Familial Hypercholesterolemia

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Keywords
Atherosclerosis · Cholesterol · Familial hypercholesterolemia · Low-density lipoprotein · Low-density lipoprotein receptor

5.1 Case Report

A 40-year-old woman with a history of cutaneous and tendon xanthoma (abnormal accumulation of lipids in macrophages of several tissues) and hypercholesterolemia attended an outpatient clinic of the Kanazawa University Hospital with progressive chest pain lasting for 1 month. The patient was diagnosed with severe hypercholesterolemia (total cholesterol, 800 mg/dL) at 3 years of age by the dermatologist examining her cutaneous xanthoma. The cholesterol biosynthesis inhibitor pravastatin was administered when she was 20 years of age; however, she discontinued the medication because it was ineffective in reducing her cholesterol levels. At an approximately 30 years of age, she experienced chest pain induced by exerting effort or cold atmosphere. Therefore, she restricted her daily activity to minimize chest pain. At 40 years of age, she visited her general physician complaining of progressive chest pain, and she was found to have severe hypercholesterolemia {total cholesterol, 450 mg/dL; normal range < 220 mg/dL, low-density lipoprotein cholesterol (LDL-C), 360 mg/dL; normal range < 140 mg/dL} in laboratory test and ST depression in electrocardiogram, suggesting myocardial ischemia.

The patient was born from a non-consanguinity marriage. Her father was diagnosed with hypercholesterolemia. Her mother died from colon cancer, and no blood cholesterol data was obtained. However, her mother's elder brother had xanthelasma on his eyelids, suggesting the existence of family history of maternal hypercholesterolemia. The patient's son and daughter were also diagnosed with hypercholesterolemia; however, their cholesterol levels were milder than that of the proband. She had no coronary risk factors, such as hypertension, impaired glucose tolerance, and smoking habit, other than hypercholesterolemia. She had xanthelasma on her eyelids, xanthoma on her bilateral third and fourth extensor tendons of the hands, tuberculous skin xanthoma on her bilateral elbows, and Achilles tendon xanthomas, which were 18 mm thick (Fig. 5.1). She also had surgical scars from resection of skin xanthoma on her wrists. She had a grade 4/6 late systolic murmur (abnormal sound heard during auscultation of the heart) on the

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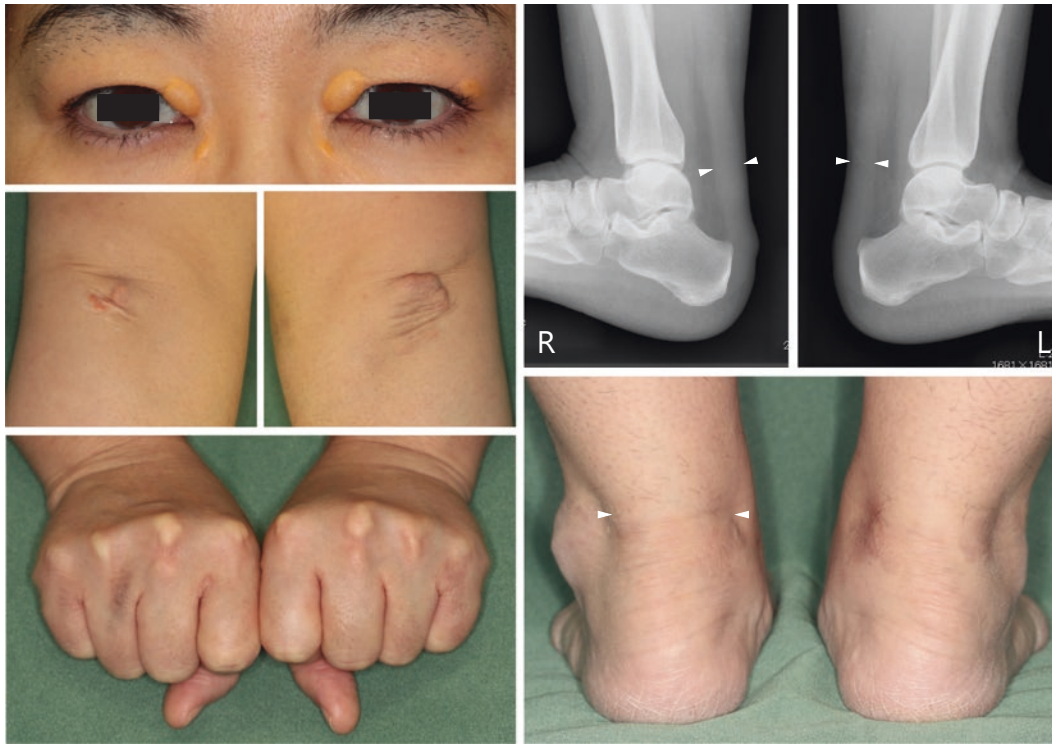

Fig. 5.1 Physical findings of a patient with compound heterozygous familial hypercholesterolemia
The presented case had xanthelasma on her eyelids (top left), tuberculous skin xanthoma on her bilateral elbows (middle left), xanthoma on her bilateral third and fourth extensor tendons of the hands (bottom left), and Achilles tendon xanthoma (18 mm thick in side view of roentgenograph; top and bottom right). The thickness of Achilles tendon on the roentgenograph is to be measured perpendicularly at its thickest part (arrow head)

second right sternal border radiating to her neck and bilateral carotid bruits (abnormal sound heard during auscultation of the vessels suggesting its narrowing). A cardiac echocardiography revealed shrinkage of the aortic root and narrowing of the ascending aorta just above the valve (supravalvular aortic stenosis), and Doppler and planimetric analysis suggested severe aortic stenosis (blockage), resulting in a significant pressure gradient. Carotid ultrasonography revealed approximately 50% and 75% stenosis in the right and left common carotid artery, respectively; cardiac catheterization revealed moderate coronary atherosclerosis. Her chest pain resolved successfully following aortic valve replacement surgery.

Her serum levels of total cholesterol, LDL-C, and high-density lipoprotein (HDL) cholesterol (normal range; ≥ 40 mg/dL) were 523, 429, and 43 mg/dL, respectively, and her triglyceride level (normal range; < 150 mg/dL) was 122 mg/dL. Her serum level of apolipoprotein B (apoB) (normal range; < 100 mg/dL) was 269 mg/dL. Genetic analysis revealed three mutations in the LDL receptor gene: two (c.904 T > G and c.907C > T) were identified in her father, both of which were changed amino acid sequences. A large deletion in exon 6 of the same gene was found in her maternal family member including uncle. Finally, she was diagnosed with compound heterozygous familial hypercholesterolemia [(FH) HeFH].

