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REVIEW ARTICLE

Unraveling the complex genetics of familial combined hyperlipidemia

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Abstract
Familial combined hyperlipidemia (FCHL) constitutes a substantial risk factor for atherosclerosis since it is observed in about 20% of coronary heart disease (CHD) patients under 60 years. FCHL, characterized by elevated levels of total cholesterol (TC) and triglycerides (TGs), or both, is also one of the most common familial hyperlipidemias with a prevalence of 1%–6% in Western populations. Numerous studies have been performed to identify genes contributing to FCHL. The recent linkage and association studies and their replications are beginning to elucidate the genetic variations underlying the susceptibility to FCHL. Three chromosomal regions on 1q21–23, 11p and 16q22–24.1 have been replicated in different study samples, offering targets for gene hunting. In addition, several candidate gene studies have replicated the influence of the lipoprotein lipase (*LPL*) gene and apolipoprotein A1/C3/A4/A5 (*APOA1/C3/A4/A5*) gene cluster in FCHL. Recently, the linked region on chromosome 1q21 was successfully fine-mapped and the upstream transcription factor 1 (*USF1*) gene identified as the underlying gene for FCHL. This finding has now been replicated in independent FCHL samples. However, the total number of variants, the risk related to each variant and their relative contributions to the disease susceptibility are not known yet.

Key words: *Association study, complex disease, coronary heart disease (CHD), familial combined hyperlipidemia (FCHL), genetics, linkage study*

Introduction

Familial combined hyperlipidemia (FCHL), first described among young survivors of myocardial infarction in 1973 (1–3), is the most common familial dyslipidemia predisposing to coronary heart disease (CHD). FCHL is a common disease with an estimated population prevalence of 1%–6% (3,4). Elevated levels of serum total cholesterol (TC), triglycerides (TGs), or both characterize the FCHL disorder. FCHL also features low levels of high-density lipoprotein cholesterol (HDL-C), elevated levels of serum apolipoprotein B (apoB), and glucose intolerance as component traits (2,3,5).

It has been evident for 30 years that FCHL has a strong genetic component (2,3,6). The genetic component in FCHL has been suggested by familial aggregation of dyslipidemia (2) and in fact, FCHL was originally suggested to be inherited as an autosomal dominant disorder due to the vertical transmission pattern (1). A more complex polygenic background is, however, likely, as suggested by metabolic (7) and segregation studies (8,9). Furthermore, whole-genome searches and candidate gene studies performed in FCHL families originating from different populations have identified several putative loci for FCHL (10–16). The recent findings and replications of the linkage and association studies are beginning to identify the DNA sequence variations contributing to FCHL. Fine-mapping of one of the linked regions recently resulted in the characterization of the first positionally cloned gene for FCHL, upstream transcription factor 1 (*USF1*) (17). Since *USF1* is a transcription factor known to regulate the expression of a number of genes participating in glucose and lipid metabolism, it provides an excellent candidate for FCHL. A recent study also indicates that common variants and haplotypes in the hepatic nuclear factor 4 alpha (*HNF4A*) gene are associated with high serum lipid

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Key messages

- Familial combined hyperlipidemia (FCHL) is a common complex disorder with both genetic and environmental factors affecting the disease susceptibility.
- Most likely the genetic susceptibility to FCHL is determined by multiple DNA sequence variants and their interactions.
- This review focuses on the recent findings that are beginning to elucidate the FCHL susceptibility genes, including the upstream transcription factor 1 (*USF1*) and lipoprotein lipase (*LPL*) genes, as well as the apolipoprotein A1/C3/A4/A5 (*APOA1/C3/A4/A5*) gene cluster.

levels and the metabolic syndrome in FCHL families (18). Interestingly, cooperative effects of *USF1* and *HNF4A* have been suggested to control the regulation of multiple genes, including apolipoprotein A2 (*APOA2*) and apolipoprotein C3 (*APOC3*) (19–21). Interactions between DNA sequence variants are likely to contribute to the complex pathogenic mechanism(s) of common cardiovascular traits, raising the possibility that variants in *USF1*, *HNF4A* and apolipoproteins may interactively confer susceptibility to FCHL. To summarize, these accumulating data indicate that multiple variants contribute to the susceptibility to FCHL.

Both rare and common variants have been suggested to contribute for example to low plasma levels of HDL-C in the general population (22,23). However, the extent to which each group influences this susceptibility is not known. It is thus likely that multiple DNA sequence variants, both common and rare, underlie the genetic susceptibility to FCHL. In addition to the human DNA sequence and variation data produced by the Human Genome Project and the HapMap Project, recent advances in genotyping technologies and statistical approaches should enable an accelerated investigation of the sequence variations at the genomic level to identify all the variants involved in the disease susceptibility of FCHL.

