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Metabolic and genetic aspects of familial combined hyperlipidaemia with emphasis on low-density lipoprotein heterogeneity

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Introduction

The relationship between elevated plasma cholesterol and the risk of coronary artery disease is now definitively established [1-5]. Accumulating evidence indicates that the total amount of triglyceride-rich lipoprotein particles, i.e. chylomicon remnants, very low-density lipoproteins (VLDLs) and intermediate-density lipoproteins (IDLs), also determines the risk of developing cardiovascular disease [6-9]. This would explain the benefit of cholesterol-lowering therapy observed in the majority of patients with coronary disease who have only marginally elevated plasma cholesterol levels but may exhibit other lipid abnormalities [4].

In patients suffering from familial combined hyperlipidaemia (FCH), elevated levels of triglyceride-rich lipoproteins mainly determine the presenting lipid phenotype. Because FCH appears to be the most common form of hyperlipidaemia in young survivors of myocardial infarction [10-12], causing an estimated 10% of all premature coronary heart disease [13,14], recent research has been focused on the pathophysiological mechanism underlying premature atherogenesis in FCH. In this review, hypotheses concerning the metabolic and genetic basis of FCH and its related entities, as well as the origin of LDL heterogeneity associated with these lipid disorders, will be discussed.

Phenotypic diagnosis of FCH

In 1973, FCH was first described by Goldstein et al. [10], Rose et al. [13] and Nikkila and Aro [11], shortly followed by others [15,16], as a new autosomal dominant inherited lipid disorder characterized by elevated plasma cholesterol and triglyceride levels in first-degree relatives and strongly associated with premature cardiovascular disease. At that time, the recognition of FCH confounded the previously formulated Fredrickson classification of hyperlipoproteinaemias by the presence of first-degree relatives exhibiting different lipid phenotypes within one single family.

Because there is no specific marker for the disorder whereas the lipid phenotypic expression among affected individuals may show some variation with time, the diagnosis is necessarily based on family investigation to demonstrate a so-called 'mixed hyperlipidaemia' with either hypercholesterolaemia, hypertriglyceridaemia or combined hyperlipidaemia in first-degree relatives. Criteria supporting the FCH diagnosis are presented in Table 1. Nowadays, it is common sense that all main inclusion criteria, in the absence of all exclusion criteria, should be met for a true diagnosis, whereas the diagnostic value of the mentioned additional inclusion criteria is still under debate.

Although FCH patients frequently exhibit elevated plasma apo B concentrations [14,17] when compared with their normolipidaemic relatives, the interpretation of total plasma apolipoprotein B (apo B) levels as a diagnostic criterion is still open for discussion. A plasma apo B100 level above 130 mg dL⁻¹, standardized according to the radioimmunoassay method of the International Union of Immunological Societies, may contribute to defining FCH patients [18,19]. Considering that the lipid to protein ratio of VLDL and LDL particles is relatively constant even in FCH patients [14,20,21], total plasma apo B could be derived from the strong correlation that appears to exist between VLDL- plus LDL-cholesterol and plasma apo B [17]. Therefore, plasma apo B is strongly correlated with the presented FCH lipid phenotype based on elevated VLDL and/or LDL concentrations [17].

For reasons that are not clear, the manifestation of hyperlipidaemia in childhood, as frequently observed in familial hypercholesterolaemia, rarely occurs in FCH [22]. However, hyperapobetalipoproteinaemia, a feature associated with FCH, has been detected in children of parents with premature cardiovascular disease [23,24].
Table 1. Inclusion and exclusion criteria supporting the diagnosis familial combined hyperlipidaemia

**Main inclusion criteria**
- Presence of a multiple type hyperlipidaemia in first-degree relatives of a single family comprising hypertriglyceridaemia, hypercholesterolaemia, and combined hyperlipidaemia, as defined by fasting plasma cholesterol and/or plasma triglyceride concentrations above the 90th percentile for age and gender
- Autosomal dominant inheritance of the hyperlipidaemia
- Presence of premature atherosclerosis (before age of 60 years) in first-degree relatives

