4245791) captured rs6544713 and rs4299376, while tag SNP *ABCG8* (rs3795860) captured rs13390041 (**Table 1**). For SNPs in *ABCG5* (**Figure S3a**) and *NPC1L1* (**Figure S3b**), no high LD was found (all r^2 < 0.70).



(**a**) (**b**)

Figure 1. Pairwise LD among (**a**) 7 SNPs in *ABCG8* and (**b**) 4 SNPs in *HMGCR* is indicated in the diamond shapes. The triangles mark the two haplotype blocks within this region (based on the confidence interval of D'). The shading with a dark grey to white gradient indicates the level of higher to lower LD between each pair of SNPs based on the r^2-value. The LD plot was created by Haploview version 4.1 (35).

Table 1. Tag SNPs and their captured SNPs with their corresponding r^2-values.

Gene	Tag SNP	Captured SNP	R^2-Value
ABCG8	rs4245791	rs6544713	0.995
	rs4245791	rs4299376	0.919
	rs3795860	rs13390041	0.982
DHCR24	rs6676774	rs7551288	0.906
HMGCR	rs12916	rs12654264	0.872
	rs12916	rs3846662	0.862
	rs12916	rs3846663	0.879

TagSNPs and their captured SNPs were selected using the Tagger program within Haploview version 4.1. (35).

3.4. Linkage Disequilibrium and Tagging for SNPs in Genes Related to Endogenous Cholesterol Synthesis

All SNPs in *HMGCR* were in (borderline) LD (all $r^2 \geq 0.75$) and consequently all SNPs were included in one single haplotype block (**Figure 1b**). One tag SNP in *HMGCR* was selected (rs12916), which captured rs12654264, rs3846662, and rs3846663 (**Table 1**). For *DHCR24*, rs6676774 and rs7551288 were in high LD ($r^2 = 0.90$) and *DHCR24* (rs6676774) was selected as a tag SNP for rs7551288 (**Figure S4c**; **Table 1**). None of the other SNPs in *DHCR24,* as well as the SNPs in *LBR,*-were in pairwise LD (all $r^2 < 0.80$) (**Figure S4**).

3.5. Associations between SNPs in ABCG5, ABCG8, and NPC1L1 with TC-Standardized Serum Non-Cholesterol Sterol Levels and Serum LDL-C Concentrations

Significant associations were found for a SNP in *ABCG8* (rs4245791; $p < 0.001$) with both TC-standardized serum campesterol and TC-standardized serum sitosterol levels. *ABCG5* (rs4245786) was also significantly associated with TC-standardized sitosterol levels ($p = 0.041$). In addition, two SNPs in *NPC1L1* (rs217429 and rs217416) were significantly related with TC-standardized serum lathosterol levels ($p < 0.05$) (**Table 2**). After Benjamini–Hochberg multiple testing correction, all associations remained significant. Results for SNPs with a genotype group <12 participants are presented in **Table S6**. A recessive model was built for *NPC1L1* (rs217429 and rs217416) with lathosterol levels (**Figure S5**). The additive models for *ABCG5* (rs4245786) with sitosterol, and for *ABCG8* (rs4245791) with sitosterol and campesterol levels can be found in **Table S7**. No significant associations were observed between SNPs in *ABCG5*, *ABCG8,* or *NPC1L1* with serum LDL-C concentrations (all $p > 0.05$) (**Table 2**) or TC concentrations (all $p > 0.05$) (**Table S8**).

3.6. Associations between SNPs in CYP51A1, DHCR24, HMGCR, HSD17B7, LBR, and MSMO1 with TC-Standardized Serum Non-Cholesterol Sterol Levels and Serum LDL-C Concentrations

None of the SNPs in genes essential in endogenous cholesterol synthesis showed a significant association with TC-standardized campesterol, sitosterol or lathosterol serum levels (all $p > 0.05$). Significant associations were reported for *HMGCR* (rs12916) and *LBR* (rs12141732) with serum LDL-C concentrations (all p-value < 0.05) (**Table 3**). Dominant models for these SNPs can be found in **Figure S6**. SNPs in *CYP51A1, DHCR24, HSD17B7,* and *MSMO1* were not significantly associated with serum LDL-C concentrations (all $p > 0.05$). **Table S9** presents

associations for SNPs with a genotype group <12 participants. Results for serum TC concentrations (**Table S10**) are comparable to these of serum LDL-C concentrations (**Table 3**).

Table 2. Associations between various SNPs in cholesterol absorption genes with serum TC-standardized campesterol, sitosterol and lathosterol levels (N = 455), and serum LDL-C concentrations (N = 456).

Gene	SNP	Genotype	N	Campesterol 10^2×*µmol/mmol TC	P-value	Sitosterol 10^2×µmol/mmol TC	P-value	Lathosterol 10^2×µmol/mmol TC	P-value	N	LDL-C mmol/L	P-value
				Mean (95% CI)		Mean (95% CI)		Mean (95% CI)			Mean (95% CI)	
ABCG5	rs4245786	AA	266	252 (236–267)	0.074	152 (142–162)	0.041[$]	120 (112–129)	0.959	266	3.44 (3.30–3.57)	0.306
		AG	160	230 (212–249)		136 (124–148)		120 (110–131)		161	3.34 (3.18–3.50)	
		GG	29	259 (222–296)		154 (130–178)		123 (103–144)		29	3.23 (2.90–3.55)	
	rs7599296	AA	15	261 (210–312)	0.228	164 (131–197)	0.173	109 (81–137)	0.653	15	3.40 (2.95–3.85)	0.98
		AG	141	255 (236–274)		152 (140–165)		119 (108–130)		141	3.38 (3.21–3.54)	
		GG	299	239 (224–254)		143 (133–152)		122 (113–130)		300	3.39 (3.26–3.52)	
	rs4148184	TT	74	232 (207–256)	0.297	142 (126–158)	0.803	117 (103–130)	0.217	74	3.30 (3.08–3.51)	0.561
		TC	219	251 (235–268)		148 (137–159)		117 (108–126)		219	3.42 (3.28–2.57)	
		CC	161	242 (223–260)		146 (134–158)		126 (116–137)		162	3.39 (3.23–3.55)	
	rs13396273	TT	53	236 (207–264)	0.431	144 (126–163)	0.819	116 (101–132)	0.526	53	3.36 (3.11–3.60)	0.922
		TC	214	251 (234–267)		148 (138–159)		119 (109–128)		214	3.40 (3.26–3.55)	
		CC	188	240 (222–257)		145 (133–156)		124 (114–134)		189	3.38 (3.22–3.53)	
ABCG8	rs4148207	TT	156	249 (231–268)	0.757	151 (139–163)	0.364	121 (111–131)	0.713	157	3.34 (3.18–3.50)	0.53
		TC	227	243 (226–259)		145 (123–155)		121 (112–130)		227	3.43 (3.29–3.58)	
		CC	72	241 (216–266)		139 (123–155)		116 (102–129)		72	3.35 (3.13–3.57)	
	rs3795860[+]	TT	128	253 (234–273)		154 (141–167)		120 (109–131)		129	3.32 (3.15–3.50)	
		TC	233	244 (228–260)	0.342	146 (135–156)	0.174	123 (114–131)	0.515	233	3.46 (3.32–3.60)	0.175
		CC	94	234 (211–257)		138 (123–152)		115 (102–127)		94	3.29 (3.09–3.49)	
	rs4077440	TT	92	256 (233–279)	0.129	154 (140–169)	0.125	120 (107–132)	0.378	92	3.38 (3.18–3.58)	0.252
		TC	217	249 (232–266)		149 (138–159)		124 (115–133)		218	3.45 (3.31–1.60)	
		CC	145	232 (213–251)		138 (126–150)		116 (105–126)		145	3.30 (3.13–3.46)	
	AX_82902928	--	197	248 (231–265)	0.752	151 (140–161)	0.334	120 (111–130)	0.955	197	3.40 (3.25–3.55)	0.145
		-AC	192	240 (223–258)		141 (130–165)		120 (110–130)		193	3.43 (3.28–3.58)	
		ACAC	66	246 (219–272)		147 (130–165)		122 (108–137)		66	3.19 (2.60–3.42)	
	rs4245791[+]	TT	206	221 (205–237)[A]		130 (120–141)[A]		123 (114–132)		206	3.32 (3.17–3.47)	
		TC	215	256 (239–272)[B]	<0.001[$]	153 (143–164)[B]	<0.001[$]	119 (109–128)	0.642	216	3.46 (3.31–3.61)	0.239
		CC	34	315 (282–349)[C]		180 (176–219)[C]		117 (97–136)		34	3.34 (3.04–3.65)	

Table 2 (continued). Associations between various SNPs in cholesterol absorption genes with serum TC-standardized campesterol, sitosterol and lathosterol levels (N = 455), and serum LDL-C concentrations (N = 456).

Gene	SNP	Genotype	N	Campesterol 10^2×*µmol/mmol TC	P-value	Sitosterol 10^2×µmol/mmol TC	P-value	Lathosterol 10^2×µmol/mmol TC	P-value	N	LDL-C mmol/L	P-value
				Mean (95% CI)		Mean (95% CI)		Mean (95% CI)			Mean (95% CI)	
NPC1L1	rs217429	AA	259	239 (223–254)	0.19	142 (132–152)	0.134	119 (110–128)[A]	0.017[#]	259	3.37 (3.23–3.50)	0.825
		AC	169	256 (238–275)		154 (142–166)		117 (107–127)[A]		170	3.42 (3.26–3.58)	
		CC	27	238 (200–276)		146 (121–170)		149 (128–170)[B]		27	3.39 (3.06–3.73)	
	rs217416	TT	239	240 (223–256)	0.208	143 (132–153)	0.236	119 (110–127)[A]	0.020[#]	239	3.40 (3.26–3.54)	0.922
		TC	189	254 (237–272)		153 (141–164)		118 (108–128)[A]		190	3.38 (3.23–3.54)	
		CC	25	228 (188–267)		140 (114–165)		149 (128–171)[B]		25	3.33 (2.98–3.67)	
	rs11763759	TT	208	244 (227–261)	0.961	145 (134–156)	0.938	120 (111–130)	0.953	209	3.42 (3.27–3.56)	0.084
		TC	202	246 (229–263)		147 (136–158)		120 (111–129)		202	3.31 (3.16–3.46)	
		CC	43	242 (211–273)		149 (128–169)		123 (106–140)		43	3.62 (3.35–3.89)	
	rs2072183	CC	18	260 (213–307)	0.314	154 (123–184)	0.361	121 (95–147)	0.862	18	3.33 (2.91–3.75)	0.93
		CG	173	254 (235–272)		152 (140–164)		122 (112–133)		174	3.40 (3.24–3.57)	
		GG	263	240 (225–255)		143 (134–153)		119 (111–128)		263	3.38 (3.25–3.52)	

Abbreviations: LDL-C = low-density lipoprotein cholesterol; N/A = not applicable; SNP = single-nucleotide polymorphism; TC = total cholesterol. Note: All analyses were adjusted for the factor study. Data are presented as estimated marginal means (95% CI). Non-cholesterol sterol levels were missing for N = 1. Different letters between genotypes within a SNP indicate significantly different non-cholesterol sterol levels between the genotypes based on a Bonferroni post-hoc test. Significant *p*-values remained significant after adjustment for multiple testing by calculating ~~q~~–critical values for each p-value using the Benjamini–Hochberg principle. [+] Indicates a tag SNP. [#] Recessive models are presented in the supplemental material (**Figure S5**). [$] Additive models are presented in the supplemental material (**Table S7**).

Table 3. Associations between various SNPs in endogenous cholesterol synthesis genes with serum TC-standardized campesterol, sitosterol and lathosterol levels (N = 455), and serum LDL-C concentrations (N = 456).

Gene	SNP	Genotype	N	Campesterol 10^2×*µmol/mmol TC	P-value	Sitosterol 10^2×µmol/mmol TC	P-value	Lathosterol 10^2×µmol/mmol TC	P-value	N	LDL-C mmol/L	P-value
				Mean (95% CI)		Mean (95% CI)		Mean (95% CI)			Mean (95% CI)	
CYP51A1	rs35968894	AA	161	240 (222–258)	0.239	142 (131–154)	0.334	115 (104–124)	0.066	161	3.40 (3.24–3.56)	0.976
		AG	223	241 (224–258)		146 (135–157)		127 (118–136)		224	3.38 (3.23–3.53)	
		GG	71	262 (238–287)		156 (140–172)		117 (103–131)		71	3.39 (3.17–3.60)	
DHCR24	rs6676774[+]	AA	75	231 (207–256)	0.436	144 (128–160)	0.887	120 (106–134)	0.535	75	3.42 (3.20–3.63)	
		AG	208	246 (230–263)		146 (135–157)		123 (114–132)		208	3.30 (3.16–3.45)	0.122
		GG	172	249 (230–267)		148 (136–160)		117 (107–127)		173	3.48 (3.33–3.64)	
	rs718265	AA	43	231 (200–263)	0.292	143 (123–164)	0.794	117 (98–134)	0.57	43	3.35 (3.07–3.62)	0.46
		AG	190	252 (235–269)		149 (138–160)		123 (114–133)		190	3.34 (3.19–3.49)	
		GG	222	240 (223–257)		145 (134–156)		118 (109–127)		223	3.44 (3.29–3.59)	
HMGCR	rs12916[+]	TT	151	240 (221–260)	0.373	145 (133–158)	0.541	122 (112–133)	0.838	152	3.22 (3.05–3.39)[A]	0.011[@]
		TC	231	242 (226–259)		145 (134–155)		119 (110–128)		231	3.49 (3.35–3.63)[B]	
		CC	73	259 (234–284)		154 (138–170)		122 (108–135)		73	3.35 (3.13–3.56)	
HSD17B7	rs77482353	AA	156	241 (222–259)	0.676	142 (130–154)	0.516	121 (111–131)	0.889	156	3.40 (3.24–3.56)	0.07
		AG	227	250 (233–266)		150 (139–160)		120 (111–130)		228	3.32 (3.18–3.47)	
		GG	68	246 (220–272)		150 (133–167)		117 (103–132)		68	3.60 (3.72–3.83)	
		CC	34	251 (216–286)		159 (136–182)		114 (95–134)		34	3.50 (3.20–3.81)	

Table 3 (continued). Associations between various SNPs in endogenous cholesterol synthesis genes with serum TC-standardized campesterol, sitosterol and lathosterol levels (N = 455), and serum LDL-C concentrations (N = 456).

