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MINIREVIEW

Genetic Dissection of Familial Combined Hyperlipidemia

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Familial combined hyperlipidemia (FCHL) is the most common genetic hyperlipidemia in man. FCHL is characterized by familial clustering of hyperlipidemia and clinical manifestations of premature coronary heart disease, i.e., before the age of 60. Although FCHL was delineated about 25 years ago, at present the FCHL phenotype and its complex genetics are not fully understood. Initially, the familial aggregation of high plasma total cholesterol and triglyceride levels, with a bimodal distribution of triglycerides, was taken as evidence of a dominant mode of inheritance. However, it is now clear that the genetics of FCHL is more complex, and it has been suggested that FCHL is heterogeneous. Several approaches can be taken to identify genes contributing to the disease phenotype in complex genetic disorders either by studying the disease in the human situation or by using animal models. Recent reports have shown that a combination of genetic linkage studies, association studies, and differential gene expression studies provides a useful tool for the genetic dissection of complex diseases. Therefore, the genetic strategies that will be used to dissect the genetic background of FCHL are reviewed.

Key Words: **FCHL; genome-wide screens; animal models; differential gene expression.**

Familial Combined Hyperlipidemia

Familial combined hyperlipidemia (FCHL; MIM144250) is the most common genetic hyperlipidemia in man. FCHL is characterized by familial clustering of hyperlipidemia, with clinical manifestations of premature coronary heart disease, i.e., before the age of 60. Although FCHL was delineated about 25 years ago (1), at present the complex FCHL phenotype is not fully understood. Hypercholesterolemia, hypertriglyceridemia, elevated apolipoprotein (apo) B (apoB), and apoCIII concentrations are found in FCHL families. Several studies have shown abnormalities in lipoprotein metabolism, including hepatic hypersecretion of apolipoprotein B containing lipoproteins (very-low-density lipoproteins (VLDL); the large VLDL1 and smaller VLDL2) and delayed clearance of atherogenic triglyceride-rich lipoprotein remnants, such as VLDL remnants (intermediate-density lipoproteins) and chylomicron remnants (2,3). Increased hepatic VLDL secretion contributes to elevated plasma triglycerides (TG), apoB, and total cholesterol (TC) (Fig. 1) (4). It has been suggested recently that both VLDL1 and VLDL2 are oversecreted and that an increased VLDL1 residence time in the circulation contributes to hypertriglyceridemia in FCHL (4). In addition to abnormalities in lipid metabolism, insulin resistance of adipose tissue and muscle has been documented in FCHL as well (5). Adipose tissue is one of the major contributors of free fatty acids (FFA) in the circulation. High levels of FFA in the circulation may lead to both a decrease in insulin-stimulated