Genes conferring the susceptibility to FCHL

USF1 *identified as the gene underlying the linkage signal on chromosome 1q21*

Previously a locus for FCHL was identified on human chromosome 1q21–q23 in FCHL families originating from the genetically relatively isolated population of Finland (10). Since then, this finding has been replicated in several FCHL samples, originating from other, more heterogeneous populations (15,24–26). Linkage has been observed to FCHL, as well as to several FCHL component traits, including TG, TC and apoB levels (10,15,25,26). On 1q21 no significant evidence of genetic heterogeneity was observed in the Finns (17). However, in Mexican, German and Chinese samples the proportion of families contributing to linkage ranged from 22% to 71% (24,26). Interestingly, the same markers in the 1q21 region have also been linked to type 2 diabetes mellitus (T2DM) in numerous studies (27–33). Most recently, 1q23–31 was also shown to be linked to the metabolic syndrome (34). The evidence for linkage obtained for 1q21 has varied in these FCHL and T2DM studies, most likely reflecting the underlying genetic heterogeneity, as well as population-based and diagnostic differences. Interestingly, many of the critical metabolic features of FCHL, e.g. hypertriglyceridemia and insulin resistance, also represent trait components of T2DM. Taken together, these linkage findings suggest that one or

more genes in this particular chromosomal region predispose to both FCHL and T2DM, two clinical phenotypes with overlapping component traits and shared diagnostic features.

The upstream transcription factor 1 (*USF1*) was recently linked and associated with FCHL in 60 extended Finnish FCHL families with 721 genotyped individuals ($P=0.00002$) (17). The evidence for association was strongest among males for high serum TGs ($P=0.0000009$) and extended to a ~46-kb region, containing also the adjacent F11 receptor (*F11R*) gene (17) (Figure 1). An association was also observed for apoB, TC and the LDL peak particle size (17). The known functions of F11R, related to regulation of tight junction assembly in epithelia, pathogenesis of viral infections, and transendothelial migration of certain T cell types (35–37), make it a less likely candidate gene for FCHL than *USF1*. *USF1* is a ubiquitously expressed transcription factor of the basic helix-loop-helix leucine zipper family. It forms homo- and hetero-dimers (with USF2) and recognizes a CACGTG motif termed E box, resulting in activation of the gene transcription and enhanced expression in response to various stimuli such as glucose and dietary carbohydrates (38). *USF1* regulates the expression of several genes participating in glucose and lipid metabolism, such as apolipoprotein C3 (*APOC3*), apolipoprotein A2 (*APOA2*), apolipoprotein A5 (*APOA5*), apolipoprotein E (*APOE*), hormone sensitive lipase (*LIPE*), hepatic lipase (*LIPC*), glucokinase (*GCK*), islet-specific glucose-6-phosphatase catalytic-subunit-related protein (*IGRP*), insulin (*INS*), glucagon receptor (*GCGR*), ATP-binding cassette transporter A1 (*ABCA1*), fatty acid synthase (*FAS*), acetyl-CoA carboxylase alpha (*ACACA*) and plasminogen activator inhibitor-1 (*PAI1*) (20,21,38–51). A more complete list of the *USF1* target genes is available in a recent paper (52).

In the original study, genetic data were supported by preliminary functional data, as the *USF1* risk haplotype had an effect on the expression profiles of fat biopsies (17). Expression profiles of fat biopsies of FCHL cases seemed to differ depending on their carrier status for the associated *USF1* haplotype (17). A total of 25 genes were significantly upregulated and 73 genes downregulated in the susceptibility haplotype carriers (17). The upregulated genes belonged to functional classes mainly related to fat metabolism, and the downregulated genes included several functional classes related to immune response. Interestingly, the upregulated genes included the stearoyl-CoA desaturase (*SCD*) gene (17). The SCD activity has been shown to be associated with plasma TG levels (53). The downregulated genes also included several known important atherosclerosis-related genes, such as *APOE*,
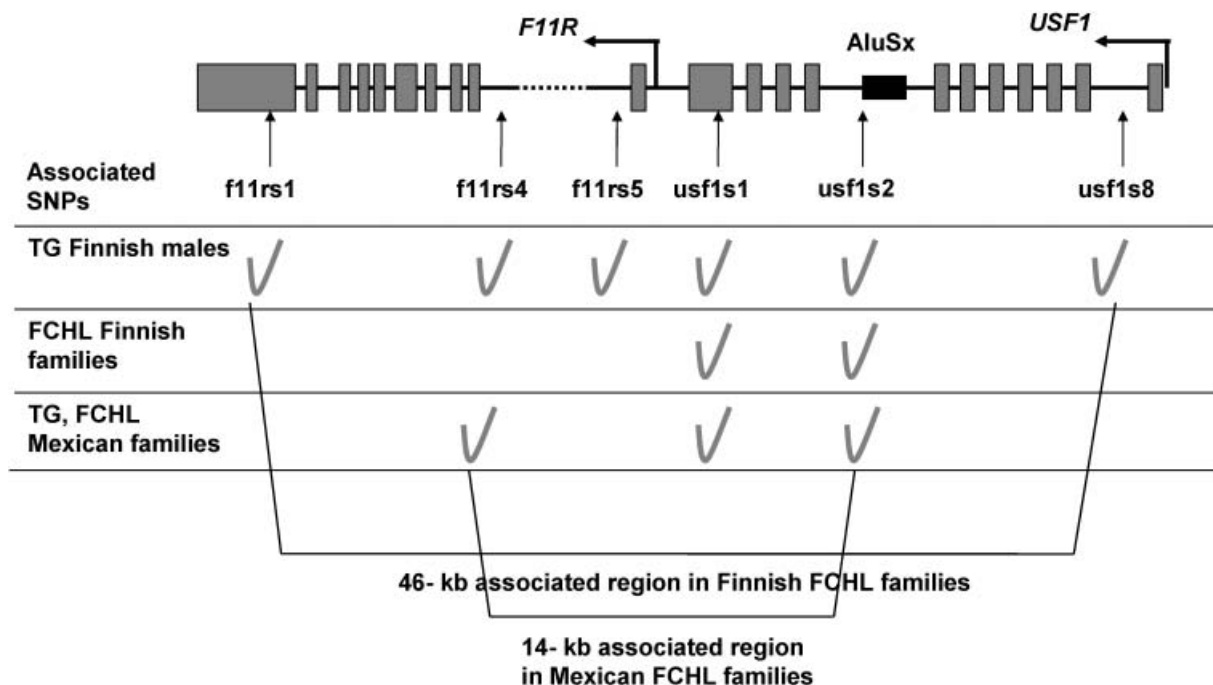
Figure 1. Overview of the associated *USF1* region in Finnish and Mexican familial combined hyperlipidemia (FCHL) families. The associated region was restricted by ~70% (from 46 kb to 14 kb) using two different populations, the Finns and Mexicans (17,26). The rs numbers of the single nucleotide polymorphisms (SNPs) are as follows: f11rs1 (rs836), f11rs4 (hCV1459766), f11rs5 (rs4339888), usf1s1 (rs3737787), usf1s2 (rs2073658), usf1s8 (rs2516838).