**Additional inclusion criteria**
- An elevated total plasma apolipoprotein-B concentration
- A LDL subfraction profile predominated by small, dense LDL particles
- Manifestation of the hyperlipidaemia in adolescence

**Exclusion criteria**
- Presence of any form of xanthoma in first-degree relatives
- Presence of a secondary cause for the hyperlipidaemia in affected relatives
- Presence of the Apo e2/e2 genotype in first-degree relatives

FCH and its related lipid phenotypes

Initially FCH was thought to be inherited as a single-gene disorder with a major effect on triglyceride levels [10]. Recently, evidence for a major gene effect on triglyceride levels was again provided by complex segregation analysis in British FCH families [25]. Although this supposed gene mutation has still not been located, other studies have indicated that a variety of metabolic and biochemical defects predispose for the FCH phenotype, suggesting that the genetic basis of this trait is heterogeneous and may even involve several defects in one family. According to these reports FCH may be considered more as a 'syndrome', showing overlapping characteristics with other entities (Fig. 1) such as (a) hyperapobetalipoproteinaemia (hyperapoB) defined by a normal LDL-cholesterol level with an increased LDL protein (apo B) content [26]; (b) the 'atherogenic lipoprotein phenotype' (ALP) characterized by increased triglyceride and apo B levels, decreased HDL levels and a predominance of small, dense LDL [27]; (c) familial dyslipidaemic hypertension (FDH), a syndrome of mixed lipid abnormalities resembling the FCH phenotype, associated with mild hypertension [28]; and (d) the insulin resistance syndrome, which is associated with increased VLDL production and impaired clearance of triglyceride-rich particles, also key features of FCH [29,30].

Pathophysiology of FCH

In general, FCH is thought to be caused by hepatic overproduction of VLDL with or without impaired clearance of triglyceride-rich lipoproteins [31,32]. As no single metabolic defect detected so far can fully account for the FCH phenotype, it has been hypothesized that a number of defects are involved. It still has to be determined whether these defects are causal of the disorder or are a regulatory consequence of an underlying metabolic defect. In general, circulating triglyceride-rich lipoproteins are of exogenous or endogenous origin. For better comprehension, these pathways are described in more detail below.

**The exogenous pathway**

This pathway involves the transport of dietary lipids from the intestine to the liver by apo B48-containing chylomicrons. As a result of the action of the enzyme lipoprotein lipase (LpL), activated by co-factor apo CII, fatty acids are liberated from chylomicrons and pass to the adipose tissue or skeletal muscle cells to be oxidized or stored. Reduced LpL activity as a result of LpL gene mutations has been reported repeatedly in subsets of FCH populations [33,34] and may result in impaired clearance of chylomicrons. For storage in adipocytes, free fatty acids (FFAs) are intracellularly re-esterified to triglycerides, a process that is mediated by the action of a basic protein called acylation stimulating protein (ASP) [35]. Owing to impaired ASP activity, as reported in hyperapoB, a reduced rate of FFA uptake into adipocytes may result in an increased flux of FFA to the liver and
consequently in increased hepatic VLDL synthesis [36]. After the release of FFAs, the remaining chylomicron remnant particles are taken up by the liver via a specific remnant receptor that only recognizes apo E as ligand [37]. A delayed clearance of atherogenic chylomicron remnants, possibly due to competition between chylomicrons and endogenous VLDL for available LpL activity and competition for remnant receptor capacity, has been reported to exist in FCH patients [38]. In the hepatocytes, all components of the remnants are hydrolysed in the lysosomal compartment and a part of this material is re-used to form nascent VLDL particles entering the endogenous pathway. Intracellular increase in cholesterol in hepatocytes may increase plasma LDL-cholesterol concentration as a result of hepatic LDL receptor down-regulation.