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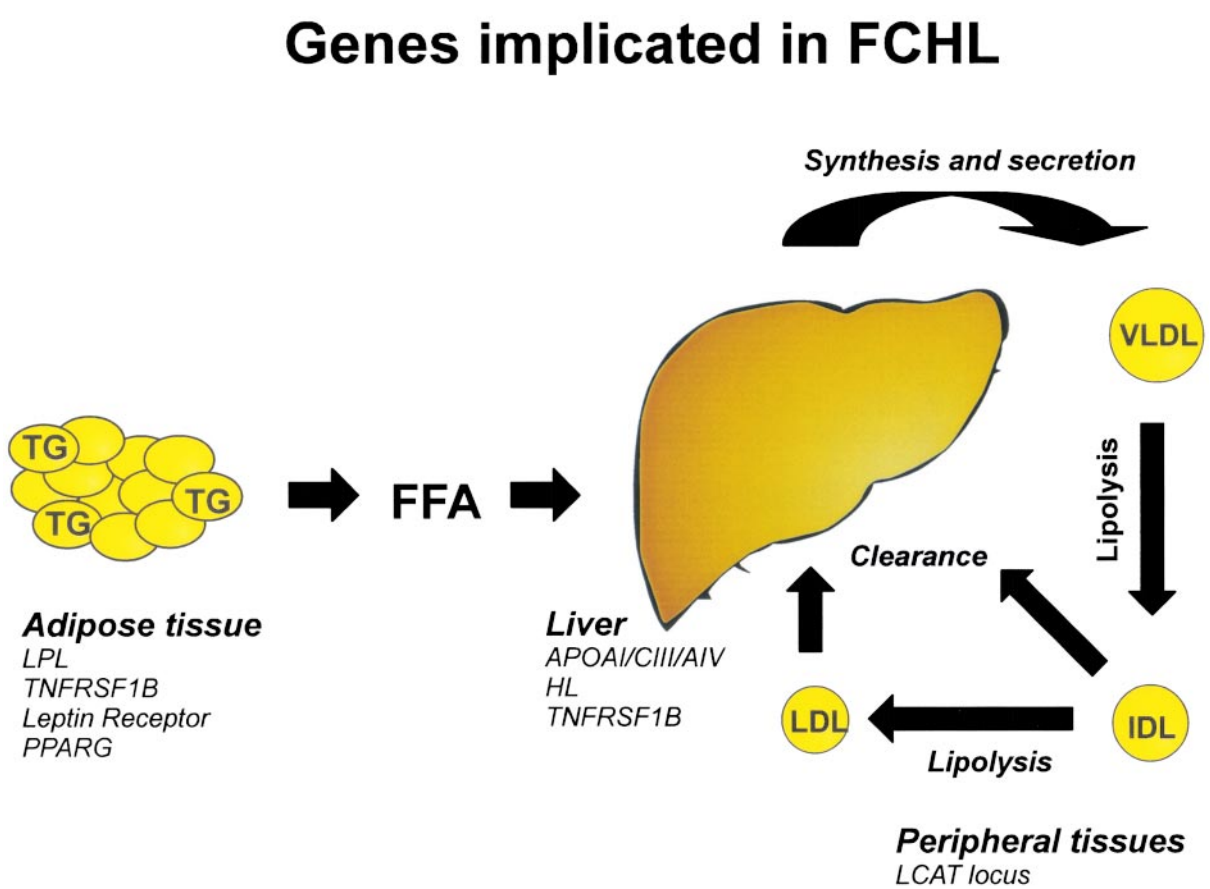

FIG. 1. Model for familial combined hyperlipidemia. Increased hepatic VLDL production in FCHL results in an increased flux of atherogenic IDL and LDL particles, and higher concentrations of TC, TG, apoB, and apoCIII. In addition, insulin resistance of adipose tissue results in an increased FFA flux toward the liver, potentially contributing to hepatic VLDL production. The gene-gene and gene-environment interactions contributing to the FCHL phenotype remain to be elucidated.

glucose uptake in skeletal muscle and an increase in hepatic lipoprotein synthesis, both characteristics of FCHL (6,7). Therefore, liver, adipose tissue, and muscle are interesting target tissues for differential gene expression studies.

Initially, the familial aggregation of traits (i.e., high plasma TG and TC levels) and bimodal distribution of plasma TG levels were taken as evidence of a dominant mode of inheritance (1). However, it is now clear that the genetics of FCHL is more complex (8–12), and it has been suggested that FCHL is heterogeneous (13). It is possible that the complex FCHL phenotype results from a defect in multiple genes or, alternatively, that the disorder results from one or more major genes and gene-environment interactions. This review will discuss genetic strategies to dissect the genetic background of FCHL in both humans and animal models.