She was treated with a low-fat, low-cholesterol, restricted caloric diet and 20 mg daily of rosuvastatin (maximum doses in Japan), a cholesterol biosynthesis inhibitor stronger than old generation statin, such as pravastatin. Her serum levels of LDL-C and apoB reached to 257 mg/dL and 165 mg/dL, respectively. The selective cholesterol absorb inhibitor ezetimibe was also introduced. Following treatment, her LDL-C and

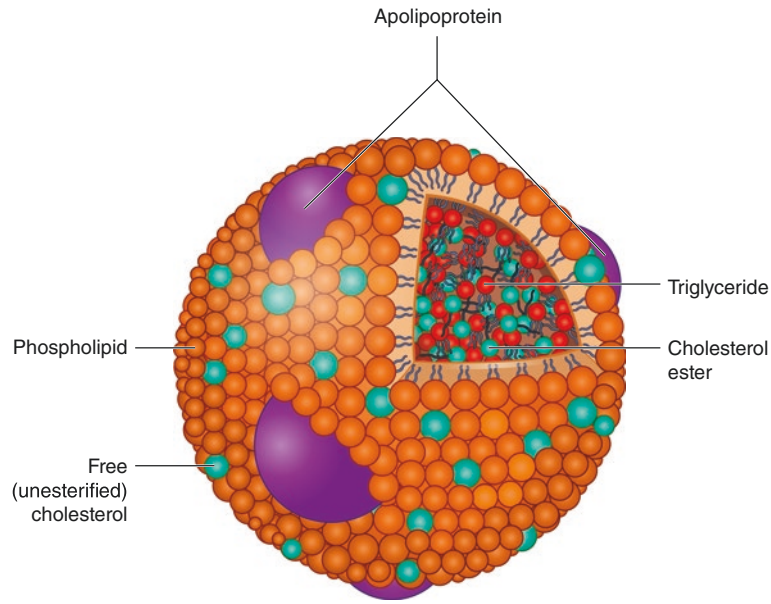

Fig. 5.2 Basic structure of lipoprotein Lipoproteins are composed of a core neutral lipid, mainly cholesteryl ester and triglycerides, surrounded by free cholesterol, phospholipids, and apolipoproteins

apoB levels decreased to approximately 240 and 155 mg/dL, respectively. Because these medicines were only partially effective, LDL-apheresis therapy, which is extracorporeal selective adsorption of apoB-containing lipoproteins, was introduced along with the anti-proprotein convertase subtilisin/kexin type 9 (PCSK9) antibody evolocumab, which inhibits intracellular degradation of LDL receptor. Finally, her serum levels just before LDL-apheresis of LDL-C and apoB reached approximately 170 and 130, respectively. However, even after these intensive cholesterol-lowering therapies, her serum LDL-C levels did not reach to the target level for secondary prevention of coronary artery disease (100 mg/dL): therefore, microsomal triglyceride transfer protein inhibitor is the next treatment option.

5.2 Diagnosis

5.2.1 Compound Heterozygous Familial Hypercholesterolemia

Lipids including cholesterol are hydrophobic; thus, they are bound with apolipoproteins to create lipoproteins that circulate in the blood stream. Lipoproteins are composed of a core neutral lipid, mainly cholesteryl ester and triglycerides, surrounded by free cholesterol, phospholipids, and apolipoproteins (Fig. 5.2).

Cells require cholesterol as an essential material for its membrane. The liver synthesizes bile acid from cholesterol, which is required for uptake lipid from the intestine (exogenous pathway). In some endocrine organs, such as adrenal gland, testis, and ovary, cholesterol is also used as a substrate for steroids, such as testosterone, androsterone, estradiol, and progesterone. Cells can synthesize cholesterol from acetyl-CoA; however, the quantity is not sufficient for the cells, except for hepatocytes. Thus, cholesterol is transported from the liver to the other cells mainly by LDL via the LDL receptor (endogenous pathway). Cholesterol is returned from the peripheral cells including macrophages in atherosclerotic lesion to the liver by HDL (reverse cholesterol transport pathway) (Fig. 5.3).

FH is the most severe form of monogenic hypercholesterolemia and is mainly caused by mutations in the LDL receptor gene (Rader et al. 2003, Mabuchi 2017). Endogenous cholesterol is secreted from hepatocytes (liver cells) into circulation as very low-density lipoprotein (VLDL), dilipidated by lipoprotein and hepatic lipases, and consequently metabolized to LDL through intermediate-density lipoprotein (IDL) (Fig. 5.3).