phospholipid transfer protein (*PLTP*), macrophage scavenger receptor 1 (*MSP1*), arachidonate 5-lipoxygenase (*ALOX5*), and complement component 3a receptor 1 (*C3AR1*).

A recent study further demonstrated differential expression of known *USF1* target genes, *APOE*, *ABCA1* and angiotensinogen (*AGT*), between *USF1* risk allele carriers and non-carriers using a larger number of fat biopsies of FCHL and low HDL-C-affected patients (*n*=19) (52). No differences in the characteristically low *USF1* transcript levels were observed between carriers of the *USF1* risk versus non-risk alleles in the original (17) or in this subsequent study (52). However, it is possible that even a subtle difference may have significant effects on the expression of *USF1* target genes.

Hoffstedt et al. studied the effect of *USF1* variants on lipolysis in adipocytes (54). Lipolysis is a critical aspect of adipocyte function and manifests large differences between individuals potentially due to genetic differences. In this study including fat biopsies from 196 normolipidemic obese women, the usf1s2 (rs2073658) variant was associated with the maximum lipolytic action in response to stimulation by noradrenaline, as well as by β_1-, β_2- and β_3- adrenergic receptor agonists (dobutamine, terbutaline, CGP12177 and forskolin). Importantly, the carriers of the protective allele of the usf1s2 variant had a significantly increased maximum lipolytic activity in response to these drugs. This association between the *USF1* variant and the maximum lipolytic activity was observed in normolipidemic women without any disease such as FCHL, CHD or T2DM. The result may imply that the defect caused by *USF1* is present already before the clinical manifestation of the disease. Defect in *USF1* may ultimately lead to disease when other genetic or environmental factors accumulate in the same individual.

Since the original finding in Finnish FCHL families, the association between the DNA sequence variations in *USF1* and FCHL has been replicated in several study samples (26,55–57) (Table I). The first replication study including 24 extended multi-generational Mexican FCHL families, replicated the association, and moreover the associated region was restricted to 14 kb (26) versus more than 46 kb in the Finns (17) (Figure 1). These data provides a shorter region with fewer variants available for functional analyses. No gender differences were observed in Mexican FCHL families (26). The most significantly associated single nucleotide polymorphisms (SNPs), usf1s1 (rs3737787) and usf1s2 (rs2073658), or SNPs in linkage disequilibrium (LD) with usf1s1/s2, were investigated in three other study

samples ascertained for CHD or for family history of CHD (55–57). In extended Utah pedigrees with family members suffering early death due to CHD, early strokes, or early onset hypertension, the *USF1* SNPs and haplotype were associated with FCHL, TG and low-density lipoprotein cholesterol (LDL-C) levels (57). In the European Atherosclerosis Research Study II, Putt et al. examined the lipid levels before and after meal and after oral glucose tolerance test (56). They found differences in the correlation between body mass index (BMI), fasting LDL-C and glucose levels according to the *USF1* genotypes. It is worth noting that as in Finns (17) the common allele of usf1s1 (rs3737787), usf1s2 (rs2073658) or SNPs in LD with usf1s1/s2 represents the associated allele in all of these studies, replicating the original study. It can be concluded that these SNPs seem to capture the disease-associated signal, although their direct relationship to the functional defect contributing to FCHL pathogenesis is not known yet.