Genetic Dissection of Complex Diseases

Several approaches can be taken to identify genes contributing to the disease phenotype in complex genetic disorders either by studying the disease in the human situation or by using animal models. First of all, genome-wide screens can be used to identify chromosomal regions potentially containing genes that contribute to the expression of the complex phenotype. Second, candidate gene studies can be used to identify genes either by linkage or by association. Finally, in tissues from affected subjects compared to tissue from appropriate controls, differential gene expression studies can be used to yield results that can be translated to chromosomal regions identified by the first two approaches. The advantage of this strategy is that it can be used in human as well as animal models. Recent reports have shown that this strategy can be useful in the

TABLE 1
Major Results from FCHL Linkage Studies Presented as LOD Scores

		Traits						
Chromosome	Marker	FCHL	TG	TC	apoB	Leptin	SBP[a]	Reference
1	D1S104 to D1S1677	5.9	3.3					(10)
	D1S104 to D1S1677	3.3	1.9					(21)
	D1S194	2.6						(20)
	D1S1665					3.4		(23)
2	D2S1391		2.3					(12)
4	D4S2369						4.6	(35)
10	D10S1220		3.2					(12)
	D10S169		3.3	2.7				(23)
11	D11S1985 to D11S1324	2.6						(11)
	D11S4127	3.1						(21)
21	D21S1437				2.2			(12)

[a] SBP, systolic blood pressure.

genetic dissection of complex diseases such as insulin resistance, which resulted in the identification of the human resistin gene (14,15).

Animal Models

At present, two spontaneous animal models are available to assist in the genetic dissection of FCHL. The St. Thomas mixed hyperlipidemic rabbit is characterized by increased plasma levels of TC, TG, and hepatic overproduction of apoB-containing lipoproteins, such as VLDL (16), thereby resembling the FCHL phenotype. The HcB19 mice model is characterized by a combination of hypertriglyceridemia, hypercholesterolemia, and elevated plasma apoB levels (17). Like FCHL patients, the hyperlipidemia in HcB19 mice is progressive with increasing age (17). In HcB19 mice, a novel gene, *Hyplip1*, has been mapped to a portion of the mouse genome that is orthologous to human chromosome 1q21–q23 (10,18). This region has recently been shown to be associated with FCHL (17).

Mapping of genetic loci that contribute to the specific phenotype in rodent animal models is considered a more feasible undertaking than in humans. The genetic background is more homogeneous in animals and, therefore, identification of specific genes is within reach. For this reason several investigators advocate the combined animal-human approach to elucidate a complex human disease (14,15,18,19). Therefore the HcB19 mouse, as well as the St. Thomas rabbit, may prove to be of great value in elucidating the genetic background of FCHL.

FCHL Genome Scans

At present, several genome scans of FCHL families have been published and the results of new genome screens are awaited (11,12,20,21). The genome-wide screens showed linkage of the affected FCHL status to several loci the most prominent of which, in terms of log of odds (LOD) score, were chromosomal region 1q21–23 (LOD 5.93), in a Finnish population (10), and chromosomal region 11p with a LOD score of 2.6, in a Dutch population (Table 1) (11). Several other loci with LOD scores between 1 and 3 have been reported (20,21) (Table 1).

The analysis in Finnish FCHL families, yielding the LOD of 5.93 on chromosome 1q21–23, was done according to a parametric model that used the affected-only strategy. This model favors the detection of dominant genes and elegantly avoids the problem of reduced penetrance (10). The diagnosis of FCHL in the Finnish population was according to the 90th population percentiles for plasma TC and TG. Obesity and type 2 diabetes were not specifically excluded (10). Initial analyses in Dutch FCHL families used nonparametric linkage methods (not favoring a dominant or recessive model of inheritance) following "identity-by-descent" principles of mathematical genetic analyses. This approach may also reveal recessive genes (22). FCHL was analyzed as either a qualitative or a quantitative trait (22). The inclusion criteria for FCHL in the Dutch study required a proband with a cholesterol >6.5 mmol/L and/or TG >2.3 mmol/L, whereas probands with a BMI >30, type 2 diabetes, hypothyroidism, renal insufficiency,

and hepatic disease were excluded. In addition, individuals on lipid-lowering medication or glucocorticoid treatment were excluded from analysis (11). In addition to the locus on chromosome 11p in Dutch FCHL families, a chromosomal locus harboring a gene contributing to adiposity and leptin levels was identified on chromosome 1p31 (D1S1665, LOD 3.4) (23). The locus on chromosome 1 harbors the leptin receptor gene and contributed to plasma leptin concentrations, a marker of adipose tissue mass.

