Genetic Disorders of Lipoprotein Metabolism: Diagnosis and Management

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Abstract

Disorders of lipoprotein metabolism are major contributors to cardiovascular disease (CVD). Dyslipidemia refers to elevated LDL-C levels, triglycerides, or remnant cholesterol, and decreased HDL-C. Most cases of CVD are multifactorial and/or polygenic in origin. However, (mono)genic causes can be suspected in individuals with early-onset CVD or with specific clinical hallmarks. The first step in the diagnostic workup of dyslipidemias is to exclude secondary dyslipidemias by obtaining a medical history and through biochemical testing. Specialized biochemical tests or genetic tests can often help in establishing a definite diagnosis.

Treatment consists of lifestyle modifications, usually in combination with pharmacological agents such as statins. However, advances in gene technologies have enabled a rapid increase in the repertoire of available treatment options.

This chapter provides an extensive overview of lipoprotein metabolism, followed by an overview of mono- and polygenic disorders of lipoprotein metabolism, including underlying causes, clinical- and diagnostic characteristics, and available treatment options.

Abbreviations

ABC	Adenosine triphosphate (ATP) binding cassette
ACAT	Acyl-coenzyme A: cholesterol O-acyltransferase
Apo-A1	Apolipoprotein A1
BASs	Bile acid sequestrants
CAD	Coronary artery disease
CE	Cholesterylester
CETP	Cholesterylester transfer protein
cIMT	Carotid intima media thickness
CHD	Coronary heart disease
CVD	Cardiovascular disease
EMA	European Medicines Agency
FCH	Familial combined hyperlipidemia
FD	Familial dysbetalipoproteinemia
FDA	Food and Drug Administration
FDB	Familial defective apolipoprotein B
FH	Familial hypercholesterolemia
FHTG	Familial hypertriglyceridemia
HDL-C	High-density lipoprotein cholesterol
HL	Hepatic lipase
HMG-CoA	3-Hydroxyl-3-methylglutaryl coenzyme A
IDL	Intermediate-density lipoprotein
LCAT	Lecithin: cholesteryl acyltransferase
LDL-C	Low-density lipoprotein cholesterol
LDL-R	Low-density lipoprotein receptor
LDLRAP	LDL-receptor-adapting protein
LIPC	Gene-encoding hepatic lipase
LIPG	Gene-encoding endothelial lipase
LPL	Lipoprotein lipase
NPC1L1	Niemann-Pick C1 like 1
PCSK9	Proprotein convertase subtilisin/kexin type 9
PLTP	Phospholipid transfer protein

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RCT	Reverse cholesterol transport
SNP	Single nucleotide polymorphism
SR-B1	Scavenger receptor B1
TC	Total cholesterol
VLDL	Very low-density lipoprotein

Introduction

Atherosclerosis, leading to ischemic manifestations in different vascular beds, is the leading cause of morbidity and mortality worldwide. It is a multifactorial disease, driven by a combination of genetic, environmental, and behavioral factors. The process of atherosclerosis accelerates in the presence of classical risk factors such as *dyslipidemia*, hypertension, diabetes mellitus, obesity, and smoking. Dyslipidemia is one of the major contributors to atherosclerosis and includes both elevated low-density lipoprotein cholesterol (*LDL-C*) and remnant cholesterol levels, as well as decreased high-density lipoprotein cholesterol (*HDL-C*) levels [1]. The crucial role of increased plasma LDL-C levels in the pathogenesis of atherosclerosis has been well established. This also applies to the pharmacological reduction of plasma LDL-C levels accomplished by 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or *statins*. A large prospective meta-analysis including over 90,000 individuals demonstrated that an LDL-C reduction of 1 mmol/L is associated with a 21 % reduction in major cardiovascular events [2]. In addition, decreased plasma HDL-C levels are an independent predictor of cardiovascular disease (CVD), as has been unequivocally established by numerous epidemiological studies. Almost 40 % of patients with premature coronary artery disease (CAD) have low HDL-C levels, either alone or in conjunction with hypertriglyceridemia or combined hyperlipidemia [3]. Furthermore, it has been estimated that each 0.03 mmol/L (1 mg/dL) increase in HDL-C is associated with a 2 % reduction CAD risk in men and a 3 % reduction in women [4]. However, whether raising HDL-C by pharmacological means will result in cardiovascular benefit is questionable. A recent meta-regression analysis of 108 randomized controlled trials, including more than 300,000 patients using several lipid-modifying interventions, did not show a relationship between treatment-induced increases in HDL-C and a decrease in coronary heart disease events or deaths when corrected for concurrent LDL-C reductions [5]. Nevertheless, this study does not prove that increasing HDL-C in selected patients with low HDL-C levels has no value [6]. In addition, these studies evaluated only HDL-C concentrations and did not address HDL functionality.

Finally, *hypertriglyceridemia* also influences CVD risk. Several epidemiological and genetic studies have indicated elevated plasma triglyceride levels as an independent risk factor for CVD [7–13]. This also applies to remnant cholesterol [11]. For individuals with high triglycerides in the general population, the risks for myocardial infarction, ischemic heart disease, ischemic stroke, and all-cause mortality are significantly increased [11].

Although dyslipidemia has a largely polygenic background, a number of *monogenetic disorders* have been identified. This chapter provides an overview of genetic causes underlying disturbances in lipid and lipoprotein metabolism, in which the focus will be primarily on these monogenetic disorders. The chapter starts with a global overview of lipid and lipoprotein metabolism, followed by the genetic background of disturbances in LDL-C and HDL-C levels, respectively. Finally, genetic causes of disorders in triglyceride metabolism are also discussed. For each of these categories, genetics, clinical phenotype, diagnosis, and management will be addressed.

Structure of Lipids and Lipoproteins

Cholesterol and *triglycerides* exert essential functions in body cell membranes and in hormone and energy homeostasis. Due to their hydrophobic properties, cholesterol and triglycerides are transported in large macromolecular complexes, the so-called lipoproteins. *Lipoproteins* contain a core of hydrophobic lipids surrounded by hydrophilic molecules such as phospholipids, unesterified cholesterol, and *apolipoproteins*. The latter are proteins that provide structural integrity to the lipoprotein and serve as ligands for binding to specific receptors. Based on their relative density, lipoproteins can be categorized into five major classes: chylomicrons, very low-density lipoproteins (*VLDL*), intermediate-density lipoproteins (*IDL*), *LDL*, and *HDL*. The first two categories are large, buoyant triglyceride-rich particles, whereas the latter three are dense, cholesterol-rich particles. When fasting, plasma cholesterol levels are usually a reflection of the amount of LDL in the plasma, whereas plasma triglyceride levels reflect the amount of VLDL.

Lipid and Lipoprotein Metabolism

The liver and the intestine are the most important sources of lipoproteins. Their transport and metabolism is generally divided into three systems: absorption of exogenous and endogenous lipids and lipoproteins, endogenous synthesis of lipids and lipoproteins, and *reverse cholesterol transport (RCT)*. These processes are depicted in Fig. 21.1.

Absorption of Exogenous and Endogenous Lipids

The average Western diet consists of a daily intake of approximately 100 g of fat and 500 mg of cholesterol. Phospholipids