Most recently, Komulainen et al. investigated the role of *USF1* variants and haplotypes as a risk factor for cardiovascular disease events at the population level (55) (Table I). Importantly, they observed that female carriers of a *USF1* risk allele had a two-fold risk of a cardiovascular event and an increased risk of all-cause mortality during the follow-up period in a Finnish prospective population cohort consisting of 14,000 individuals, followed up for cardiovascular events in a period of 7–10 years (55).

Two different studies have focused on the contribution of *USF1* SNPs to T2DM (58,59) (Table I). In the first study by Ng et al., a significant association of *USF1* polymorphisms with T2DM and the metabolic syndrome-related traits was observed in Chinese T2DM families, although no single SNP explained the previous linkage to the 1q21 (58). In the Chinese case-control sample, the population-based hospital cases of T2DM were, however, not associated with usf1s1 (58). In the second study, Gibson et al. did not find differences in allele frequencies between French diabetics and healthy controls (59). To summarize, the results for *USF1* with T2DM and the metabolic syndrome have included both positive and negative associations (58,59). These data imply that there are also other regional genes on 1q21 that contribute to the linkage signals of these two disorders.

Interestingly, a gene for combined hyperlipidemia (Hyplip1) in mouse was mapped to a region on chromosome 3 in the HcB-19/Dem mouse that was orthologous to human chromosome 1q21 (60). The underlying gene, thioredoxin interacting protein (*TXNIP*), was recently identified as a gene for

Table I. Summary of the genetic studies on the *USF1* gene, the first gene identified for familial combined hyperlipidemia (FCHL) using positional cloning approach.

Trait	Study sample	Marker/SNP	Positive (+)/negative (−) results	Reference
FCHL and/or TGs	Finnish FCHL families	rs836	+	(17)
		rs790056	+	
		hCV1459766	+	
		rs4339888	+	
		rs3737787	+	
		rs2073658	+	
		rs2516839	+	
		rs2516838	+	
FCHL and/or TGs	Mexican FCHL families	rs3737787	+	(26)
		rs2073658	+	
		hCV1459766	+	
FCHL, TGs and/or LDL-C	Utah families with premature CHD, stroke or hypertension	rs3737787	+	(57)
		rs2073658	+	
LDL-C and/or glucose	Caucasian males with family history of CHD and controls	rs3737787	+	(56)
		rs2073658	+	
Cardiovascular event and/or all cause mortality (association with individual SNPs or 6-SNP haplotype)	Finnish CHD cases and population-based cohort	rs10908821	+	(55)
		rs2073658	+	
		rs2774276	+	
		rs2516839	+	
		rs1556259	+	
		rs2774279	+	
Lipolytic activity in adipose tissue	Caucasian obese women	rs3737787	+	(54)
		rs2073658	+	
T2DM and/or metabolic syndrome	Chinese T2DM families	rs3737787	+	(58)
		rs2516841	+	
		rs2516839	+	
T2DM and/or metabolic syndrome	Chinese cases and controls	rs3737787	−	
		rs2516841	−	
		rs2516839	−	
T2DM	Cases and controls for T2DM	rs2516837	−	(59)
		rs1556259	−	
		rs2516838	−	
		rs2073653	−	
		rs2774276	−	
		rs2516841	−	
		rs2073658	−	
		rs3737787	−	

CHD=coronary heart disease; LDL-C=low-density lipoprotein cholesterol; SNP=single nucleotide polymorphism; T2DM=type 2 diabetes mellitus; TG=triglycerides.

combined hyperlipidemia in mouse (61). *TXNIP* provided thus a strong positional candidate for human FCHL (62). However, several recent studies show that variations in the *TXNIP* gene do not confer the susceptibility to FCHL (17,63,64). Regarding the possibility whether the identified *USF1* risk haplotype would have a long-range effect on the expression of *TXNIP*, the *TXNIP* expression profiles of fat biopsies from affected Finnish FCHL family members having the *USF1* risk haplotype were compared with affected FCHL family members, homozygous for the putative protective haplotype. No haplotype-dependent difference in *TXNIP*

expression was detected (17). To summarize, it seems unlikely that *TXNIP* accounts for the observed evidence of linkage between FCHL and the 1q21 region.

Variants in the apolipoprotein gene cluster APOA1/C3/A4/A5 *are implicated in FCHL*

Multiple studies predict the importance of the *APOA1/C3/A4* gene cluster as a modifier gene complex in the development of FCHL (65–73) (Table II), although not all studies have shown the connection (74–76). The most investigated

Table II. Genes associated with familial combined hyperlipidemia (FCHL) in previous studies.