The National Heart Lung and Blood Institute Family Heart Study group published replication of linkage of FCHL to chromosome 1q21 (marker D1S104), with an additional heterogeneous effect of the apo AI-CIII-AIV gene cluster on chromosome 11 (21) (Table 1). The same dominant model was used as in the Finnish study and FCHL was also defined in a similar way. Subjects with type 2 diabetes, obesity, and the use of lipid lowering drugs were not excluded. A combined German-Chinese study also confirmed linkage of FCHL to region 1q21–q23 (LOD 2.6) (Table 1) (20). This study followed the same selection criteria for FCHL subjects as the Finnish study, while they excluded individuals with type 1 or 2 diabetes, renal disease, or thyroid disorders. In this study linkage was found with marker D1S194 that is closely situated to marker D1S104. Marker D1S194 is adjacent to the gene for the retinoid receptor (RXR), and that was suggested as an attractive candidate gene for FCHL (20).

Recently, the region on chromosome 1q has been narrowed down to 1q21 by fine-mapping the *Hyplip1* locus in the mouse and defining the borders of the region of conserved synteny between human and mouse (18). This region lies approximately within 5–10 Mb of the peak marker (D1S104) for linkage to FCHL. Recent data suggest that in addition to *Hyplip 1* other interesting candidate genes exist in this region.

The fact that linkage of region 1q21–q23 to FCHL has been reconfirmed provides strong evidence that it harbors a strong candidate gene for FCHL. Therefore it is surprising that this result was not obtained in the Dutch study. As noted above, differences in genetic analytical methodology and subject selection criteria between the Dutch study and the studies supporting linkage to chromosome 1q could explain this difference. Work in progress, in collaboration with the laboratories from Drs. Peltonen, Lusis, and Rotter (UCLA, Los Angeles, CA), seems to prove this explanation since analyses of the Dutch genome-wide scan data, after applying the same criteria for the affected status and use of the affected-only analytical model (10) yielded a LOD of 2.0 with marker D1S104 (unpublished observation). Overall, the data obtained in the different populations support the concept that FCHL is a complex and heterogeneous genetic disease.

Candidate Gene Studies

Linkage methods may have limited power in detecting genes with minor contribution to the complex disease phenotype, whereas association studies have more power (24). According to this view, association studies are useful for confirming conceptual and positional candidate genes that have been chosen for further elucidation, based on their biological function, or results from linkage approaches. Of the candidate studies up to this time, the lipoprotein lipase (*LPL*) gene (25–28), the *APOAI-CIII-AIV* gene cluster (29,30), the hepatic lipase (*HL*) gene (22,31), the peroxisome proliferator-activated receptor gamma (*PPARγ*) gene (32), the tumor necrosis factor receptor superfamily member 1B (*TNFRSF1B*) gene (33), and the lecithin:cholesterol acyltransferase (*LCAT*) locus (22) have been shown to have some effect on the FCHL phenotype, but these genes are not likely the primary cause of FCHL. Obvious and less obvious candidate genes have also been excluded such as the manganese superoxide dismutase (*SOD2*), VLDL-receptor (*VLDLR*), low-density lipoprotein receptor (*LDL-R*), and *APOB* gene (22). These results show that candidate gene studies have been very useful in identifying modifier genes.

Differential Gene Expression Studies

Differential gene expression studies present a powerful tool for identifying genes involved in genetically complex diseases such as FCHL. Prompted by completion of the human genome project, rapid progress has been made in the identification of genes involved in complex metabolic and cardiovascular diseases. A good example is the identification of the hormone resistin, which functions as a bridge molecule between obesity and diabetes (15). In our laboratory, we use three complementary strategies to identify adipose tissue genes specifically up- or down-regulated in FCHL. First of all the suppression subtractive hybridization technique (SSH) is used to isolate differentially expressed sequences from FCHL adipose tissue samples (34). SSH is based on a suppression PCR effect in which long inverted terminal repeats on target DNA result in

the repression of undesirable sequences during PCR. SSH enables the generation of a differential cDNA library using only 50 ng of total RNA, which makes it possible to analyze RNA levels from small amounts of human tissue samples (34).