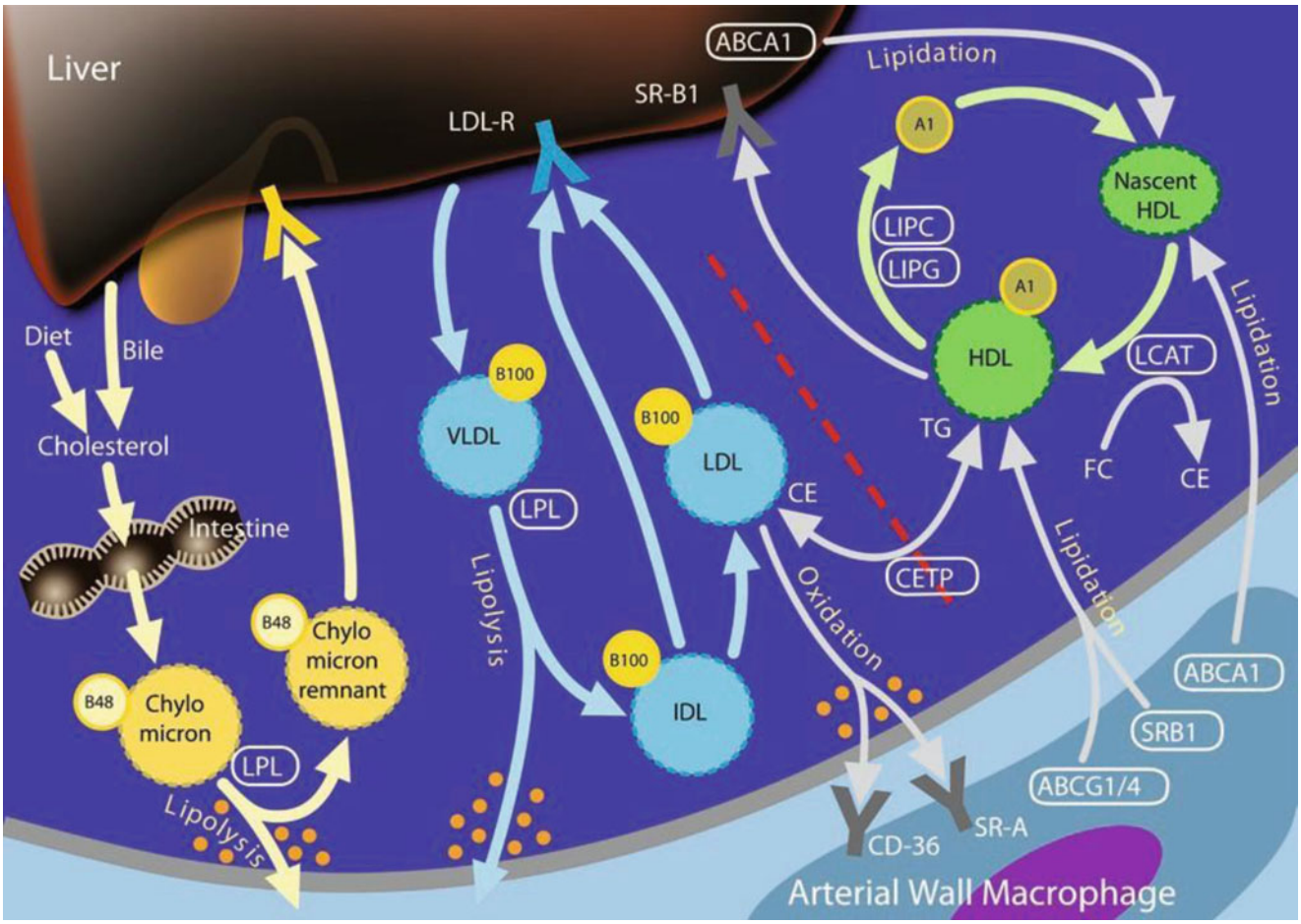

Fig 21.1 Overview of lipoprotein metabolism. Dietary lipids and cholesterol from the hepatic bile are absorbed in the intestine, packaged into chylomicrons, and secreted into the lymph, which drains into the systemic circulation. In the bloodstream, the triglyceride-rich (*TG*) chylomicrons are hydrolyzed through the action of lipoprotein lipase (*LPL*) and the removed TGs and free fatty acids are taken up by extrahepatic tissues such as the liver and muscles. The chylomicrons remnants are taken up by the liver for further processing. In the fasting state, the liver assembles TG-rich very low-density lipoprotein (*VLDL*). Also VLDL are hydrolyzed by LPL and thereby transformed to smaller VLDL remnants, IDL. Half of the IDL are directly taken up by the liver through binding of the LDL-R, whereas the other half is converted to cholesterol-rich LDL. Most of the plasma LDL-C is cleared from the circulation by binding to the LDL-R of the liver. Of the remaining LDL, some subfractions are especially prone to oxidative modification and then taken up by scavenger receptors (CD-36) of arterial wall macrophages resulting in foam cells and atherosclerotic plaques. High-density lipoprotein (*HDL*) is responsible for the RCT from extrahepatic tissues to the liver. Nascent HDL is formed from lipid-poor Apo-A1, which is secreted by the liver and intestine and which is lipidated through interaction with ABCA1. Nascent HDL is also generated from surface components shed during lipolysis of TG-rich lipoproteins by LPL (not depicted). After lipidation, LCAT esterifies free cholesterol (*FC*) to cholesterylesters (*CE*) which migrates into the core of the HDL making them larger spherical particles. These larger HDL particles acquire additional lipids from extrahepatic tissues, including arterial wall macrophages, by receptor-mediated pathways such as ABCG1, ABCG4, and SR-B1, as well as from lipolysis of TG-rich lipoproteins and passive diffusion (not depicted). The HDL particles can be metabolized in several ways. First, they can deliver CE to the liver by binding to SR-B1 on the hepatocyte surface. In the liver, the cholesterol can be processed and eliminated. Alternatively, CE in HDL can be exchanged for TG in apo-B-containing lipoproteins, by the action of CETP. The TG-enriched HDL is hydrolyzed by LIPC and LIPG to smaller HDL and lipid-poor Apo-A1 particles. These can be either recycled to acquire cholesterol or excreted from the body through the kidneys

and bile acids, present in hepatic bile, emulsify lipids from food to form micelles within the intestinal lumen. Hepatic bile also delivers significant amounts of unesterified cholesterol to these micelles.

Pancreatic lipases secreted into the intestinal lumen digest dietary lipids to chemical entities that can be absorbed by enterocytes. Fatty acids and monoacylglycerides are almost entirely absorbed through both passive diffusion and carrier-mediated processes [14]. By contrast, *cholesterol absorption* is an active process, mediated by several transporter proteins which are located at the intestinal brush-border membrane. Cholesterol and sterols derived from plants are taken up by the enterocyte through the recently identified Niemann-Pick C Like 1 (NPC1L1) transporter [15], whereas the ATP-binding cassette transporters (ABC) G5 and G8 actively secrete plant sterols, and to a lesser extent cholesterol, back into the intestinal lumen [16]. Of note, NPC1L1 and ABCG5 and G8 are also located in the liver, where they are involved

in hepatic cholesterol trafficking to the bile [16, 17]. Intestinal cholesterol absorption exhibits on average about 50 % efficiency, with large interindividual variation, ranging from 20 % to 80 % [18]. Free cholesterol that has entered the enterocyte is either reesterified intracellularly by Acyl-coenzyme A: cholesterol acyltransferase (ACAT) 2 and then packaged into chylomicrons, or trafficked toward the basolaterally located ATP-binding cassette transport protein A1 (ABCA1) protein for HDL formation.

Chylomicrons consist for approximately 80–95 % of triglycerides and apolipoprotein B48 (apo-B48) as their structural surface protein. They are secreted into the lymph, which drains directly into the systemic circulation. In the bloodstream, chylomicrons are hydrolyzed, that is, triglycerides and free fatty acids (FFAs) are removed from the core of the chylomicrons, by *lipoprotein lipase (LPL)*, thereby generating remnant particles. LPL is anchored to the endothelial surface by proteoglycans and/or by the recently identified anchoring protein GPIHBP1 (glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1) [19]. LPL requires apolipoprotein CII as a cofactor for adequate hydrolysis. The removed triglycerides and FFAs are taken up by the liver and muscle, whereas the chylomicrons remnants are taken up by the liver for further processing, as described below. Chylomicrons have a short half-life in the circulation, averaging approximately 10–20 min, provided that clearance is undisturbed. Hence, chylomicrons are not present in the bloodstream in the fasting state. However, when postprandial levels of chylomicrons and their remnants remain high, due to intestinal overproduction or delayed clearance, this can promote delivery of chylomicrons and their remnants to the arterial endothelium, with subsequent generation of *foam cells* and fatty streaks and eventually *atherosclerotic plaque formation*.

Endogenous Synthesis of Lipids and Lipoproteins

In the fasting state, the liver assembles VLDL-C by combining triglycerides, phospholipids, apolipoprotein B100 (apo-B100), and cholesterylesters (CEs). The latter originate either from de novo synthesis and subsequent esterification by ACAT2 or from remnant particles that have been taken up from the circulation. Like chylomicrons, VLDLs are triglyceride-rich particles secreted into the bloodstream, where they are hydrolyzed by LPL and thereby transformed to smaller and denser VLDL remnants, IDL, and finally LDL particles. In general, half of the VLDL remnants are directly taken up by the liver through binding to the *LDL receptor* (LDL-R), whereas the other half is converted to LDL.

LDL is the most abundant cholesterol-carrying particle in humans and accounts for more than 75 % of plasma cholesterol. Mediated by *apo-B100*, most of plasma LDL is cleared from the circulation by the LDL-R, which is located at the surface of hepatocytes and internalized entirely (lipoprotein + receptor) upon binding of LDL. The remaining LDL particles are delivered to peripheral tissues such as the adrenals and gonads for the synthesis of steroid and sex hormones. In hepatic endosomes, LDL is degraded to amino acids and free cholesterol, whereas the LDL-R is scavenged back to the cell surface for the uptake of additional LDL particles. Approximately 70–80 % of the LDL catabolism takes place via the LDL-R. The remaining part is cleared via non-specific routes.

The recently identified proprotein convertase subtilisin/kexin type 9 (*PCSK9*) protein plays a pivotal role in LDL metabolism by promoting degradation of LDL-R instead of recycling it back to the cell surface, thereby reducing the number of available LDL receptors at the surface of hepatocytes [20]. It primarily does so by acting on the LDL receptor as a secreted factor and the expression is – similar to LDL-R – modulated by intracellular cholesterol levels. Because of this effect of cholesterol at the transcriptional level, statins increase *PCSK9* expression, thereby partially counteracting their effect in terms of upregulating LDL receptor expression. Therefore, inhibiting PCSK9 could lower LDL-C levels by increasing the available pool of LDL receptors and work synergistically with statins. PCSK9 inhibitors have emerged as the prime candidate to further reduce CVD risk, as will be discussed in section, "Management."

Finally, LDL is not a homogeneous lipoprotein fraction, as it consists of several subfractions with varying mass and density. *Small-dense LDL* is particularly associated with atherosclerotic disease. This subfraction is mostly prevalent in subjects with elevated triglyceride levels. Small-dense LDL particles are prone to oxidative modification, resulting in uptake by scavenger receptors of arterial macrophages, which express a strong affinity for these so-called ox-LDL particles. Since a negative feedback system for these scavenger receptors is lacking, unlimited amounts of ox-LDL can be taken up by these macrophages, which transform into foam cells and atherosclerotic plaques.

HDL Metabolism and RCT

HDL is a highly heterogeneous class of lipoprotein particles that differ in protein component and lipid composition, size, shape, density, and charge. In addition to the observational support for the atheroprotective role of HDL, numerous *in vitro* and *in vivo* animal studies have demonstrated various mechanisms through which HDL exerts its beneficial effects on the arterial wall. The most widely acknowledged mechanism is its role in *RCT*. This involves the ability of HDL to

stimulate efflux of cholesterol from peripheral tissues, transport in the plasma, and uptake by the liver, followed by biliary excretion and elimination via the feces. Specifically, the efflux of cholesterol from macrophage foam cells in the artery wall is thought to be central to the antiatherogenic properties of HDL. In addition, putative atheroprotective properties of HDL include its ability to improve endothelial function, inhibit LDL oxidation, and induce several anti-apoptotic, anti-inflammatory, and antithrombotic effects. However, whether stimulation of these processes results in clinical benefit in humans is as of yet not resolved [21].