Gene	Ethnicity	Replicated (at least once)	Reference
USF1	Finnish, Mexican and Caucasian (Utah) FCHL families	Yes	(17,26,57)
APOA1/C3/A4/A5	Caucasian (Dutch, Spanish, Northern European, European)	Yes	(65–73,88–90)
LPL	Caucasian (Dutch and Italian)	Yes	(96–105)
HNF4A	Finnish and Mexican FCHL families	No (only one study)	(18)
CD36/FAT	Caucasian (Dutch)	No (only one study)	(135)
TNFRSF1B	Caucasian (Dutch)	No (only one study)	(126)
LEPR	Caucasian (Dutch)	No (only one study)	(127)

polymorphisms of this gene cluster are three restriction enzyme polymorphisms, XmnI and MspI residing upstream to the apolipoprotein A1 (APOA1) gene and the SstI site in the 3′ untranslated region of exon 4 of the APOC3 gene (77). The positive studies include a Dutch study, in which the minor alleles of these polymorphisms were associated with elevated plasma TC, TG, LDL-C, apoB, and apoC3 levels in Dutch FCHL families (67). Furthermore, a suggestive evidence for linkage between the MspI minor allele and plasma LDL-C levels was detected (67). Based on the results, Dallinga-Thie et al. suggested that the APOAI/C3/A4 gene cluster is not the primary cause of FCHL, but rather it has a specific modifying effect on plasma TG and LDL-C levels in this lipid disorder (67). The negative studies include a Finnish study in which the MspI polymorphism was associated with serum TC and apoB levels in spouses, but no evidence of direct involvement of the APOAI/C3/A4 loci or haplotypes in the expression of FCHL in the Finnish FCHL families was found (76).

Recently, APOA5 gene was added as part of the APOA1/C3/A4 gene cluster (78). Variants of this gene cluster have been linked to high TGs in both the general population (78–87) and in FCHL (88–90). In the Dutch FCHL families, APOA1/C3/A4/A5 showed an association with TG levels and LDL particle size, and the strongest evidence of association was obtained with SNPs in APOA1 and APOA5 (89). Ribalta and colleagues also suggested a potential implication of APOA5 in the hypertriglyceridemia present in FCHL, because in hyperlipidemic patients with FCHL, the carriers of the minor allele of a SNP in APOA5 had significantly increased plasma TG levels when compared with the carriers of the common allele (90). In British FCHL families, alleles of the APOA1/C3/A4/A5 gene cluster were overtransmitted to subjects with FCHL, and the transmission of the common APOA1/C3/A4/A5 haplotype to the affected subjects was reduced (88). Several studies also imply that APOA5 is a potential risk factor for cardiovascular disease (82,91,92).

APOA5 is suggested to reduce plasma TGs by inhibiting lipidation of apoB and thus reducing the hepatic very low-density lipoprotein (VLDL) production rate, as well as by stimulating LPL-mediated clearance of TG-rich lipoproteins (93). Overexpression of APOA5 has been shown to lower TGs in mice and ApoA5 knockout mice have severe hypertriglyceridemia (78,94). Recently, a study by Nowak et al. implied that APOA5 is regulated by insulin (46). Interestingly, insulin induces a dose-dependent downregulation of APOA5 expression by reducing the binding of upstream stimulatory factors USF1 and USF2 to E-Box (5′-CACGTG-3′) of the APOA5 promoter. It was also suggested, that the inhibitory effect of insulin on the APOA5 transcription involves a phosphorylation mechanism of USF that modulates their binding to the APOA5 promoter and results in APOA5 transrepression (46). The downregulation of APOA5 by insulin could explain the association between hypertriglyceridemia and hyperinsulinemia. Interestingly, APOA5 is also a highly responsive target gene of the peroxisome proliferator-activated receptor alpha and may act as a major mediator for fibrates in reduction of plasma TGs (95).

Lipoprotein lipase and hepatic lipase genes in FCHL

The variants in the LPL gene have been of interest in FCHL because LPL catalyzes the hydrolysis of TGs of VLDL and chylomicrons and thus delivers fatty acids to tissues (Table II). Nevin et al. identified variations in the coding region of the LPL gene in 6 of 20 FCHL patients, the variations including Asp9Asn, Val108Val and Ser447stop (96). Reymer et al. found a common Asn291Ser variant of the LPL gene in FCHL patients and observed an association with FCHL and HDL-C levels, as well as with catalytic activity of LPL (97). Since these studies, the Asn291Ser and Asp9Asn variants have been associated with reduced HDL-C, elevated TG, apoB and/or insulin levels in FCHL patients in several

different studies (98–103). In addition to coding variants in *LPL*, variations in the promoter region have been identified in FCHL patients (101,102,104,105). Yang et al. reported a heterozygous carrier of a promoter variant (−39C/T) in *LPL* when screening 20 FCHL probands for mutations in the putative *LPL* promoter region (105). The variant resulted in diminished transcriptional activity of the *LPL* gene, potentially by abolishing the transcription factor Oct-1 binding site. The −93G/T and −53C/G variants were also reported to affect promoter activity (104). However, not all studies have supported the role of *LPL* in FCHL (74,106,107). In addition to FCHL, variations in *LPL* have been associated with CHD (108). Evidence for linkage to the hepatic lipase (*LIPC*) locus ($P < 0.003$) and to the lecithin:cholesterol acyltransferase (*LCAT*) locus ($P < 0.0006$) has also been observed (13) and a coding variant in *LIPC* was associated with elevated TC and apoB levels in the Dutch FCHL families (109). However, the linkage to *LIPC* locus was not confirmed in the Finnish FCHL families (106).