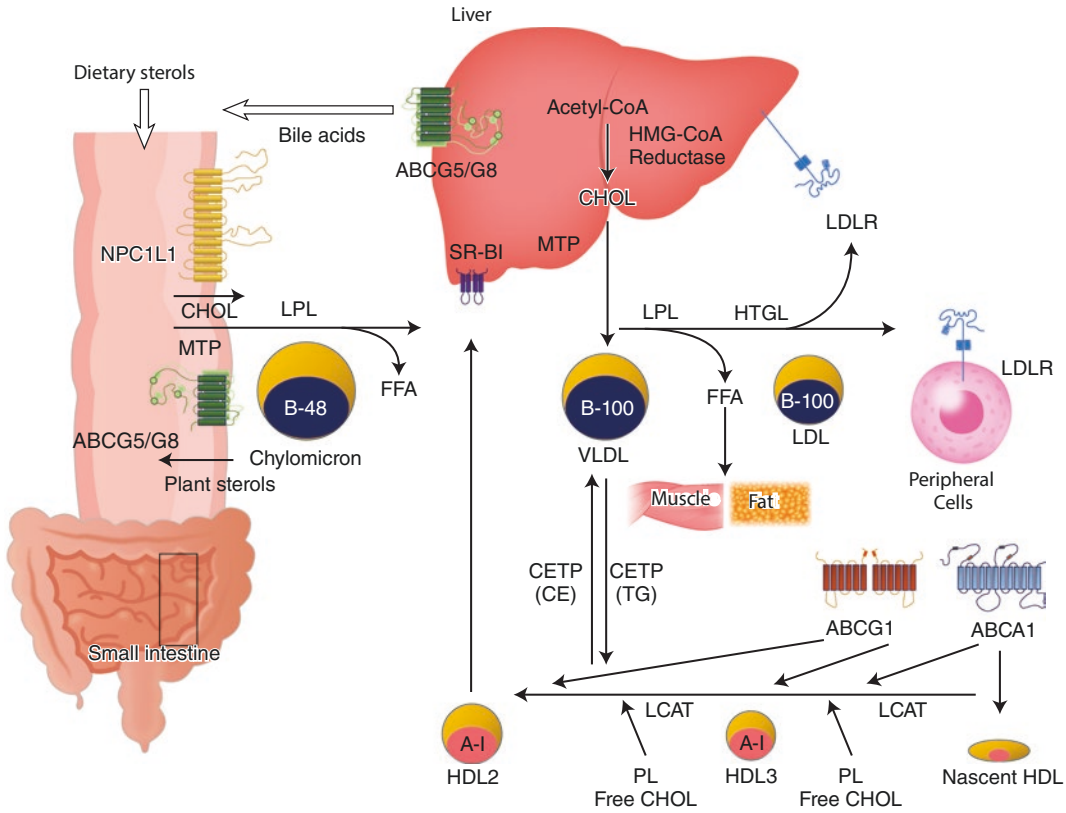

Fig. 5.3 Lipoprotein metabolism
Human lipoprotein metabolism is divided into three categories; the exogenous pathway (chylomicron), the endogenous pathway (mainly LDL), and the reverse cholesterol transport pathway (HDL). LDL receptor, the causal protein of FH, is the main regulator of human cholesterol levels
ABCA1 adenosine triphosphate-binding cassette sub-family A member 1, *ABCG1* adenosine triphosphate-binding cassette sub-family G member 1, *ABCG5G8* adenosine triphosphate-binding cassette sub-family G member 5/sub-family G member 8, *CE* cholesteryl ester, *CETP* cholesteryl ester transfer protein, *CHOL* cholesterol, *FFA* free fatty acid, *HDL* high-density lipoprotein, *HTGL* hepatic triglyceride lipase, *HMG-CoA* hydroxymethylglutaryl-CoA, *LCAT* Lecithin-cholesterol acyltransferase, *LDL* low-density lipoprotein, *LDLR* low-density lipoprotein receptor, *LPL* lipoprotein lipase, *MTP* microsomal triglyceride transfer protein, *NPC1L1* Niemann-Pick C1-like 1, *PL* phospholipid, *SR-BI* scavenger receptor class B type 1, *TG* triglyceride

LDL is the major transport particle of cholesterol to peripheral cells via the LDL receptor, and the remaining LDL particles in circulation are cleared by the LDL receptor on hepatocytes. LDL particles of FH without functional LDL receptors are congested in circulation because of the inability of the LDL receptor to clear the particles (Fig. 5.3).

FH is a common autosomal dominant hereditary disorder, and there are two forms of FH: individuals with two mutated LDL receptor alleles (homozygous FH: HoFH) and those with a single mutated LDL receptor allele (heterozygous FH: HeFH). The blood total cholesterol levels of HoFH, HeFH with LDL receptor gene mutation, and their normal siblings are trimodal as follows: 713 ± 122, 332 ± 60, and 179 ± 26 mg/dL, respectively (Fig. 5.4) (Mabuchi 2017). Initially, the frequency of HeFH was reported as 1 in 500 general populations; recent advances in genetic analysis have expanded the frequency to 1 in 200–300 general populations (Mabuchi 2017, Nordestgaard et al. 2013). Accordingly, the frequency of HoFH is estimated as 1 in

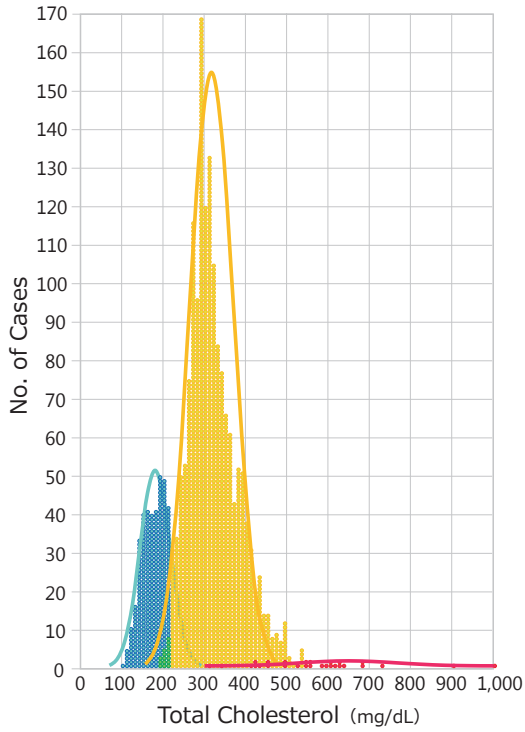

Fig. 5.4 Total cholesterol levels of HoFH, HeFH, and their normal siblings
The blood total cholesterol levels of HoFH, HeFH with LDL receptor gene mutation, and their normal siblings are trimodal as follows: 713 ± 122, 332 ± 60, and 179 ± 26 mg/dL, respectively (Mabuchi 2017)

160,000–360,000 general populations. In certain populations, such as Afrikaners in South Africa, French Canadians, and the Lebanese, the frequency of HeFH is substantially higher than in other regions because of the founder gene effect, whereby these population groups originated from a small number of individuals with a specific LDL receptor gene mutation at a higher frequency than that of the general population.

HoFHs cause the development of systemic cutaneous xanthoma and atherosclerosis from childhood; cutaneous xanthomas between the hand fingers and buttocks are specific physical findings for HoFH. Interestingly, as in the case presented here, the manifestation of early atherosclerosis in HoFH is often due to the formation of atheroma along with aortic root, aortic valve, and coronary ostia, which extends to coronary and systemic atherosclerosis thereafter. Supravalvular and aortic valve stenosis can cause acute heart failure or sudden cardiac death (Mabuchi 2017). The development of coronary artery disease (CAD) is decided by the cumulative values of LDL-C levels (Nordestgaard et al. 2013). Thus, HoFH could cause the development of premature CAD before 20 years of age along with extremely high LDL-C levels from the birth if effective cholesterol-lowering therapy is not administered. Thus, early surgical intervention including aortic valve replacement or a Bentall procedure, which is the composite graft replacement of aortic valve, aortic root, and ascending aorta, and coronary artery bypass surgery may be needed. Although it is also important to optimize the other cardiovascular risk factors, such as blood pressure and diabetes, these have minimal impact on the clinical course of HoFH.

HeFHs also initially present as tendon xanthomas in the backs of hands and Achilles' tendons, and cutaneous tuberculous xanthomas occur during and after the second decade of life. Arcus cornealis and xanthelasma on eyelids are characteristics of FH; however, they are not specific or suitable for the diagnosis of FH. Coronary atherosclerosis detected by coronary computed tomography starts to develop at 23 and 33 years in male and female HeFH patients, respectively (Tada et al. 2015b). Clinically significant CAD usually manifests after 30 years of age for men and 40 years for women.