Gene	SNP	Genotype	N	Campesterol 10^2×*µmol/mmol TC	P-value	Sitosterol 10^2×µmol/mmol TC	P-value	Lathosterol 10^2×µmol/mmol TC	P-value	N	LDL-C mmol/L	P-value
				Mean (95% CI)		Mean (95% CI)		Mean (95% CI)			Mean (95% CI)	
LBR	rs6678087	TT	141	247 (228–267)	0.367	147 (134–160)	0.988	120 (109–131)	0.997	141	3.41 (3.24–3.58)	0.97
		TC	223	248 (232–265)		147 (136–157)		121 (112–130)		223	3.39 (2.25–3.53)	
		CC	90	232 (209–254)		146 (131–161)		120 (108–133)		91	3.39 (3.19–3.59)	
	rs12141732	TT	226	241 (224–258)	0.706	144 (133–155)	0.453	121 (111–130)	0.799	227	3.50 (3.35–3.65)[A]	0.027[@]
		TC	194	248 (232–265)		147 (136–158)		121 (112–130)		194	3.28 (3.13–3.43)[B]	
		CC	34	251 (216–286)		159 (136–182)		114 (95–134)		34	3.50 (3.20–3.81)	
MSMO1	rs17046216	AA	53	237 (209–266)	0.112	147 (128–165)	0.347	113 (97–128)	0.542	53	3.63 (3.38–3.88)	0.101
		AG	205	236 (219–253)		142 (131–153)		121 (112–131)		206	3.35 (3.21–3.50)	
		GG	197	256 (239–273)		151 (140–162)		122 (112–131)		197	3.36 (3.21–3.51)	

Abbreviations: LDL-C = low-density lipoprotein cholesterol; N/A = not applicable; SNP = single-nucleotide polymorphism; TC = total cholesterol. Note: All analyses were adjusted for the factor study. Data are presented as estimated marginal means (95% CI). Non-cholesterol sterol levels were missing for N = 1. Different letters between genotypes within a SNP indicate significantly different LDL-C concentrations between the genotypes based on a Bonferroni post-hoc test. Significant *p*-values remained significant after adjustment for multiple testing by calculating ~~q~~-critical values for each p-value using the Benjamini–Hochberg principle. [+] Indicates a tag SNP. [@] Dominant models are presented in the supplemental material (**Figure S6**).

4. Discussion

Large inter-individual variation in intestinal cholesterol absorption and endogenous cholesterol synthesis exists, which may relate to differences in genetic background. Indeed, we found that SNPs in *ABCG5* and *ABCG8* were associated with intestinal cholesterol absorption, while SNPs in *NPC1L1* were significantly associated with endogenous cholesterol synthesis. However, none of the SNPs that were associated with intestinal cholesterol absorption or endogenous synthesis were associated with serum LDL-C concentrations, whereas SNPs in *HMGCR* and *LBR* did show such a relation. No associations were found for SNPs in *CYP51A1*, *DHCR24*, *HSD17B7*, and *MSMO1* with either one of the evaluated parameters.

ABCG5 (rs4245786) was significantly related with TC-standardized serum sitosterol levels, a marker for intestinal cholesterol absorption. To the best of our knowledge, this association has not been reported before. *ABCG8* (rs4245791) had tagged rs6544713 and rs4299376, which all showed significant associations with intestinal cholesterol absorption markers. A previous study in a European cohort has also reported that SNPs in *ABCG8* were associated with cholesterol absorption (19). In that study, the minor allele of rs41360247 was negatively related to cholesterol absorption and the minor allele of rs4245791 positively (19), which is in agreement with our findings.

For genes encoding enzymes of the endogenous cholesterol synthesis pathways, no significant associations with TC-standardized serum lathosterol levels were reported. Lathosterol is an intermediate in the Kandutsch–Russell pathway. To what extent the selected SNPs that are essential in endogenous cholesterol synthesis are associated with cholesterol synthesis rates in the Bloch pathway is not clear. For this, serum desmosterol should have been measured, which is specific for the Bloch pathway, whereas we analyzed lathosterol which is only part of the Kandutsch–Russell pathway. An explanation for the non-significant relations for the SNPs in the endogenous cholesterol synthesis genes that were selected in our study may be that other SNPs in these genes are associated with endogenous cholesterol synthesis, which were not included in the present study. Another explanation might be that the regulation of endogenous cholesterol synthesis is more complex and does not relate to one single SNP, as many enzymes are involved in the endogenous cholesterol synthesis pathway. In contrast to the absence of an association with lathosterol levels, SNPs in *LBR*

(rs12141732) and *HMGCR* (rs12916) were significantly related with serum LDL-C concentrations. *HMGCR* (rs12916) was selected as tag SNP for *HMGCR* (rs12654264, rs3846662, and rs3846663), which also showed significant associations with serum LDL-C concentrations. For *HMGCR* (rs12654264, rs3846662, rs3846663, and rs12916) these associations with LDL-C concentrations agree with previous studies in Asian and European populations (38–42). Although intestinal cholesterol absorption and endogenous cholesterol synthesis play a key role in the regulation of plasma LDL-C concentrations (2), they do not explain the significant associations between SNP in *HMGCR* and *LBR* with serum LDL-C concentrations. It is likely that other genes that are involved in cholesterol homeostasis have contributed to these findings.

Interestingly, SNPs in genes involved in intestinal cholesterol absorption were not exclusively associated with markers for their postulated physiological process. However, the cholesterol absorption genes *ABCG5*, *ABCG8,* and *NPC1L1* are not only expressed in the human intestine, but also in the liver (43,44). On hepatocytes, ABCG5/G8 regulates the secretion of cholesterol into bile and NPC1L1 facilitates hepatic cholesterol re-uptake, thereby finetuning an otherwise potentially large biliary and fecal loss of cholesterol (45). In transgenic mice, overexpression of human *ABCG5* and *ABCG8* in the liver and small intestine reduced plasma plant sterol levels and fractional cholesterol absorption as measured by the fecal dual-isotope radio method (46). In contrast, plasma lathosterol and liver mRNA levels of *HMGCR* were increased. Additionally, in vivo cholesterol synthesis was increased in the liver, possibly to compensate for the elevated biliary cholesterol secretion rates in these transgenic mice (46). This animal study thus shows that *ABCG5* and *ABCG8* expression influences endogenous cholesterol synthesis which confirms our observations. Moreover, in our cohort, we noticed a similar association for an absorption gene, i.e., two SNPs in *NPC1L1* (rs217429 and rs217416) were associated with endogenous cholesterol synthesis. The question remains whether these associations between SNPs in intestinal cholesterol absorption genes and lathosterol only show the reciprocal phenomenon or should also be interpreted as a possible direct effect of the SNP on hepatic cholesterol synthesis. Temel et al. have shown that hepatic *NPC1L1* expression in transgenic mice increased hepatic cholesterol levels by enhancing the reuptake of cholesterol from the bile (47). It may be that SNPs in *NPC1L1* have increased the expression or activity of NPC1L1 in the liver, which in turn impacts serum lathosterol levels. Furthermore, the SNPs in *ABCG5* and *ABCG8* that showed an association with intestinal cholesterol

absorption were not associated with serum LDL-C concentrations and also did not show an inverse association with endogenous cholesterol synthesis. This may suggest that the cholesterol has been eliminated from the body, via for example hepatobiliary cholesterol excretion involving ABCG5/G8 or transintestinal cholesterol efflux (2,48).

There are some points that should be considered while interpreting our data. Firstly, it should be noted that almost all selected SNPs were located in intron regions. In general, SNPs in introns do not induce changes in protein-coding sequences, suggesting that they are potentially of less functional relevance than SNPs located in exons. However, SNPs in the intron regions can impact the protein via alternative regulation of splicing (49). This can lead to incorrectly spliced mRNA, which may ultimately affect mRNA translation and result in non-functional proteins and can also have clinical consequences (50). SNPs in introns could also serve as markers for other functionally relevant SNPs, as should be indicated by high LD between the SNPs. Secondly, significant differences were found between all baseline characteristics, except for gender distribution, between the five different studies. This heterogeneity between study populations was taken into account by correcting for the factor study in our analyses. In addition, only European individuals were included, which has further minimized this heterogeneity. In four studies, only individuals with a stable body weight (weight gain or loss of <3 kg for studies 1, 2 and 3 and <2 kg for study 5) could participate. For study 4, a stable body weight was not an inclusion criterion. It is therefore possible that some of the participants lost or gained some weight in the months preceding the study. However, it is not expected that possible changes in weight were related to a specific genotype group and therefore biased the results. Thirdly, this study had a relatively small sample size. This suggests that the significant findings that we found reflect strong associations. Our results can therefore help to determine whether individuals with specific genotypes are more sensitive to specific nutritional and pharmacological interventions, such as foods enriched with plant sterols or stanols, or ezetimibe and statin treatment. To illustrate, 4-week statin treatment in women with familiar hypercholesterolemia resulted in a significantly smaller percentage reduction in LDL-C concentrations in women with the AA genotype of *HMGCR* (rs3846662) compared to women with the other genotypes. Moreover, statin efficacy was significantly decreased in the AA group compared with women with the other genotypes (51). This suggests that genotyping SNPs, even those located in the intron region, may play an important role in the development of more personalized treatment. Finally, an independent cohort in

which we could replicate the positive findings was unavailable. Therefore, an additional study is needed to reach greater validity.

5. Conclusions

This study showed that several SNPs in genes that are essential in intestinal cholesterol absorption were associated with serum markers for intestinal cholesterol absorption and/or endogenous cholesterol synthesis. In addition, a number of SNPs in genes that are essential in endogenous cholesterol synthesis were associated with serum LDL-C concentrations in a European cohort.

Supplemental data

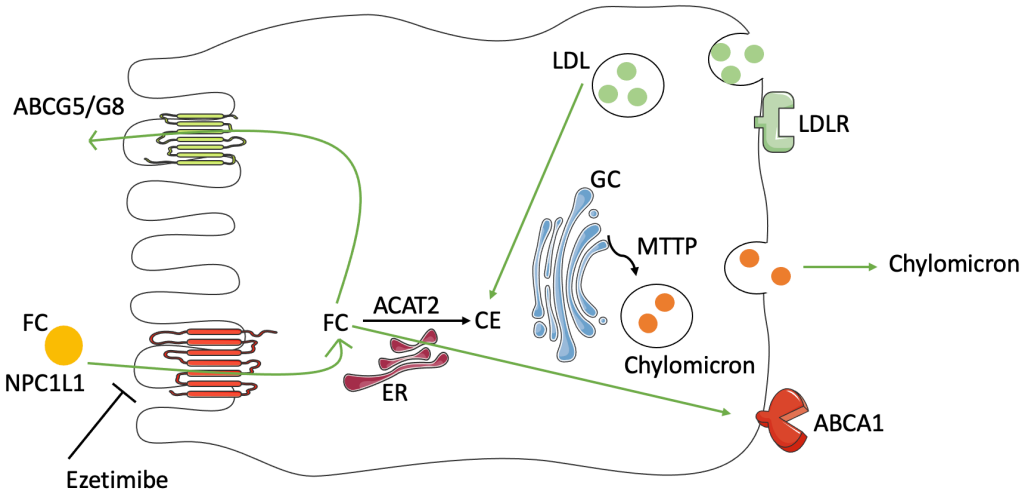


Figure S1. Schematic overview of the intestinal cholesterol absorption pathway. Free cholesterol (FC) enters the enterocyte via Niemann-Pick C1-like 1 (NPC1L1) and is esterified by Acetyl-CoA Acetyltransferase 2 (ACAT2) in the endoplasmic reticulum (ER). FC can also be transported back into the intestinal lumen via ATP-binding cassette member 5 and 8 (ABCG5/G8). Low-density lipoprotein (LDL) is taken up from the basolateral side via LDL receptor-mediated endocytosis. FC is also used by ATP-binding cassette A1 (ABCA1) to form high-density lipoprotein. Cholesterol ester (CE) is further processed in the Golgi complex (GC) with other components to form chylomicrons which are further transported to the lymphatic system. The drug ezetimibe is a NPC1L1 blocker and thus inhibits intestinal cholesterol absorption. Note: Single-nucleotide polymorphisms in genes in bold have been included in the present study.

