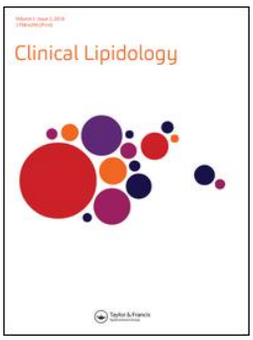

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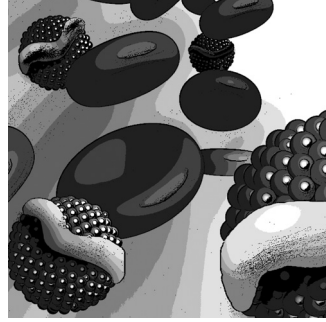

Clinical and genetic factors influencing cardiovascular risk in patients with familial hypercholesterolemia

Patients with familial hypercholesterolemia (FH) have high levels of LDL-C, owing to defective uptake of these particles by LDL receptors in the liver. Consequently, FH patients have a high risk of cardiovascular disease (CVD). However, among these patients, there is marked variance in age of onset of CVD and a proportion of untreated patients do not develop CVD at all. Statin treatment can greatly reduce risk, and since it is not yet possible to precisely predict which FH patients will develop CVD, all patients initiate treatment once the diagnosis has been made. The purpose of this article is to provide an update of clinical and genetic risk factors influencing CVD risk in FH patients, and to discuss future lines of research that could uncover improved methods of treatment for heterozygous FH patients.

KEYWORDS: cardiovascular disease ▪ coronary heart disease ▪ familial hypercholesterolemia ▪ risk factor

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Familial hypercholesterolemia (FH; OMIM #143890) [101] is an autosomal dominant disorder present in 1:500 Caucasians. FH is caused by defective LDL receptors, leading to the diminished uptake of LDL-C from the plasma by the liver. As a result, FH patients have high LDL-C levels, tendon xanthomas and a high risk of cardiovascular disease (CVD) [1]. FH can be diagnosed on the basis of clinical criteria **(Table 1)** or by detection of the causal mutation in the LDL-receptor gene [2,3,102]. Overlapping phenotypes can be caused by familial defective ApoB (FDB), familial defective protein convertase-subtilin kexin 9 (PSCK9), autosomal recessive familial hypercholesterolemia or by familial combined hyperlipidemia [4,5].

Despite the homogenous background of hypercholesterolemia, the onset and severity of CVD among FH patients varies considerably. This article provides an update of the clinical and genetic factors influencing CVD risk in heterozygous FH patients. Here, we describe studies focusing on CVD overall and those focusing solely on coronary heart disease (CHD), because the CVD risk in FH patients is mainly determined by CHD. In addition, we discuss the efficacy of statin treatment and what future research is needed to further improve the prognosis of patients with heterozygous FH.

Clinical characteristics influencing cardiovascular risk in familial hypercholesterolemia

■ Classical risk factors

Most of the classical CVD risk factors found in the general population also contribute to CVD risk in FH patients; age, male gender, BMI, hypertension, diabetes mellitus and HDL-C levels are all associated with CVD in FH [6–8]. Smoking is associated with a higher risk of CVD up to 9 years after quitting [9]. However, not all of these risk factors are independently associated in all studies, probably owing to small sample sizes and limited numbers of events during short follow-up [10].

In order to overcome the costly and time-consuming follow-up required in hard end point studies, several studies in FH patients examined intermediate end points such as intima–media thickness (IMT). Follow up of IMT progression is used as a surrogate marker for generalized atherosclerosis [11]. In FH patients, the following classical risk factors are significantly associated with this marker: age, BMI, waist circumference, systolic and diastolic blood pressure, and triglyceride level [12,13].

■ 'Novel' risk factors

In addition to these classical factors, the influence of several biomarkers on cardiovascular risk was studied. Similar to the situation in the general

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Table 1. Diagnostic criteria of familial hypercholesterolemia.