There are substantial interindividual differences in LDL-C levels among patients with HoFH and HeFH. HoFH is classically divided into two categories: receptor-negative FH with no or minimum LDL receptor activity and receptor-defective FH with substantial residual LDL receptor activity. Although LDL receptor activity can be measured in vitro using skin fibroblasts, it is not routinely performed in clinical practice. The clinical course of receptor-negative FH is usually worse compared with that of receptor-defective FH because of substantially higher cumulative LDL-C levels. Standard medical cholesterol-lowering therapies, which eventually increase hepatic LDL receptor, do not effectively reduce LDL-C levels in patients with receptor-negative HoFH but can substantially

reduce LDL-C levels in patients with receptor-defective HoFH.

The diagnosis of HeFH is made by combining elevated LDL-C levels, clinical manifestations (particularly xanthomas), and family history of hypercholesterolemia and/or premature CAD. Although several different clinical diagnostic criteria of FH have been proposed, the existence of tendon or skin xanthomas, which are the reflection of continuous hypercholesterolemia from birth, is essential for the clinical diagnosis of FH. Of those, the following three diagnostic criteria are major: (a) Simon Broome Register Diagnostic Criteria for FH, (b) Make Early Diagnosis to Prevent Early Death (MedPed) Program Diagnostic Criteria for FH, and (c) Dutch Lipid Clinic Network Diagnostic Criteria for FH (Table 5.1a, 5.1b, and 5.1c). The diagnosis of HoFH is made based on markedly elevated LDL-C levels and a family history of parental HeFH on both sides, except for very rare recessive hereditary conditions.

A genetic diagnosis is considered to be definitive; however, the annotation of the specific mutation is not always easy. Very recently, comprehensive genetic analysis of known causal genes (*LDL receptor, apoB, PCSK9, and LDL receptor adaptor protein 1*) has become readily available. Thus, it can be speculated that genetic analysis will play a greater role in future diagnostic criteria of FH. Regarding the patient in the case report, three different mutations in the LDL receptor gene were detected, two (c.904 T > G and c.907C > T) were of a paternal origin and the other (a large deletion in exon 6) was of a maternal origin. Thus, the final diagnosis of the case was compound heterozygous FH, which has a similar significance as HoFH in the clinical setting.

5.3 Differential Diagnosis

1. Familial defective apolipoprotein B-100 (FDB or FH2)
2. Autosomal dominant hypercholesterolemia due to gain-of-function mutations in proprotein convertase subtilisin/kexin type 9 (PCSK9) gene (FH3)

Table 5.1a Simon Broome Register Diagnostic Criteria for FH

Definite FH is defined as	
(a)	Total cholesterol > 260 mg/dL or LDL-cholesterol above 155 mg/dL in a child < 16 years
	Total cholesterol > 290 mg/dL or LDL-cholesterol above 190 mg/dL in an adult
	(levels either pretreatment or highest on treatment)
	plus
(b)	Tendon xanthoma in patient
	or in 1st-degree relative (parent, sibling, child)
	or in 2nd-degree relative (grandparent, uncle, aunt)
	or
(c)	DNA-based evidence of an LDL receptor mutation or familial defective apoB-100
Possible FH is defined a	
(a)	Total cholesterol > 260 mg/dL or LDL-cholesterol above 155 mg/dL in a child < 16 years
	Total cholesterol > 290 mg/dL or LDL-cholesterol above 190 mg/dL in an adult
	(Levels either pretreatment or highest on treatment)
	plus one of (d) or (e)
(d)	Family history of myocardial infarction
	below age of 50 in 2nd-degree relative
	below age of 60 in 1st-degree relative
(e)	Family history of raised cholesterols
	> 290 mg/dL in adult in 1st- or 2nd-degree relative
	> 260 mg/dL in child or sibling under 16

Table 5.1b Make Early Diagnosis to Prevent Early Death (MedPed) Program Diagnostic Criteria for FH

Age (years)	First-degree relative with FH	Second-degree relative with FH	Third-degree relative with FH	General Population
<20	220	230	240	270
20–29	240	250	260	290
30–39	270	280	290	340
≥40	290	300	310	360

FH is diagnosed if total cholesterol exceeds these cut points in mg/dL
The total cholesterol cut points for FH is dependent upon the confirmed cases of FH in the family
If FH is not diagnosed in the family, then the cut point for diagnosis is as per "general population"

Table 5.1c Dutch Lipid Clinic Network Diagnostic Criteria for FH

Criteria		Points
Family history		
	First-degree relative with known premature* coronary and vascular disease, OR	1
	First-degree relative with known LDL-C level above the 95th percentile	
	First-degree relative with tendinous xanthomata and/or arcus cornealis, OR	2
	Children aged less than 18 years with LDL-C level above the 95th percentile	
Clinical history		
	Patient with premature* coronary artery disease	2
	Patient with premature* cerebral or peripheral vascular disease	1
Physical examination		
	Tendinous xanthomata	6
	Arcus cornealis prior to age 45 years	4
Cholesterol levels (mg/dL)		
	LDL-C ≥ 330 mg/dL	8
	LDL-C 250 – 329 mg/dL	5
	LDL-C 190 – 249 mg/dL	3
	LDL-C 155 – 189 mg/dL	1
DNA analysis		
	Functional mutation in the LDL receptor, apoB or PCSK9 gene	8
Diagnosis (diagnosis is based on the total number of points obtained)		
	Definite FH	≥ 8
	Probable FH	6 - 8
	Possible FH	3 - 5
	Unlikely FH	< 3

*Premature means < 55 years in men; < 60 years in women

3. Autosomal recessive hypercholesterolemia (ARH)
4. Sitosterolemia
5. Secondary hyperlipidemia, such as hypothyroidism

5.4 Biochemical and Molecular Perspectives

Brown and Goldstein received the Nobel Prize in Physiology or Medicine in 1985 for discovering the mechanisms of receptor-mediated endocytosis and the disruption of the LDL receptor as the cause of FH (Brown and Goldstein 1983). To date, dysfunctions of the LDL receptor pathway are recognized as the causes of FH, including FH2, FH3, and ARH.