The process of RCT starts by lipidation of *apolipoprotein A1* (apo-A1) through interaction with the ABCA1. Apo-AI is the most important structural protein of HDL and comprises approximately 70 % of the proteins in HDL-C. It is synthesized by the liver and intestine and released into the circulation either in a free non-lipidated form or incorporated in small discoid particles, rich in phospholipids and poor in cholesterol, the so-called nascent or pre-β-HDL. Nascent HDL is also generated from redundant surface components shed during lipolysis of triglyceride-rich lipoproteins such as chylomicrons and VLDL by LPL. The *ABCA1 transporter* resides at the cellular membrane and facilitates the transfer of free cholesterol and phospholipids from intracellular lipid pools to apo-A1. New insights suggest that, in contrast to previous opinion, hepatic ABCA1 appears to be critical for the initial lipidation of lipid-poor apo-A1, protecting it from rapid degradation and allowing it to go on to form mature HDL. Conversely, macrophage ABCA1 appears to contribute little to bulk lipidation of HDL and therefore to plasma HDL-C levels, but does seem to be important for protection against atherosclerosis [22]. After lipidation, lecithin: cholesteryl acyltransferase (*LCAT*) subsequently esterifies the externalized free cholesterol to cholesterylesters on the surface of HDL on activation by its cofactor apo-A1. The esterified cholesterol then migrates into the core of the HDL and as larger amounts of cholesterylesters become incorporated, HDL becomes a larger spherical particle. These larger so-called HDL-3 and HDL-2 particles acquire additional free cholesterol and phospholipids from extrahepatic tissues, including macrophage foam cells, by means of passive diffusion or receptor-mediated pathways, such as ABCG1, ABCG4, and scavenger receptor B1 (SR-B1), as well as from lipolysis of triglyceride-rich lipoproteins [23]. The HDL-3 and HDL-2 particles can be metabolized in several ways. First, they can directly deliver cholesterylesters to the liver by binding to *SR-B1* on the hepatocyte surface. In the liver, the cholesterol can be processed and eliminated as bile or converted to cholesterol-containing steroids. Once the HDL particle is delipidated, it dissociates from SR-B1 and can then reinitiate another cycle of RCT. Alternatively, cholesterylesters in HDL can be exchanged for triglycerides in apo-B-containing lipoproteins such as LDL, by the action of *cholesterylester transfer protein (CETP)*, after which these cholesterylesters are available for hepatic clearance via the LDL receptor. However, if a population of apo-B-containing lipoproteins enriched with cholesterylesters by CETP interacts with macrophages in arterial walls and promotes net cholesterol uptake, this process is potentially atherogenic. Whether the sum effect of CETP activity in humans is pro- or antiatherogenic is not clear. Most experimental evidence in animals favors a pro-atherogenic role for CETP. The triglyceride-enriched HDL is a substrate for hydrolysis by *hepatic lipase* (LIPC) while phospholipids are mainly hydrolyzed by *endothelial lipase* (LIPG). In this way, HDL is remodeled to lipid poor Apo-A1 and smaller HDL particles which can either be recycled to acquire cholesterol from extrahepatic tissues or dissociated apo-A1 is excreted from the body through the kidneys. *Phospholipid transfer protein (PLTP)* also plays a major role in HDL metabolism in various ways. PLTP facilitates the transfer of phospholipids from triglyceride-rich lipoproteins during lipolysis and evidence has accumulated over the years that PLTP can also remodel HDL particles [24].

Genetic Causes of Elevated LDL-C Levels

Mutations in genes involved in LDL metabolism can result in increased plasma LDL-C concentrations. Three genes have been characterized: the *LDL-R* gene, the *Apo-B* gene, and most recently the *PCSK9* gene [25]. These genes are involved in *autosomal dominant hypercholesterolemia*. The single known *autosomal recessive* form of *hypercholesterolemia* (ARH) is caused by failing internalization of the LDL-R/LDL particle complex in the hepatocytes and is caused by a mutation in the ARH gene [26, 27].

Familial Hypercholesterolemia

Familial hypercholesterolemia (FH) is the most common autosomal dominant inherited disorder of metabolism. Approximately 1:500 people are affected, resulting in almost ten million patients worldwide. In some populations, this prevalence is higher due to a founder effect [28]. Homozygosity is rare, with an average of one per 160,000–300,000, with higher frequencies in populations where a founder effect or high rates of consanguinity are present [29]. FH subjects are characterized by plasma LDL-C levels above the 95th percentile for age and gender, due to impaired internalization of LDL particles caused by functional alterations in the LDL receptor [30]. Moreover, the decrease in the hepatic cholesterol pool stimulates cholesterol synthesis, resulting in increased production of VLDL, which further increases LDL-C levels.

Genetics

The molecular defect underlying FH most often consists of a mutation in the *LDL-R gene*, located on chromosome 19p13 [30]. At present, over 1000 different mutations in the LDL-R or promoter region leading to an FH phenotype have been described [31], 91 % of which are point mutations [32].

In addition, mutations in the *PCSK9 gene* on chromosome 1 are a rare cause of the FH phenotype, accounting for <1 % of cases [33]. To date, eight hypercholesterolemic missense mutations in *PCSK9* have been reported [34]. These "gain-of-function" mutations cause hypercholesterolemia due to PCSK9-induced enhanced degradation of LDL receptors, thereby decreasing the available pool of hepatic LDL receptors. Finally, mutations in the LDL-R-binding domain of apo-B100 can result in a phenotype which resembles FH, approximately in 5 % of FH cases [33]. This is outlined in the next paragraph on familial defective apolipoprotein B (FDB).

Clinical Characteristics

A hallmark of FH is plasma LDL-C levels above the 95th percentile for age and gender. This induces accelerated deposition of cholesterol in arterial walls and other tissues, resulting in the clinical hallmarks of FH: premature atherosclerosis, *tendon xanthomas, xanthelasma*, and corneal arcus [30]. However, these clinical characteristics (Figs. 21.2 and 21.3) are not exclusively associated with or may not be present in every patient with FH. Moreover, the age of appearance of these symptoms varies depending on the severity of the phenotype. If untreated, approximately 50 % of male and 30 % of female heterozygous FH patients will develop symptomatic CVD before the age of 50 years [35]. However, the onset and progression of atherosclerotic disease varies considerably between FH individuals and within families. It was shown that event-free survival depends more on actual LDL-C levels caused by the mutation, rather than the type of mutation itself [36].

Patients with homozygous FH have plasma cholesterol levels >13 mmol/L. If untreated, patients suffer from CVD before 20 years of age and generally do not survive past 30 years of age [29].

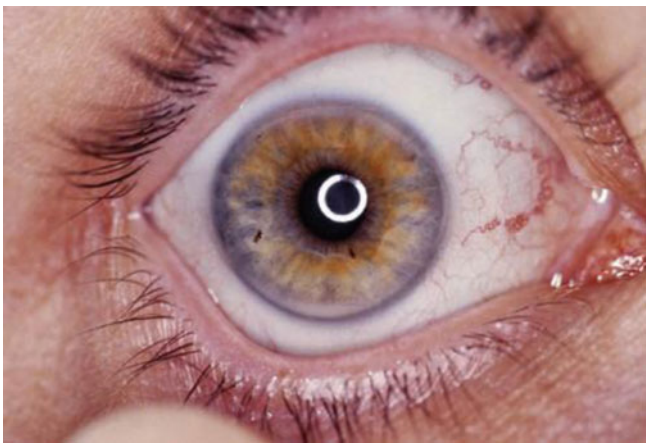

Fig. 21.3 Arcus lipoides

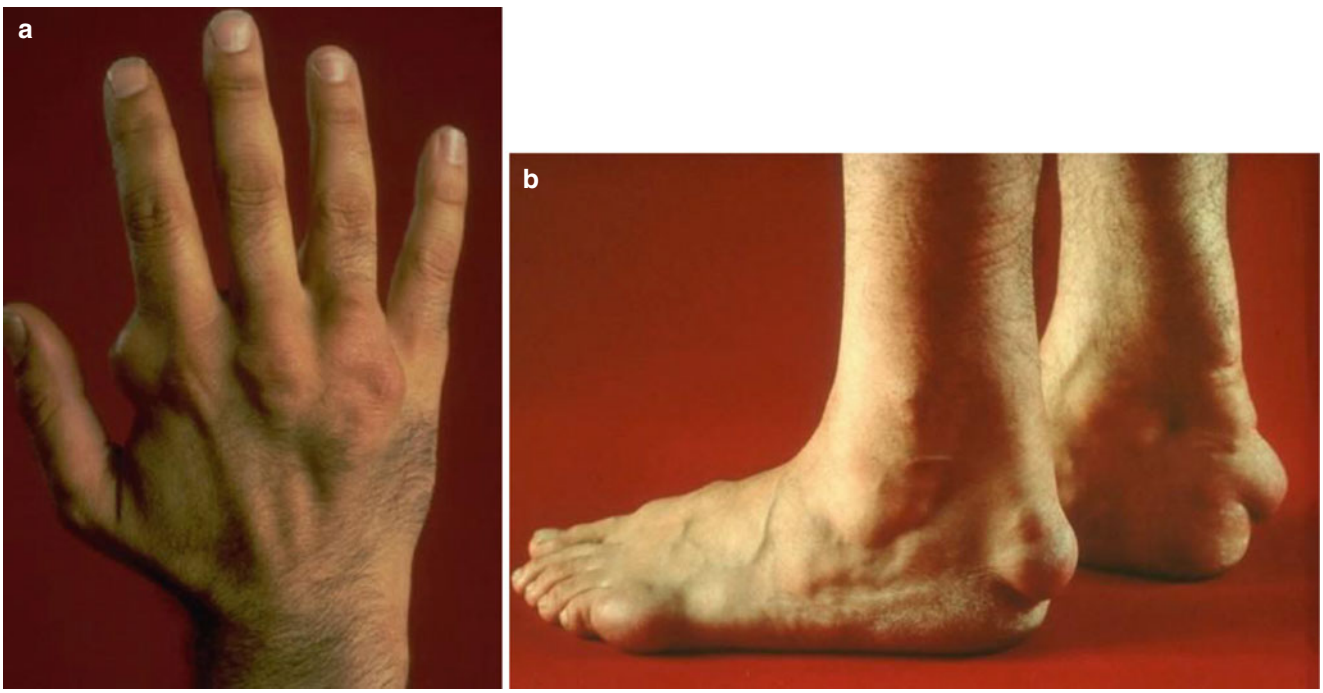

Fig. 21.2 Skin xanthomas (**a**) Xanthomas of the hand and (**b**) Achilles tendon xanthomas

Although cardiovascular events are rare in children heterozygous for FH, affected children were already shown to have an impaired endothelial function [35], as well as an increased *carotid intima-media thickness (cIMT)* [37], when compared to their unaffected siblings, which is indicative for an early onset of subclinical atherosclerosis. Based on these findings, current guidelines advise early pharmacological cholesterol-lowering in children with FH, as discussed below.

Diagnosis

FH is usually diagnosed on the basis of clinical features. Several clinical tools have been developed, with different diagnostic criteria, some of which combined with DNA analysis (reviewed in Reference ([38])). In the Netherlands, the algorithm of the Dutch Lipid Network is used, as shown in Fig. 21.4. The primary clinical diagnostic criteria are elevated LDL-C levels above the 95th percentile for age and gender, the presence of tendon xanthomata in the patient or a

Family history*	
I First-degree relative with CVD < 60 years of age	1
II First-degree relative with plasma LDL-cholesterol levels > 5 mmol/l	
III First-degree relative with an corneal arcus < 45 years and/or tendon xanthomas IV Children < 18 years of age with plasma LDL-cholesterol levels > 3.5 mmol/l	2
Personal Medical History	
I First-degree relative with CHD < 60 years of age	2
II Cerebro-vascular event or peripheral arterial disease < 60 years of age	1
Physical Examination	
I Presence of tendon xanthomas	6
II Presence of corneal arcus < 45 years of age	4
Laboratory parameters	
I LDL-cholesterol > 8.5 mmol/l	8
II LDL-cholesterol 6.5 -8.4 mmol/l	5
III LDL-cholesterol 5.0 -6.4 mmol/l	3
IV LDL-cholesterol 4.0 -4.9 mmol/l	1
FH DIAGNOSIS	
Almost certain score = 8 Likely score= 6-7 Possible score= 3-5	
Additional DNA testing is advised if the score > 6	

* In this category, only the highest applicable number should be scored; the highest score for family history is 2.