The endogenous pathway

This pathway involves the assembly of formed endogenous cholesterol and triglycerides in the core of VLDL followed by excretion into the circulation. In vitro studies show that in HepG2 cells intracellular triglyceride biosynthesis, but not the rate of synthesis of cholesterol or cholesteryl esters, determines the secretion rate of VLDL-apo B [39]. The triglyceride biosynthesis itself depends on the availability of required FFAs, which are either released from adipocytes, stored intracellularly or converted from dietary carbohydrates [40]. The release of required FFAs from visceral adipocytes is mediated by the action of the enzyme hormone-sensitive lipase (HSL) [41,42]. Post-prandial hyperinsulinaemia plays a regulatory role because it inhibits the lipolytic effect of HSL to allow FFA uptake by adipocytes [43]. FCH is associated with increased insulin resistance, which would allow for increase in VLDL production by net increase of serum FFAs [44,45]. Without merging with lipids to allow the formation of a VLDL particle, nascent apoprotein B100 is degraded. This process is catalysed by the action of microsomal triglyceride transfer protein (MTP), referring to its site of action in the hepatic endoplasmic reticulum [46,47]. Recently, abetalipoproteinemia, the metabolic 'opposite' of FCH, was found to be caused by MTP absence [48]. Consequently, it has to be established whether MTP overexpression could also play a role in VLDL-apo B overproduction of FCH.

The continuous hydrolysis of core triglycerides in FFAs by LpL converts VLDLs into smaller apoprotein B100-containing VLDL remnants, IDL and LDL [40,49,50]. Recent reports suggest that a heterozygous state for one of the mutations found in the LpL gene, affecting its activity, may result in a lipoprotein pattern classified as FCH [33,34,51]. However, it remains unclear whether this phenomenon is more pronounced in hyperlipidaemic subjects than in normolipidaemic individuals without an underlying metabolic defect. Accurate data on the prevalence of these mutations in different populations may help to interpret the observed influences. Increased apo CIII levels are associated with impaired clearance of triglyceride-rich lipoprotein due to direct inhibition of LpL by apo CIII [52]. Interestingly, linkage between the FCH phenotype and the AICIII/AIV gene cluster has been reported [53]. However, this finding could not be confirmed by others, although several polymorphisms in the gene cluster were recently found to amplify the phenotypic expression in FCH [54].

A portion of small VLDL, i.e. IDL, is catabolized after apoprotein E-mediated binding to hepatic LDL or B/E receptors, which differ from the chylomicron remnant receptor. Affinity for the B/E receptor depends on the apo E isoform (i.e. high for apo E3 and E4, but low for apo E2). A recent study on the effects of apo E polymorphism on presenting lipid phenotype in FCH suggested that differences in apo E isoform-related clearance may only contribute to the hyperlipidaemia as a result of other defects [55]. Further hydrolysis of triglycerides, predominantly by hepatic triglyceride lipoprotein lipase (HtgL), processes remaining IDL into LDL particles that then mainly consist of cholesteryl esters and apoprotein B100 [56]. Exchange of LDL cholesteryl esters with VLDL triglycerides mediated by cholesteryl ester transfer protein (CETP) activity determines in part the observed heterogeneity of LDL particles [57], as will be discussed later.

FCH and LDL heterogeneity

Introduction

It has been recognized for a number of years that LDL particles are markedly heterogeneous in physical and chemical properties [58–62]. In FCH, these properties of LDL are reported to be different from normal [20,63,64]. The LDL subspecies in FCH are heterogeneous with a propensity towards small, dense particles [14,20,65]. The predominance of small, dense LDL subfractions in FCH family members may not be fully explained by metabolic processing alone. Direct, as yet unclarified, genetic influences on the distribution are proposed as well [17,66,67]. Also, it is only recently that the relationship between qualitative features of LDL particles and cardiovascular disease has attracted considerable interest. This interest was raised by reports that certain LDL classes may be more atherogenic than others owing to differences in susceptibility towards oxidative modification [59,68–71]. Recent prospective studies supported this evidence of a role for small, dense LDL particles in the aetiology of atherosclerosis [72–74]. However, major questions about origin, structural variation and biological function of LDL subspecies are still only partly understood.