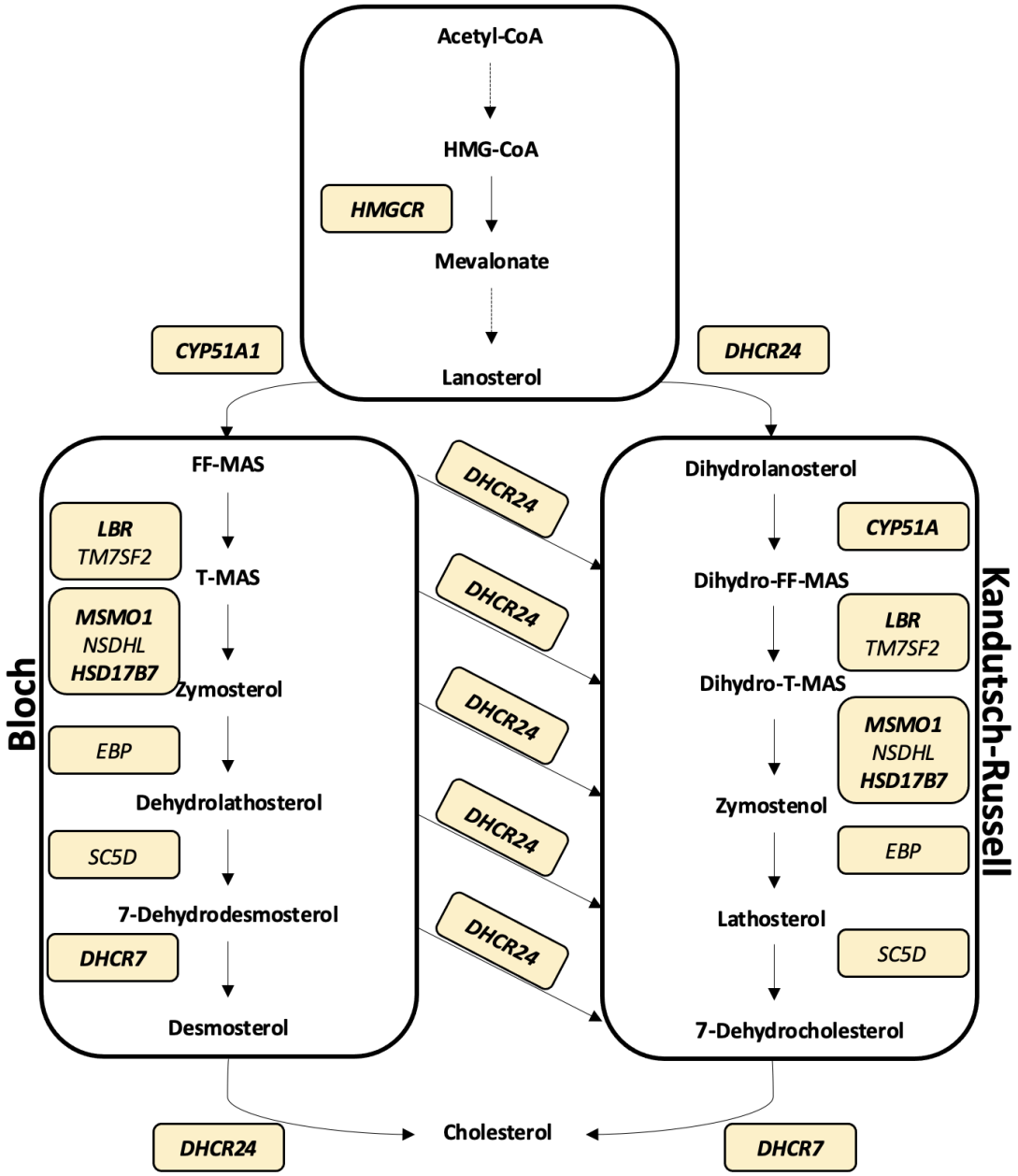


Figure S2. The endogenous cholesterol synthesis pathway. Cholesterol is synthesized via the Bloch and/or Kandutsch-Russell pathway. Similar enzymes are involved in these pathways, but intermediates differ. Note**:** Single-nucleotide polymorphisms in genes in bold have been included in the present study.

Table S1. List of full names of genes included in the present study.

Gene symbol/ HGNC	Approved gene name in HGNC
Cholesterol absorption genes	
ABCG5	ATP binding cassette subfamily G member 5
ABCG8	ATP binding cassette subfamily G member8
NPC1L1	NPC1 like intracellular cholesterol transporter 1
Cholesterol synthesis genes	
CYP51A1	Cytochrome P450 family 51 subfamily A member 1
DHCR7	7-dehydrocholesterol reductase
DHCR24	24-dehydrocholesterol reductase
HMGCR	3 -hydroxy-3-methylglutaryl-CoA reductase
HSD17B7	Hydroxysteroid 17-beta dehyrogenase 7
LBR	Lamin B receptor
MSMO1	Methylsterol monooxygenase 1

Abbreviation: HGNC = Human Genome Organisation (HUGO) Gene Nomenclature Committee.

Table S2. Information given by the Precision Medicine Research Array for the two SNPs in *ABCG8* with an unknown rs-number.

Affymetrix SNP ID	Transcript ID Ensembl	SNP Location	Accession Number Nucleotide Database NCBI	Gene		
				Full name	Abbreviation	NCBI Gene ID
AX_11180448	ENST00000272286	Missense	NM_022437	ATP-binding cassette, sub-family G (WHITE), member 8	ABCG8	64241
AX_82902928	ENST00000272286	Intron	NM_022437	ATP-binding cassette, sub-family G (WHITE), member 8	ABCG8	64241

Accession Number Nucleotide Database NCBI = reference sequence of mRNA which links to the nucleotide database of NCBI; Affymetrix SNP ID = a unique Affymetrix identifier for the SNP; NCBI Gene ID = ID for a specific gene provided by NCBI; Transcript ID Ensembl = an identifier for the transcripts in the Ensembl database.

Table S3. Baseline characteristics for all participants and stratified by study.

	All subjects (N = 456)	Study 1 (N = 108)	Study 2 (N = 34)	Study 3 (N = 39)	Study 4 (N = 257)	Study 5 (N = 18)	P-value
Age (years)	45.4 ± 15.3	33.0 ± 14.9	31.2 ± 13.8	50.0 ± 11.9	53.0 ± 10.1	33.0 ± 12.2	**<0.001**
Body mass index (kg/m²)*	25.1 ± 3.6	23.1 ± 2.9	22.8 ± 2.5	25.3 ± 3.0	26.3 ± 3.6	23.9 ± 2.8	**<0.001**
Underweight	7 (1.5)	3 (2.8)	1 (2.9)	0 (0.0)	3 (1.2)	0 (0.0)	
Normal weight	225 (49.3)	76 (70.4)	27 (79.4)	18 (46.2)	92 (35.8)	12 (66.7)	
Overweight	179 (39.3)	27 (25.0)	6 (17.6)	21 (53.8)	119 (46.3)	6 (33.3)	**< 0.001**
Obesity class I	28 (6.1)	2 (1.9)	0 (0.0)	0 (0.0)	26 (10.1)	0 (0.0)	
Obesity class II	6 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	6 (2.3)	0 (0.0)	
Obesity class III	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Females	254 (55.7)	69 (63.9)	23 (67.6)	23 (59.0)	128 (49.8)	11 (61.1)	0.06
Smoking**	55 (12.1)	21 (19.4)	N/A	4 (10.3)	30 (11.7)	0 (100)	**<0.001**
Lipids							
TC	5.50 ± 1.02	4.95 ± 0.79	4.97 ± 0.91	5.95 ± 0.84	5.73 ± 1.05	5.38 ± 0.83	**<0.001**
HDL-C	1.46 ± 0.41	1.60 ± 0.38	1.42 ± 0.34	1.46 ± 0.44	1.40 ± 0.43	1.59 ± 0.33	**<0.001**
LDL-C	3.54 ± 0.95	2.93 ± 0.76	3.05 ± 0.89	3.93 ± 0.83	3.82 ± 0.91	3.20 ± 0.76	**<0.001**
TAG	0.97 (0.93 – 1.01)	0.82 (0.75 – 0.90)	0.99 (0.86 – 1.15)	1.05 (0.88 – 1.26)	1.00 (0.94 – 1.06)	1.25 (1.06 – 1.48)	**<0.001**
Non-cholesterol sterols***							
Lathosterol	107 ± 55	104 ± 35	131 ± 40	113 ± 54	101 ± 61	153 ± 70	**<0.001**
Sitosterol	137 ± 65	121 ± 44	153 ± 66	166 ± 54	136 ± 71	155 ± 60	**<0.001**
Campesterol	214 ± 120	321 ± 100	241 ± 126	256 ± 96	156 ± 95	249 ± 92	**<0.001**

Abbreviations: HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TAG = triacylglycerol; TC = total cholesterol. Note: Categorical data are presented as n (%), and continuous data as mean ± SD. For TAG, the mean and (95% CI) are presented after back-transformation of the log-transformed values. Lipids are presented in mmol/L and the non-cholesterol sterols in $10^2 \times$ µmol/mmol cholesterol. * BMI data presented for N = 445, as data were unavailable for N = 11 in Study 4. BMI categories: underweight <18.5 kg/m^2, normal weight 18.5 – 24.9 kg/m^2, overweight 25.0 – 29.9 kg/m^2, obesity class I 30.0 – 34.9 kg/m^2, obesity class II 35.0 – 39.9 kg/m^2, and obesity class III ≥40 kg/m^2 (26). ** Smoking data presented for N = 410, because data were unavailable for N = 34 in S2 and for N = 12 in Study 4. *** Non-cholesterol sterol levels presented for N = 455, because data were unavailable for N = 1 in Study 2.

Table S4. Associations between intestinal cholesterol absorption markers, an endogenous cholesterol synthesis marker and serum LDL-C concentrations.

Independent Variable	Dependent Variable	t	β	95 % CI for β		P-value
				Lower Bound	Upper Bound	
Sitosterol	Campesterol	42.424	1.39	1.321	1.449	**<0.001**
Sitosterol	Lathosterol	−2.253	−0.09	−0.169	−0.012	**0.025**
Campesterol	Lathosterol	−3.733	−0.10	−0.146	−0.045	**<0.001**
Campesterol	LDL-C	0.335	0.00	−0.001	0.001	0.738
Sitosterol	LDL-C	0.277	0.00	−0.001	0.001	0.782
Lathosterol	LDL-C	−0.403	0.00	−0.002	0.001	0.687

Abbreviations: LDL-C = low-density lipoprotein cholesterol. Note: Non-cholesterol sterols are presented in $10^2 \times$ µmol/mmol cholesterol and LDL-C in mmol/L. All results were obtained from a linear regression analysis adjusted for the factor study.

Table S5. The location and allele frequencies for various SNPs in intestinal cholesterol absorption and endogenous cholesterol synthesis genes for 456 participants.

	Gene	SNP	Location	Call rate (%)	Alleles	Frequenc		HWE
						Our cohort	European Cohort*	
		rs-number			(Ref/Alt)	(Ref/Alt)	(Ref/Alt)	P-value
Cholesterol absorption								
	ABCG5	rs10208987	Intron	99.8	T/G	0.938/0.062	0.928/0.072	0.065
		rs4148189	Intron	100	C/T	0.899/0.101	0.888/0.112	0.741
		rs4245786	Intron	100	G/A	0.240/0.760	0.236/0.764	0.487
		rs7599296	Intron	100	G/A	0.813/0.188	0.832/0.168	0.751
		rs4148184	Intron	99.8	C/T	0.597/0.403	0.619/0.381	0.999
		rs13396273	Intron	100	C/T	0.649/0.351	0.640/0.360	0.518
	ABCG8	AX_11180448**	Missense	100	G/C	0.932/0.068	-	0.033
		rs4148207	Intron	100	T/C	0.593/0.407	0.610/0.390	0.532
		rs4299376	Intron	98.2	G/T	0.320/0.680	0.323/0.677	0.004
		rs41360247	Intron	100	T/C	0.939/0.061	0.937/0.063	0.008
		rs6544713	Intron	100	T/C	0.310/0.690	0.322/0.678	0.019
		rs4245791	Intron	100	C/T	0.311/0.689	0.327/0.673	0.021
		rs13390041	Intron	100	A/G	0.543/0.457	0.554/0.446	0.694
		rs6709904	Intron	100	A/G	0.902/0.098	0.884/0.116	0.158
		rs4077440	Intron	99.8	T/C	0.442/0.558	0.439/0.561	0.511
		rs3795860	Intron	100	T/C	0.538/0.462	0.559/0.441	0.581
		AX_82902928**	Intron	100	AC/-	0.356/0.644	-	0.089
		rs55924588	Intron	100	T/C	0.950/0.050	0.935/0.065	0.257
	NPC1L1	rs217429	Intron	100	A/C	0.754/0.246	0.751/0.249	0.898

Table S5 (continued). The location and allele frequencies for various SNPs in intestinal cholesterol absorption and endogenous cholesterol synthesis genes for 456 participants.