Criteria	Points
Family history	
First-degree relative with known premature (men <55 years; women <60 years) coronary and vascular disease, or first-degree relative with known LDL-C >95th percentile	1
First-degree relative with tendon xanthomas and/or arcus cornealis, or children aged less than 18 years with LDL-C >95th percentile	2
Clinical history	
Patient with premature (men <55 years; women <60 years) coronary heart disease	2
Patient with premature (men <55 years; women <60 years) cerebral, or peripheral vascular disease	1
Physical examination	
Tendon xanthomas	6
Arcus cornealis at less than 45 years of age	4
Cholesterol levels (mmol/l)	
LDL-C ≥8.5	8
LDL-C 6.5–8.4	5
LDL-C 5.0–6.4	3
LDL-C 4.0–4.9	1
DNA analysis: functional mutation in the LDL-receptor gene	8

Diagnosis: definite familial hypercholesterolemia: >8 points; diagnosis: probable familial hypercholesterolemia: 6–8 points; diagnosis: possible familial hypercholesterolemia: 3–5 points. Data taken from [102].

population, high-sensitivity CRP (hsCRP), a marker of inflammation, is an independent predictor of higher IMT, coronary calcium score and arterial stiffness in FH patients [12,14,15]. An atheroprotective biomarker in the general population is adiponectin, a hormone that is excreted by fat tissue [16,17]. Low plasma levels of this hormone also increased the risk of CHD in an FH population [18]. However, the effects of both biomarkers need to be replicated in prospective analyses.

Small-isoform lipoprotein (a) (Lp[a]) levels are associated with CHD and CVD risk [7,19,20]. This was also demonstrated by a study performed in children with FH; while having similar cholesterol levels, FH children with a family history of CHD had higher levels of small-isoform Lp(a) than those without a family history of CHD [21,22]. The size of specific lipoproteins appears to influence CVD risk in FH as well [23–26]. This size is related to the lipoprotein composition and atherogenity – the relative amount of HDL_3 and highly atherogenic small-dense LDL are associated with CVD risk [27,28].

■ Treatment

Lipid-lowering treatment, more specifically statin treatment, has a large impact on CVD risk in FH patients. Before the widespread introduction of statins, treatment of FH patients mainly focused on reduction of cholesterol uptake in the intestine. These uptake-inhibitors, so-called bile-acid sequestrants, resulted in relatively moderate cholesterol lowering and CVD risk reduction [29,30]. Owing to gastrointestinal side effects, compliance to this treatment was low, in contrast to well-tolerated statins. Statins inhibit HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis. This leads to upregulation of LDL receptors and therefore statins can be considered as causal treatment for FH. The CHD risk in asymptomatic FH patients treated long term with these drugs was not significantly higher than that of the general population, even when only moderate statin doses were used [31]. This was confirmed for primary prevention in a large observational registry study in the UK [32]. In addition, the IMT of FH patients treated with statins was in the normal range at baseline of the Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression (ENHANCE) trial [33]. In this trial, adding ezetimibe on top of statin treatment did not reduce IMT, which could most likely be explained by the fact that these patients were optimally treated with statins and further lowering of LDL-C could not decrease IMT anymore. The authors of ENHANCE proposed two alternative explanations: IMT may not be a sensitive measurement of the atherosclerotic burden in patients whose risk has been minimized; or ezetimibe may not be a very potent antiatherogenic drug. In favor of this latter explanantion, Rizzo *et al.* recently summarized a number of studies demonstrating that ezetimibe decreases LDL-C levels, but the atherogenic LDL subclass profile remained unchanged [34].

Genetic factors influencing the familial hypercholesterolemia phenotype

■ Type of LDL-receptor mutation

Whether the type of LDL-receptor mutation influences the severity of the FH phenotype has been debated for several years. LDL-receptor mutations can be classified into receptor-negative mutations with no residual function and receptor-defective mutations resulting in LDL receptors with residual function. A number of studies have consistently demonstrated a more severe phenotype in patients with receptor-negative mutations. These patients had higher LDL-C levels, higher CVD risk and higher femoral IMT thicknesses [35–39]. In a pravastatin trial, it was shown that children with a

receptor-negative mutation had higher levels of LDL-C and a greater IMT than children with receptor-defective mutations [40]. However, in a later parent–offspring study, there was no difference in CVD risk between receptor-negative and receptor-defective mutations when the mild receptor-defective mutation N543H/2393*del9* was excluded [41]. Therefore, the presence of certain mild mutations in the receptor-defective group might also explain the differences between both mutation groups found in previous studies. Finally, mortality studies demonstrated that there is large variation in risk among carriers of an identical mutation, suggesting that other risk factors are important [42]. Whether identification of the underlying LDL-receptor mutation could be omitted at all is another issue that is particularly crucial with regard to family screening. Screening using genetic testing is highly cost effective and identifies family members who could have been misclassified by cholesterol measurement alone leading to undertreatment (false-negatives) and treatment without indication (false-positives) in a substantial percentage of family members (at least 36%) [43,44].