In our laboratory, the suppression subtractive hybridization experiments resulted in the generation of an up-regulated FCHL adipose tissue cDNA library as well as a down-regulated FCHL cDNA library from total RNA obtained from one FCHL patient versus one age-, BMI-, and gender-matched control subject. Samples were prepared from subcutaneous abdominal adipose tissue biopsies. At present, 128 clones have been isolated from the up-regulated library, 68 of which represent known gene products, 25 represent ESTs, and 35 represent rRNA sequences. From the down-regulated cDNA library 147 clones have been isolated, 75 of which represent known gene products, 63 represent ESTs, and 9 represent rRNA sequences (unpublished results). Confirmation experiments, using approaches such as quantitative RT-PCR and microarray analyses, are currently being carried out.

As a second approach, commercially available cDNA expression filters (Atlas Human cDNA expression array, Clontech, Palo Alto, CA) have been used to study the differences in adipose tissue gene expression between FCHL patients and controls. This array consists of 588 genes involved in several cellular processes and can be classified as (a) oncogenes, tumor-suppressor genes, and cell cycle regulators, (b) ion channels, transporters, modulators, effectors, intracellular transducers, and stress response, (c) apoptosis, DNA synthesis, repair, and recombination, (d) transcription factors and DNA-binding proteins, (e) receptors, cell surface antigens, and cell adhesion proteins, and (f) growth factors, cytokines, chemokines, interleukins, interferons, and hormones. The major advantage of these arrays is that they are ready to use and require only 2 μg of total RNA for a good hybridization signal, based on the fact that a gene-specific oligo(dT) primer mix is used for labeling. It is important to note that a limited number of genes can be studied by this system, and therefore interesting genes can be missed.

In three experiments (3 FCHL subjects versus 2 controls), total RNA samples were hybridized to the Atlas Human cDNA expression arrays. This resulted in identification of 47 up-regulated genes and 16 down-regulated genes. Preliminary analyses showed that these genes are equally distributed over the functional classes spotted on the filters.

In the near future, DNA microarray technology will be implemented to identify novel genes in FCHL. This technology is ideal for the analyses of large amounts of genetic data in a single experiment and will therefore be especially suited to perform large-scale "expression profiling" and "comparative genomics." DNA microarrays will be constructed for human and mouse genetic profiling experiments. It is of interest to note that the HcB19 mice provide interesting target tissues, such as liver and muscle, for differential gene expression studies. Arrays containing conceptual candidate FCHL genes and SSH genes will be screened in parallel to characterize known and unknown genes in the pathogenesis of this disorder. Functional experiments can be performed subsequently with genes of interest, that are identified in the above-noted lines of research, to gain more insight into their effect on FCHL pathogenesis and particularly on adipocytes. Array experiments are specifically important in combination with genome scan data, to evaluate the relevance of the pathway(s) identified. A combination of these microarray strategies will provide powerful tools for further genetic dissection of FCHL.

Conclusion

At present the genetics of the complex FCHL phenotype are still a scientific challenge. The near future will show whether major gene(s) in combination with a number of minor genes and environmental factors generate the FCHL phenotype, or multiple gene-gene and gene-environment interactions. Until now the strongest candidate region for FCHL, situated on chromosome 1q21–23, has been identified using linkage approaches in five different populations. In addition important candidate genes have been involved, including the *LPL* gene, *HL* gene, the *APOAI-CIII-AIV* gene cluster, and the *LCAT* locus, whereas obvious candidates such as the *APOB*, *VLDLR*, and *LDLR* genes have been excluded. Differential gene expression studies will prove to be of additional value for dissecting the genetic background of FCHL, because in combination with genome scan data the relevance of identified pathways can be established. The presently described genetic strategy has been shown to be successful in other complex diseases and is expected to yield more insight in the genetics of FCHL in the near future.

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