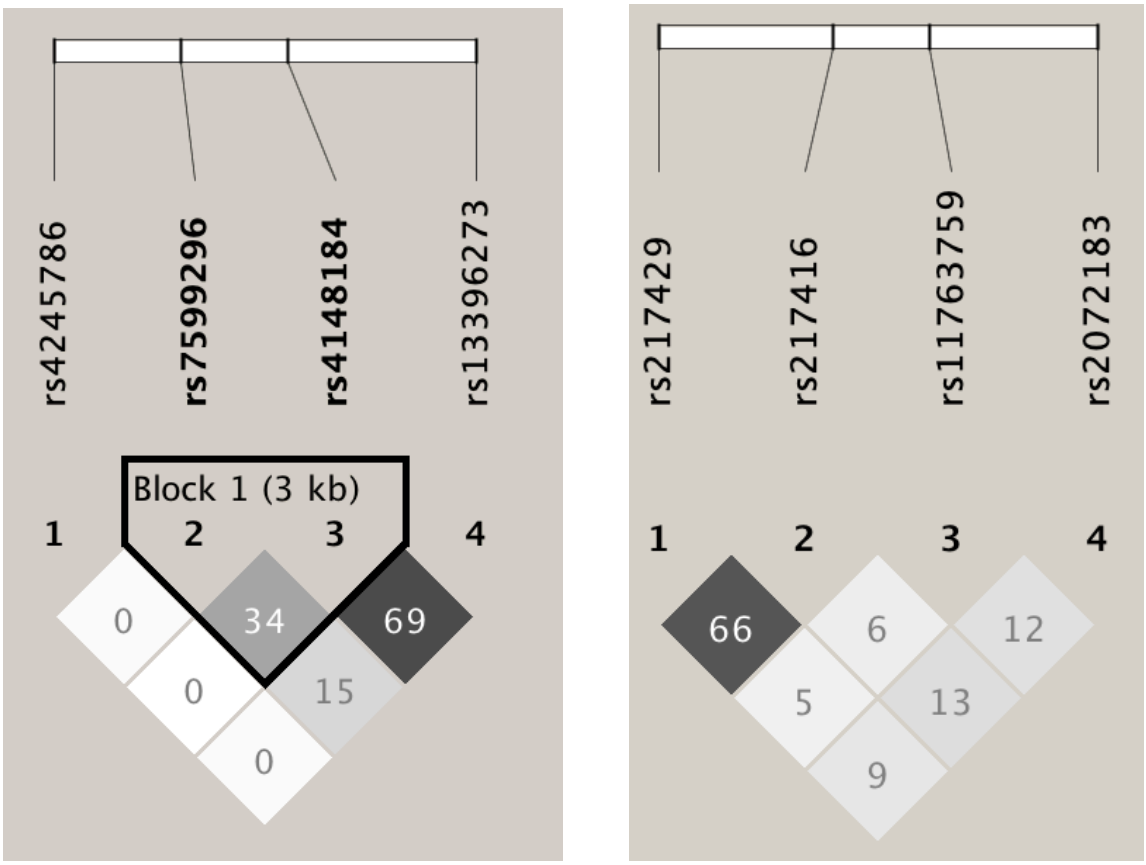
	Gene	SNP	Location	Call rate (%)	Alleles	Frequencies		HWE
						Our cohort	European Cohort*	
		rs-number			(Ref/Alt)	(Ref/Alt)	(Ref/Alt)	P-value
		rs217416	Intron	99.6	T/C	0.736/0.264	0.737/0.263	0.105
		rs11763759	Intron	99.6	T/C	0.683/0.317	0.697/0.303	0.562
		rs2072183	Synonymous	99.8	G/C	0.769/0.231	0.774/0.226	0.100
Cholesterol synthesis								
	CYP51A1	rs35968894	Intron	100	A/G	0.599/0.401	0.626/0.374	0.634
	DHCR7	rs1792275	Intron	99.8	C/T	0.053/0.947	0.054/0.946	0.235
		rs72954301	Upstream	100	G/T	0.894/0.106	0.913/0.087	0.288
	DHCR24	rs77668549	Intron	99.8	A/G	0.866/0.134	0.884/0.116	0.379
		rs7553385	Intron	100	A/G	0.938/0.062	0.942/0.058	0.300
		rs7551288	Intron	99.8	A/G	0.403/0.597	0.430/0.570	0.243
		rs11206456	Intron	100	C/T	0.917/0.083	0.905/0.095	0.512
		rs111480286	Intron	100	ACAG/-	0.934/0.066	0.941/0.059	0.434
		rs6676774	Intron	100	G/A	0.607/0.393	0.609/0.391	0.865
		rs718265	Synonymous	100	A/G	0.303/0.697	0.310/0.690	0.784
	HMGCR	rs12654264	Intron	100	A/T	0.620/0.380	0.617/0.383	0.232
		rs3846662	Intron	100	A/G	0.553/0.447	0.564/0.436	0.319
		rs3846663	Intron	99.8	C/T	0.619/0.381	0.618/0.382	0.155
		rs12916	Prime UTR	100	T/C	0.587/0.413	0.594/0.06	0.342

Table S5 (continued). The location and allele frequencies for various SNPs in intestinal cholesterol absorption and endogenous cholesterol synthesis genes for 456 participants.

Gene	SNP	Location	Call rate (%)	Alleles	Frequencies		HWE
	rs-number			(Ref/Alt)	Our cohort (Ref/Alt)	European Cohort* (Ref/Alt)	P-value
HSD17B7	rs77482353	Intron	99.1	A/G	0.597/0.403	0.649/0.351	0.302
LBR	rs6678087	Intron	99.8	T/C	0.163/0.837	0.585/0.415	0.868
	rs12141732	Intron	99.8	T/C	0.712/0.288	0.700/0.300	0.395
	rs4653635	Intron	100	A/G	0.163/0.837	0.151/0.849	0.688
	rs12410357	Intron	100	G/A	0.889/0.111	0.875/0.125	0.449
MSMO1	rs17585739	Synonymous	100	G/A	0.944/0.056	0.938/0.062	0.611
	rs17046216	Intron	100	T/A	0.658/0.342	0.671/0.329	0.939

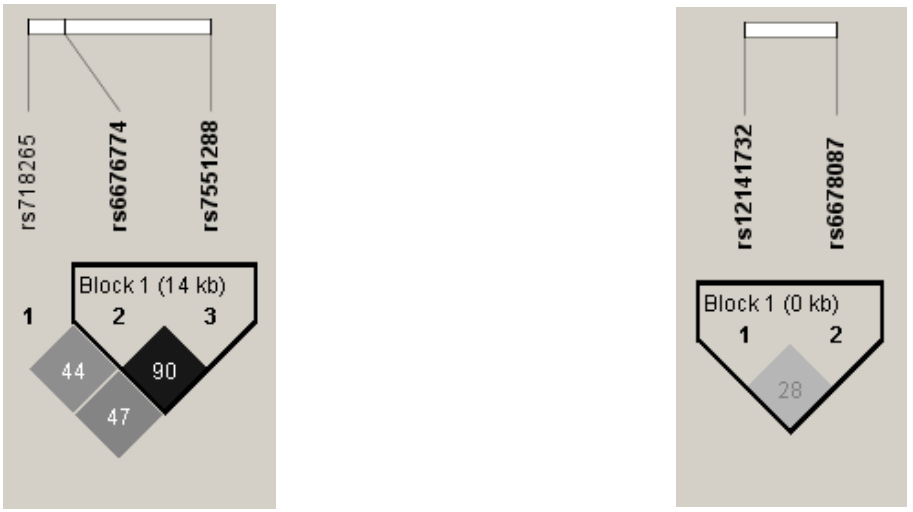
Abbreviations*:* Alt = Alternative allele; HWE = Hardy-Weinberg Equilibrium; Ref = Reference allele; SNP = single-nucleotide polymorphism.

* European cohort data (release version: 20201027095038) were obtained on January the 28th, 2021 from NCBI (37). *HSD17B7* (rs7748253) was merged into rs11590043 on July the 1st, 2015. ** SNPs with unknown rs-numbers and European cohort frequencies.



(**a**) (**b**)

Figure S3. Pairwise LD among SNPs in (**a**) *ABCG5* and (**b**) *NPC1L1* is indicated in the diamond shapes. The triangle marks the haplotype block within each region (based on the confidence interval of D'). The shading with a dark grey to white gradient indicates higher to lower LD between each pair of SNPs based on the r^2-value The LD plots were created by Haploview version 4.1 (35).



(**a**) (**b**)

Figure S4. Pairwise LD among SNPs in (**a**) *DHCR24*, and (**b**) *LBR* is indicated in the diamond shapes. The triangle marks the haplotype block within each region (based on the confidence interval of D'). The shading with a dark grey to white gradient indicates higher to lower LD between each pair of SNPs based on the r^2-value The LD plots were created by Haploview version 4.1 (35).

Table S6. Associations between various SNPs in cholesterol absorption genes, that were either captured by a tag SNP or contained a genotype group < 12 individuals, with serum TC-standardized campesterol, sitosterol and lathosterol levels (N = 455), and serum LDL-C concentrations (N = 456).

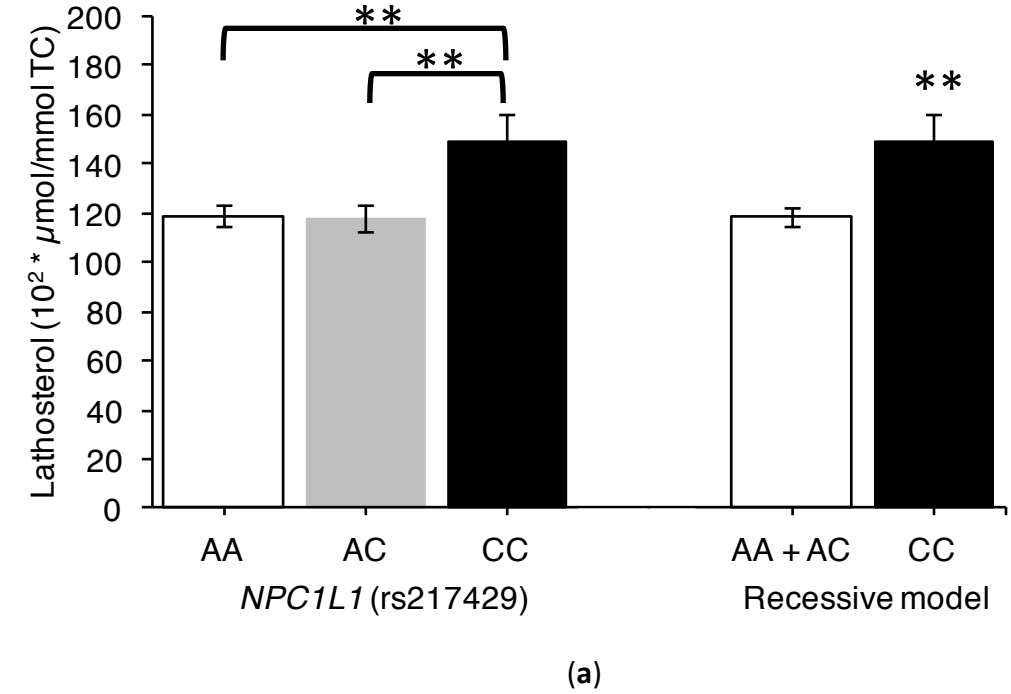
Gene	SNP	Genotype	N	Campesterol 10^2×µmol/mmol TC		Sitosterol 10^2×µmol/mmol TC		Lathosterol 10^2×µmol/mmol TC		N	LDL-C mmol/L	
				Mean (95% CI)	**P-value**	**Mean (95% CI)**	**P-value**	**Mean (95% CI)**	**P-value**		**Mean (95% CI)**	**P-value**
ABCG5	rs10208987	TT	403	249 (235 – 263)		149 (140 – 158)[A]		119 (111 – 127)		403	3.39 (3.27 – 3.51)	
		TG	47	214 (184 – 244)	**0.04**	124 (104 – 143)[B]	**0.02**	131 (115 – 148)	0.34	48	3.38 (3.12 – 3.64)	0.9
		GG	4	195 (98 – 292)		112 (50 – 175)		124 (70 – 178)		4	3.19 (2.33 – 4.05)	
	rs4148189	TT	4	290 (192 – 388)		158 (94 – 221)		114 (60 – 167)		4	2.94 (2.08 – 3.80)	
		TC	83	227 (204 – 251)	0.14	136 (121 – 151)	0.24	137 (125 – 150)[A]	**0.01**	84	3.43 (3.22 – 3.63)	0.53
		CC	368	249 (234 – 263)		149 (140 – 158)		116 (108 – 124)[B]		368	3.38 (3.25 – 3.51)	
ABCG8	AX_11180448	CC	5	193 (106 – 280)		126 (70 – 182)		150 (102 – 198)		5	3.05 (2.28 – 3.81)	
		CG	51	217 (189 – 245)	**0.04**	125 (107 – 143)[A]	**0.02**	117 (101 – 132)	0.43	52	3.28 (3.03 – 3.52)	0.38
		GG	399	250 (236 – 264)		150 (141 – 159)[B]		121 (113 – 129)		399	3.41 (3.29 – 3.54)	
	rs4299376*	TT	194	220 (204 – 237)[A]		130 (119 – 140)[A]		124 (114 – 133)		194	3.34 (3.19 – 3.49)	
		TG	220	256 (239 – 272)[B]	**<0.001[$]**	153 (143 – 164)[B]	**<0.001[$]**	118 (109 – 128)	0.58	221	3.41 (3.27 – 3.56)	0.66
		GG	33	320 (286 – 254)[C]		201 (179 – 222)[C]		117 (98 – 137)		33	3.36 (3.05 – 3.67)	
	rs41360247	TT	405	249 (235 – 264)		150 (141 – 159)		120 (113 – 128)		405	3.42 (3.29 – 3.54)	
		TC	45	216 (187 – 246)	0.05	124 (105 – 144)	**0.03**	119 (102 – 135)	0.47	46	3.22 (2.97 – 3.48)	0.24
		CC	5	193 (106 – 280)		126 (70 – 182)		150 (102 – 198)		5	3.05 (2.28 – 3.81)	

Table S6 (continued). Associations between various SNPs in cholesterol absorption genes, that were either captured by a tag SNP or contained a genotype group < 12 individuals, with serum TC-standardized campesterol, sitosterol and lathosterol levels (N = 455), and serum LDL-C concentrations (N = 456).