■ Candidate gene studies

As not all variance in the phenotype and complications of FH can be explained by the classical risk factors previously mentioned, numerous studies have focused on identifying genetic risk variants other than the LDL receptor mutation in order to explain the variance of CVD risk. Here we discuss the genetic variants studied in candidate genes per pathogenetic pathway. The genetic variants that were most significantly associated with atherosclerosis within each pathway are summarized in **Table 2**.

■ Lipid metabolism

Lipid metabolism pathways are one of the most important pathways in the development of atherosclerosis and several genetic variants involved in lipid metabolism have been studied. Variants in genes encoding proteins interacting with the LDL receptor, such as *ApoB* and *ApoE*, did not associate with CHD in FH [45–47]. Variants in the *PCSK9* gene were associated with a more favorable lipid profile. However, it is unknown if this also leads to a reduction in CVD risk [48]. Variants of genes involved in reverse cholesterol transport have been studied more extensively. Reverse cholesterol transport is an antiatherogenic pathway by which cholesterol that has accumulated in macrophages is returned to the liver. This is facilitated by ATP-binding cassette (ABC)A1 transporter. ABCA1 enhances the cholesterol flux from macrophages to HDL particles. Then cholesteryl ester transfer protein (CETP) transfers this cholesterol from the HDL particles to LDL particles, which are cleared by LDL receptors in the liver. Genetic variants in *ABCA1* and *CETP* influenced CVD and CHD risk [49–52].

The metabolism of chylomicrons and very-low-density lipoproteins is controlled by ApoAIV, ApoCIII and lipoprotein lipase (LPL). Genetic variants in the genes encoding these proteins are associated with CVD in FH [46,53–56].

Genetic variants in the *ABCG8* transporter gene were examined because mutations in this gene cause siterosterolemia, resulting in impaired plant sterol metabolism and premature CVD. One of the two variants examined was associated with higher risk of CHD in FH patients [57].

LDL oxidation & inflammation

LDL is more atherogenic when it is oxidized [58]. Paraoxonase 1 and 2 (PON1 and PON2) hydrolyze oxidized LDL, and may therefore protect against CVD. Variants in genes encoding these proteins indeed associated with both IMT and CVD [46,59–62]. If the oxidized LDL is not hydrolyzed by these paraoxonases, this modified LDL can induce macrophages to release cytokines leading to local inflammation. Inflammation is a major pathogenetic pathway in atherosclerosis in FH [63,64]. Therefore, multiple candidate genes in the inflammation pathway were studied. One of them, encoding 5-lipoxygenase-activating protein (*ALOX5AP*) is involved in the biosynthesis of the proinflammatory leukotrienes and a haplotype in this gene increased the risk of CHD [65]. The inflammation process and the final development of atherosclerosis may be linked through activation of the complement system. Complement factor H prevents uncontrolled complement activation and FH carriers of the CC genotype of the genetic variant *rs1061170* are protected against CVD **(Table 2)** [66].

Blood pressure regulation

Variations in genes known to be involved in blood pressure regulation have also been associated with CVD in FH, although an effect on blood pressure could not often be demonstrated. The main blood pressure regulatory pathway studied is the renin–angiotensin–aldosterone system (RAAS). Several genetic risk factors were identified in the genes encoding

Table 2. Most significant genetic variants and cardiovascular disease-related risk in familial hypercholesterolemia patients.

