Coronary heart disease is the leading cause of death in developed countries. This alarming statistic is partly attributable to lifestyle, and partly due to the genetic factors that make humans highly susceptible to atherosclerotic vascular disease. The principal metabolic causes of atherosclerosis include hyperlipidemia, hypertension, obesity, insulin resistance and diabetes mellitus. Here we discuss the aetiology of familial combined hyperlipidemia (FCHL), a highly atherogenic disorder affecting 1–2% of the Western world. Genome-wide linkage studies indicate that more than three genes contribute to the pernicious lipid profile of FCHL, and that these genes reside within the 1q21–23, 11p14.1–q12.1 and 16q22–24.1 chromosomal regions. Other loci include 1p31, 6q16.1–16.3 and 8p23.3–22, but the linkage data for these are not yet persuasive. Combined linkage and association analyses provide compelling evidence for the involvement of two distinct alleles at the \( APOA1/C3/A4/A5 \) gene cluster in the transmission of FCHL. An important lesson arising from the study of a complex genetic disorder, such as FCHL, that lacks a consensus on diagnostic criteria, is that an understanding of complex genetic disorders can derive from comparative analyses of genome-wide linkage data generated from conditions that share phenotypic overlap. The identification of potential genetic overlap between FCHL and the Metabolic Syndrome, which is estimated to affect 47 million Americans, promises to deliver new targets for reducing the risk of important conditions such as cardiovascular disease and stroke.

**INTRODUCTION**

Coronary heart disease (CHD), already the major cause of mortality in developed countries, is on track to become the world’s most common cause of disease-related disability and death by the year 2020 (1,2). This review focuses on the genetic basis of familial combined hyperlipidemia (FCHL), a relatively common condition that confers a substantially increased risk of CHD (3–5). The term FCHL was coined in 1973 to describe a mixed pattern of lipid abnormalities in 47 Seattle pedigrees (6), which was subsequently observed in many cohorts throughout the world (5,7–12). The Seattle families were ascertained through survivors of myocardial infarction who had hyperlipidemia. Families in which there was a predominance of elevated cholesterol or triglyceride levels were assigned to groups termed familial hypercholesterolemia and hypertriglyceridemia, respectively. Pedigrees containing members with hypercholesterolemia and hypertriglyceridemia were said to have FCHL (OMIM 44250). In these families, members characteristically had high blood levels of both cholesterol and triglyceride. However, increases in either cholesterol or triglyceride level alone were also frequently observed. In humans, serum cholesterol and triglyceride levels are primarily determined by a series of metabolic pathways, ligands and receptors that operate in the small intestine, liver, adipose tissue and skeletal muscle (Fig. 1). Dietary lipids initially enter the circulation in the form of chylomicron (Cm) particles, where they may provide peripheral tissues with an important source of energy through the \( \beta \)-oxidation of fatty acids. In the post-prandial period, a proportion of these dietary lipids re-enter the circulation in the form of very low density lipoprotein (VLDL) particles (13), which are assembled in the liver (Fig. 1). VLDL also transports non-dietary lipids formed from the catabolism of dietary carbohydrate, the recycling of cellular membranes and the esterification of free fatty acids, that may derive from adipose tissue (14–16). The small intestine and liver additionally secrete nascent high density lipoproteins (HDL), which returns excess cholesterol from diverse sources to the liver for excretion from the body in bile (17). These sources include macrophages/foam cells (18,19), a cellular component of atheromatous plaque, peripheral tissues, and the products of triglyceride-rich lipoprotein catabolism, namely Cm remnant particles, intermediate density lipoproteins and low density lipoproteins (LDL). The increased transfer of...
cholesteryl esters from HDL to intermediate density lipoproteins (IDL) and remains with these particles throughout their catabolism. The lower molecular weight apolipoproteins, A1, C3 and A4 readily exchange between lipoproteins of different classes. For simplicity the mechanisms regulating the transport of lipids other than cholesterol and triglyceride are not shown. CETP = cholesterol ester transfer protein; CmR = chylomicron remnant particle; FFA = non-esterified fatty acids; LCAT = lecithin-cholesterol acyltransferase; \( \gamma \) = receptors for lipoprotein and lipid uptake; asterisk = apoA1.