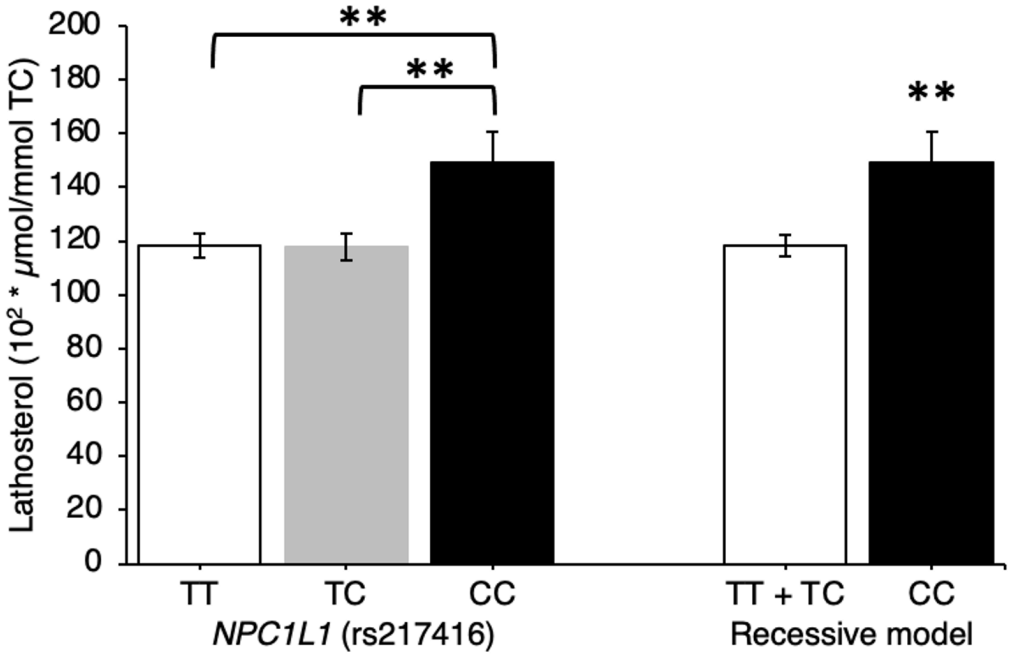
Gene	SNP	Genotype	N	Campesterol 10^2×µmol/mmol TC		Sitosterol 10^2×µmol/mmol TC		Lathosterol 10^2×µmol/mmol TC		N	LDL-C mmol/L	
				Mean (95% CI)	P-value	Mean (95% CI)	P-value	Mean (95% CI)	P-value		Mean (95% CI)	P-value
ABCG8	rs6544713*	TT	33	316 (282 – 350)^{A}		198 (176 – 220)^{A}		117 (98 – 137)		33	3.38 (3.08 – 3.69)	
		TC	216	256 (239 – 272)^{B}	**<0.001**$^{\$}$	153 (143 – 164)^{B}	**<0.001**$^{\$}$	118 (109 – 128)	0.65	217	3.45 (3.31 – 3.60)	0.29
		CC	206	221 (205 – 237)^{C}		130 (120 – 141)^{C}		123 (114 – 132)		206	3.32 (3.17 – 3.47)	
	rs6709904	AA	374	246 (231 – 260)		147 (137 – 156)		121 (113 – 129)		374	3.41 (3.28 – 3.54)	
		AG	74	243 (219 – 268)	0.44	145 (130 – 161)	0.95	116 (103 – 129)	0.58	75	3.33 (3.21 – 3.54)	0.31
		GG	7	198 (124 – 272)		139 (91 – 187)		135 (95 – 176)		7	2.94 (2.29 – 3.59)	
	rs55924588	TT	409	247 (232 – 261)		147 (138 – 156)		121 (113 – 128)		410	3.38 (3.26 – 3.50)	
		TC	46	223 (192 – 254)	0.12	136 (116 – 156)	0.25	117 (100 – 134)	0.68	46	3.45 (3.18 – 3.71)	0.63
		CC	0	N/A		N/A		N/A		0	N/A	
	rs13390041*	AA	131	252 (232 – 272)		153 (140 – 166)		120 (109 – 131)		132	3.33 (3.15 – 3.50)	
					0.38		0.17		0.53			0.18
		AG	213	245 (229 – 261)		146 (136 – 157)	0.17	123 (114 – 132)		231	3.46 (3.32 – 3.60)	
		GG	93	233 (210 – 256)		136 (122 – 151)		115 (103 – 128)		93	3.29 (3.09 – 3.49)	

Abbreviations: LDL-C = low-density lipoprotein cholesterol; N/A = not applicable; SNP = single-nucleotide polymorphism; TC = total cholesterol.

Note: All analyses were adjusted for the factor study. Data are presented as estimated marginal means (95% CI). Non-cholesterol sterol levels were missing for N = 1. Different letters within a SNP indicate significantly different non-cholesterol sterol levels between the genotypes based on a Bonferroni post-hoc test. * Indicates a SNP captured by a tag SNP. $^{\$}$ Additive models are presented in the supplemental material (**Table S7)**.



(**a**)



(**b**)

Figure S5. Association between SNPs (**a**) *NPC1L1* (rs217429) and (**b**) *NPC1L1* (rs217416) with serum levels of cholesterol-standardized lathosterol using recessive models. All values were adjusted for the factor study and presented as estimated marginal means ± SE. The black bars refer to the least frequent homozygous genotype, and the white bars refer to the most frequent homozygous genotype. * p-value ≤ 0.05, ** p-value ≤ 0.01.

Table S7. Associations between SNPs in intestinal cholesterol absorption genes with TC-standardized non-cholesterol sterols using additive models (N = 455).

Gene	SNP	Alleles (Ref/Alt)	Marker	t	β	95 % CI for β		P-value
						Lower Bound	Upper Bound	
ABCG5	rs4245786	G/A	Sitosterol	-1.4	-6.9	-16.5	2.7	0.161
ABCG8	rs6544713	T/C	Campesterol	5.7	41.5	27.3	55.8	**<0.001**
ABCG8	rs6544713	T/C	Sitosterol	6.2	28.7	19.6	37.9	**<0.001**
ABCG8	rs4245791	C/T	Campesterol	5.8	41.4	27.2	55.5	**<0.001**
ABCG8	rs4245791	C/T	Sitosterol	6.2	28.6	19.5	37.7	**<0.001**
ABCG8	rs4299376	G/T	Campesterol	5.8	42.9	28.5	75.4	**<0.001**
ABCG8	rs4299376	G/T	Sitosterol	6.3	29.7	20.4	38.9	**<0.001**

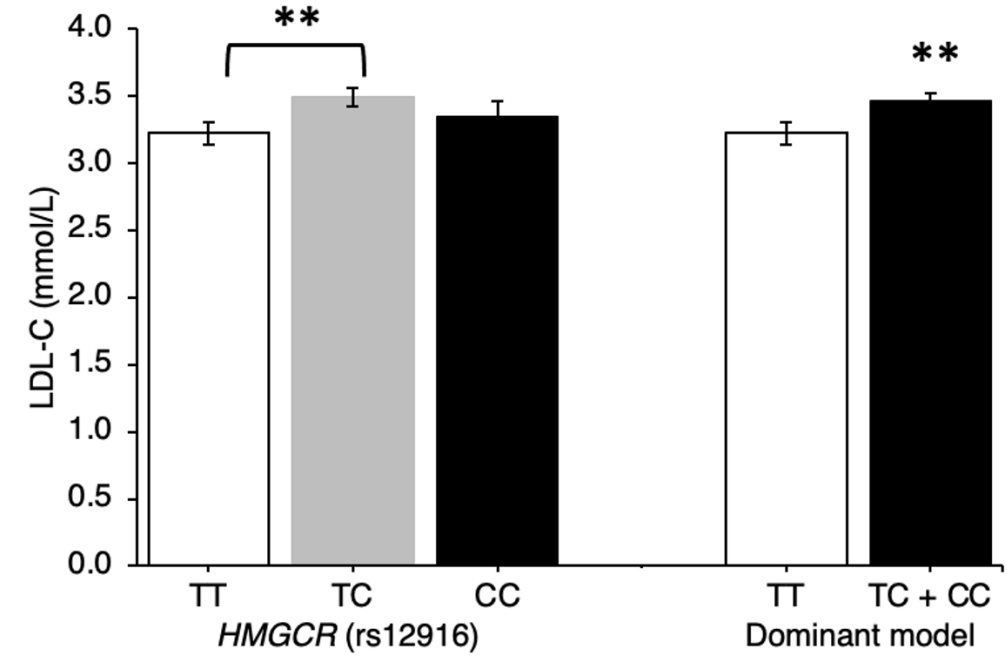
Abbreviations: Alt = alternative allele; Ref = reference allele; SNP= single-nucleotide polymorphism. Note: Non-cholesterol sterols are presented in $10^2 \times$ μmol/mmol total cholesterol. All results were obtained from a linear regression analysis adjusted for the factor study. The alternative allele was used as reference in the model; each copy of the reference allele changes the outcome parameter (marker) with β.

Table S8. Associations between various SNPs in genes involved in intestinal cholesterol absorption with serum total cholesterol concentrations (N = 456).

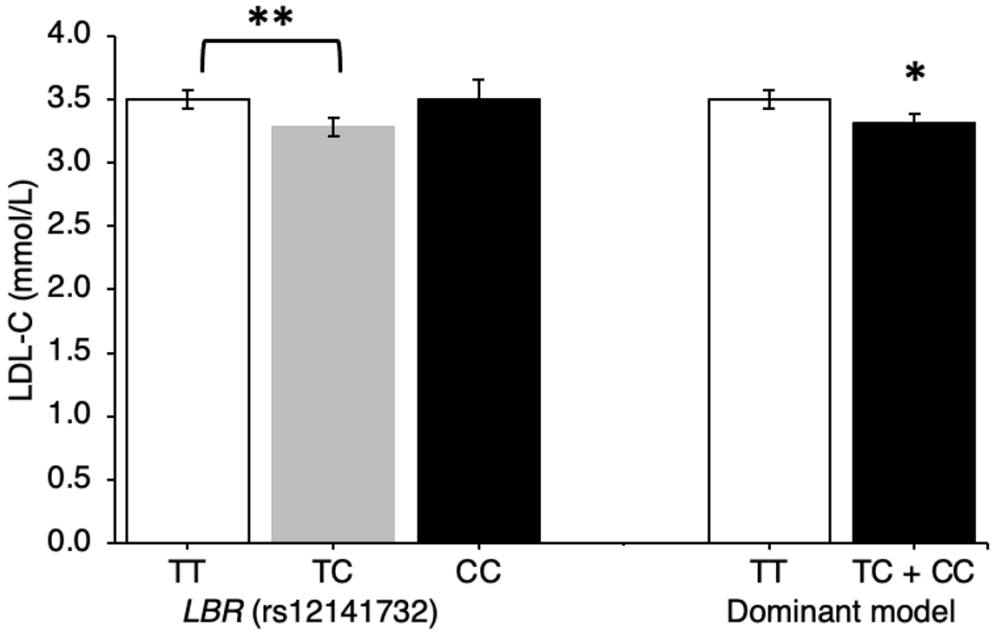
Gene	SNP	Genotype	N	Total cholesterol mmol/L	
				Mean (95% CI)	**P-value**
ABCG5	rs10208987	TT	403	5.40 (5.26 – 5.53)	
		TG	48	5.40 (5.11 – 5.69)	0.906
		GG	4	5.19 (4.24 – 6.14)	
	rs4148189	TT	4	5.13 (4.81 – 6.09)	
		TC	84	5.43 (5.21 – 5.66)	0.803
		CC	368	5.39 (5.25 – 5.53)	
	rs4245786	AA	266	5.45 (5.29 – 5.60)	
		AG	161	5.35 (5.18 – 5.53)	0.356
		GG	29	5.21 (4.85 – 5.58)	
	rs7599296	AA	15	5.16 (4.66 – 5.66)	
		AG	141	5.42 (5.23 – 5.60)	0.616
		GG	300	5.40 (5.26 – 5.55)	
	rs4148184	TT	74	5.22 (4.98 – 5.46)	
		TC	219	5.41 (5.24 – 5.57)	0.182
		CC	162	5.47 (5.29 – 5.65)	
	rs13396273	TT	53	5.25 (4.98 – 5.53)	
		TC	214	5.39 (5.23 – 5.55)	0.376
		CC	189	5.46 (5.29 – 5.63)	
ABCG8	AX_11180448	CC	5	5.16 (4.31 – 6.01)	
		CG	52	5.27 (5.00 – 5.54)	0.463
		GG	399	5.42 (5.29 – 5.56)	
	rs4148207	TT	157	5.43 (5.25 – 5.61)	
		TC	227	5.42 (5.26 – 5.58)	0.408
		CC	72	5.26 (5.02 – 5.50)	
	rs4299376	TT	194	5.33 (5.16 – 5.50)	
		TG	221	5.42 (5.25 – 5.58)	0.467
		GG	33	5.52 (5.18 – 5.86)	
	rs41360247	TT	405	5.44 (5.30 – 5.57)	
		TC	46	5.18 (4.90 – 5.47)	0.203
		CC	5	5.16 (4.31 – 6.01)	
	rs6544713	TT	33	5.54 (5.21 – 5.88)	
		TC	217	5.46 (5.30 – 5.63)	0.151
		CC	206	5.30 (5.14 – 5.47)	
	rs4245791	TT	206	5.31 (5.14 – 5.47)	
		TC	216	5.47 (5.31 – 5.63)	0.163
		CC	34	5.51 (5.18 – 5.85)	
	rs13390041	AA	132	5.41 (5.22 – 5.60)	
		AG	231	5.46 (5.30 – 5.61)	0.115
		GG	93	5.21 (4.99 – 5.44)	
	rs6709904	AA	374	5.42 (5.28 – 5.56)	
		AG	75	5.33 (5.10 – 5.56)	0.392
		GG	7	4.99 (4.27 – 5.71)	
	rs4077440	TT	92	5.49 (5.27 – 7.71)	
		TC	218	5.46 (5.30 – 5.62)	0.066
		CC	145	5.25 (5.06 – 5.43)	
	rs3795860	TT	129	5.41 (5.22 – 5.60)	
		TC	233	5.46 (5.30 – 5.61)	0.127
		CC	94	5.21 (5.00 – 5.44)	
	AX_82902928	--	197	5.45 (5.29 – 5.62)	
		-AC	193	5.41 (5.24 – 5.58)	0.057
		ACAC	66	5.13 (4.88 – 5.39)	
	rs55924588	TT	410	5.39 (5.26 – 5.53)	
		TC	46	5.46 (5.16 – 5.76)	0.645

		CC	0	N/A	
NPC1L1	rs217429	AA	259	5.39 (5.24 – 5.54)	
		AC	170	5.41 (5.23 – 5.59)	0.938
		CC	27	5.45 (5.08 – 5.83)	
	rs217416	TT	239	5.43 (5.28 – 5.59)	
		TC	190	5.36 (5.18 – 5.53)	0.698
		CC	25	5.37 (4.90 – 5.76)	
	rs11763759	TT	209	5.42 (5.26 – 5.58)	
		TC	202	5.31 (5.15 – 5.48)	**0.043**
		CC	43	5.71 (5.41 – 6.01)	
	rs2072183	CC	18	5.34 (4.88 – 5.80)	
		CG	174	5.41 (5.23 – 5.59)	0.956
		GG	263	5.40 (5.25 – 5.54)	

Abbreviations: N/A: not applicable; SNP = single-nucleotide polymorphism. Note: All analyses were adjusted for the factor study. Data are presented as estimated marginal means (95% CI). Statistical significance was set at a p-value < 0.05.