Gene	Genetic variant	Risk allele/genotype	n	MAF	Phenotype	Effect	Ref.
Lipid metabolism							
Lipoprotein lipase	*rs268* (A>G)	G allele	1045	0.07	CVD	OR: 3.88; p = 0.006	[55]
Inflammation & LDL oxidation							
5-lipoxygenase activating protein (ALOX5AP)	haplotype *rs17216473-rs10507391-rs9315050-rs17222842*	A-A-A-G	1817	0.07	CHD	HR: 1.48; p = 0.001	[65]
Blood pressure regulation							
Angiotensinogen (AGT)	haplotype *rs5049* (G>A)-*rs4762* (C>T)-*rs699* (T>C)-*rs7079* (C>A)	G-C-C-C	1785	0.15	CHD	RR: 1.45; p = 0.006	[67]
Coagulation & hemostasis							
Prothrombin (F2)	*rs1799962* (G>A)	G allele	1940	0.011	CVD	HR: 2.44; p < 0.001	[46]
Replication of GWA studies							
Near cyclin-dependent kinase N2A/B (CDKN2A/B)	*rs10757274* (A>G)	GG genotype	2145	0.47	CHD	HR: 1.39; p < 0.001	[77]

CHD: Coronary heart disease; CVD: Cardiovascular disease; GWA: Genome-wide association; HR: Hazard ratio; MAF: Minor allele frequency; OR: Odds ratio; RR: Risk ratio.

angiotensin-converting enzyme (*ACE*), angiotensinogen (*AGT*) and Ang II receptor (*AGTR1*) [67–70]. In addition to moderately increased risk by one of these risk factors, FH patients carrying five or six genetic risk variants of the RAAS pathway had a 2.3-fold higher risk of CHD than patients with none or only one risk variant [71].

Coagulation & hemostasis

Coagulation and hemostasis are important pathogenetic pathways in atherosclerosis. One variant in the prothrombin gene strongly associated with CHD in a large FH cohort [45]. However, genetic variants in other genes involved in this pathway did not show a relationship with CHD [46,72]. In a number of other genes, risk variants were identified such as the estrogen receptor, the glucocorticoid receptor-α and so on [46,73–76]. For a complete overview, we refer to **Supplementary Table 1** (see online www.futuremedicine.com/toc/clp/5/2).

■ Genome-wide association studies

In contrast to candidate gene studies, genome-wide association (GWA) studies test thousands of common genetic variants throughout the genome without a prior hypothesis. By studying patients with and without CHD, this method can lead to identification of unknown atherogenetic pathways. To limit false-positive results of GWA studies owing to multiple-testing, it is important to replicate the results in independent populations. In addition, genetic risk variants in the general population do not necessarily associate with CVD risk in FH patients. Van der Net *et al.* tested ten genetic variants found previously in several GWA studies, in a large Dutch FH cohort, and could only replicate four of them **(Table 2 & Supplementary Table 1** [see online www.futuremedicine.com/toc/clp/5/2]**)** [77].

Prediction

In an ideal situation, we would be able to precisely predict CVD risk using these classical and novel risk factors and genetic risk factors in each FH patient, and therefore prevent overtreatment and undertreatment of their hypercholesterolemia and CVD risk. However, using classical risk factors, we cannot accurately discriminate FH patients who will develop CHD from those who will not – the c-statistic of a classical risk factor prediction model was 0.75, and adding 14 genetic variants did not improve this prediction model significantly [78]. Whether IMT measurements or other surrogate markers

can improve risk predictions on top of predictions based on classical risk factors remains to be established.

Novel targets for treatment

To date, treatment with statins appears to be cost effective, efficient and may be sufficient for primary prevention of the majority of heterozygous FH patients when this treatment is initiated in a timely manner [79]. However, adding another cholesterol-lowering drug or an HDL-C-raising drug remains important in further improving the lipid profile in heterozygous patients who do not tolerate high doses of statins or have manifest CHD, and to improve the situation for homozygous FH patients. Statins are generally considered to be very safe drugs, but the effects of lifelong treatment are still unknown. Although there is no indication that LDL-C lowering will become less effective during long-term statin use, we cannot exclude that this will occur during lifelong treatment and alternative LDL-C-lowering drugs should be developed. In this context, a new squalene synthase inhibitor was tested, but it was shown to have hepatic side effects [80]. The genes involved in the function of LDL receptors, such as *ApoB* and *PCSK9* are, of course, an important target as well. Gene therapy (antisense mRNA) directed at these targets is currently being tested [81,82]. Mipomersen is antisense *ApoB* mRNA and is currently tested in a number of Phase III trials [83]. It is at least very promising for homozygous FH patients and other patients resistant to statins or experiencing severe side effects. For large-scale use, the invasive administration of these drugs may be a limitation.