For example, Pajunkata and colleagues (10,43) ascertained their families through a hyperlipidemic proband (i.e. raised serum cholesterol or triglyceride levels) with premature CHD (Fig. 2), while Naoumova et al. (12) recruited families through an index patient with primary combined hyperlipidemia (i.e. raised serum cholesterol and triglyceride levels) who had to have a blood relative with primary hyperlipidemia (i.e. raised cholesterol or triglyceride levels). Premature CHD was not an inclusion criteria, although more than 50% of the index patients had either a personal or family history of premature CHD (12). In Westernized societies, the lipid abnormalities of FCHL may occur as a manifestation of the Metabolic Syndrome (Fig. 3), and the received wisdom is that the two conditions may share aetiological overlap (48,49). However, the extent of this overlap has been difficult to define because of important differences in patient ascertainment and follow-up (Figs 2 and 3). The diagnosis of FCHL typically proceeds through an index patient with marked hyperlipidemia, and would normally exclude individuals with secondary hyperlipidemia due to obesity and/or type 2 diabetes. By contrast, a diagnosis of the Metabolic Syndrome (50–52) is designed to encompass individuals with a cluster of CHD risk factors (53), including...
impaired glucose intolerance, type 2 diabetes, dyslipidemia (i.e. raised blood triglyceride and/or low HDL-cholesterol levels), obesity and hypertension (Fig. 3). More fundamentally, an FCHL diagnosis demands that index patients have a blood relative with primary hyperlipidemia, whereas the Metabolic Syndrome does not.

Remarkably, the past 5 years have seen considerable progress in dissecting the genetics of FCHL, which has indicated where genetic overlap with the Metabolic Syndrome may exist. This success derives from advances in statistical genetics and comparative sequence methodologies, and the recognition that the inheritance of FCHL involves both disease and quantitative trait loci (QTL). In this review, we focus on data implicating the involvement of genes within the 11p14.1–q12.1 and 16q22–24.1 chromosomal regions, and distinct sequence variants at the APOA1/C3/A4/A5 gene cluster, which resides ∼70 cM downstream of the 11p14.1–q12.1 genomic interval. The 1q21–23 locus (43) has been the focal point of several previous reviews, and for this reason is not considered here (44,54,55).

REPLICATION OF CHROMOSOME 11p14.1–q12.1 FCHL LINKAGE

The evidence for linkage of the 11p14.1–q12.1 chromosomal region to FCHL was originally detected in a two-stage genome-wide screen of 35 extended FCHL Dutch families (Fig. 2) using non-parametric linkage analyses (Fig. 4). In the first stage analysis, an LOD of 2.6 was obtained for linkage of the interval to an FCHL lipid abnormality, defined as either high serum cholesterol, triglyceride or apoB (9). In a second stage, the estimated position of the causative lesion moved closer to D11S1324 at 35 cM (Fig. 4). Subsequently, genome-wide studies in white British families (Fig. 2) identified a potential QTL for serum cholesterol and, a disease locus for the triglyceride trait of FCHL in the same genomic interval (Fig. 4). In detail, binary trait analysis produced a non-parametric LOD of 2.9 for the triglyceride component of FCHL (i.e. serum triglyceride levels >90th age-sex specific percentile values) at 49 cM, which was attributable to a positive linkage score in around half of the affected families. Similarly, a parametric analysis that modelled the inheritance of the triglyceride trait of FCHL via a relatively rare allele with reduced penetrance produced a two-point heterogeneity LOD of 3.1 (α = 0.37) at 47.1 cM. Whether this allele has an appreciable impact on serum triglyceride levels, as indicated by quantitative trait linkage analysis (Fig. 4), awaits gene(s) identification.