(**a**)



(**b**)

Figure S6. Association between SNPs (**a**) *HMGCR* (rs12916) and (**e**) *LBR* (rs12141732) with serum LDL-C concentrations using dominant models. All values were adjusted for the factor study and presented as estimated marginal means ± SE. The black bars refer to the least frequent homozygous genotype, and the white bars refer to the most frequent homozygous genotype. * p-value ≤ 0.05, ** p-value ≤ 0.01.

Table S9. Associations between various SNPs in endogenous cholesterol synthesis genes, that were either captured by a tag SNP or contained a genotype group < 12 individuals, with serum TC-standardized campesterol, sitosterol and lathosterol levels (N = 455), and serum LDL-C concentrations (N = 456).

Gene	SNP	Genotype	N	Campesterol 10^2×µmol/mmol TC		Sitosterol 10^2×µmol/mmol TC		Lathosterol 10^2×µmol/mmol TC		N	LDL-C mmol/L	
				Mean (95% CI)	P-value	Mean (95% CI)	P-value	Mean (95% CI)	P-value		Mean (95% CI)	P-value
DHCR7	rs1792275	TT	406	243 (229 – 256)	0.27	145 (136 – 154)	0.25	123 (115 – 130)	**0.02**	407	3.37 (3.24 – 3.49)	0.08
		TC	48	259 (230 – 289)		156 (137 – 175)		104 (88 – 120)		48	3.60 (3.34 – 3.86)	
		CC	0	N/A		N/A		N/A		0	N/A	
	rs72954301	TT	3	207 (94 – 320)	0.54	121 (48 – 194)	0.76	137 (75 – 200)	0.83	3	3.35 (2.36 – 4.34)	0.84
		TG	91	253 (230 – 276)		148 (133 – 163)		122 (109 – 135)		91	3.34 (3.14 – 3.54)	
		GG	361	243 (229 – 258)		146 (137 – 155)		120 (112 – 128)		362	3.40 (3.27 – 3.52)	
DHCR24	rs77668549	AA	339	244 (229 – 258)	0.35	145 (136 – 155)	0.46	119 (111 – 127)	0.65	339	3.39 (3.26 – 3.52)	0.77
		AG	109	252 (230 – 273)		152 (138 – 166)		125 (113 – 137)		110	3.36 (3.17 – 3.55)	
		GG	6	194 (115 – 274)		124 (72 – 176)		134 (90 – 178)		6	3.62 (2.93 – 4.32)	
	rs7551288*	AA	80	232 (208 – 256)	0.38	144 (128 – 159)	0.8	119 (106 – 133)	0.52	80	3.40 (3.19 – 3.61)	
		AG	207	251 (234 – 267)		148 (138 – 159)		123 (114 – 133)		207	3.33 (3.18 – 3.48)	0.36
		GG	167	244 (225 – 262)		145 (133 – 157)		117 (107 – 127)		168	3.46 (3.30 – 3.62)	
	rs7553385	AA	401	246 (232 – 250)	0.49	147 (138 – 156)	0.73	121 (113 – 128)	0.92	402	3.39 (3.26 – 3.51)	0.21
		AG	51	229 (200 – 259)		139 (121 – 158)		119 (103 – 135)		51	3.34 (3.09 – 3.60)	
		GG	3	261 (149 – 374)		144 (72 – 217)		109 (46 – 171)		3	4.25 (3.26 – 5.23)	
	rs11206456	TT	2	224 (86 – 362)	0.61	137 (48 – 226)	0.61	140 (64 – 216)	0.32	2	3.88 (2.67 – 5.09)	0.6
		TC	72	255 (230 – 280)		153 (137 – 170)		129 (115 – 143)		72	3.33 (3.11 – 3.55)	
		CC	381	243 (229 – 257)		145 (136 – 154)		119 (111 – 127)		382	3.40 (3.27 – 3.52)	
	rs111480286	--	3	261 (149 – 374)	0.51	145 (72 – 217)	0.73	109 (46 – 171)	0.89	3	4.25 (3.26 – 5.23)	0.15
		-ACAG	54	230 (202 – 259)		140 (121 – 158)		118 (103 – 134)		54	3.29 (3.04 – 5.53)	
		ACAGACAG	398	246 (232 – 260)		147 (138 – 156)		121 (113 – 129)		399	3.39 (3.27 – 3.52)	

Table S9. Associations between various SNPs in endogenous cholesterol synthesis genes, that were either captured by a tag SNP or contained a genotype group < 12 individuals, with serum TC-standardized campesterol, sitosterol and lathosterol levels (N = 455), and serum LDL-C concentrations (N = 456).

Gene	SNP	Genotype	N	Campesterol 10^2×µmol/mmol TC		Sitosterol 10^2×µmol/mmol TC		Lathosterol 10^2×µmol/mmol TC		N	LDL-C mmol/L	
				Mean (95% CI)	P-value	Mean (95% CI)	P-value	Mean (95% CI)	P-value		Mean (95% CI)	P-value
DHCR24	rs4653635	AA	11	244 (185 – 304)	0.43	148 (110 – 187)	0.35	100 (68 – 133)	0.5	11	3.05 (2.53 – 3.57)	0.28
		AG	127	235 (214 – 255)		139 (126 – 152)		121 (109 – 132)		127	3.46 (3.28 – 3.63)	
		GG	317	248 (233 – 263)		149 (139 – 158)		121 (113 – 129)		318	3.37 (3.24 – 3.50)	
	rs12410357	AA	4	284 (186 – 382)	0.73	138 (75 – 202)	0.45	125 (71 – 179)	0.69	4	4.10 (3.24 – 4.95)	0.22
		AG	93	244 (221 – 267)		139 (124 – 154)		125 (112 – 138)		93	3.34 (3.13 – 3.54)	
		GG	358	245 (230 – 259)		148 (139 – 157)		119 (112 – 127)		359	3.39 (3.27 – 3.52)	
HMGCR	rs12654264*	AA	168	240 (221 – 259)	0.7	144 (132 – 157)	0.91	112 (112 – 133)	0.82	169	3.24 (3.08 – 3.40)[A]	**0.02**
		AT	227	245 (229 – 262)		147 (137 – 158)		119 (110 – 128)		227	3.48 (3.34 – 3.62)[B]	
		TT	60	253 (226 – 279)		147 (130 – 165)		122 (107 – 136)		60	3.40 (3.17 – 3.63)	
	rs3846662*	AA	134	239 (218 – 259)		144 (131 – 157)		117 (106 – 129)		134	3.20 (3.02 – 3.38)[A]	
		AG	235	243 (227 – 258)	0.34	145 (134 – 155)	0.43	121 (112 – 130)	0.72	236	3.46 (3.32 – 3.59)[B]	**0.02**
		GG	86	258 (235 – 281)		154 (140 – 169)		123 (110 – 136)		86	3.43 (3.23 – 3.63)	
	rs3846663*	TT	59	245 (218 – 272)	0.81	143 (126 – 160)	0.91	122 (107 – 137)	0.81	59	3.39 (3.16 – 3.63)	**0.02**
		TC	229	244 (228 – 260)		146 (136 – 157)		119 (110 – 128)		229	3.48 (3.34 – 3.62)[A]	
		CC	166	238 (220 – 257)		144 (132 – 156)		122 (112 – 133)		167	3.23 (3.07 – 3.94)[B]	
LBR	rs4653635	AA	11	244 (185 – 304)	0.43	148 (110 – 187)	0.35	100 (68 – 133)	0.5	11	3.05 (2.53 – 3.57)	0.28
		AG	127	235 (214 – 255)		139 (126 – 152)		121 (109 – 132)		127	3.46 (3.28 – 3.63)	
		GG	317	248 (233 – 263)		149 (139 – 158)		121 (113 – 129)		318	3.37 (3.24 – 3.50)	

Table S9 (continued). Associations between various SNPs in endogenous cholesterol synthesis genes, that were either captured by a tag SNP or contained a genotype group < 12 individuals, with serum TC-standardized campesterol, sitosterol and lathosterol levels (N = 455), and serum LDL-C concentrations (N = 456).

Gene	SNP	Genotype	N	Campesterol 10^2×µmol/mmol TC		Sitosterol 10^2×µmol/mmol TC		Lathosterol 10^2×µmol/mmol TC		N	LDL-C mmol/L	
				Mean (95% CI)	P-value	Mean (95% CI)	P-value	Mean (95% CI)	P-value		Mean (95% CI)	P-value
LBR	rs12410357	AA	4	284 (186 – 382)	0.73	138 (75 – 202)	0.45	125 (71 – 179)	0.69	4	4.10 (3.24 – 4.95)	0.22
		AG	93	244 (221 – 267)		139 (124 – 154)		125 (112 – 138)		93	3.34 (3.13 – 3.54)	
		GG	358	245 (230 – 259)		148 (139 – 157)		119 (112 – 127)		359	3.39 (3.27 – 3.52)	
MSMO1	rs17585739	AA	2	231 (91 – 371)	0.87	140 (50 – 231)	0.76	91 (14 – 168)	0.64	2	3.23 (2.01 – 4.46)	0.97
		AT	47	238 (231 – 260)		140 (120 – 159)		125 (109 – 142)		47	3.40 (3.14 – 3.67)	
		TT	406	246 (231 – 250)		147 (138 – 156)		120 (112 – 128)		407	3.39 (3.26 – 3.51)	

Abbreviations: LDL-C = low-density lipoprotein cholesterol; N/A = not applicable; SNP = single-nucleotide polymorphism; TC = total cholesterol.

Note: All analyses were adjusted for the factor study. Data are presented as estimated marginal means (95% CI). Non-cholesterol sterol levels were missing for N = 1. Different letters within a SNP indicate significantly different non-cholesterol sterol levels or LDL-C concentrations between the genotypes based on a Bonferroni post-hoc test. * Indicates a SNP captured by a tag SNP.

Table S10. Associations between various SNPs in genes involved in endogenous cholesterol synthesis with serum total cholesterol concentrations (N = 456).

Gene	SNP	Genotype	N	Total cholesterol mmol/L	
				Mean (95% CI)	**P-value**
CYP51A1	rs35968894	AA	161	5.40 (5.22 – 5.57)	
		AG	224	5.39 (5.23 – 5.56)	0.993
		GG	71	5.41 (5.17 – 5.65)	
DHCR7	rs1792275	TT	407	5.37 (5.24 – 5.51)	
		TC	48	5.63 (5.35 – 5.92)	0.075
		CC	0	N/A	
	rs72954301	TT	3	5.50 (4.40 – 6.59)	
		TG	91	5.28 (5.06 – 5.51)	0.453
		GG	362	5.43 (5.29 – 5.56)	
DHCR24	rs77668549	AA	339	5.41 (5.27 – 5.55)	
		AG	110	5.36 (5.15 – 5.57)	0.738
		GG	6	5.64 (4.87 – 6.41)	
	rs7553385	AA	402	5.40 (5.26 – 5.53)	
		AG	51	5.38 (5.09 – 5.66)	0.347
		GG	3	6.20 (5.11 – 7.29)	
	rs7551288	AA	80	5.44 (5.21 – 5.67)	
		AG	207	5.36 (5.19 – 5.52)	0.700
		GG	168	5.43 (5.25 – 5.61)	
	rs11206456	TT	2	5.81 (4.47 – 7.15)	
		TC	72	5.34 (5.10 – 5.59)	0.725
		CC	382	5.41 (5.27 – 5.54)	
	rs111480286	--	3	6.20 (5.11 – 7.29)	
		-ACAG	54	5.31 (5.03 – 5.58)	0.276
		ACAGACAG	399	5.40 (5.27 – 5.54)	
	rs6676774	AA	75	5.45 (5.21 – 5.68)	
		AG	208	5.34 (5.18 – 5.50)	0.427
		GG	173	5.46 (5.28 – 5.63)	
	rs718265	AA	43	5.30 (5.00 – 5.61)	
		AG	190	5.36 (5.20 – 5.53)	0.437
		GG	223	5.45 (5.29 – 5.62)	
HMGCR	rs12654264	AA	169	5.26 (5.08 – 5.44)^{A}	
		AT	227	5.51 (5.35 – 5.66)^{B}	**0.037**
		TT	60	5.34 (5.08 – 5.60)	
	rs3846662	AA	134	5.24 (5.04 – 5.44)	
		AG	236	5.47 (5.31 – 5.62)	0.087
		GG	86	5.41 (5.18 – 5.63)	
	rs3846663	TT	59	5.34 (5.08 – 5.60)^{A}	
		TC	229	5.51 (5.35 – 5.66)^{B}	**0.034**
		CC	167	5.26 (5.08 – 5.44)	
	rs12916	TT	152	5.24 (5.06 – 5.43)^{A}	
		TC	231	5.51 (5.36 – 5.67)^{B}	**0.022**
		CC	73	5.32 (5.08 – 5.55)	
HSD17B7	rs77482353	AA	156	5.39 (5.21 – 5.57)	
		AG	228	5.34 (5.18 – 5.50)^{A}	0.103
		GG	68	5.62 (5.37 – 5.87)^{B}	
LBR	rs6678087	TT	141	5.41 (5.22 – 5.60)	
		TC	223	5.36 (5.20 – 5.52)	0.530