The size and densities of the different classes of lipoproteins are determined by their composition; larger lipoproteins are buoyant and have more triglycerides and less apolipoproteins, while smaller lipoproteins are dense and have less triglycerides and more apolipoproteins (Table 5.2). Surface charge revealed by agarose gel electrophoresis varies between lipoproteins according to the amount of charged lipids and the conformations of their apolipoproteins. Hence, lipoproteins can be isolated by numerous techniques including size exclusion chromatography, nuclear magnetic resonance, ultracentrifugation, agarose gel electrophoresis, and polyacrylamide

Table 5.2 Properties of major lipoprotein classes

Lipoproteins	Diameter (nm)	Density (g/mL)	Electrophoretic mobility	Composition (%)					Major apolipoproteins
				Core		Surface			
				CE	TG	FC	PL	Protein	
Chylomicrons	80–500	< 0.93	Origin	3	86	2	7	2	B-48, E, A-I, A-II, A-IV, C
VLDL	30–80	0.95–1.006	Pre-beta	12	55	7	18	8	B-100, C-I, C-II, C-III, E
IDL	25–35	1.006–1.019	Slow pre-beta	29	23	9	19	19	B-100, E
LDL	21.6	1.019–1.063	Beta	42	6	8	22	22	B-100
HDL_2	10	1.063–1.125	Alpha	17	5	5	33	40	A-I, A-II
HDL_3	7.5	1.125–1.210	Alpha	13	3	4	25	55	A-I, A-II
Lp(a)	30	1.055–1.085	Slow pre-beta	33	3	9	22	33	B-100, apo(a)

CE cholesteryl ester, *FC* free cholesterol, *PL* phospholipid, *Lp(a)* lipoprotein (a)

gel electrophoresis. Specific lipoproteins are associated with specific apolipoproteins; however, there is some overlaps (Table 5.2). For example, apoB-100 is associated with all lipoproteins except for chylomicron and HDL, and apoA-I is mainly associated with HDL. LDL is the most abundant lipoproteins in humans.

The LDL receptor is a cell surface glycoprotein, which mediates the removal of LDLs and remnant lipoproteins from circulation by binding to apoB and apoE and plays a major role in blood cholesterol level regulation. The extracellular component of the LDL receptor comprises the following domains: a ligand-binding domain; an epidermal growth factor (EGF) precursor homology domain, which contains a six-bladed beta-propeller flanked by cysteine-rich EGF domain; and an O-liked sugar-rich domain (Fig. 5.5) (Gidding et al. 2015). LDL receptors are synthesized in the endoplasmic reticulum (ER) and glycosylated in the Golgi apparatus and then transported to clathrin-coated pits on the cell surface. After LDL binds to the ligand-binding domain on the LDL receptor, the LDL-LDL receptor complex is internalized and delivered to endosomes. In the acidic environment of the endosome, the LDL particle is dissociated from the receptor, which is recycled back to the cell surface, and the particle is degenerated for the storage of intracellular cholesterol (Fig. 5.6).

Numerous mutations (more than 1100) at the LDL receptor locus have been described as the cause of FH (http://www.ucl.ac.uk/fh). Interindividual phenotypic variation among patients with HeFH and HoFH is at least partially explained by LDL receptor activity and their causal mutations. The LDL receptor gene contains 18 exons and 17 introns and encodes 860 amino acids (Fig. 5.5). Mutations in the LDL receptor gene can be divided into five different functional classes; Class 1 mutations (null alleles) result in LDL receptor synthesis alteration. These mutations include nonsense mutations with a premature stop codon occurring early in the protein, large rearrangements including many bases, and insertions or deletions causing frameshift. Class 2 mutations cause an alteration in receptor transport to the Golgi apparatus or to the plasma membrane (completely, Class 2A; partially, Class 2B). Class 3 mutations result in an alteration of binding to apoB-100 despite normal transport to the plasma membrane. Class 4 mutations result in an endocytosis alteration despite normal binding to LDL, and class 5 mutations cause an alteration in the recycle mechanism (Fig. 5.6). Class 1 and class 2A are usually associated with null or less than 2% LDL receptor activity in cultured fibroblasts (receptor negative), while other classes are associated with reduced LDL receptor activity (receptor defective). Although the LDL receptor activity of the present case was not determined in vitro, it can be speculated that her LDL receptor function was preserved because cholesterol-lowering medication, including statin, was partially effective.

ApoB-100 is the ligand for the LDL receptor, and initially a missense mutation in the LDL

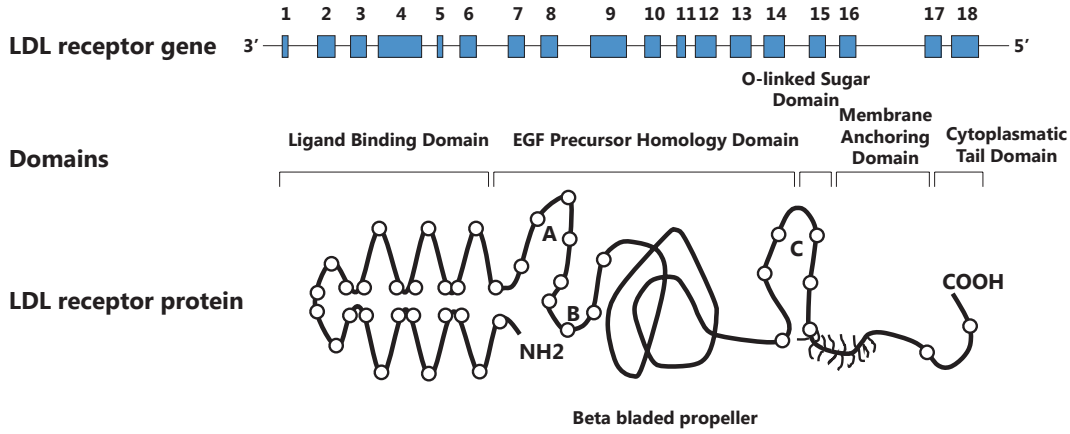

Fig. 5.5 Protein and domains of the LDL receptor
The different domains in LDL receptor protein are encoded by specific regions in the LDL receptor gene