Identification and characterization of LDL subfractions

Since the first reports on measurements of LDL heterogeneity, two basically different techniques have been used to identify LDL subspecies: (a) non-denaturing gel electrophoresis (GGE) of whole plasma or of isolated LDL, which separates several LDL subfractions based on...
Metabolic aspects of LDL subfractions

Electrophoretic technique may combine the advantages than 90% of the sera [81]. However, different LDL continuous variable research in our laboratory. This method reveals up to prestained whole plasma has been the basis of the used and ultracentrifugation performed, up to 15 fractions can be isolated within the LDL density of 1.019 – 1.063 g mL\(^{-1}\). A single-spin DGUC procedure using prestained whole plasma has been the basis of the research in our laboratory. This method reveals up to five different LDL subfractions. Quantification by densitometric scanning allows the calculation of a continuous variable \(K\) describing the relative contribution of each LDL peak height to the total LDL [80]. In a comparison study, the number of LDL subfractions detected by GGE or DGUC was the same for more than 90% of the sera [81]. However, different LDL subfractions were less well separated by GGE than by DGUC. Furthermore, an advantage of the DGUC is that isolated samples of subclasses are available for further biochemical analysis. In the near future, a capillary electrophoretic technique may combine the advantages of a fast procedure and small amount of required plasma to discriminate between different LDL subfractions [82].

Metabolic aspects of LDL subfractions

The intravascular formation of LDL subfractions involves the conversion of VLDL precursors [83,84], and possibly also a direct hepatic secretion of different IDL [85] or LDL subspecies [31,86]. Previously, it was postulated that exchange of LDL cholesteryl ester for VLDL triglyceride, mediated by the CETP, results in a net transfer and a significant enrichment of the LDL with triglyceride [87–89]. The subsequent action of lipoprotein lipase (LpL) or hepatic lipase (HtgL) results in hydrolysis of a significant amount of LDL triglycerides and thereby a decrease in particle size [90–93]. The rate and magnitude of exchange may depend upon the relative pool size of triglyceride-rich lipoproteins vs. the cholesteryl ester-rich lipoproteins. In general, this hypothesis of exaggerated triglyceride transfer and lipolysis can explain the predominance of small, dense LDL in any form of hypertriglyceridaemia, but in FCH the pool of triglyceride-rich lipoproteins is primarily enlarged and consists of chylomicron remnants as well [38]. However, few data, including our own, also support the presence of a predominance of dense particles in FCH family members who display primarily elevated cholesterol levels [66,67,94]. Furthermore, VLDL heterogeneity may also underlie LDL heterogeneity. Large triglyceride-rich VLDL, resembling chylomicrons in patients with LpL deficiency, were found to be rapidly removed from the circulation [50]. Several studies demonstrated that predominantly small VLDL particles secreted into the circulation are converted into LDL [49,95]. Using stable isotopes, it was recently demonstrated that, in subjects with predominantly dense LDL, both an increased production and reduced clearance of large VLDL occurred, which then undergo intravascular catabolism to successively smaller remnant particles, a pathway not apparent in subjects with larger, more buoyant LDL [96]. This and numerous other studies demonstrate the complexities of apo B particle metabolism. However, all these studies have in common that the metabolic actions of lipid transfer proteins and lipases, eventually combined with substrate specificity as well as heterogeneity among apo B precursor particles, could account for the multiple different LDL subfractions observed in normal and hyperlipidaemic subjects.

Genetic aspects of LDL heterogeneity

Accumulating data suggest that the formation of LDL subfraction profiles is influenced genetically [97]. In particular, the finding of inherited LDL subfraction profiles in normolipidaemic families [80,98] strongly suggests a genetic background. Initially, Fisher et al. [99] reported a single genetic locus without dominance thought to be responsible for inheritance of LDL molecular weight quality in five families. Complex segregation analysis in healthy families [98] and in families of probands with familial combined hyperlipidaemia [66] have indicated that LDL subclass pattern B, as assessed by gradient gel electrophoresis, is under the influence of a major gene or genes with a dominant or additive mode of inheritance. Two recent studies, a study in normolipidaemic families [80] and a study in FCH families [67] from our laboratory, in which LDL subfractions were detected by DGUC, confirmed a major gene effect. In contrast to the previous studies of Austin et al. [66,98], we observed a recessive mode of inheritance and gene frequencies significantly different between the normolipidaemic and FCH population [67,80]. However, all these studies, including a recently performed heritability analysis of a continuous LDL peak particle diameter performed in twins [100], have indicated that genetic factors could account for at least 40% of the variation in LDL particle size and density in both normolipidaemic and hyperlipidaemic subjects, with the remaining 60% due to non-genetic or environmental influences. Among these environmental factors are age, gender, body mass index, smoking habits, hormonal status in women (combined estimated effect of 20%) and lipid and lipoprotein levels (estimated effect of 40%) [67,80].