		CC	91	5.49 (5.27 – 5.71)	
	rs12141732	TT	227	5.52 (5.36 – 5.44)[A]	
		TC	194	5.28 (5.12 – 5.88)[B]	**0.032**
		CC	34	5.54 (5.20 – 286)	
	rs4653635	AA	11	5.11 (4.53 – 5.69)	
		AG	127	5.41 (5.21 – 5.60)	0.600
		GG	318	5.40 (5.26 – 5.55)	
	rs12410357	AA	4	6.34 (5.39 – 7.29)	
		AG	93	5.33 (5.10 – 5.55)	0.111
		GG	359	5.41 (5.27 – 5.54)	
MSMO1	rs17585739	AA	2	5.54 (4.18 – 6.90)	
		AT	47	5.43 (5.13 – 5.72)	0.956
		TT	407	5.39 (5.26 – 5.53)	
	rs17046216	AA	53	5.68 (5.41 – 5.96)	
		AG	206	5.39 (5.23 – 5.55)	0.060
		GG	197	5.33 (5.17 – 5.50)	

Abbreviations: N/A: not applicable; SNP = single-nucleotide polymorphism. Note: All analyses were adjusted for the factor study. Data are presented as estimated marginal means (95% CI). Different letters within a SNP indicate significantly different TC concentrations between the genotypes based on a Bonferroni post-hoc test. Statistical significance was set at a p-value < 0.05.

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CHAPTER 4

Associations of genetic variants with intestinal cholesterol absorption markers in a European population

Fatma B. A. Mokhtar, Jogchum Plat, Herman E. Popeijus, Marcus E. Kleber, Dieter Lütjohann Ronald P. Mensink

CHAPTER 5

Effects of plant stanol ester consumption on mRNA gene expression profiles in duodenum and jejunum biopsies differ between humans pre-classified as high-cholesterol or low-cholesterol absorbers

Fatma B. A. Mokhtar, Susan L.M. Coort, Ronald P. Mensink, Els Desmet, Joshua J. M. Zimmerman, Tom Pauly, Rogier de Ridder, Dieter Lütjohann, Jogchum Plat

CHAPTER 6

General Discussion

APPENDICES

Impact

This dissertation focused on associations between genetic variants with cholesterol metabolism, in particular with intestinal cholesterol absorption. It was shown that different single nucleotide polymorphisms (SNPs) were associated with intestinal cholesterol absorption. These SNPs were located in or close to the following genes: ABCG5, *ABCG8, EIF2B5, EPHB3, C4orf26, CDKL2, NR3C2, LOC285626, BMP6, HLA-G, HLA-H, WBSCR27, WBSCR28, TMTC4, and COL4A2.* Moreover, to better understand the complex network of intestinal cholesterol absorption, an intestinal cholesterol absorption network was built. Furthermore, in an intervention study, the effects of a non-nutrient that lowers intestinal cholesterol absorption on gene expression profiles were investigated. It was found that gene expression profiles of high-cholesterol and low-cholesterol absorbers were distinct after the intake of plant stanol esters. Altogether, results have contributed to a better understanding of the complex intestinal cholesterol network. In the following paragraphs, the potential impact of the main findings in terms of societal, economic, and scientific relevance will be addressed. Finally, possible implications for the translation of the findings into practice will be discussed.

Societal and economic relevance

Despite the significant advancement over the past decades in its prevention and treatment, cardiovascular disease (CVD) continues to be the primary cause of morbidity and mortality globally (1). Moreover, its burden on the EU economy is substantial and - according to estimations of the European Heart Network - imposes annually an economic burden of over €200 billion (2). A well-known risk marker for the development of CVD is the concentration of cholesterol in the blood. Many processes play a role in cholesterol homeostasis, including intestinal cholesterol absorption. It is widely recognized that the amount of cholesterol absorbed by the small intestine differs between individuals due to various factors, including genetic background (3). In fact, individuals can be categorized as high-cholesterol or low-cholesterol absorbers, and individuals with a high cholesterol absorption rate may exhibit a more favorable response to interventions aimed at reducing intestinal cholesterol uptake compared to those with a low cholesterol absorption rate (4). Importantly, the rate of

cholesterol absorption has been associated with different metabolic diseases (5). Therefore, it is important to understand in more detail 1) the mechanism of intestinal cholesterol absorption and 2) associations between genetic variants related to the amount of cholesterol absorbed.

Translation into practice

Currently, there is a high demand for tools and methods that can support and enhance the interpretation of the physiological and metabolic effects of carrying genetic variants, particularly SNPs. These SNPs are common genetic variations found in the human genome and are crucial for the emerging field of precision nutrition and medicine. As the business activity surrounding genetic testing rapidly grows, numerous companies are providing genetic tests for various purposes, surpassing the traditional roles of healthcare professionals. Therefore, the need for robust tools and methods to analyze and understand the biological implications of genetic variants under a non-business influence is of utmost importance.

Healthcare professionals can benefit from a better understanding of the mechanism of intestinal cholesterol absorption and the relationship between genetic variants with intestinal cholesterol absorption. With the knowledge that high levels of LDL-C have limited life quality and increase the chance to develop different metabolic diseases, the practical need to understand this variation is crucial. This dissertation now provides evidence that genetic variation affects intestinal cholesterol absorption. However, it is unclear if these SNPs also link to the efficacy of LDL lowering interventions. If not, it is even possible that the characteristic of having a high cholesterol absorption is a risk factor independent of (changes in) serum LDL cholesterol concentrations. To date, however, there is no (genetic) test that differentiates high-cholesterol absorbers from those of low-cholesterol absorbers. Finding a tool that can distinguish these two groups from each other may help in the future healthcare professionals to optimize the treatment for individuals using precision nutrition and medicine. Since genetic tests like SNPs are more robust and easier to interpret as compared to analyzing serum non-cholesterol sterols, this may be a more suitable strategy. Therefore, a validated SNP or set of SNPs that can be linked to these characteristics is a promising tool for future precision nutrition approaches.

Scientific relevance

Other researchers can build upon the scientific conclusions of this dissertation which provides a foundation for further research. However, it is crucial to critically evaluate and validate the results before drawing any definitive conclusions. Therefore, further experimental studies are needed to determine the functional effects of the identified genes and genetic variants on intestinal cholesterol absorption and on other pathways affected. For instance, to investigate the effect of particular genetic variants on cholesterol absorption, CaCo-2 cells cultured on a transwell system can be used as it serves as a model for intestinal epithelial cells. These cells can be transfected with genes of interest and used in a cholesterol absorption assay. Also, studies are needed the establish relationships between (a set of) genetic variants with SNPs LDL-C responses. Also, the causal connection between cholesterol absorption and various metabolic disease warrants further study. Our findings may ultimately contribute to improve the management of elevated blood cholesterol concentrations.

In conclusion, the research presented in this thesis has made a valuable contribution to the scientific community. The findings have undergone or are undergoing the process of publication in peer-reviewed scientific journals. Findings have also been presented at scientific meetings. As a result, the knowledge obtained is readily available to scientists, facilitating further investigations into the mechanism of intestinal cholesterol absorption and the influence of genetic variation on cholesterol absorption levels.

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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.

Samenvatting in het Nederlands

Verhoogde concentraties cholesterol in het bloed, ook bekend als hypercholesterolemie, verhogen het risico op het ontwikkelen van hart- en vaatziekten (HVZ). Vele factoren spelen een belangrijke rol bij het tot stand komen van de cholesterolconcentraties in het bloed, zoals bijvoorbeeld de hoeveelheid cholesterol die in de darm wordt opgenomen. Deze hoeveelheid varieert aanzienlijk tussen - maar minder binnen - individuen. Dit suggereert dat variaties in cholesterolopneming in de darm, ook wel cholesterolabsorptie genoemd, verband houden met verschillen in genetische achtergrond. Mensen kunnen dan ook worden gekarakteriseerd als hoog-cholesterol of laag-cholesterol “absorbers”. Dit is een belangrijk concept, omdat variatie in cholesterolabsorptie is geassocieerd met de aanwezigheid van verschillende metabole stoornissen. Ook betekent dit dat laag-cholesterol en hoog-cholesterol “absorbers” op een verschillende manier kunnen reageren op behandelingen, die erop gericht zijn om het cholesterolgehalte in het bloed te verlagen. Hoog-cholesterol “absorbers” kunnen bijvoorbeeld beter reageren op interventies die de opname van cholesterol in de darmen remmen dan laag-cholesterol “absorbers”. Het belangrijkste doel van de studies beschreven in dit proefschrift was dan ook om (i) redenen voor de grote variatie in cholesterolabsorptie tussen personen en (ii) het complexe cholesterolabsorptie-netwerk in de darm beter te begrijpen.

In **Hoofdstuk 2** werd op gestructureerde wijze een literatuuronderzoek uitgevoerd om verbanden tussen genetische variaties met de cholesterolopname in de darm in kaart te brengen. De resultaten lieten zien dat genetische varianten in zeven genen waren geassocieerd met cholesterolabsorptie; deze genen waren *ABCG5*, *ABCG8*, *ABO*, *APOE*, *MTTP*, *NPC1L1* en *LDLR*. In dit hoofdstuk werd ook een cholesterolabsorptie-netwerk gemaakt, gebruik makend van de resultaten van het literatuuronderzoek. Het bouwen van dit netwerk gebeurde met behulp van de GeneMANIA Cytoscape-plug-in. Het geconstrueerde netwerk liet zien dat de cholesterolabsorptie in de darm zeer complex is. Er werd dan ook geconcludeerd dat verder onderzoek nodig is om dit netwerk te valideren en te verbeteren, hetgeen uiteindelijk zou kunnen resulteren in een beter begrip van de verschillen in

cholesterolabsorptie tussen mensen en het adviseren van gepersonaliseerde behandelings-interventies, die erop gericht zijn om de cholesterolabsorptie te veranderen.

Single nucleotide polymorfismen (SNPs) zijn een bepaalde vorm van genetische variatie en kunnen invloed hebben op vele processen, waaronder de cholesterolstofwisseling. In **Hoofdstuk 3** en in **Hoofdstuk 4** is dan ook in een dwarsdoorsnede-onderzoek gekeken of bepaalde SNPs gerelateerd zijn aan de cholesterolopneming in de darm. In **Hoofdstuk 3** werden SNPs geselecteerd van genen, waarvan reeds bekend was dat zij coderen voor eiwitten betrokken bij de opneming van cholesterol in de darm. Omdat cholesterolabsorptie in de darm en cholesterolsynthese door het lichaam in het algemeen negatief gerelateerd zijn, werden ook SNPs in genen betrokken bij de cholesterolsynthese geselecteerd. Voor de geselecteerde SNPs werden verbanden onderzocht met cholesterolabsorptie-markers, endogene cholesterolsynthesemarkers en het cholesterolgehalte in de lage-dichtheidslipoproteïnen (LDL-C). Een SNP in *ABCG5* (rs4245786) en de tag SNP *ABCG8* (rs4245791) waren significant geassocieerd met cholesterolabsorptiemarkers. Daarentegen waren SNPs in *NPC1L1* (rs217429 en rs217416) significant geassocieerd met de cholesterolsynthese door het lichaam. Ten slotte waren de tag SNP in *HMGCR* (rs12916) en een SNP in *LBR* (rs12141732) significant geassocieerd met serum LDL-C concentraties. Opmerkelijk was dat die andere SNPs in de cholesterolabsorptie- of cholesterolsynthese-genen niet geassocieerd waren met serum LDL-C-concentraties.

In **Hoofdstuk 4** zijn associaties van een groot gegevensbestand met meer dan 160.000 SNPs met markers voor de absorptie van cholesterol in de darm bestudeerd. Hiervoor is de associatie tussen totaal cholesterol-gestandaardiseerde (TC-gestandaardiseerde) campesterol- en sitosterolniveaus (gevalideerde markers voor de absorptie van cholesterol in de darm) bestudeerd bij 457 personen van Europese afkomst. Alleen die SNPs die een associatie vertoonden met zowel campesterol als sitosterol werden als relevant beschouwd. Deze SNPs bevonden zich in of tussen de genen *ABCG8*, *EIF2B5*, *EPHB3*, *C4orf26*, *CDKL2*, *NR3C2*, *LOC285626*, *BMP6*, *HLA-G*, *HLA-H*, *WBSCR27*, *WBSCR28*, *TMTC4* en *COL4A2*. Deze genen werden gebruikt om een eiwit-eiwit-interactienetwerk (PPI-netwerk) te construeren, dat kon worden gekoppeld aan 30 unieke WikiPathways. Deze studie suggereerde dat nog vele

genen en pathways mogelijk geassocieerd zijn met de absorptie van cholesterol in de darm, hetgeen verder onderzoek rechtvaardigt.