Fig. 21.4 Diagnostic algorithm for familial hypercholesterolemia

first-degree relative, and a pattern of autosomal dominant inheritance of premature coronary heart disease or hypercholesterolemia. The diagnosis can be confirmed through genetic testing of the *LDLR, APO-B, and PCSK9* genes. However, careful selection of patients is important given the costs of genetic analysis. Although the current yield is relatively low (between 54 % and 70 %), tools to aid physicians in the decision for referral are currently being developed [39].

Early diagnosis – preferably during childhood – of FH is recommendable to enable prompt treatment. Nationally organized genetic cascade-screening programs have been implemented in the Netherlands, Spain, and Wales, in addition to initiatives on a smaller scale in various other countries [40].

Management

Treatment with *high-dose statins* is currently the most effective strategy to reduce CVD risk in FH patients [41]. Lowering LDL-c levels remains the primary target for therapy. Recent studies have confirmed that the inverse relationship between LDL-c and CVD risk persists even at very low LDL-c levels, without apparent adverse effects. In response, updated clinical guidelines no longer advocate the use of LDL-c treatment targets but instead argue for treatment with high-intensity statins from the start of therapy [42]. In addition, patients are treated in combination with lifestyle modifications aimed to reduce the risk of other atherogenic factors. Finally, several new pharmacological agents have been developed to optimize cholesterol-lowering treatment in those who do not reach acceptable LDL-C levels or who are unable to tolerate high doses of statins. However, current guidelines do not recommend the routine use of nonstatin drugs in addition to high-intensity statin therapy.

PCSK9 inhibition represents the latest advancement in cholesterol-lowering drugs [43]. Although several approaches to PCSK9 inhibition are currently being developed and evaluated in clinical studies, monoclonal antibodies are most advanced in clinical development. By virtue of their ability to prevent lysosomal degradation of the LDL receptor, PCSK9 inhibitors increase the available pool of hepatic LDL receptors. Recent meta-analyses [44, 45] of clinical trials comprising >10,000 individuals have unequivocally demonstrated the efficacy of PCSK9 inhibitors in improving lipid profiles: mean LDL reductions were observed of 50 %, whereas HDL increased by 6 % and Lp(a) decreased by 26 %. Preliminary evidence suggests that these improved lipid profiles translate into improved CVD outcomes, and large-scale clinical outcome trials are currently ongoing to substantiate these findings. Importantly, PCSK9 inhibitors have shown to be generally safe and well tolerated. Based on these promising results, both the Food and Drug Administration (FDA) and European Medicines Agency (EMA) have approved alirocumab and evolocumab for several categories of patients including hetero- and homozygous FH patients who fail to meet LDL goals as well as statin-intolerant patients.

Ezetimibe is a cholesterol absorption-inhibiting compound, which acts by blocking the intestinal NPC1L1 protein. As of yet, ezetimibe is the only nonstatin drug which has been shown to improve clinical outcomes when added to statins in clinical trials (Improve it). These results were somewhat surprising, given that earlier studies, using surrogate end points by ultrasound (ENHANCE), did not find a benefit in terms of primary end points [46].

Bile acid sequestrants (BASs) bind bile acids in the intestine and subsequently increase hepatic conversion of cholesterol into bile acids. The resulting decrease in hepatic cholesterol content results in increased hepatic LDL-R expression. *Colesevelam* is a novel BAS with a more favorable side-effect profile, as it is thought to bind with higher affinity compared to other BAS [47]. It is currently being evaluated in patients with FH with higher than acceptable LDL-C levels, despite a maximally tolerated and stable dose of statin and ezetimibe. Although Colesevelam effectively lowered LDL-C levels, to date no data on clinical end points are available [46]. Moreover, it showed adverse effects similar to other BASs, including increased serum triglyceride levels and reduced intestinal uptake of several drugs, which means that these drugs should be taken more than 4 h before Colesevelam [46].

Selective inhibition of apo-B100 mRNA synthesis by *antisense oligonucleotides (ASOs)* is an entirely new approach to lower cholesterol levels. ASOs bind to a complementary mRNA sequence by Watson-Crick hybridization, resulting in selective degradation of the targeted mRNA sequence and thereby in a reduction in apo-B100 synthesis. The drug is administered subcutaneously once a week or less and induces an approximately 50 % LDL-C reduction in FH [48]. The most common adverse events are mild injection-site reactions, as well as modest increases in liver enzymes, as seen with all other lipid-lowering drugs. Mipomersen is an ASO that in 2013 was approved by the FDA for use in patients with homozygous FH (HoFH), although it is still not approved in Europe. It lowered LDL-C with 25 % in patients already receiving lipid-lowering drugs, while also reducing Apo-B and Lp(a) and having no effect on HDL-C levels. Benefits in terms of clinical outcomes remain to be confirmed in clinical trials. Side effects, including flu-like symptoms and injection-site reactions, as well as adverse hepatic effects, could reduce compliance [46].

Gene therapy is currently under investigation as treatment option in HoFH. While the first trial in the early 1990s showed disappointing results, new advances paved the road to new trials. In gene therapy, patients are being treated with a recombinant adeno-asssociated virus (AAV) vector loaded with a functional transgene, for example, an LDL-R expressing transgene. The newest vector AAV8 is of interest because of its strong liver tropism and relatively low seroprevalence in Western populations, which is important because of immune responses. Clinical testing in mice showed promising results, with a total cholesterol of 227 versus 1032 mg/dL at day 56 post-vector administration. Metabolic effects were

maintained for up to 20 weeks. Preliminary clinical trials with HoFH patients are currently ongoing. Of note, an AAV1 vector expressing LPL was the first gene therapy agent approved in the Western world to treat LPL-deficient patients (reviewed below) [49].

Lomitapide is an oral small-molecule inhibitor of the microsomal triglyceride transfer protein (MTP), which facilitates the assembly of apo-B-containing lipoproteins. This leads to reductions in lipoprotein secretion and lowers LDL-C levels. After a single-arm, open-label, 78-week phase 3 trial including 29 patients, which showed promising results [50], Lomitapide was approved for treatment of HoFH. A larger registry (LOWER) was started in 2014 to evaluate the clinical long-term safety and effectiveness, including at least 300 patients for follow-up for at least 10 years [51].

Finally, with respect to treatment of *children with FH*, several statin trials have been performed over the past decade [52]. On the basis of these studies, showing that statin treatment lowers LDL-C safely and effectively in children with FH [52, 53], and a study demonstrating reduced cIMT progression in FH adolescents on statin therapy [54], current guidelines in the USA and Europe recommend initial statin treatment in children with heterozygous FH from the age of 10 years [33]. Another international workforce published a consensus-based guideline applicable for most patients, which advocates for lifestyle modifications and consideration of the use of statin monotherapy starting from the age of 8 years and the eventual addition of ezetimibe or a BAS from the age of 10 years. For patients with homozygous FH, treatment with statins should start as early as possible [55]. In the USA, pravastatin is approved from the age of 8 years, while in Europe rosuvastatin is approved from age 6 years. Ezetimibe is approved from age 10 years in both the USA and Europe. Pediatric trials of PCSK9 inhibitors are underway or planned. In the event of homozygous FH and rapidly progressive atherosclerosis, lomitapide and mipomersen should be considered, although both drugs yet have to be tested in children, especially when apheresis is not an option [56].

In addition to pharmacological treatment of hypercholesterolemia, management should also comprise screening of first-degree relatives (referral to review [33]).

Familial Defective Apolipoprotein B

Familial Defective Apolipoprotein B (FDB) is an autosomal dominant disorder, which resembles the clinical phenotype of FH. The mechanism underlying the hypercholesterolemia is defective binding of apo-B100 of the LDL particle to the LDL receptor. The estimated prevalence is 1:500 in Central Europe and 1:700 in Northern America. Due to founder effects, prevalence up to 1:200 was observed in certain regions of Europe [57]. However, the exact prevalence remains unknown, since it frequently overlaps with that of FH, Therefore, the prevalence of the phenotype of FH due to mutations in *LDLR, PCSK9, or Apo-B100* is around 1:250.

Genetics

FDB is caused by mutations in the *apo-B100* gene located on chromosome 2p23–24. So far, 11 functional mutations at the *apo-B* locus have been identified. The R3500Q mutation is the most frequent one, with a prevalence of 1:600–700 in Caucasians [32].

Clinical Characteristics, Diagnosis, and Management

FDB is clinically indistinguishable from FH [58], although with slightly lower LDL-C levels [59]. FDB is diagnosed by genotyping or according to clinical diagnostic criteria for FH. Equal to FH, patients with FDB are treated with lipid-lowering medication combined with lifestyle modification.

Autosomal Recessive Hypercholesterolemia

ARH is the single known recessive disorder causing hypercholesterolemia. Only about 50 individuals with ARH have been identified worldwide, although the disease is not uncommon on the island of Sardinia with a frequency of 1:40.000 for homozygotes and compound heterozygotes and even 1:143 for heterozygotes [60]. In ARH, hepatic endocytosis of the LDL-R/LDL particle complex, mediated by the *LDL-R-adapting protein (LDLRAP)* is disrupted [27, 61].

Genetics

To date, 17 mutations in the *ARH* gene, located on chromosome 1p35–36.1, have been identified, the great majority being truncating mutations [26].

Clinical Characteristics

ARH is characterized by a phenotype, which resembles homozygous FH, consisting of severe hypercholesterolemia, large xanthomas, and premature CVD, although the phenotype in ARH is slightly milder, since patients tend to have higher HDL-C levels and are more responsive to lipid-lowering therapy and express a longer event-free survival when compared to homozygous FH patients [62]. The presence of residual LDL-R activity, as demonstrated in skin fibroblasts, might explain the favorable plasma cholesterol concentrations and the response to cholesterol-lowering medication in patients with ARH [63]. In general, no clinical symptoms before the age of 20 years are present in ARH. Heterozygous carriers of the *ARH* mutation have slightly elevated lipid levels within the normal range.

Diagnosis

ARH is diagnosed by genetic testing. Affected individuals meet clinical criteria for homozygous FH, as described in

section, "Clinical Characteristics"; however, based on the clinical evaluation of first-degree relatives, a lipid disorder of recessive origin, rather than homozygous FH, should be considered.