EGF endothelial growth factor

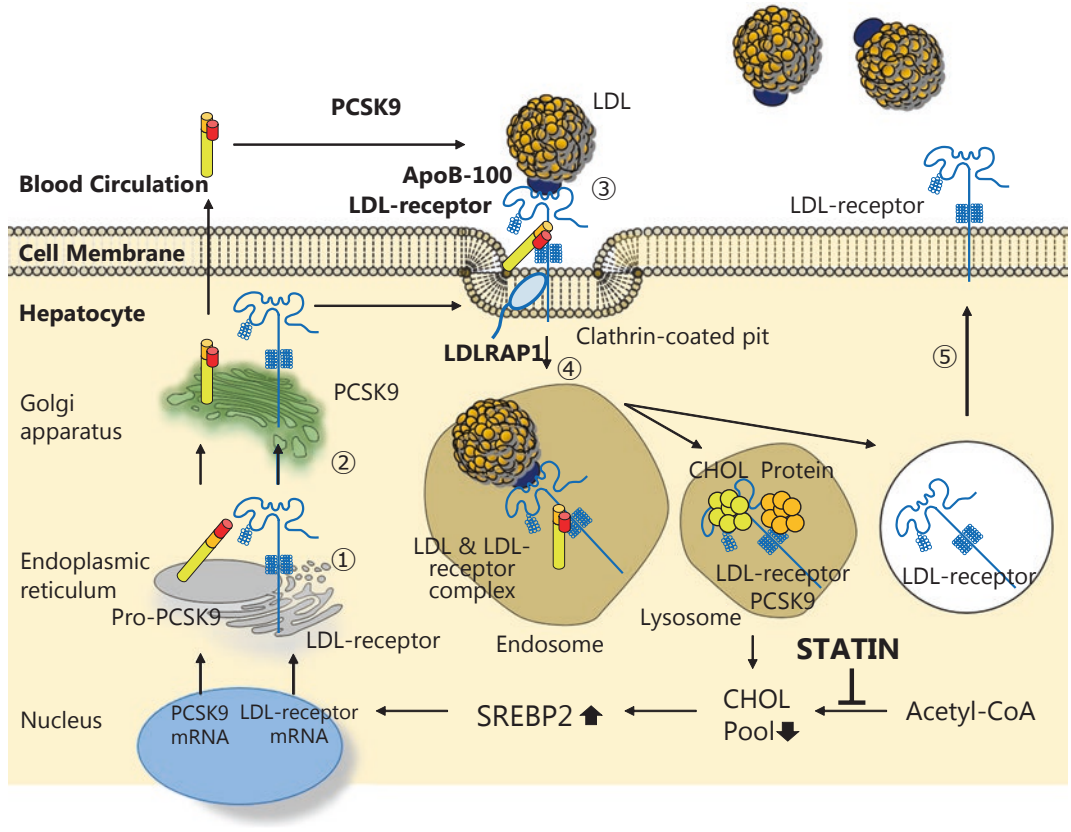

Fig. 5.6 Regulation of the LDL receptor
Both the LDL receptor and proprotein convertase subtilisin/kexin type 9 (PCSK9) are regulated by sterol regulatory element-binding protein 2 (SREBP2). The numbers indicate the sites of functional classes of LDL receptor gene mutations.
LDLRAP1 LDL receptor adaptor protein 1

receptor-binding domain of apoB-100 (Arg3500Gln) was described, followed by another mutation in the same codon (Arg3500Trp), as a cause of familial defective apolipoprotein B-100 (FDB) or FH2 (Soria et al. 1989). Both mutations are thought to reduce binding activity with the LDL receptor; eventually LDL particles in circulation are not cleared by the receptor. There is a clear localization of FDB; the frequency in Western countries is 1 in 1000 in the general population, and no cases of FDB have been reported in Japan so far (Nohara et al. 1995). Although clinical manifestations of FDB are usually milder than those of FH with the LDL receptor gene mutation (FH1), it is impossible to clinically distinguish FDB from FH1 without genetic analysis.

The proprotein convertase subtilisin/kexin type 9 (PCSK9) is the 9th member of the proprotein convertase family, which was the third cause of FH (Abifadel et al. 2003). After PCSK9 is synthesized as pre-pro PCSK9 in the ER, signal peptide (aa 1-30) is cleaved, followed by cleavage of the pro-domain (aa 31-152) by its own enzymatic activity. The pro-domain non-covalently binds to mature PCSK9 (aa 153-692) at the catalytic subunit, thereafter losing its enzymatic activity; this mature form is then secreted into circulation (Fig. 5.6). The pro-segment-PCSK9 complex binds to the EGF domain of the LDL receptor, and PCSK9 enters into cells via clathrin-coated vesicles as a chaperon with the receptor. In the acidic environment of endosomes, PCSK9 tightly binds to the LDL receptor, and PCSK9-LDL receptor complex is escorted to lysosomes for degradation (Fig. 5.6). PCSK9 regulates plasma LDL-C levels posttranscriptionally; thus, gain-of-function mutations in the *PCSK9* gene result in FH (FH3). The LDL-C levels of patients with gain-of-function mutations in *PCSK9* are widely distributed (Hopkins et al. 2015); thus, clinical distinguishing FH1 from FH3 is also difficult without measuring PCSK9 activity or genetic analysis. If PCSK9 is absent or inactivated, the LDL receptor effectively returns to the cell surface for recycling, eventually causing hypocholesterolemia.

Interestingly, the LDL receptor and PCSK9 are regulated by the same transcriptional factor, sterol regulatory element-binding protein 2 (SREBP2). SREBPs are inactive proteins bound to the ER. In the ER, SREBP binds to the SREBP cleavage-activating protein (SCAP), which is a sensor of intracellular sterols. When cellular cholesterol content is low, the SCAP-SREBP complex is able to move to the Golgi apparatus, where SREBP is sequentially cleaved by two proteases, S1P and S2P, and the NH2 terminal of SREBP is released to the cytoplasm. SREBP then translocates to the nucleus, where it activates transcription by binding to SRE-1 in the promoter regions of many genes encoding proteins involved in the homeostasis of cholesterol and other lipids. Statin, which is an inhibitor of cholesterol biosynthesis, upregulates SREBP2, increases the transcriptions of LDL receptors, and then increases PCSK9, which degrades LDL receptors. In contrast, when the cellular cholesterol content is in excess, SCAP undergoes conformational changes, which inhibits the transport of the SCAP-SREBP complex to the Golgi apparatus. As a result, the NH2 terminal of SREBP is not cleaved, and SREBP does not translocate to the nucleus to activate the target genes.

ARH is a recessive form of hypercholesterolemia. Fibroblasts from a patient with ARH have normal LDL receptor activity. The cholesterol turnover after LDL-apheresis using a rebound curve results in a defect in the degradation of cholesterol in ARH (Harada-Shiba et al. 1992). Subsequently, the cause of ARH was revealed as a deficiency in the adopter protein of the LDL receptor (LDL receptor adaptor protein 1), which is important for the internalization of the LDL receptor. Thus, the phenotype of ARH resembles HoFH with Class 5 mutations (Tada et al. 2015a). No family history of hypercholesterolemia suggesting FH is clinically important to distinguish ARH from HoFH.

Sitosterol is a type of plant sterol, which enters an enterocyte via the Niemann-Pick C1-like 1 (NPC1L1) protein together with cholesterol, and is excreted from the adenosine triphosphate-binding cassette sub-family G member 5/sub-family G member 8 (ABCG5/G8) (Fig. 5.3).

Sitosterolemia is a rare autosomal recessive hyperlipidemia caused by dysfunctional mutations in ABCG5 or ABCG8. Sitosterolemia can exhibit severe hypercholesterolemia as well as tendon and skin xanthoma, resembling HoFH. In particular, infantile sitosterolemic patients with severe hypercholesterolemia sometimes exhibit systemic intertriginous xanthomas, which diminish quickly when weaned from breastfeeding (Tada et al. 2015c). Detecting hypersitosterolemia and determining the absence of family history of severe hypercholesterolemia are important to distinguish sitosterolemia from HoFH.

It is known that double heterozygous FH, which has two different mutations in FH-causing genes (i.e., LDL receptor and apoB or PCSK9), resembles HoFH but with somewhat milder clinical manifestations.