The evidence for the chromosome 11p14.1–q12.1 region containing a distinct QTL for the cholesterol component of FCHL is tantalizing because of findings from several other groups. Klos et al. (56) found support for a cholesterol QTL (LOD, 1.84) at 35 cM in 232 multigenerational pedigrees, ascertained without regard to lipid levels, but no such evidence for a triglyceride QTL. Similarly, the genome-wide scans of Reed et al. (57) and Coon et al. (58), which produced robust evidence for a LDL-cholesterol QTL (Fig. 4), found no evidence for a triglyceride QTL. The first study, which ascertained 62 nuclear families through two obese subjects, produced a two-point LOD of 2.7 at 54 cM. The second study analysed 500 families from the National Heart, Lung and Blood Institute Family Heart Study, a population-based sample investigating genetic and non-genetic causes of CHD. A peak LOD of 3.7 was obtained at 56 cM. However, because the estimated position of a QTL may vary markedly (59), the premise that the 11p14.1–q12.1 genomic interval contains separate susceptibility loci for the total cholesterol, LDL-cholesterol and triglyceride traits of FCHL requires further evaluation.

The chromosome 11p14.1–q12.1 FCHL locus may contain a genetic lesion that primarily affects insulin metabolism, and secondarily affects serum triglyceride levels. The evidence for...

Figure 2. Clinical and lipid criteria applied in the recruitment of families for three different FCHL genome-wide scans. BMI = body mass index.
this currently derives from a single study of 159 Japanese families with type 2 diabetes, that were ascertainment through probands with two or more affected siblings (60). A 10 cM genome-wide scan of 359 non-obese affected family members (i.e. BMI = 22.7 ± 2.8 and 23.2 ± 3.6 kg/m² for men and women, respectively) produced a LOD of 3.1 within 5 cM of the peak of linkage for the triglyceride trait of FCHL (Fig. 4). Why a convincing linkage signal should have been detected here, when a large number of genome-wide scans have produced no evidence for linkage of the 11p14.1–q12.1 interval to type 2 diabetes, is uncertain (55,61). A trivial explanation would be chance association. However, the biologically more intriguing possibility is that the aetiologies of FCHL and of type 2 diabetes in non-obese subjects share genetic overlap. In these subjects, a growing body of data indicates that the propensity to diabetes is primarily influenced by pancreatic β-cell dysfunction, rather than resistance of end-organs to insulin, which ensues once the β-cell can no longer secrete sufficient insulin to maintain normal blood glucose levels (62,63).

**CHROMOSOME 16q22–24.1 LOCUS CONTAINS AN HDL-CHOLESTEROL QTL**

Data from four genome-wide scans (10,44,64,65), combined with some early linkage results (66–68), provide convincing evidence that the 16q22–24 chromosomal region contains a QTL(s) that contributes to the development of low HDL-cholesterol levels in FCHL. Two of the genome-wide scans were concerned with the genetics of the lipid abnormalities in FCHL (28,44), whereas three studies centred on families affected with premature CHD (64,66,68), in which the phenotype of low HDL-cholesterol often figures (69–71). Figure 5 summarizes the various study designs and resultant LOD scores from these datasets.

Pajukanta et al. (28) combined data from a Dutch (9) and Finnish (10) FCHL genome-scan, and obtained a multipoint LOD score of 2.7, close to D16S3096, for linkage of the low HDL-cholesterol trait (i.e. Finnish <10th age-sex-specific percentile) in their families to the 16q22–24.1 chromosomal region. The LOD score increased to 3.4 for a HDL-cholesterol QTL following the inclusion of data from an additional 25 Finnish families (Fig. 5), who had been ascertained through index patients with premature CHD and low serum HDL-cholesterol levels (64). Similarly in 1984, an analysis of a large pedigree (n = 200 family members), characterized by a high incidence of premature CHD, with the genetic marker haptoglobin, which resides within 4 Mb of the gene encoding lecithin-cholesterol acyltransferase (LCAT), produced nominal evidence for linkage of the 16q22–24.1 genomic interval to low HDL-cholesterol levels (Fig. 5). The maximum parametric LOD score was 1.8 at a recombination fraction of 0 in males, and 0.16 in females. In a marginally larger study, involving 30 multigenerational Caucasian pedigrees, ascertained through two family members with premature CHD (as defined by angiography, myocardial infarction or coronary artery bypass surgery), a suggestive LOD score of 2.1 was obtained with marker D16S3131, which resides within 8 Mb of LCAT (Fig. 5).