Management

Patients with ARH are sensitive to treatment with statins and a cholesterol-lowering diet [64]. A case study of a Sardinian patient treated with rosuvastatin 60 mg combined with ezetimibe showed an 81 % reduction of LDL-C. Although it is just one case, it is in line with another Lebanese patient being treated with rosuvastatin 80 mg and ezetimibe, showing a reduction of even 90 % [60].

Familial Combined Hyperlipidemia

Familial combined hyperlipidemia (FCH) is a relatively common lipoprotein disorder, with a prevalence of 1:200. The disease is based on increased *VLDL synthesis*, due to an overproduction of apo-B100, sometimes combined with delayed hepatic clearance of VLDL [65].

Genetics

Initially, FCH was considered an autosomal dominant monogenetic disorder; however, the hereditary background might be *polygenetic*, as a handful of families display a convincingly autosomal dominant mode of inheritance, whereas in others, a multifactorial basis is considered to be more likely. FCH might be best conceptualized as a phenotype with a common clinical presentation but with variable predisposing causes. Rare large-effect mutations are found in a fraction of patients, while multiple independently segregating small-effect mutations accumulate in a patient's genome, thereby raising LDL-C and triglycerides further [66].

Clinical Characteristics

FCH is phenotypically heterogeneous and, in most individuals, not manifest until adulthood. It is characterized by elevated LDL-C and/or triglyceride levels, a tendency to decreased HDL-C levels, and is often accompanied by central obesity, insulin resistance, and hypertension. In addition, there are no clinical stigmata such as in FH. Different phenotypes can be expressed within members of one affected family. In most cases, apo-B100 levels are elevated above 1.2 g/L and plasma triglycerides are mildly to moderately increased; however, cholesterol and triglyceride levels can vary over time within affected individuals. FCH patients have an increased risk of premature CVD [67].

Diagnosis

FCH is diagnosed based on a combination of plasma lipid abnormalities and a positive family history of dyslipidemia: either solitary elevated LDL-C, triglycerides or both, with or without premature CVD in the index patient or family members. A nomogram has been developed to aid physicians in estimating the probability of FCH based on clinical criteria [68].

Management

Untreated FCH patients are prone to premature CVD; therefore, aggressive lipid-lowering treatment equal to that for FH is required. Most FCH patients are treated with high-dose statins, with a target LDL-C of 2.5 or 1.8 mmol/L in subjects with CVD. In case triglyceride levels are elevated as well, patients can be treated with a *fibrate* on top of the statin, but not Gemfibrozil, since that combination increases the risk of rhabdomyolysis [69]. In addition, FCH patients should be treated with lifestyle modification, also in order to target the accompanying symptoms of obesity, insulin resistance, and hypertension. Finally, since FCH patients have functioning LDL receptors, the response to dietary interventions and pharmacological cholesterol lowering is generally better than observed in FH.

Sitosterolemia

Sitosterolemia is a rare autosomal recessive disorder characterized by premature atherosclerosis. Although hypercholesterolemia is not obligatory, elevations in LDL-C levels may be observed. The underlying defect in sitosterolemia is hyperabsorption of *plant sterols* and *sterols* and decreased biliary secretion of both cholesterol and plant sterols [70]. Plant sterols are structurally similar to cholesterol and are derived solely from the diet. Normally, plasma plant sterol levels in humans are extremely low due to active efflux, as achieved by the *ABCG5/G8 transporters*. In sitosterolemia, this mechanism is disrupted. The exact prevalence is unknown; approximately 50 cases have been described worldwide.

Genetics

The *ABCG5* and *ABCG8* transporter genes are arranged in a head-to-head configuration on chromosome 2p21 [71]. Mutations in either the *ABCG5* or the *ABCG8* gene can cause sitosterolemia [70, 72]. All of the missense mutations in either *ABCG5* or *ABCG8* studied to date either prevent the formation of the obligate heterodimer or block the efficient trafficking of the heterodimer to the plasma membrane [16].

Clinical Characteristics

Sitosterolemia is characterized by xanthomas, arthralgias, anemia, and premature atherosclerosis [73]. Plasma cholesterol levels are not necessarily elevated; however, affected individuals are highly sensitive to dietary cholesterol and

become markedly hypercholesterolemic when fed a high-cholesterol diet [74].

Diagnosis

The disease should be suspected in patients who develop xanthomas in early childhood, despite normal or only moderately elevated plasma cholesterol concentrations. Sitosterolemia can be diagnosed by genetic analysis or by plasma plant sterol levels exceeding 0.024 mM (1 mg/dL).

Management

Affected individuals should be restricted from a cholesterol- and plant sterol-rich diet, as well as from plant sterol-enriched food products. In addition, subjects benefit from treatment with ezetimibe, a cholesterol absorption inhibitor, which also inhibits intestinal absorption of plant sterols [75], alone or combined with a BAS [76]. Statins are not effective in sitosterolemia.

Genetic Causes of HDL-C Disorders

Disorders of HDL-C identified in humans may result from interaction between genetic and environmental factors. Plasma HDL-C levels are under strong genetic influence, with heritability estimates ranging between 40 % and 60 % [77]. Several monogenetic defects in various proteins involved in HDL metabolism have been identified in humans to date. The genes encoding apolipoprotein-AI, ABCA1, and LCAT are essential for the de novo synthesis of HDL. A complete lack of any of these factors confers severe HDL deficiency, which is referred to as familial hypoalphalipoproteinemia. By contrast, CETP deficiency mostly induces accumulation of HDL in the circulation, the so-called hyperalphalipoproteinemia. However, the vast majority of cases with HDL-C deficiency, defined as an age- and sex-adjusted plasma HDL-C concentration below the tenth percentile, are polygenic and/or multifactorial in origin. Decreased HDL levels are often found in patients with genetically disturbed metabolic pathways such as hypertriglyceridemia, diabetes mellitus type 2, and obesity and metabolic syndrome [78]. In addition, multiple other factors have been identified to negatively influence HDL-C levels, such as smoking, physical inactivity, anabolic steroids, and certain medication or diseases such as rheumatoid arthritis and systemic lupus erythematosus [79].

The first step in the diagnostic workup of HDL deficiency consists of exclusion of these underlying conditions. Patients with a virtual absence of HDL must undergo careful physical examination to unravel the clinical characteristics of certain HDL deficiency syndromes as described below. In addition, family studies should be initiated to show segregation of low HDL in the family. Definitive diagnosis requires specialized biochemical tests and the demonstration of a functionally relevant mutation in an HDL gene [78].

To date, no routinely used drug is able to increase HDL-C in patients with specific familial HDL deficiency syndromes so that the prevention of CVD in these patients must be focused on the avoidance and treatment of additional risk factors. In general, several lifestyle and pharmacologic interventions have shown to modestly increase HDL, although the impact of these interventions on the functional quality of HDL is unclear. Lifestyle modifications such as weight reduction, exercise, and smoking cessation can increase HDL levels by approximately 10–15 %. In addition, pharmacologic therapies with niacin, fibrates, and statins, alone or in combination, raise HDL. Niacin therapy is the most effective pharmacological agent currently available and results in significant HDL increases of 15–35 %. Several mechanisms have been suggested, although it is not exactly clear how niacin raises HDL. The most common reason for treatment failure is inability to tolerate cutaneous flushing. This can be reduced by prescribing the long-acting form, or by administering premedication with aspirin, or may diminish spontaneously after several days of therapy, as patients develop tolerance. Niacin used as monotherapy has shown benefit with regard to CHD risk reduction [80]. However, two recent clinical trials (AIM-HIGH, ([81]) HPS2 THRIVE [82]) in which niacin was added to statins in patients with established CVD and well-controlled LDL failed to confirm the clinical benefit in terms of CVD prevention, despite markedly increasing HDL levels. Fibric acid derivatives increase the synthesis of apo-A1, enhancing the formation of new HDL particles and raising HDL by 5–20 %, with the largest increases seen in patients with hypertriglyceridemia. Triglycerides are reduced by 20–50 %, but LDL is changed minimally, if at all, and are sometimes increased. To date, trial results are mixed, with two trials reporting a significant reduction in their primary outcome, while three others did not. Overall, patients with high triglyceride and low HDL cholesterol seem to benefit from fibrates, but it is debatable whether this is due to an increase in HDL concentration [83].

Current treatment guidelines do not recommend specific HDL treatment goals, because it remains to be determined whether pharmacologically increasing HDL will translate into clinically meaningful CVD risk reduction [84]. However, promising new agents, which target both quantity and quality of HDL particles, are currently under development including CETP inhibitors, apo-A1 and HDL mimetics, intravenous apo-A1 (Milano) infusion, and agonists of PPAR-alpha, LRH-1 and LXR [85]. CETP inhibitors, like torcetrapib, dalcetrapib (JTT- 705), evacetrapib and anacetrapib, are powerful HDL raisers. However, all CETP inhibitor clinical trials have failed [86]. A phase III trial on anacetrapib is underway and should be completed in 2017 [83].

Table 21.1 Clinical hallmarks of familial HDL deficiency syndromes [78]

Apo-A1 deficiency		Tangier disease	Fish-eye disease	Familial LCAT deficiency
Affected gene	*APO-A1*	*ABCA1*	*LCAT*	*LCAT*
Enlarged tonsils	No	Occasionally	No	No
Hepato/splenomegaly	No	Occasionally	No	No
Neuropathy	No	Occasionally	No	No
Corneal opacities	+++	+	+++	+++
Xanthomas	Occasionally	No	No	Occasionally
Xanthelasma	Occasionally	No	No	No
Nephropathy	No	No	No	Yes
Hemolytic anemia	No	No	No	Yes

In this section, we focus on the established monogenetic disorders of HDL metabolism including Apo-A1, ABCA1, and LCAT. Also genetic disorders of CETP will be discussed.

Apolipoprotein AI Deficiency

Apo-A1 is the major protein component of HDL-C in plasma and plays a central role in cholesterol efflux from tissues to the liver for excretion. Apo-A1 deficiency is a rare autosomal recessive inherited disorder characterized by decreased HDL-C levels.

Genetics

The *apo-A1* gene is located on the long arm of chromosome 11, adjacent to the genes encoding the apolipoproteins C-III and IV. Of the approximately 70 reported distinctive mutations of this gene, mostly found in heterozygous state, some are functionally relevant, that is, are associated with reduced levels of apo-A1 and HDL-C [87].