Additional genetic studies have linked candidate genes to the small, dense LDL phenotype. Although reported
only once, remarkably strong linkage (LOD score of 4.43) of pattern B to a gene locus near the LDL receptor on chromosome 19p was found in 51 family members of nine probands with an 'atherogenic lipoprotein phenotype' (ALP), whereas weaker linkage was observed with the insulin receptor locus on the same chromosome 19p [102]. Recently, in subjects of the San Antonio Heart Study, variations in apo E polymorphism on LDL heterogeneity were not observed [55]. In another study, the apo B100 EcoRI polymorphism, previously associated with variation in plasma lipids, was found to play a role in the susceptibility to the development of dense LDL in visceroally obese hyperinsulinaemic men [103]. Evidence for linkage of pattern B to three markers on the LDL receptor itself – the apo CIII gene on chromosome 11, the CETP gene on chromosome 16p and the manganese superoxide dismutase (MnSOD) gene on chromosome 6q – has also been reported. However, no linkage was observed for other candidate loci tested: apo B, apo AI, apo (a), apo E/CII/CIII, LpL and HDL-binding protein [104,105]. Although the investigated genetic loci have been identified by polymorphic DNA markers, which do not necessarily indicate the presence of causative mutations, it is remarkable that the protein products of genes with observed linkage have connections with metabolic pathways possibly involved in the generation or an impaired clearance of small, dense LDL.

1 Because small, dense LDL particles have been shown to have reduced affinity for the LDL receptor [106–108], altered LDL receptor function or regulation could result in further impairment of plasma clearance of these LDL or their metabolic products.

2 Apo CIII gene haplotypes are associated with variation in plasma triglyceride levels [52], which in turn could affect levels of small, dense LDL.

3 CETP may facilitate lipolytic conversion of larger to smaller LDL particles by promoting triglyceride transfer into the LDL core [109].

4 A possible mechanism associated with MnSOD activity is unclear, but it is conceivable that defective function of MnSOD results in increased lipid hydroperoxides in plasma lipoproteins, with a concomitant increase in oxidative susceptibility, or otherwise alters lipoproteins in a manner leading to formation of small, dense, more oxidizable LDL [71,105,110].

Although genetically influenced factors resulting in retardation of catabolism of triglyceride-rich lipoproteins or their remnants may have an aetiological or contributory role in the formation of small, dense LDL, it is striking that mutations in the gene coding for LpL were not linked to the pattern B [105] even so, because the LpL Asn291→Ser was recently found to be significantly linked to the presenting lipid phenotype in FCH families [51].

Altogether, LDL heterogeneity results from a variety of environmental influences and probably also from direct genetic factors. In a family, one or more defect may be responsible for the major gene and additive effects identified by segregation analysis.

**FCH and premature atherogenesis**

In spite of often mildly elevated lipid levels compared with other lipid disorders, a high prevalence of cardiovascular diseases occurs in FCH families. The explanation for premature cardiovascular disease in FCH may be attributed to the increased prevalence of small, dense LDL [27,111].