Arguably, the best evidence that the 16q22–24.1 chromosomal region contains a genetic lesion that has an appreciable impact on HDL-cholesterol levels comes from the data of Mahaney et al. (65). These authors examined 10 extended families (472 participants) constructed around Mexican American probands, randomly ascertained without respect to disease status and phenotype values. In their dataset, only chromosome 16 exhibited convincing evidence for a HDL-cholesterol QTL, with a peak multipoint LOD of 4.3, between
marker D16S2624 and D16S518 (Fig. 5). These markers reside within 10 Mb of the gene encoding LCAT, the enzyme that esterifies free cholesterol at the surface of lipoproteins (Fig. 1). In serum, LCAT preferentially binds nascent HDL to promote the formation and accumulation of cholesteryl esters into the core of HDL (72). This reaction, which serves to remove cholesterol from the surface of HDL, also promotes the flux of cholesterol from cell membranes into HDL. Importantly for the identification of sequence variants that underlie the 16q22–24.1 linkage signal, most evidence suggests that LCAT regulates HDL-cholesterol levels through mechanisms that primarily affect the surface of its lipoprotein substrates (73,74). In other words, genetic lesions affecting the transcriptional activity of LCAT are unlikely to be the major cause of low HDL-cholesterol levels in FCHL, as suggested by the study of Ribalta et al. (75). That said, we recall that the overexpression of human LCAT in rabbits promotes the formation of high levels of serum HDL-cholesterol, the catabolism of non-HDL-cholesterol, as well as attenuating the development of diet-induced atherosclerosis (76,77).

The position of the chromosome 16q22–24.1 linkage signal appears to exclude the cholesteryl ester transfer protein (CETP) gene as a major locus contributing to the development of low HDL-cholesterol levels in FCHL (Fig. 5). However, we note that Blankenberg et al. (78) have recently reported associations between the less common allele at the CETP<sup>c</sup>-629C>A locus, lower CETP activity and higher HDL-cholesterol levels in a population of 1211 German CHD patients, prospectively followed up for a median of 4.1 years. In this data set, there was also a significant association between the CETP<sup>c</sup>-629C>A genotype and the risk of future cardiovascular death, consistent with data from the West of Scotland Coronary Prevention Study (79). In the German study, mortality decreased from 10.8% in CETP<sup>c</sup>-629C homozygote patients (low HDL-cholesterol) to 4.6 and 4.0% in heterozygote and homozygote CETP<sup>c</sup>-629A patients, respectively. As importantly, homozygote patients with the high-risk CETP<sup>c</sup>-629C genotype were found to derive greater clinical benefit from lipid-lowering medication than homozygote patients with the low risk CETP<sup>c</sup>-629C genotype, supporting data from two slightly differently designed studies (80,81). Significantly, haplotype analyses have now established that the CETP<sup>c</sup>-629A allele is in strong linkage disequilibrium (LD) with the less common allele at the much-studied CETP Taq1B polymorphic site (79,82–85). This is important because a large number of studies have reported an association of this allele, which is created by a single-nucleotide polymorphism (SNP) within intron 1 of CETP, with low CETP and/or high HDL-cholesterol concentrations (80,84,86–99). We also note the results from transient transfection studies which show that the CETP<sup>c</sup>-629A allele...
resides within one of the three Sp1 and Sp3 binding sites regulating the activity of the CETP promoter (100,101). To sum up, we suggest that it will be important to establish whether sequence variation at the CETP locus makes a modest, rather than major, contribution to the overall variance in HDL-cholesterol levels in families with FCHL and low HDL-cholesterol concentrations.