Clinical Characteristics

Heterozygous carriers of a functionally relevant mutation usually present with half normal apo-A1 and HDL-C levels. Some mutations can even lead to more pronounced decreases. In most cases, heterozygous carriers of apo-A1 variants do not present with specific clinical symptoms. Important exceptions are some structural apo-A1 variants with amino acid substitutions in the N-terminus, which have been detected in patients with familial amyloidosis [88]. Surprisingly, susceptibility for premature coronary heart disease has been shown to differ markedly between apo-A1 variants. Low HDL-C levels due to heterozygosity for a specific apo-A1 mutation (p.L178P) were associated with vascular dysfunction, accelerated carotid arterial wall thickness, and an increased incidence of premature vascular events compared with their family controls [89]. By contrast, despite very low HDL levels, carriers of the *apo-A1* (p.R173C) Milano mutant did not differ from controls in terms of vascular function [90] and arterial wall thickness [91]. These differences are likely due to the profoundly different effects of the mutations at the protein level.

Patients with complete apo-A1 deficiency, due to homozygosity or compound heterozygosity, present with a virtually absent HDL-C. In adult patients, variable clinical manifestations have been described, such as abnormalities of the skin (xanthelasma and xanthomas) and/or eyes (corneal opacities) [78] (see Table 21.1). Remarkably, only 11 of the 25 reported cases with complete apo-A1 deficiency suffered from premature cardiovascular events. However, the remaining 14 cases were almost all below the age of 50 and may have been too young for clinical manifestations of atherosclerosis to occur. In addition, this small number of cases and differences in the type of apo-A1 gene defect makes conclusions on the susceptibility to premature coronary heart disease in these specific patients difficult [92]. Mendelian randomization studies suggest no relationship between HDL-C levels and CAD [93].

Diagnosis

The diagnosis of apo-A1 deficiency requires sequencing of the apo-A1 gene and the demonstration of a functionally relevant mutation.

Management

Since no routinely used drug is able to increase HDL-C levels in patients with familial low HDL cholesterol, the prevention of CVD in these patients must be focused on the avoidance and treatment of additional risk factors and the use of statins to obtain very low LDL-C levels [78].

ABCA1 Deficiency and Tangier Disease

ABCA1 mediates the efflux of cholesterol and phospholipids from peripheral tissues to lipid-poor apo-A1 in plasma and thereby plays a central role in forming HDL. Functionally relevant mutations in the *ABCA1* gene lead to cholesterol efflux defects, which subsequently cause low HDL-C and apo-A1

levels. Complete ABCA1 deficiency is the underlying cause of *Tangier disease*. This rare autosomal recessive disorder has been diagnosed in about 70 patients worldwide.

Genetics

The *ABCA1* gene resides on chromosome 9q31. To date, more than 90 mutations and several common and rare variants have been described in the *ABCA1* gene, with a wide range of biochemical and clinical phenotypes [94]. Several recent genome-wide association studies have identified common variants in *ABCA1* as a significant source of variation in plasma HDL cholesterol levels across multiple ethnic groups [95, 96] establishing *ABCA1* as a major gene influencing HDL levels in humans [97].

Clinical Characteristics

Heterozygote carriers of functionally relevant *ABCA1* mutations can present with a broad range of plasma HDL-C levels ranging from 30 to 83 % of age- and sex-matched controls [97]. However, the majority of these mutations are associated with an approximately 50 % reduction in serum HDL-C and apo-A1 levels and increased triglycerides. LDL levels are typically within the normal range.

Tangier disease, which is caused by complete ABCA1 deficiency due to homozygosity or compound heterozygosity, is characterized by profoundly decreased HDL-C plasma and apo-A1 levels. Frequently, serum levels of total and LDL cholesterol are also low, whereas serum levels of triglycerides are mildly elevated. The clinical presentation of Tangier disease varies considerably and if present clinical symptoms can be isolated or combined (see Table 21.1). It is likely that this phenotypic heterogeneity might at least in part be accounted for by the nature of the mutation and its effect on the protein [98]. Presenting features of Tangier disease include enlarged orange tonsils, hepatomegaly, and splenomegaly. Also, lymph nodes can have bright yellow streaks and morphologic characteristics as those present in the tonsils. A symptom with significant implications for quality of life is a peripheral neuropathy, which, however, has a highly variable expression. These clinical symptoms result from the accumulation of cholesterylesters in reticuloendothelial cells, that is, macrophages, Kupffer cells or histiocytes, leading to the accumulation of these cells in various organs [78]. Despite the known role of ABCA1 in determining plasma HDL levels, the impact of ABCA1 on atherosclerosis remains controversial and incompletely understood [99]. Prior to the identification of *ABCA1* mutations as the genetic basis of Tangier disease in 1999, patients were identified based on their clinical phenotype, that is, extremely low HDL cholesterol in homozygotes, with the offspring and parents of homozygotes being obligate heterozygotes. Considering the wide variation in phenotype, misclassification of patients was likely and this complicated accurate CAD risk estimation. Since the assignment of disease has been based on genotype, allowing a more unambiguous diagnosis, several studies have addressed the risk for CAD in these patients. Large family studies, studying several mutations, showed a more than threefold excess of CAD and increased carotid arterial wall thickness in affected family members when compared to unaffected members [100, 101]. In both studies, levels of cholesterol efflux correlated well with HDL-C levels and there was a strong correlation between levels of cholesterol efflux and CAD and/or carotid arterial wall thickness. However, these family studies potentially suffer from selection bias, as only families with the most severe phenotypes may have presented at clinics. Also, CAD risk estimates were based on few individuals and were not adjusted for age and other cardiovascular risk factors. Bypassing these problems, seven different *ABCA1* mutations were studied in two different population cohorts and a large case-control study, including a total of 109 heterozygotes, 6666 ischemic heart disease cases, and a total of 41,961 participants [102]. Four mutations were found to be associated with an average of 30 % reduction in HDL-C and decreased cholesterol efflux. Carriers of these four mutations, however, did not display an increased risk of CVD. However, this conclusion should also be interpreted with caution as the variants studied were mild mutations, giving relatively small reductions in HDL cholesterol levels and cholesterol efflux [103]. The findings are also conflicting with several reports showing that common genetic variations of the ABCA1 gene influence the risk of CAD in the general population [102, 104, 105]. Interestingly, these associations with atherosclerosis are independent of effects on HDL levels. These findings, of an altered risk for CAD but without corresponding differences in lipid levels, suggest that although ABCA1 may be an important atherosclerosis susceptibility locus, the mechanism by which it exerts this effect is not necessarily by altering steady-state HDL-C levels. In conclusion, any specific *ABCA1* variant must be considered in relation to its impact on protein function, as different variants will have different effects on HDL and susceptibility to atherosclerosis [106].

Diagnosis

The findings of virtually absent HDL-C and low levels of apo-A1 are not sufficient to diagnose Tangier disease, which ultimately requires *ABCA1* gene sequence analysis. Cholesterol efflux defects can be demonstrated with the cholesterol efflux assay on cultivated skin fibroblasts. However, even in the absence of coding sequence mutations in *ABCA1*, cellular cholesterol efflux defects are a common feature in subjects with low HDL [107]. Foam cell formation, responsible for the clinical symptoms in Tangier disease, can be detected in the rectal mucosa by endoscopic examination as pale mucosa with studded 1–2-mm discrete orange brown spots [78].

Management

To date, no specific treatment for Tangier disease exists. It is advised to identify and tightly regulate modifiable cardiovascular risk factors and possibly institute statin therapy as a means to drive LDL-C levels down even further.

Familial LCAT Deficiency and Fish-Eye Disease

Lecithin: cholesterol acyltransferase (LCAT) plays a key role in the maturation of small HDL by means of esterification of free cholesterol, primarily at the surface of the HDL particle (the so-called alpha-LCAT activity) but also on lipids transported by apo-B-containing lipoproteins (the so-called beta-LCAT activity). After esterification, the CE molecules migrate to the inner core of the lipoprotein, promoting further cholesterol efflux from peripheral tissues and leading to larger, cholesterylester-enriched HDL particles. Mutations in the *LCAT* gene causing LCAT deficiency represent another rare autosomal recessive disorder that underlies HDL deficiency. Low HDL-C values result from defective HDL maturation followed by rapid clearance of nascent HDL particles from the circulation. Depending on the mutation, patients with complete LCAT deficiency present with one of the two clinical phenotypes, *familial LCAT deficiency* (FLD) or *fish-eye disease* (FED).

Genetics

The gene encoding *LCAT* is located on chromosome 16, locus 16q22.1. Mutations in *LCAT* account for approximately 4 % of low HDL [107]. Thus far, over 80 mutations in the *LCAT* gene have been described in reports that predominantly investigated single cases or small nuclear families [92].

Clinical Characteristics

Heterozygous carriers of *LCAT* mutations lack clinical symptoms, although frequently they present with half normal HDL-C levels and mild hypertriglyceridemia [92]. Homozygous or compound heterozygous patients with mutations in the *LCAT* gene present with one of two clinical phenotypes, familial LCAT deficiency or fish-eye disease. In FLD, both alpha-LCAT, which is specific for HDL, and beta-LCAT, which is specific for VLDL and LDL, are deficient, that is, the deficient esterification is generalized. By contrast, patients with FED have a selective alpha-LCAT deficiency. Because LCAT is still partly active, these patients have, in general, a less severe phenotype. Both FLD and FED are characterized by corneal opacifications, which become apparent after the third decade (see Table 21.1). In addition, FLD is characterized by hemolytic anemia, and the deposition of foam cells in bone marrow, spleen, and particularly in kidneys. Progressive renal disease, with proteinuria and hematuria, which progresses to terminal renal insufficiency, has been described in a high percentage of these patients [65] (see Table 21.1). Biochemically, FLD and FED are both characterized by variable loss of LCAT activity and *HDL deficiency* (5–10 % of normal HDL-C levels). Serum levels of apo-A1 are usually decreased but not as low as in patients with apo-A1 deficiency or Tangier disease. Additionally, hypertriglyceridemia is observed [92].

The association between *LCAT* gene mutations and atherosclerosis is still controversial, both because of the limited number of carriers and because of variable results in the studies performed. A 25-year follow-up of nine heterozygote family members [108], as well as a large family study, including 68 carriers of *LCAT* defects of which 59 heterozygotes and 74 family controls [92] which measured carotid arterial wall thickness indicated that heterozygous carriers of *LCAT* defects may have an increased risk of atherosclerotic vascular disease. Another study including 45 carriers of *LCAT* mutations reported an increase in aortic pulse wave velocity with both ultrasound and MRI, indicating increased arterial stiffness and carotid wall thickening [109]. However, a study including 540 carriers from the IMPROVE study who underwent ultrasound showed no increase in intima wall thickening [110].