Variants in HNF4A *gene associated with lipid levels in FCHL*

A region on chromosome 20q12–q13 has been linked to T2DM and obesity in numerous studies (110–114). Recently, several independent groups have identified associations between SNPs in the *HNF4A* gene residing in this 20q12–q13.1 region and T2DM (115–119). Previously mutations in *HNF4A* have been demonstrated to cause maturity onset diabetes of the young type I (MODY1) (120). Interestingly, the same chromosomal region on 20q12–q13 has also been linked to TGs and low HDL-C in FCHL families (11,121,122). Subsequently, evidence for linkage has been found with high TGs in other populations (123,124). There is a clear phenotypic overlap between FCHL and T2DM, patients with T2DM exhibiting often hypertriglyceridemia and patients with FCHL glucose intolerance and/or insulin resistance (5,125). Both diseases also predispose to CHD.

Considering this clear phenotypic overlap between T2DM and FCHL, and the fact that both disorders have been linked to the same chromosomal region on 20q (11,110,111,113,114,121,122), it is possible that *HNF4A* contributes to linkage signals in both diseases. Recently common *HNF4A* variants and their haplotypes were investigated for association in Finnish and Mexican FCHL families, comprising 1020 subjects (18) (Table II). The common

HNF4A variants and haplotypes were associated with elevated serum lipid levels, the metabolic syndrome as well as with elevated glucose parameters in the Finnish FCHL families (18). Importantly, both the Finnish and Mexican FCHL families shared two common lipid-associated *HNF4A* haplotypes (18). This is the first study demonstrating that common *HNF4A* variants and their haplotypes are associated with high plasma lipid levels and the metabolic syndrome.

HNF4A is a transcription factor, regulating several genes in lipid and glucose metabolism. Interestingly, it has been shown that cooperative binding of *USF1* and *HNF4A* drives the transcription of the human *APOA2* gene (20). In addition, sequences from sites bound by *HNF4A* and *USF1* were demonstrated to show significant overlap in HepG2 cells (19). As interactions between DNA sequence variants are suggested to be critical in the etiology of complex traits such as FCHL, it is possible that DNA sequence variants in *USF1* and *HNF4A* interactively influence the susceptibility to FCHL.

TNFRSF1B *and* LEPR *as candidate genes for QTL affecting apoB levels on chromosome 1*

A genome-wide scan in 18 Dutch FCHL families revealed a quantitative trait locus (QTL) affecting apoB levels on chromosome 1 short arm (15). The candidate genes associated with FCHL in this region include tumor necrosis factor receptor superfamily, member 1B (*TNFRSF1B*) (126) and leptin receptor (*LEPR*) (127). *TNFRSF1B*, located on 1p36.2, was associated with susceptibility to FCHL in the Dutch family sample (126). For further support, the levels of soluble extracellular domain of TNF-R p75 were lower in the hyperlipidemic than in the normolipidemic relatives of FCHL patients (128). A polymorphism in the coding region of the *LEPR* gene (Gln223Arg), located on 1p31 was associated with an increased risk of FCHL and a difference in HDL-C levels (127). To determine whether one or both of these genes explain the linkage signal, or if there are additional regional genes involved, requires further investigation.

Other candidate genes investigated for FCHL include manganese superoxide dismutase locus on chromosome 6, which showed suggestive evidence of linkage in Dutch FCHL families with FCHL, as well as with related traits such as TC, apoB and apoC3 levels ($P < 0.02$) (13). Biochemical and genetic associations of plasma apoA2 levels with FCHL have also been suggested (129). The role of *APOA2* in FCHL has been discussed recently (130,131).

Gene expression profiling provides novel candidate genes for FCHL

Gene expression is the major determinant of the phenotype and function of a living organism. The profile of expressed genes in a particular cell is highly dynamic and changes rapidly in response to cellular events and external stimuli. For long, methods have been available to measure the gene expression of a limited number of genes at a time. Recently, DNA microarray technology has provided a tool to monitor the expression pattern of a whole genome simultaneously on a single chip (132). This technology provides a useful tool to profile gene expression at the genomic level, and to determine sets of genes expressed or turned off together. Consequently, these techniques allow for identification of the genes and metabolic pathways that are differentially expressed in healthy and diseased individuals and, thus, provide an alternative approach to identify novel genes and pathways involved in FCHL.

Three studies have been performed to identify the differential gene expression between FCHL patients and healthy controls (133–135). One of the studies investigated lymphoblastic cell lines (133), and two others focused on adipose tissue (134,135). The first study by Erlings et al. detected 25 differentially expressed genes out of 588 genes in subcutaneous adipose tissue obtained from 5 unrelated FCHL and 4 control individuals (134). Many of the differentially expressed genes had been implicated in the activation of the adipocyte cell cycle. Expression of tumor necrosis factor, alpha (*TNFA*) was upregulated in FCHL patients, which is of particular interest since *TNFRSF1B* variants and decreased levels of soluble extracellular domain of TNF-R p75 have been observed to be associated with FCHL previously (126,128).