Ten slotte werden in **Hoofdstuk 5** genexpressieprofielen geanalyseerd van een gerandomiseerde, dubbelblinde cross-overstudie met deelnemers van Europese afkomst. De verschillend tot expressie gebrachte genen (Differentialy Expressed Genes: DEGs) van deelnemers die vooraf waren geclassificeerd als hoog-cholesterol of laag-cholesterol absorbers, werden geanalyseerd voor en na de inname van plantenstanolesters. Dit zijn stoffen die de absorptie van cholesterol in de darm remmen. Hiervoor werd materiaal gebruikt afkomstig van twee verschillende delen van de dunne darm: de twaalfvingerige darm en het jejunum. In de twaalfvingerige darm werden 181 DEGs in de hoog-cholesterol absorbers en 482 DEGs in de laag-cholesterol absorbers geïdentificeerd. In het jejunum waren de overeenkomstige getallen 366 en 316 DEGs. Bovendien verschilde de genregulatie tussen de twee groepen: in het algemeen waren veranderingen in genexpressie in de tegenovergestelde richting in de laag-cholesterol absorbers en de hoog-cholesterol absorbers. De DEGs werden vervolgens gebruikt om processen te identificeren, waarvan deze genen onderdeel van uitmaakten. Hiervoor werd gebruik gemaakt van WikiPathways, KEGG en Reactome. Uit deze studie blijkt duidelijk dat veranderingen in genexpressieprofielen verschillen tussen proefpersonen die a priori waren gedefinieerd als lage of hoge cholesterolabsorbers. Deze verschillen leiden tot een beter begrip van de moleculaire kenmerken van lage en hoge cholesterol absorbers, voor en na blootstelling aan een interventie die de opname van cholesterol in de darm verandert. Volgende stappen moeten uitwijzen of deze patronen ook kunnen worden gebruikt om de oorzaak van metabole ziekten die mogelijk verband houden met een veranderde cholesterolabsorptie beter te begrijpen.

Samenvattend kan worden gesteld dat de studies beschreven in dit proefschrift hebben bijgedragen aan een beter begrip van factoren die verschillen in cholesterolabsorptie in de darm van gezonde mensen kunnen verklaren. In hoeverre bevindingen ook van toepasing zijn op andere populaties en kunnen worden bevestigd in grotere cohorten, verdient verder onderzoek. Ook is de meerderheid van de geïdentificeerde SNPs of genen die deel uitmaakten van de gecreëerde netwerken niet eerder in verband gebracht met de cholesterolabsorptie in

de darm. Toekomstige studies moeten dus worden uitgevoerd om een beter begrip te krijgen van de relatie tussen deze genen en de absorptie van cholesterol in de darmen.

في الختام، قد أسهمت هذه الدراسة في تعميق فهمنا لشرح الاختلاف الكبير بين الأفراد في امتصاص الكوليسترول عن طريق الأمعاء الدقيقة لدى الأفراد الأصحاء. إن مدى تطبيق النتائج على السكان الأخرين وتأكيدها في مجموعات أكبر يستدعى دراسات إضافية. علاوة على ذلك، معظم ال SNPs أو الجينات المحددة التي كانت جزءا من الشبكات المنشأة لامتصاص الكوليسترول في الأمعاء الدقيقة لم ترتبط بهذا الامتصاص من قبل. وبالتالي، ينبغي إجراء دراسات مستقبلية للحصول على فهم أعمق للعلاقة بين هذه الجينات وامتصاص الكوليسترول عن طريق الأمعاء الدقيقة.

لقد تبين أن SNP في (rs4245786) *ABCG5* و ال (rs4245791) *ABCG8* tag SNP مرتبطة بشكل ملحوظ بعلامات امتصاص الكوليسترول عن طريق الأمعاء. بالمقابل، كانت SNPs في (rs217416, rs217429) *NPC1L1* مرتبطة بشكل ملحوظ بتخليق الكوليسترول الذاتي. واخيراً, تم ارتباط ال tag SNP في (rs12916) *HMGCR* و (rs12141732) *LBR* بتركيزات الليبوبروتين ذو الكثافة المنخفضة في الدم. على كلا، بقية SNPs في الجينات التي تشفر البروتينات المشاركة في امتصاص الكوليسترول عن طريق الأمعاء الدقيقة، أو تخليق الكوليسترول الذاتي لم تكن مرتبطة بتركيزات الليبوبروتين ذو الكثافة المنخفضة.

في **الفصل الرابع**، تم تحليل قاعدة بيانات كبيرة تحتوي على أكثر من 1600 SNPs ودراسة علاقتها بعلامات امتصاص الكوليسترول في 457 فردا من أصل أوروبي. تم اختبار مستويات الكامبيسترول والسيتوسترول المعيارية الكلية للكوليسترول، وهي علامات معتمدة لامتصاص الكوليسترول عن طريق الأمعاء الدقيقة. تم فقط اعتبار ال SNPs التي أظهرت ارتباطا متوافقا في كلا العلامات (الكامبيسترول والسيتوسترول المعيارية الكلية للكوليسترول) بارتباطها بامتصاص الكوليسترول. هذه ال SNPs وجدت في أو بين الجينات التالية: *ABCG8, EIF2B5, EPHB3, C4ORF26, CDKL2, NR3C2, LOC285626, BMP6, HLA-G, HLA-H, WBSCR27, WBSCR28, TMTC4, COL4A2*. تم استخدام هذه الجينات لإنشاء شبكة تفاعل البروتين-البروتين (PPI)، والتي أمكن ربطها بثلاثين مسارا فريدا في ويكيباثويس. أبرزت هذه الدراسة اكتشاف العديد من الجينات والمسارات الغير مكتشفة بعد والتي قد تكون مرتبطة بامتصاص الكوليسترول عن طريق الأمعاء الدقيقة وتستحق مزيدا من الاستقصاء.

في **الفصل الخامس** تم تحليل التنميط التعبير الجيني لدراسة سابقة لأفراد ينحدرون من أصول أوربية، واعتمدت هذه الدراسة على تصادف عشوائي وتعمية مزدوجة للمشاركين. تم تحليل الجينات المعبرة بشكل مختلف (DEGs) في المشاركين المصنفين مسبقا كممتصي كوليسترول عالي وممتصي كوليسترول منخفض قبل وبعد تناول استرات الستانول النباتية، والتي تعرف بفاعليتها في تثبيط امتصاص الكوليسترول عن طريق الأمعاء الدقيقة. تم تحليل الجينات في جزئين من الأمعاء الدقيقة، وهما: الاثنا عشر والصائم. في الاثنا عشر، تم تحديد 181 جينا معبرا بشكل مختلف في ممتصي الكوليسترول العالي و 482 جينا في ممتصي الكوليسترول المنخفض. في الصائم، كانت الأعداد المقابلة 366 و 316 DEGs. بشكل عام كانت التغيرات في تعبير الجينات في اتجاه معاكس بين ممتصي الكوليسترول المنخفض وممتصي الكوليسترول العالي. تم استخدام ال DEGs الناتجة من هذه الدراسة لتحليل الإثراء باستخدام ويكيباثويس، وكيغ، ورياكتوم. من هذه الدراسة يتضح ان استجابات التنميط التعبير الجيني تختلف بين الأفراد المصنفين مسبقا كممتصي كوليسترول منخفض أو مرتفع. هذه الاختلافات توفر مؤشرات لفهم أعمق للخصائص الجزيئية للأمعاء المتعلقة بامتصاص الكوليسترول المنخفض مقارنة بامتصاص الكوليسترول المرتفع، قبل وبعد التعرض لتدخل يقلل من امتصاص الكوليسترول في الأمعاء الدقيقة. على كلا هناك حاجة للمزيد من البحوث لإمكانية استعمال هذه النتائج لفهم أفضل لمسببات الأمراض الأيضية التي قد تكون مرتبطة بامتصاص الكوليسترول عن طريق الأمعاء الدقيقة.

ملخص الأطروحة

إن التركيزات المرتفعة في الدم، والمعروفة أيضًا باسم فرط الكوليسترول في الدم، تزيد من خطر الإصابة بأمراض القلب والأوعية الدموية. توجد عدة عوامل تؤثر في مستوى الكوليسترول في الدم، ومن أحد هذه العوامل هي امتصاص الكوليسترول عن طريق الأمعاء الدقيقة. إن كمية امتصاص الكوليسترول عن طريق الأمعاء الدقيقة تختلف بين الأفراد، ولكنها شبه غير متغيرة لنفس الفرد. وهذا يشير إلى أن التباينات في امتصاص الكوليسترول ترتبط بالفروق في الخلفية الوراثية. وبناء على ذلك يمكن تصنيف الأفراد إما أفراد ذو امتصاص عالي للكوليسترول، أو أفراد ذو امتصاص منخفض للكوليسترول. إن هذا المفهوم مهم جدا، حيث أن الاختلاف في امتصاص الكوليسترول يرتبط بالاضطرابات الأيضية. كما يمكن تصور أن الأفراد ذوي امتصاص عالي أو منخفض للكوليسترول عن طريق الأمعاء الدقيقة، يستجيبون بشكل مختلف لعلاجات خفض الكوليسترول. فمثلاً، قد يستجيب الأفراد ذو الامتصاص العالي للكوليسترول بشكل أفضل للتدخلات التي تمنع امتصاص الكوليسترول في الأمعاء الدقيقة مقارنة بالأفراد ذوي الامتصاص المنخفض. لذلك، كان الهدف الرئيسي لهذه الأطروحة: أولا فهم أسباب هذا التباين بين الأفراد في امتصاص الكوليسترول عن طريق الأمعاء الدقيقة، وثانياً فهم الشبكة المعقدة لامتصاص الكوليسترول عن طريق الأمعاء الدقيقة.

في **الفصل الثاني**، تمت مراجعة العلاقة بين الاختلافات الجينية وامتصاص الكوليسترول بطريقة منهجية. من هذه الدراسة المنهجية تبين أن الاختلافات الجينية في سبع جينات كانت مرتبطة بامتصاص الكوليسترول عن طريق الأمعاء الدقيقة، وهذه الجينات هي: *ABCG5*, *ABCG8*, *ABO*, *APOE*, *MTTP*, *NPC1L1*, *LDLR* في هذا الفصل، تم أيضا انشاء شبكة لامتصاص الكوليسترول عن طريق الأمعاء الدقيقة باستخدام هذه الجينات السبع بمساعدة GeneMania Cytoscape. لقد كشفت هذه الشبكة عن الطبيعة المعقدة لامتصاص الكوليسترول عن طريق الأمعاء الدقيقة. وتم استنتاج انه هناك حاجة ماسة للتحقق من صحة هذه الشبكة وتطويرها، والذي بدوره سيساعد في فهم أفضل لاختلافات معدلات امتصاص الكوليسترول وصياغة تدخلات علاجية شخصية.

تم ربط العلاقة بين تعدد أشكال النيوكليوتيد المفردة SNPs في بعض الجينات بأيض الكوليسترول وقد تفسر هذه SNPs جزئيا التباين الفردي الكبير في امتصاص الكوليسترول عن طريق الأمعاء الدقيقة. في **الفصل الثالث والرابع**، تمت دراسة العلاقة بين ارتباط SNPs وامتصاص الكوليسترول عن طريق الأمعاء في دراسة عرضية. أولاً في **الفصل الثالث**، تم اختيار SNPs في الجينات التي تشفر البروتينات المشاركة في امتصاص الكوليسترول عن طريق الأمعاء الدقيقة، ونظرا لأن امتصاص الكوليسترول وتخليق الكوليسترول الذاتي عموماً مترابطان ترابطا سلبيا، تم أيضا اختيار SNPs للجينات المشاركة في تخليق الكوليسترول الذاتي. لهذه SNPs المختارة، تم حساب العلاقة بين علامات امتصاص الكوليسترول عن طريق الأمعاء، وعلامات تخليق الكوليسترول الذاتي و كوليسترول الليبوبروتين ذو الكثافة المنخفضة في الدم LDL-C.

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About the author

Fatma Bashir Abulgasem Mokhtar was born on the 31st of December 1987 in Al Zawia, Libya. After graduating from high school Life Sciences (Al Whda, Al Zawia) in 2006, Fatma started her Bachelor of Pharmacy at the University of Zawia. Her bachelor's thesis focused on assessing the understanding of Attention Defect Hyperactivity Disorder (ADHD) in children among pediatricians and psychiatrists in Libya under the supervision of Dr. Abdulbaset Elfituri. After obtaining her bachelor's degree in 2011, she worked as a pharmacology lab assistant at the College of Pharmacy at Zawia University. In 2016, Fatma continued her education in the Netherlands, where she obtained her master's in biomedical sciences at Maastricht University at the Faculty of Health Medicine and Life Sciences. She did her final internship at the Department of Nutrition and Movement Sciences at Maastricht University under the supervision of dr. Herman E. Popeijus and prof. dr. Jogchum Plat. She was incorporated in a project that examined the impact of short-chain fatty acids on the inflammatory pathways in relation to ApoA-I transcription in inflamed hepatic cells.

After obtaining her master's degree in 2018, Fatma started her Ph.D. at the Department of Nutrition and Movement Sciences at Maastricht University under the supervision of prof. dr. Ronald P. Mensink and prof. dr. Jogchum Plat. Her Phd. research focused on Intestinal cholesterol absorption in humans, particularly emphasizing genetic variation and molecular pathways.

List of publications

Tayyeb JZ, Popeijus HE, Mensink RP, Konings MC, **Mokhtar FBA**, Plat J. Short-Chain Fatty Acids (Except Hexanoic Acid) Lower NF-kB Transactivation, Which Rescues Inflammation- Induced Decreased Apolipoprotein A-I Transcription in HepG2 Cells. Int. J. Mol. Sci. 2020. [doi: 10.3390/ijms21145088].

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