Diagnosis

The identification of LCAT deficiency needs either genetic testing or measurement of LCAT activity. Depending on the kind of mutation, immunoassays of LCAT detect either no, or slightly reduced concentrations of LCAT protein in plasma. Routine lipid and lipoprotein analyses do not help to distinguish patients with FLD and FED. However, patients with FLD show an increased proportion of unesterified cholesterol in plasma (80–100 % instead of normal <30 %). By contrast, the plasma of patients with FED has a normal or slightly elevated (up to 70 %) unesterified cholesterol/cholesterol ester ratio [78].

Management

Only symptomatic treatments exist for LCAT deficiency. Because deposition of highly abnormal apo-B- containing lipoproteins in the kidneys of FLD patients has been implicated as the pathogenetic factor in the formation of renal disease, therapies that reduce the concentration of apo-B-containing lipoproteins (such as a fat-restricted diet and statins) are at least theoretically useful [78]. Recombinant LCAT therapy has been suggested as possible acute treatment for acute coronary syndrome [111]. The effects of long-term rLCAT therapy have not been investigated yet.

Genetic Disorders of CETP

As a regulator of cholesterol flux through the RCT system, CETP, may be viewed as potentially having both pro-atherogenic and antiatherogenic properties (see Fig. 21.1). By facilitating the exchange of cholesterylesters for triglycerides between HDL and Apo-B-containing lipoproteins (LDL and VLDL), CETP may decrease direct RCT via the HDL/hepatic SR-B1 route. In addition, pro-atherogenic effects of CETP activity may result from a reduction in overall HDL levels, potentially reducing cellular cholesterol efflux from the arterial wall, and from an increase in atherogenic LDL levels. However, the potentially pro-atherogenic activities of CETP may, to a large extent, be neutralized by an increase in indirect RCT via the LDL/hepatic LDL receptor route [112].

Genetics

CETP is encoded by a gene located on the long arm of chromosome 16. Several mutations of the *CETP* gene have been associated with altered CETP activity and HDL-c levels. Recent genome-wide association studies have reported that *CETP* genotypes are associated with HDL-C levels more strongly than any other locus across the genome [95, 96].

Clinical Characteristics

Significant differences between ethnic groups with regard to allele frequencies of *CETP* polymorphisms exist [113]. Particularly in Japan, *CETP* gene defects are common and there are appreciable numbers of individuals who are homozygous for mutations in the *CETP* gene. Not surprisingly, functional mutations of the *CETP* gene can produce significant changes in lipid and lipoprotein metabolism. Not all *CETP* gene mutations have an as-dramatic effect on CETP protein levels as the ones described above. Various single nucleotide polymorphisms of the *CETP* gene are associated with only small changes in plasma CETP levels and subsequently HDL-C levels, in either direction [113]. Consequently, the role of *CETP* mutations on cardiovascular risk profiles is complex. Although sparse, there is evidence emerging from clinical trials that elevated CETP levels, regardless of the cause, are associated with an increased risk of CVD [114–116]. However, studies on individuals with CETP protein deficiency, arising from different genetic mutations, have reported ambiguous findings on the relationship between CETP protein deficiency and CAD risk. In some studies, CETP-deficient patients were thought to have an increased CAD risk [117] but, conversely, this concept was not supported by others [118, 119]. In addition, a recent meta-analysis was published that involved a total of more than 113,000 individuals and six *CETP* polymorphisms [120]. Three common *CETP* gene variants (TaqIB, I405V, and −629C > A) were consistently associated with a decreased CETP concentrations, modestly increased HDL-C and apo-A1 levels, and weakly decreased triglycerides and coronary risk. Data were insufficient for informative per-allele estimates in relation to three uncommon *CETP* variants (p. D442G, p.−631C > A, and p.R451Q). However, they were associated with mean differences in HDL-C of 13.4 %, −0.7 %, and −8.8 %, respectively, compared with controls. Thus, from the results of studies on individuals with CETP protein deficiency arising from genetic mutations, the relationship between CETP and the risk of CVD is not entirely conclusive. Overall cardiovascular risk is presumably dependent not only on the effect of CETP deficiency on overall levels of HDL-C but also on the effect on functionality of the HDL particles. Moreover, additional factors affecting the metabolic setting of the *CETP* gene mutation probably also play an important role. It was shown that high HDL-C resulting from simultaneous presence of *CETP*- and *LIPC* gene variants did not protect against CAD. By contrast, an increased risk for CAD was found in these patients [121]. In addition, high triglyceride levels have been suggested to enhance the effect of CETP concentration on CHD risk [116]. Also, potential joint effects of *CETP* genotypes with environmental determinants of HDL-C levels (e.g., exercise and alcohol) on the risk of coronary disease have been reported [122].

Genetic Causes of Elevated Triglycerides

Severely elevated *triglyceride* concentrations are a risk factor for developing *pancreatitis* and in the absence of other causes such as diabetes mellitus, alcohol abuse, chronic renal failure, or hypothyroidism, generally point to genetic disorders of triglyceride-rich lipoprotein-modulating enzymes or apolipoproteins. Mutations in several genes have been described, of which the *LPL*, *apo-CII*, and *Apo-E* genes are the most important ones. Very recently, the *GPIHBP1 gene* has been introduced as a contributor to *primary hypertriglyceridemia* [19, 123]. Two other new contributors described in a few families are the *APO-A5 gene* and the *LMF1 gene* [124]. On the other hand, loss-of-function mutations in apoC3 are associated with low levels of triglycerides and a reduced risk of CVD [13]. Next to the monogenic causes, polygenetic causes are also familial due to clustering of genetic mutations within families. Susceptibility then results from the accumulation of these mutations, because individual variants are insufficient to actually raise triglyceride levels significantly [124].

Regardless of its origin, the management of hypertriglyceridemia consists of therapeutic lifestyle changes aiming at dietary and weight control, as well as pharmacological treatment with fibrates, niacin, or high doses of fish oil, alone or in various combinations. In case triglyceride levels exceed

10 mmol/L (800 mg/dL), combinations of different drugs are usually required, in order to reduce the risk of pancreatitis [125]. The benefit of treating mild-to-moderate triglyceride elevations is less clear [126]. If hypertriglyceridemia is a comorbidity, statins can lower triglyceride levels by 20–40 % [127]. *Fibrates* lower triglyceride levels by approximately 40–60 % and modestly raise HDL-C levels by approximately 15–25 % [127]. Patients who do not respond to *fibrates* can be treated with *niacin*, which lowers triglyceride levels by 30–50 %, raises HDL-C levels by 20–30 %, and lowers LDL-C levels by 5–25 % [127, 128]. They are reviewed above. Fish oil with 2–4 g of *omega-3 fatty acids* daily can reduce triglyceride levels by 15–50 %, depending on the dosage and different formulations [129]. Moreover, few adverse effects have been reported, mostly gastrointestinal. Over-the-counter preparations usually contain far less than these required amounts [130]. The new drug *lomitapide* (reviewed above) has also been shown to reduce triglyceride levels up to 40 % [124]. Loss-of-function mutations of Apolipoprotein CIII (APOCIII) are associated with low levels of triglycerides and decreased incidence of CVD [13]. As a result, a new second-generation ASO inhibiting APOCIII is currently under investigation in phase 2 studies [131]. Of note, in patients with *diabetes mellitus*, optimizing glycemic control might help to lower triglyceride levels without additional medication for hypertriglyceridemia.

LPL Deficiency and Apo-CII Deficiency

Plasma LPL and its cofactor apo-CII are involved in the hydrolysis of triglyceride-rich particles such as chylomicrons and VLDL. Genetic *LPL deficiency* is a rare autosomal recessive disorder causing severe hypertriglyceridemia. Estimations of prevalence vary between 1:500,000 in the general population and 1:5000 in French Quebec. The incidence of apo-CII deficiency is even lower than that of LPL deficiency.

Genetics

The *LPL* gene is located on chromosome 8p22 [132]. More than 114 mutations have been described [124]. The *apo-CII* gene is located on chromosome 19, in which at least 13 mutations have been described [133].

Clinical Characteristics

Affected individuals have insufficient capacity to hydrolyze triglycerides, resulting in extremely high plasma triglyceride concentrations, often accompanied by recurrent episodes of *pancreatitis*. LPL deficiency typically manifests itself in early childhood with severe and repetitive colicky pain in the abdomen, acute pancreatitis, and failure to thrive. Eruptive xanthomas (Fig. 21.4), lipemia retinalis, and hepatosplenomegaly can also be present. Plasma is lipemic, reflecting increased plasma levels of both chylomicrons and VLDL. Loss-of-function mutations in *LPL* are associated with an increased risk of CVD, while gain-of-function mutations are protective [134–136]. To date, the only *apo-CII* mutation described to cause early atherosclerosis is the *apo-CII* St Michel mutation [137].

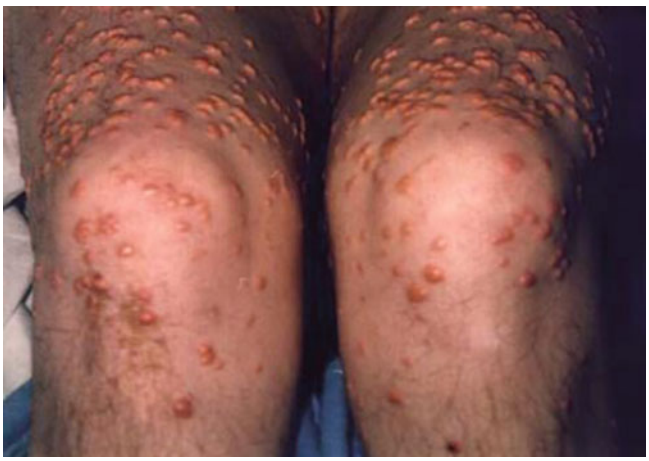

Fig. 21.5 Eruptive xanthomas

Diagnosis

Genetic *LPL* and *apo-CII deficiency* are diagnosed by genotyping, combined with the phenotype as described above. Apo-CII deficiency can also be diagnosed by a post-heparin LPL activity assay, in which the patient's post-heparin plasma is mixed with that of a nonaffected individual. In this experiment, triglyceride levels will decrease rapidly in an apo-CII-deficient patient, in contrast to subjects with LPL deficiency.