Morello et al. studied the gene expression in immortalized lymphoblastic cells obtained from FCHL cases and their normolipidemic spouses and relatives (133). Of the 7647 genes expressed in these samples, 166 genes were differentially expressed between cases and controls. Categorizing the differentially expressed genes according to biological processes that the genes were involved in revealed that almost half of the genes were taking part in metabolism. Interestingly, the early growth response 1 gene (*EGR-1*) encoding a transcription factor was upregulated in lymphoblasts (133), as well as in the adipocytes derived from FCHL patients in the previous study (134). Of the 166 genes differentially expressed in the lymphoblastic cell lines, surrounding sequences of 16 genes contained the EGR-1 consensus sequence (5′-CGCCCCCGC-3′) (133).

Meex et al. (135) measured the expression levels of 640 genes in 5 unrelated FCHL patients and 10 control individuals. Initially, 27 genes were identified differentially expressed between FCHL cases and controls. After validation *CD36/FAT* was shown to be upregulated in the original sample of FCHL cases using quantitative reverse transcription polymerase chain reaction (RT-PCR), as well as in five additional pairs of FCHL cases and controls. *CD36/FAT* is a multiligand class B scavenger receptor that has been implicated in the transmembrane transport of long-chain fatty acids and in the regulation of angiogenesis. Interestingly, Meex et al. found a significant correlation between the *CD36/FAT* expression and esterification of fatty acids into phospholipids and TGs, suggesting that this gene has a functional role in lipid metabolism (135).

To summarize the candidate gene studies and the evidence obtained in replication studies (Table II), it seems evident that DNA sequence variants at least in *USF1*, *LPL* and the *APOA1/C3/A4/A5* gene cluster confer the susceptibility to FCHL. However, none of the candidate genes investigated so far account solely for a major genetic component in FCHL, further confirming the current understanding that the complex FCHL phenotype is an end result of genetic and environmental interplay of multiple factors (Figure 2).

Chromosomal loci identified for FCHL

To identify novel loci for FCHL, genome-wide scans have been performed in the Finnish (11,122), Dutch (14,15) and British families with FCHL (16). Loci on chromosomes 1q21, 2p, 2q31, 8q, 10p11.2, 10q11.2–10qter, 16q, 20q and 21q21 were identified in the Finnish FCHL families (10,11,122), and loci on chromosomes 1p, 2p, 11p, 16q and 19q in the Dutch FCHL families (14,15). A combined genome scan of the Dutch and Finnish families with FCHL identified three loci for HDL-C on 2p, 9p and 16q (12). In addition, a recent genome scan in the British FCHL families replicated the 11p locus and identified two novel candidate loci on 6q and 8p (16).

Three of these chromosomal regions, 1q21–23, 11p and 16q22–24.1, have been replicated in several FCHL study samples. The 1q21 locus has been observed in the Finnish and Dutch genome scans, as well as in independent studies of Chinese, German, US Caucasian and Mexican families with FCHL (10,11,15,24–26). Another replicated locus is the 11p region, which has been detected in the Dutch and British FCHL families (14,16). This region
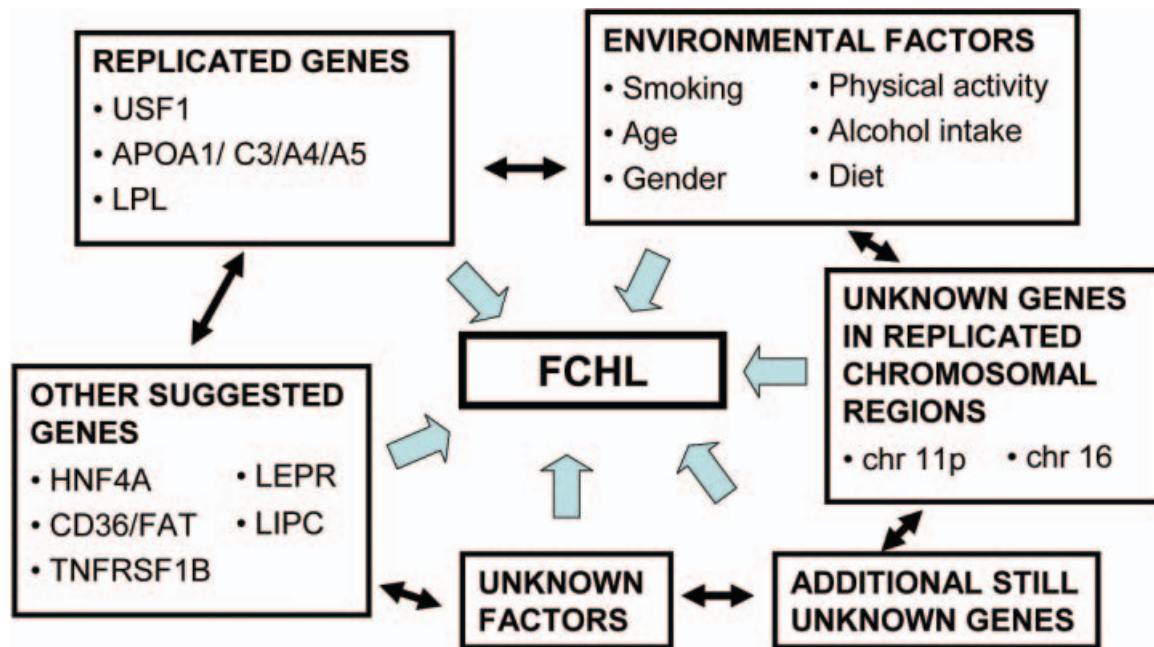
Figure 2. Multiple genetic and environmental factors confer the susceptibility to familial combined hyperlipidemia (FCHL).