One of the earliest events in the formation of atherosclerotic plaques is the massive accumulation of cholesterol in so-called scavenger cells to convert into foam cells in the artery wall [112,113]. As normal receptor-mediated endocytosis of cholesterol via the LDL receptor initiates intracellular processes that prevent further LDL uptake, alternative mechanisms are necessary to explain the foregoing intracellular cholesterol accumulation [114]. Many lines of evidence support the hypothesis that oxidative modification of LDL plays a pivotal role in atherogenesis [115–118]. However, this theory cannot be considered proven for the human disease [119]. The oxidative modification hypothesis proposes that oxidative damage to LDL generates a series of modified forms of LDL (oxLDL) that are in a number of ways more atherogenic than native LDL. In contrast to native LDL, oxidized LDL (oxLDL) is recognized and rapidly internalized by macrophage scavenger receptors [115], exhibits marked effects on the viability of endothelial cells and smooth muscle cells and alters the chemotactic activity of monocytes and monocyte-derived macrophages, all features which have been implicated in atherogenesis [115]. Special oxLDL receptors on the macrophages may not be down-regulated by the endocytosis of several forms of modified LDL and facilitate intracellular accumulation of oxLDL [120–123]. Small, dense LDL, being more susceptible to oxidative modification [64,71,124], may increase the supply of oxLDL to these receptors. Recently, in subjects with FCH, total LDL was found to be more prone to in vitro oxidation owing to the predominance of dense LDL particles. In addition, it was suggested that the decreased redox status of coenzyme Q10 in LDL from subjects with a dense LDL subtraction profile reflected the presence of already in vivo modified LDL owing to lipid peroxidation in the circulation [125,126].

**Therapeutic options in FCH**

Because of the up to 10-fold increased incidence of cardiovascular diseases in FCH patients [10,14], family screening and lipid-lowering treatment should be initiated. Both lowering of the total amount of atherogenic lipoproteins, i.e. LDL-cholesterol and triglyceride-rich lipoproteins, as well as a reduction in the atherogenicity of LDL, i.e. reduction in the total amount of small, dense...
LDL particles, should be aimed at. For this purpose, diet and lifestyle changes are usually insufficient and, consequently, drug therapy is frequently indicated. In the last decade, a spectrum of effective lipid-lowering drugs became available. The HMG-CoA reductase inhibitors are highly effective in reducing LDL-cholesterol in patients with primary hypercholesterolaemia [127]. Although these drugs show some triglyceride reduction as well, less effect is observed in reduction of the amount of small, dense LDL particles [19,128,129]. Fibrates show a primary triglyceride-lowering effect [130]. Convincingly related to this effect on triglyceride levels, a reduction in the size of the small, dense LDL subtraction and normalization of the LDL subtraction profile is observed, whereas the total amount of LDL-cholesterol is unaffected or may even increase [19,131,132]. Nicotinic acid, which especially reduces triglyceride levels by modifying the amount of FFA entering the liver, can be very useful in FCH [3,133,134]. However, because of several side-effects it is prescribed less often in Europe. bile acid-binding resins are frequently contraindicated in very useful in FCH [13,133,134]. However, because of several side-effects it is prescribed less often in Europe. bile acid-binding resins are frequently contraindicated in FCH because of an increase in VLDL concentration [135,136]. The place of antioxidants, i.e. vitamins E and C, β-carotene and flavonoids, to prevent LDL particles from oxidative modification and cardiovascular disease is still under investigation. Although a reduction in in vitro LDL oxidizability has been observed [137–140] and a reduced risk of coronary heart disease was found [141–146], the results of these studies are not totally consistent.

A direct comparison of the HMG-CoA reductase inhibitor simvastatin and the fibrate gemfibrozil in the treatment of FCH subjects with a combined hyperlipidaemic phenotype demonstrated the specific effect of both drugs. However, none of these agents alone completely normalized the lipid and lipoprotein profiles. Interestingly, an overall dense LDL subtraction profile, although less pronounced, persisted despite substantial triglyceride-lowering [19]. This finding therefore supports the hypothesis of small, dense LDL being present in FCH subjects irrespective of metabolic influences.

So far, the use of drugs should be based on which lipoprotein fraction is elevated the most, and probably a combination of a statin and a fibrate may be the therapy of choice in selected FCH patients with a high risk of cardiovascular disease. In future, possibly more potent HMG-CoA reductase inhibitors, such as atorvastatin, which also have a strong triglyceride-lowering effect, may become the drug of choice [147,148].

Conclusion
Because of its large impact on total cardiovascular mortality, knowledge of the pathogenesis of the heterogeneous FCH syndrome as well as the cause of the associated premature atherogenesis is essential. A major difficulty arises in identifying affected subjects, because a specific marker for the disorder is still lacking. Therefore, family investigation should be performed to verify the diagnosis in a patient with combined hyperlipidaemia and/or premature cardiovascular disease. Until now, this is the only way to prevent affected relatives from premature cardiovascular disease.

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