5.5 Therapy

The primary goal for HeFH, and even for HoFH, is to have the same life expectancy and the same frequency of cardiovascular events as normal subjects. Most of hyperlipidemia other than FH develops after the second decade of life; thus, cumulative LDL-C from the birth is higher in FH than in other hyperlipidemia, even when the LDL-C levels after adulthood were the same. Therefore, intense LDL-C-lowering therapy for FH from an adequate age is crucial. A diet rich in saturated fat and cholesterol is associated with an increased flux of fats and cholesterol from the intestine. An increased cellular cholesterol content results in the downregulation of hepatic LDL receptor expression. Therefore, diet therapy consists of an adequate amount of total calories and restriction of carbohydrate, saturated fat, and cholesterol; maintaining adequate body weight is essential.

Although diet therapy is important, it is insufficient in reducing LDL-C levels in patients with FH to adequate levels. Thus, all FH patients ultimately become candidates for medical LDL-C-lowering therapy; most LDL-C-lowering medication upregulates LDL receptor activity by reducing the intracellular cholesterol content. HeFH patients, whereby half of the LDL receptors are intact, can usually be treated with ordinal cholesterol-lowering medication to acceptable levels. On the other hand, HoFH patients, who mostly lack the LDL receptor, are highly resistant to standard medical cholesterol-lowering therapy.

Statins inhibit 5-hydroxyl-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a rate limiting enzyme of cholesterol biosynthesis in the mevalonate pathway, and reduce plasma LDL-C levels by increasing hepatic expression of LDL receptors. Statin is the most widely used LDL-C-lowering medication and is proved to reduce not only cardiovascular events but also all-cause mortality of patients at high risk of cardiovascular events. Moreover, the reduction rate of cardiovascular events and achieved LDL-C levels from several randomized-controlled studies using statin were linearly and significantly associated. Ezetimibe is the inhibitor of NPC1L1, the transmembrane protein that plays a key role in the cholesterol absorption by facilitating its uptake via vesicular endocytosis. Thus, ezetimibe also reduces the cholesterol content in hepatocytes and increase hepatic expression of LDL receptors. Ezetimibe combined with simvastatin therapy significantly reduced cardiovascular events in patients with acute coronary syndrome (Cannon et al. 2015). Bile acid sequestrants (resins) interrupt the enterohepatic circulation of bile acids, resulting in hepatic upregulation of LDL receptors. Combination of these three drugs, all of which increases hepatic LDL receptors, could decrease LDL-C levels to one third of pretreatment levels in patients with HeFH (Kawashiri et al. 2012).

PCSK9 regulates plasma LDL-C levels by directing hepatic cell surface LDL receptors to lysosomal degradation, resulting in an accumulation of LDLs in circulation because of reduced clearance. Evolocumab and alirocumab are fully human monoclonal antibody against human PCSK9, which can robustly reduce plasma LDL-C levels by more than 50% with monotherapy, and can be effectively combined with the other cholesterol-lowering drugs, such as statins and ezetimibe. Evolocumab combined with statin therapy significantly reduced cardiovascular events and ischemic strokes in patients with

atherosclerotic cardiovascular diseases compared with statin therapy alone (Sabatine et al. 2017).

Microsomal triglyceride transfer protein (MTP) is a key protein in the assembly and secretion of apoB-containing lipoproteins in the liver (VLDL) and intestine (chylomicron) (Fig. 5.3). Lomitapide, a small molecule that inhibits MTP, does not require LDL receptor to decrease apoB-containing lipoproteins; thus, it is approved in several countries as an orphan drug for HoFH. While lomitapide decreases plasma LDL-C levels by 50% in HoFH, special attention should be paid for hepatic steatosis (Cuchel et al. 2013). Mipomersen is an antisense oligonucleotide, which is a small strand of nucleic acid that binds to messenger RNA to inhibit translation, for apoB. It also does not require the LDL receptor to decrease apoB-containing lipoproteins in HoFH (Raal et al. 2010). LDL-apheresis is a procedure that removes all apoB-containing lipoproteins (VLDL, IDL, LDL, and Lp(a)), the surface of which are exceptionally charged positive, using negatively charged dextran sulfate. Although LDL-apheresis is safe and does not remove HDL nor other negatively charged proteins with the exception of apoB-containing lipoproteins, it should be repeated weekly or biweekly because of rapid reaccumulation of LDL. Furthermore, it is an invasive and time-consuming procedure only available at specialized medical centers. Liver transplantation is an alternative therapy for HoFH and effectively reduces LDL-C; however, it is highly invasive and lifelong immunosuppressive therapy is required.

Under these conditions, innovative therapies are needed for HoFH. Among future treatment options, regenerative medicine using induced pluripotent stem (iPS) cells, which can introduce auto-hepatocyte with functional LDL receptors, is a theoretically attractive approach. Liver-directed gene therapy is another attractive option, but long-term stable expression of de novo gene by the host is difficult because of its immunologic exclusion. Antisense oligonucleotide therapy is also of interest for rare inherited disorders such as HoFH; antisense oligonucleotides against Lp(a) and PCSK9 are currently under development.

End-of-Chapter Questions

1. FH is thought to be the most frequent monogenic hereditary disorder. Why do you think FH is so frequent worldwide?
2. Why is tendon xanthoma very specific for FH? Why does hyperlipidemia other than FH not induce the development of tendon xanthoma even when the plasma LDL-C levels are the same with those of FH after adulthood?
3. What are the molecular causes of FH? What are the clinical characteristics and lipid levels of both HeFH and HoFH? Please explain what the compound heterozygous FH or double heterozygous FH.
4. What is the mechanism of statins, ezetimibe, and PCSK9 inhibitors to reduce LDL-C? What are the specific and novel therapies for HoFH and how do they reduce LDL-C?

Additional Materials

Which of the following lipoproteins are *not* associated with apolipoprotein B-100?

1. Chylomicron
2. VLDL
3. LDL
4. Lp(a)

Answer 1

Which of the following combinations between lipoproteins and their role is correct?

1. Chylomicron & Endogenous cholesterol carrier
2. VLDL & Provider of free fatty acids
3. LDL & Reverse cholesterol transporter
4. HDL & Exogenous cholesterol carrier

Answer 2

Which of the following physical findings is specific for FH?

1. Achilles tendon xanthoma
2. Arcus cornealis
3. Eruptive xanthoma on the skin
4. Xanthelasma on eyelids

Answer 1

Which of the following disorders are *not* be seen more frequently in FH than in normal subjects?

1. Abdominal aortic aneurysm
2. Cerebral infarction
3. Myocardial infarction
4. Peripheral artery disease

Answer 2

Which of the following combinations between drug and target molecule is *not* correct?