showed evidence of linkage to FCHL, as well as to the TC and TG traits in the British study sample (16). No evidence of linkage for apoB levels was observed for this 11p region (14), implying that this locus may not directly contribute to the observed elevation of apoB-containing particles in FCHL. Genetic heterogeneity was demonstrated for the 11p region, as 49% of the British FCHL families were linked to this region (16).

Given the known difficulties in replicating and verifying the results of complex traits, international collaboration to replicate findings in independent and combined study samples is of utmost importance to accelerate the gene identification process. This strategy is based on increased statistical power to verify those of the identified regions that have the highest statistical likelihood to harbor causative genes. The power of this strategy has recently been demonstrated with other complex diseases, such as inflammatory bowel disease and asthma (136–138), as well as in a combined data analysis of Dutch and Finnish genome-wide scans for FCHL (12). In that study, three regions, 16q24.1, 2p25.1, and 9p23, were identified where the evidence for linkage emerged from the combined study sample (12). The region on 16q24.1 resulted in most significant evidence for linkage, a lod score of 3.4, for HDL-C, 50% of the Finnish and Dutch families being linked to this region. This very same 16q24.1 region was also recently implicated for HDL-C in Mexican Americans (139), as well as in several other independent studies (12,26,122,139–141). Different

study populations could thus be utilized first to replicate the loci that harbor susceptibility genes for FCHL, and second, to fine-map these verified regions.

It has not been directly evaluated whether the three replicated regions on 1q21, 11p and 16q explain the dyslipidemia in some or all FCHL families. These types of studies are clearly warranted to estimate the risk related to each variant, their contribution to FCHL and its component traits, as well as to investigate the likely gene-gene interactions in FCHL.

Future directions and concluding comments

FCHL is a typical complex trait with several genes, environmental factors and their interactions contributing to the disease phenotype (Figure 2). The possible high genetic complexity and the fact that the current strategies are based on functional characterization of mutations of monogenic diseases make the dissection of the wide allelic spectrum of variants conferring the susceptibility to complex diseases very challenging. These variants can result in minor changes in protein product or reside in promoters or other regulatory regions; or they can cause small changes in the binding affinity of a transcription factor or slightly alter gene expression levels, timing and tissue specificity. In all of these cases, it may be difficult to demonstrate not only the functional consequences of a single variant but also of the allelic combinations of multiple variants. Nutrients

or other environmental factors may potentially also modulate differences introduced by genetic variation. It is thus possible that each individual variant causes subtle changes in the disease phenotype. Accumulation of multiple deleterious gene variants and environmental factors in a particular individual will then ultimately lead to the expression of the disease phenotype.

Candidate gene studies, as well as positional cloning have provided novel candidate genes for FCHL of which variants in *USF1*, the *APOA1/C3/A4/A5* gene cluster and *LPL* have been replicated in several study samples from distinct populations. Many of the variants currently associated with FCHL are common and the risk involved with them at the population level is not known at the moment. Extensive population-based studies, such as recently conducted for *USF1* (55) and multiple T2DM candidate genes (142), estimating the risk related to each variant and their relative contribution to the disease susceptibility, are warranted.

Now in the post-genomic era, over a hundred different genomes including the human genome have been sequenced, and the HapMap project has described the human sequence variation throughout the human genome (143). This vast amount of information together with advancing genotyping technologies have enabled genome-wide LD and association studies using hundreds of thousands of SNPs. These novel genomic approaches are expected to facilitate the dissection of the molecular basis of human complex diseases and provide novel insights into disease pathogenesis in near future. Utilization of the HapMap data offers short-cuts to gene identification providing that common variants contribute significantly to common diseases (144–146). Moreover, strategies using tag SNPs, derived from the HapMap data, have most power to detect these common causative variants (147). Consequently, the novel genome-wide association approaches are more likely to identify common than rare variants influencing the FCHL susceptibility. Based on the recent candidate gene studies of DNA sequence variants contributing to levels of plasma HDL-cholesterol in different populations, it is, however, likely that rare DNA sequence variants also contribute to disease susceptibility (22,23). As large-scale sequencing of candidate genes in affected individuals is currently the only approach to identify new rare variants, more efficient and cost-effective sequencing approaches are warranted.

The Human Genome Project and HapMap Project have provided the basic tools for unraveling the complex genetics of FCHL and other complex traits. However, this challenging task can be accomplished only by combining the accumulating biological information with interdisciplinary knowledge and international collaboration. Skillful combining of molecular medicine and systems biology is needed to overcome the greatest challenge of any complex disease, i.e. the meaningful analysis of the enormous amount of data using tools reflecting accurately the underlying phenotypic and genetic complexity.

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