DISTINCT APOA1/C3/A4/A5 ALLELES CONTRIBUTE TO THE TRANSMISSION OF FCHL

The role of two distinct alleles at the APOA1/C3/A4/A5 gene cluster (Fig. 6A) in the transmission of FCHL has emerged from a number of studies (102–111). Recently, Eichenbaum-Voline et al. (111) performed non-parametric linkage analysis and a combined linkage and association test on a cohort of white British FCHL families (Fig. 2). The linkage analysis, based on excess allele sharing in affected siblings and relative pairs, produced a \( P \)-value of 0.023 for linkage of the APOA1/C3/A4/A5 genomic region to the triglyceride trait of FCHL, despite limited power in this data set for identifying a disease allele inherited through two or more bi-allelic loci (112). The combined test of linkage and association, which was performed with the Pedigree Disequilibrium Test, detected evidence for preferential transmission of the less common alleles at the \( APOA5^{c.56C \rightarrow G} \) and \( APOC3^{c.386C \rightarrow G} \) loci to family members with the triglyceride trait of FCHL. Importantly, the Gamete Competition Test, a generalized transmission disequilibrium test, which efficiently analyses data from pedigrees of arbitrary size and complexity (113), further supported the evidence for increased transmission of the \( APOA5^{c.56G} \) and \( APOC3^{c.386G} \) alleles in white British FCHL families (111).

Data from four independent studies indicate that a homozygote \( APOA5^{c.56G} \) genotype has a major impact on serum triglyceride level in susceptible individuals (105,107,109,111). Talmud et al. (107) studied 2808 healthy middle-aged men drawn from UK general practices, and found that the 11 individuals (0.39\% of participants) with this genotype had on average serum triglyceride levels (241.6 ± 107.1 mg/dl) that overlapped age–sex specific 90th percentile values (i.e. 236.0–251.3 mg/dl for age range studied), whereas the heterozygote individuals (10.5\%) had serum triglyceride levels that were on average only marginally increased (\( \sim 8\% \)) relative to their peers with two copies of the wild-type allele. Similarly, Pennacchio et al. (105) showed that the homozygote \( APOA5^{c.56G} \) genotype (0.71\% of participants) was associated with a 3-fold increased risk of high triglyceride levels (>90th percentile) in 2600 randomly selected participants from the Dallas Heart Disease Prevention Project. In another study, Vrablik et al. (109) reported that 3.6\% of severely hypertriglyceridemic individuals had a homozygous \( APOA5^{c.56G} \) genotype compared
with 0.3% of the control population, leading these authors to speculate that this genotype is the most important genetic determinant of serum triglyceride levels detected to date. In white British FCHL probands, Eichenbaum-Voline et al. (111) observed a comparable frequency (2.8 versus 0.7% in FCHL spouses) of the homozygote APOA5c.56G genotype, which was associated with some of the highest triglyceride levels in this dataset. Whether this genotype has a secondary effect on cholesterol levels in FCHL awaits further investigation.

The APOA5c.56G allele, which is in strong LD with the less common allele at the APOA1-3031C>T locus, is rarely seen on a haplotype containing the APOC3c.386G allele (Fig. 6B). In the Eichenbaum-Voline study, an APOC3c.386G genotype accounted for ~5% of the variance in triglyceride levels in FCHL spouses, suggesting that this allele marks/represents a triglyceride QTL as well as an FCHL disease locus (111). This proposition is consistent with a number of early studies (114), but because of strong allelic association across the APOA1/C3/A4/A5 locus it has been difficult to identify which of the sequence variants in LD with the APOC3c.386G allele (Fig. 6) might confer susceptibility to high triglyceride levels. A recent report appears to rule out the involvement of the less common allele at the APOA5c.-3A>G locus (111), while data from transgenic mice (116,117), gene expression studies (115,118, 119) and genome-wide linkage studies (12,56) suggest that the causative lesion(s) may operate through a mechanism perturbing transcription of either APOC3, APOA5 or both.