Treatment

Treatment consists of *dietary fat restriction*. Hypertriglyceridemia is treated as described above; however, in genetic *LPL* and *apo-CII* deficiency, most of these strategies do not result in a substantial reduction in triglyceride levels. Nevertheless, promising new compounds for the treatment of this patient group are under investigation, such as *ibrolipim*, a pharmacological stimulator of tissue LPL formation, *LPL gene therapy* [138], and *antisense apo-CIII therapy* [139]. *Alipogene tiparvovec* is an adeno-associated virus serotype 1-based gene therapy being the first gene therapy to be approved in the Western world. In clinical studies with 27 patients, it lowered plasma triglyceride levels for up to 26 weeks and after even 6 years of follow-up there were still clinically relevant reductions in the incidence of pancreatitis and acute abdominal pain events [140]. It is now approved for a small subset of patients with familial LPL deficiency suffering from recurrent severe pancreatitis under

a fat-restricted diet. However, clinical experience is limited and further research should be conducted to assess the long-term safety.

Familial Dysbetalipoproteinemia (Apo E2/E2 Deficiency)

Familial dysbetalipoproteinemia (FD) is characterized by the defective clearance of VLDL- and *chylomicron- remnant particles* caused by homozygosity for apoE2, the type of apoE unable to bind to its receptor. There are three common apoE isoforms: apoE3, apoE2, and apoE4 [141]. Although approximately 0.5 % of the population worldwide is homozygous for apoE2, only a small minority develops FD with an estimated prevalence of 1–2:10,000. This is due to the necessity of concomitant environmental, hormonal, and possibly genetic factors, inducing VLDL or chylomicron overproduction, such as a high caloric diet or alcohol abuse, diabetes mellitus, obesity, hypothyroidism, renal disease, or estrogen deficiency.

Genetics

Most people have an *apoE3/E3* genotype, with a ~55 % prevalence; however, *apoE4* and *apoE2* also exist, with an estimated frequency of 0.5 % for *apoE2/E2*, 15 % for *apoE2/E3*, 25 % for *apoE3/E4*, 1–2 % for *apoE4/E4*, and 3–4 % for *apoE2/E4*. *ApoE2* differs from *ApoE3* by a single substitution of cysteine for arginine at residue 158.

Less common, dominant-negative mutations may also cause the disorder (*ApoE3*-Leiden or *s*-Lys146 > Gln) [142].

Clinical Characteristics

Clinically, apoE2/E2 patients present with *tubero-eruptive xanthomas* (see Fig. 21.5), palmar streaks, elevated TC, and triglyceride concentrations, and are at a high risk for premature CVD and peripheral vascular disease [143]. Tubero-eruptive xanthomas begin as clusters of small papules on elbows, knees, or buttocks and can grow to the size of small grapes. Palmar xanthomas are orange yellow discolorations of palm and wrist creases. Both are pathognomonic for FD, but their absence does not exclude the disorder. Plasma TC concentrations usually exceed 8.0 mmol/L (300 mg/dL) and may approach 26.0 mmol/L (1000 mg/dL).

Triglyceride concentrations are within the same range. Dyslipidemia in FD rarely manifests before adulthood. The average age of clinically overt vascular disease is approximately 40 years in men and 59 in women.

Diagnosis

FD can be diagnosed either by lipoprotein ultracentrifugation and electrophoresis with a *VLDL/triglyceride ratio* >0.3 or by *apoE* genotyping. However, the absence of apoE2/E2 does not rule out the disease, as other genetic causes might also give rise to this trait.

Management

Treatment of FD is aimed at reducing the overproduction of VLDL and/or chylomicrons, by means of dietary restrictions, including alcohol intake and weight reduction, combined with pharmacological treatment with statins, alone or combined with other compounds, as described above. Recently, a European cross-sectional study including 305 patients from seven academic hospitals in four European countries found that the majority of FD patients have non-HDL-C levels above the threshold of 3.3 mmol/L. However, less than half of these patients were adequately treated, increasing their cardiovascular risk [144].

Familial Combined Hyperlipidemia

FCH is discussed in section, "Familial Combined Hyperlipidemia."

Familial Hypertriglyceridemia

Familial hypertriglyceridemia (FHTG) is a common disorder causing hypertriglyceridemia with the prevalence of 1:500. The genetic basis seems to be based on an accumulation of common and rare genetic mutations that increase susceptibility [145], and the onset of disease depends on the presence of certain lifestyle factors. FHTG is discussed due to its high prevalence. The metabolic defect is a combination of hepatic VLDL overproduction and decreased catabolism of both VLDL and chylomicrons.

Clinical Characteristics

Typically, patients have moderately elevated plasma triglycerides, 3–10 mmol/L, often accompanied by low HDL-C levels. FHTG is associated with obesity, insulin resistance, hypertension, and hyperuricemia. The onset of hypertriglyceridemia is usually in adult age, when lifestyle factors, which increase triglyceride levels, such as obesity, become more prominent. When the hypertriglyceridemia becomes more severe, the clinical picture can resemble that of LPL deficiency. The association with CVD is weak, at most.

Diagnosis

FHTG is diagnosed by exclusion of other causes of hypertriglyceridemia. A first-degree family member with the same disorder is useful for the diagnosis. FCH and FD should definitely be excluded, since these disorders are associated with a more pronounced CVD risk and therefore require a more stringent therapy.

Management

The first line of treatment is lifestyle modification, possibly combined with pharmacological treatment in case of more severe hypertriglyceridemia.

Finally, mutations in the recently identified *GPIHBP1 protein* might be a cause of severe hypertriglyceridemia, resembling LPL or apo-CII deficiency [19]. This protein is thought to anchor LPL on the luminal surface of capillaries, where lipolysis of triglyceride-rich particles takes place. At present, two mutations have been described in humans: the p.G56R [146, 147] and p.Q115P mutation [123], of which only the latter was proven to be causal. In addition, the *LMF1* and the *APO-A5* gene have also recently been identified as interesting candidates for severe *hypertriglyceridemia* [124, 148].

Summary

Disorders of lipoprotein metabolism are major contributors to CVD, a leading cause of mortality and morbidity worldwide. Dyslipidemia includes both elevated LDL-C levels, elevated triglycerides, and elevated remnant cholesterol, as well as decreased HDL-C levels.

LDL mediates cholesterol transport from the liver to peripheral tissues, including macrophages in the arterial wall, which, after uptake and accumulation of cholesterol, can transform into foam cells and atherosclerotic plaques. Conversely, HDL is thought to exert beneficial effects on the arterial wall through its role in the RCT, which involves the transport of cholesterol from peripheral tissues to the liver followed by biliary excretion and elimination via the feces.

The crucial role of increased plasma LDL-C levels in the pathogenesis of atherosclerosis has been firmly established, as well as the beneficial effects of LDL-C reduction accomplished by HMG-CoA reductase inhibitors or statins. In addition, decreased plasma HDL-C levels are an established independent predictor of CVD. However, pharmacological raising of plasma HDL levels has failed to reduce cardiovascular events thus far. It is therefore uncertain whether HDL plays a causative role in CVD protection or if it is merely an epiphenomenon or nonfunctional biomarker. Finally, the relationship between hypertriglyceridemia and CVD risk also remains to be elucidated.

Most cases of CVD are multifactorial and/or polygenic in origin. However, when CVD occurs at young age, a number of monogenetic disorders of lipoprotein are frequently seen. These monogenetic disorders of lipoproteins are the primary focus of this chapter.

Regarding LDL metabolism, mutations in four genes are currently identified to result in increased plasma LDL-C concentrations, namely the LDL-R gene, Apo-B gene, ARH gene, and most recently the *PCSK9 gene*. Clinical hallmarks of these disorders, of which familial hypercholesterolemia is the most frequent and well known, are elevated plasma LDL-C levels and, consequently, premature atherosclerosis.

Table 21.2 Underlying causes of dyslipidemias

Elevated LDL-C levels	Decreased HDL-C levels	Elevated TG levels
Hypothyroidism	Obesity	Obesity
Kidney diseases	Diabetes mellitus	Diabetes mellitus
Certain medications, e.g., Corticosteroids Thiazide diuretics	Metabolic syndrome Certain medication Underlying diseases, e.g., Rheumatoid arthritis Systemic lupus erythematosus	Metabolic syndrome Alcohol abuse Chronic renal failure Hypothyroidism

To date, several rare monogenetic defects in various proteins involved in HDL metabolism have been identified in humans. The genes encoding apolipoprotein-AI, ABCA1, and LCAT, respectively, are essential for the de novo synthesis of HDL. A complete lack of any of these factors confers severe HDL deficiency, which is referred to as familial hypoalphalipoproteinemia. By contrast, CETP deficiency mostly induces accumulation of HDL in the circulation. Although FHA patients display extremely low plasma HDL-C levels, the association of these genetic disorders with atherosclerosis is disputed. Since HDL is a heterogeneous class of lipoprotein particles, these different classes may have different associations with disease. Furthermore, the functionality of the HDL particles, rather than their abundance, may be an important determinant of their antiatherogenic effects.

Finally, severely elevated triglyceride concentrations can be induced by mutations in several genes, of which the *LPL*, *apo-CII*, and *Apo-E* genes are the most important ones. Despite the unclear role of hypertriglyceridemia in atherogenesis, severely elevated triglyceride levels confer a health risk due to the increased risk of pancreatitis.

In general, the vast majority of dyslipidemia are polygenic and/or multifactorial in origin.

The first step in the diagnostic workup of dyslipidemias consists of the exclusion of underlying conditions through careful medical history taking, physical examination, and biochemical testing (see Table 21.2). Suggestive for a genetic cause are the presence of specific clinical hallmarks (see text and Table 21.1) and/or the presence of familial dyslipidemias/premature atherosclerosis. In these cases, specialized biochemical tests and/or the demonstration of a functionally relevant mutation in the involved genes should be performed to obtain a definitive diagnosis. In addition, family studies should be initiated to evaluate the inheritance pattern of the phenotype (see Table 21.3).

Table 21.3 Steps in the diagnostic workup of dyslipidemias [65]

1. Exclude underlying conditions
2. Suspected genetic cause? Profoundly decreased HDL-C levels? (<fifth percentile adjusted for age and sex) Presence of specific clinical hallmarks? (see text and Table 21.1) Presence of familiar dyslipidemias/premature atherosclerosis?
3. Perform specialized biochemical tests and/or specific HDL gene sequencing
4. Initiate family studies

Treatment consists of lifestyle modifications such as weight reduction, exercise, and smoking cessation to improve other atherogenic risk factors, possibly in combination with pharmacological agents. High-dose statins are currently the most effective pharmacological strategy to reduce CVD risk. Also in case of low HDL and hypertriglyceridemia, statin monotherapy, possibly in combination with other agents, reduces the risk of CVD. However, it should be noted that many new therapies are becoming available that could change current treatment guidelines in the years to come.

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