1. Evolocumab & PCSK9
2. Ezetimibe & NPC1L1
3. Lomitapide & apolipoprotein B-100
4. Statins & HMG-CoA reductase

Answer 3

References

Abifadel M, Varret M, Rabès JP, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, Derré A, Villéger L, Farnier M, Beucler I, Bruckert E, Chambaz J, Chanu B, Lecerf JM, Luc G, Moulin P, Weissenbach J, Prat A, Krempf M, Junien C, Seidah NG, Boileau C (2003) Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet 34:154–156

Brown MS, Goldstein JL (1983) Lipoprotein receptors in the liver. Control signals for plasma cholesterol traffic. J Clin Invest 72:743–747

Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, Darius H, Lewis BS, Ophuis TO, Jukema JW, De Ferrari GM, Ruzyllo W, De Lucca P, Im K, Bohula EA, Reist C, Wiviott SD, Tershakovec AM, Musliner TA, Braunwald E, Califf RM, Investigators IMPROVE-IT (2015) Ezetimibe added to statin therapy after acute coronary syndromes. N Engl J Med 372:2387–2397

Cuchel M, Meagher EA, du Toit Theron H, Blom DJ, Marais AD, Hegele RA, Averna MR, Sirtori CR, Shah PK, Gaudet D, Stefanutti C, Vigna GB, Du Plessis AM, Propert KJ, Sasiela WJ, Bloedon LT, Rader DJ, Phase 3 HoFH Lomitapide Study investigators (2013) Efficacy and safety of a microsomal triglyceride transfer protein inhibitor in patients with homozygous familial hypercholesterolaemia: a single-arm, open-label, phase 3 study. Lancet 381:40–46

Gidding SS, Champagne MA, de Ferranti SD, Defesche J, Ito MK, Knowles JW, McCrindle B, Raal F, Rader D, Santos RD, Lopes-Virella M, Watts GF, Wierzbicki AS (2015) American heart association atherosclerosis, hypertension, and obesity in young committee of council on cardiovascular disease in young, council on cardiovascular and stroke nursing, council on functional genomics and translational biology, and council on lifestyle and cardiometabolic health. The agenda for familial hypercholesterolemia: a scientific statement from the the American Heart Association Circulation 132:2167–2192

Harada-Shiba M, Tajima S, Yokoyama S, Miyake Y, Kojima S, Tsushima M, Kawakami M, Yamamoto A (1992) Siblings with normal LDL receptor activity and severe hypercholesterolemia. Arterioscler Thromb 12:1071–1078

Hopkins PN, Defesche J, Fouchier SW, Bruckert E, Luc G, Cariou B, Sjouke B, Leren TP, Harada-Shiba M, Mabuchi H, Rabès JP, Carrié A, van Heyningen C, Carreau V, Farnier M, Teoh YP, Bourbon M, Kawashiri MA, Nohara A, Soran H, Marais AD, Tada H, Abifadel M, Boileau C, Chanu B, Katsuda S, Kishimoto I, Lambert G, Makino H, Miyamoto Y, Pichelin M, Yagi K, Yamagishi M, Zair Y, Mellis S, Yancopoulos GD, Stahl N, Mendoza J, Du Y, Hamon S, Krempf M, Swergold GD (2015) Characterization of autosomal dominant hypercholesterolemia caused by PCSK9 gain of function mutations and its specific treatment with Alirocumab, a PCSK9 monoclonal antibody. Circ Cardiovasc Genet 8:823–831

Kawashiri MA, Nohara A, Noguchi T, Tada H, Nakanishi C, Mori M, Konno T, Hayashi K, Fujino N, Inazu A, Kobayashi J, Mabuchi H, Yamagishi M (2012) Efficacy and safety of coadministration of rosuvastatin, ezetimibe, and colestimide in heterozygous familial hypercholesterolemia. Am J Cardiol 109:364–369

Mabuchi H (2017) Half a century tales of familial hypercholesterolemia in Japan. J Atheroscler Thromb 24:189–207

Nohara A, Yagi K, Inazu A, Kajinami K, Koizumi J, Mabuchi H (1995) Absence of familial defective apolipoprotein B-100 in Japanese patients with familial hypercholesterolaemia. Lancet 345:1438

Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, Wiklund O, Hegele RA, Raal FJ, Defesche JC, Wiegman A, Santos RD, Watts GF, Parhofer KG, Hovingh GK, Kovanen PT, Boileau C, Averna M, Borén J, Bruckert E, Catapano AL, Kuivenhoven JA, Pajukanta P, Ray K, Stalenhoef AF, Stroes E, Taskinen MR, Tybjærg-Hansen A, European Atherosclerosis Society Consensus Panel (2013) Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European atherosclerosis society. Eur Heart J 34:3478–3490a

Raal FJ, Santos RD, Blom DJ, Marais AD, Charng MJ, Cromwell WC, Lachmann RH, Gaudet D, Tan JL, Chasan-Taber S, Tribble DL, Flaim JD, Crooke ST (2010) Mipomersen, an apolipoprotein B synthesis inhibitor, for lowering of LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia: a randomised, double-blind, placebo-controlled trial. Lancet 375:998–1006

Rader DJ, Cohen J, Hobbs HH (2003) Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. J Clin Invest 111:1795–1803

Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, Sever PS, Pedersen TR, FOURIER Steering Committee and Investigators (2017) Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med 376:1713–1722

Soria LF, Ludwig EH, Clarke HR, Vega GL, Grundy SM, McCarthy BJ (1989) Association between a specific apolipoprotein B mutation and familial defective apolipoprotein B-100. Proc Natl Acad Sci U S A 86:587–591

Tada H, Kawashiri MA, Nohara A, Inazu A, Kobayashi J, Mabuchi H, Yamagishi M (2015a) Autosomal recessive hypercholesterolemia: a mild phenotype of familial hypercholesterolemia: insight from the kinetic study using stable isotope and animal studies. J Atheroscler Thromb 22:1–9

Tada H, Kawashiri MA, Okada H, Teramoto R, Konno T, Yoshimuta T, Sakata K, Nohara A, Inazu A, Kobayashi J, Mabuchi H, Yamagishi M, Hayashi K (2015b) Assessment of coronary atherosclerosis in patients with familial hypercholesterolemia by coronary computed tomography angiography. Am J Cardiol 115:724–729

Tada H, Kawashiri MA, Takata M, Matsunami K, Imamura A, Matsuyama M, Sawada H, Nunoi H, Konno T, Hayashi K, Nohara A, Inazu A, Kobayashi J, Mabuchi H, Yamagishi M (2015c) Infantile cases of Sitosterolemia with novel mutations in the ABCG5 gene: extreme Hypercholesterolaemia is exacerbated by breastfeeding. JIMD Rep 21:115–122