The chromosome 6q16.1–16.3 genomic interval may also contain a QTL controlling APOC3 levels, which in addition to predicting serum triglyceride constitute an independent factor risk for CHD (120–124). In one study, a LOD of 1.7 was obtained at 109 cM for an APOC3 QTL in 232 multi-generational pedigrees, ascertained without regard for health through households with more than two school-age children (56). This compares with a triglyceride QTL multipoint LOD of 1.4 at 107.9 cM in 113 white British FCHL families (12) and a multipoint LOD 0.9 at 111 cM for FCHL affection status in 18 extended Dutch families (9). In these studies APOC3 levels were not measured.

The APOA5c.56G allele alters codon 19 of the predicted amino-terminal signal sequence of APOA5, which substitutes a serine residue with tryptophan, and may therefore represent a genetic lesion conferring susceptibility to FCHL in certain individuals. The von Heijne formula (125) predicts that Ser19 of APOA5 occupies the –5 position of the pre-apolipoprotein (counting from the predicted cleavage site between positions 610.0x795.0

Figure 6. Allelic structure of the APOA1/C3/A4/A5 gene complex. (A) Organization of the cluster (not drawn to scale) was determined from genomic (AC007707) and cDNA sequences (117). Genes are denoted by black rectangles, with attached triangles at their 3′ ends. The APOA5c.56C>G and APOC3c.386C>G alleles alter the 19th codon of APOA5 (serine to tryptophan) and the 40th nucleotide of the 3′ non-coding region of APOC3, respectively. The single nucleotide polymorphism at the APOA5c.-3A>G locus resides within the putative Kozak sequence of APOA5 (117). Alternative nomenclature for the APOC3c.386C>G and APOA1-3031C>T alleles includes the S2/Sst I allele of APOC3 and the X2/XmnI allele of APOA1. (B) Common APOA5c.56C>G and APOC3c.386C>G haplotypes; 1 and 2 represent the common and less common alleles at each locus. The frequency data for FCHL spouses and probands are taken from the study of Eichenbaum-Voline et al. (111); UTR = untranslated region.
—1 and +1 of mature APOA5), a region that has a strong preference for specific amino acids at particular positions. For example, the residue at the —3 position must not be aromatic (e.g. Tyr, Trp), charged (e.g. Asp) or large and polar (e.g. Asn). Accordingly, missense mutations within signal sequences have been reported to cause serious forms of genetic diseases, such as Schmid metaphyseal chondrodysplasia and familial neurohypophyseal diabetes insipidus (126,127). By analogy, a tryptophan residue so close to the cleavage site of the APOA5 signal sequence could reduce the processing of this preprotein, which based on transgenic mice experiments (117) would be expected to lead to the development of increased serum triglyceride levels in humans. However, in the absence of functional data, the genetic studies as they stand still do not rule out the possibility that the APOA5c.356G allele is simply in LD with a lesion that confers susceptibility to FCHL.

**REFERENCES**


**CONCLUDING COMMENT**

The intracellular processes that regulate the transport of lipids are crucial for survival in both infancy and adulthood, but our understanding of many of the key factors regulating whole-body lipid homeostasis is limited due to the complexity of the transport system(s). In this review, we have summarized recent genetic studies that promise to lead to the identification of sequence variants that increase the transmission of FCHL, and the associated Metabolic Syndrome. An understanding of the primary metabolic pathways perturbed in these numerically important conditions will ultimately reduce the substantially increased risk of CHD attributable to high blood lipid levels, especially if a definitive diagnosis is reached in childhood.

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