Drugs raising HDL-C levels are a promising approach to further normalize CVD risk in statin-treated FH patients. However, the acyl CoA:cholesterol acyltransferase (ACAT) inhibitor pactimibe in the Carotid Atherosclerosis Progression Trial Investigating Vascular ACAT Inhibition Treatment Effects (CAPTIVATE) trial and the CETP inhibitor torcetrapib in the Randomized Assessment of Digoxin on Inhibitors of the Angiotensin-Converting Enzyme (RADIANCE) and Investigation of Lipid Level Management to Understand its Impact on Atherosclerotic Events (ILLUMINATE) study were not successful. The target proteins are also influenced by statins [84–87]. However, the main reason for the failure of torcetrapib was toxicity leading to excess mortality. Torcetrapib activated RAAS and increased systolic blood pressure. Other CETP inhibitors such as dalcetrapib are tested now and so far no serious side effects and effects on blood pressure have been reported [88].

Statins have many pleiotropic effects in addition to their cholesterol-lowering effect: for example, the statins appear to attenuate inflammation, reduce thrombotic risk and may even reduce blood pressure [89–92]. To further improve the CVD risk in FH patients, development of new drugs should perhaps fully focus on targets that complement the action of statins. An example is niacin, an HDL-C-increasing drug that targets outside of the statin pathway. In a recent secondary prevention trial, niacin caused significantly more IMT decrease on top of statins compared with ezetimibe [93]. Whether niacin can decrease IMT and CHD risk in FH patients remains to be established, but the effects on lipid levels are promising [94]. The initially very promising development of supplementing ApoA1 function by administration of ApoA1-Milano or D-4F peptides appears not to be followed by recent clinical data [95,96]. Exciting new developments exist in gold nanoparticles that mimic HDL function, but clinical testing is still far off [97].

The genes identified by GWA studies and replicated in FH populations are interesting targets for new drugs; however, for most of them little is known and extensive research with respect to their function and development of novel concepts is needed.

Conclusion & future perspective

Although patients with FH share high LDL-C levels, CVD risk varies substantially. Classical risk factors increase CVD risk in FH patients but cannot explain all of the variety in their phenotype. As yet, other unknown biological risk factors, including unidentified genetic risk variants, are expected to account for a substantial part of the risk difference, but only a limited number genetic variants have been identified so far and adding these genetic risk factors to the classical risk factors did not substantially improve risk prediction. Whether IMT measurements or other surrogate markers can improve risk prediction remains to be established.

Since no sufficient risk prediction is currently possible, all FH patients are advised to diminish their classical risk factors as much as possible (lifestyle intervention) and to be treated uniformly with statins, which fortunately largely reduce CVD risk. An early diagnosis of FH enables beginning this treatment at a young age. However, the latest findings suggest that it

is probably safe to wait until adulthood and it would be of great interest to find the optimal age of beginning treatment. Besides, a number of patients do not tolerate statins or experience side effects at nonoptimal dosages. Others, including homozygous patients, do not reach target cholesterol levels even when using a maximum dose. Therefore, combined therapy and new therapeutic options remain interesting. This would also be of major interest for secondary prevention in which normalization of CVD risk is still far out of reach. An improved prediction model may also assist in selecting the specific patients who will benefit from a specific additional treatment. Elucidating atherogenesis by identifying genetic risk factors therefore certainly remains of interest, in particular, genes outside the pathways influenced by statins may be valuable novel targets for future treatment.

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Executive summary

Background

- Familial hypercholesterolemia (FH) is characterized by high levels of LDL-C and a high cardiovascular disease (CVD) risk, but CVD risk varies considerably among FH patients.

Risk factors

- The same classical risk factors that cause CVD in the general population are associated with CVD in FH patients. Although several genetic risk variants have been identified, prediction of CVD risk is not improved by using these variants.

Treatment

- Fortunately, CVD risk in asymptomatic FH patients is largely reduced by statin treatment, which should be initiated as soon as the diagnosis of FH has been made. In addition, all classical risk factors present in an individual patient should be treated. A novel strategy is required to further improve the treatment, especially for secondary prevention in FH. Cardioprotective drugs without overlap with the (pleiotrophic) effects of statins offer the best opportunity to improve the prognosis of heterozygous FH patients.

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