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FAMILIAL HYPERCHOLESTEROLEMIA

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I. INTRODUCTION

Definition of the Syndrome

Familial hypercholesterolemia is a distinct syndrome characterized genetically by autosomal dominant inheritance; chemically by elevated concentrations in plasma of cholesterol and low density lipoprotein (LDL); and clinically by xanthomas, arcus corneae, and premature coronary heart disease. Although this syndrome was first recognized well over 100 years ago and its delineation as a specific clinical entity came about in the 1930's (1,2), it is only within the past few years that significant insight has been gained into its mode of inheritance, its relationship to other forms of familial elevation in cholesterol levels, and the underlying biochemical abnormality that results from the abnormal gene.

As a result of recent studies from several laboratories, it is now apparent that the syndrome of familial hypercholesterolemia comprises only one of several disorders commonly referred to as "familial type II hyperlipoproteinemia". In fact, only a small portion of the individuals in the general population with unexplained or primary hypercholesterolemia (as defined by a plasma total or LDL-cholesterol level above the 95th percentile) owe their elevated plasma levels of LDL-cholesterol to familial hypercholesterolemia. Nevertheless, familial hypercholesterolemia is the most clear-cut example of a simply inherited defect that leads to coronary heart disease. A thorough understanding of its pathogenesis should provide not only new insights into the normal regulation of cholesterol homeostasis, but also new knowledge regarding the biochemical nature of atherosclerosis. Three general review articles on the familial hypercholesterolemia syndrome have recently been published (3-5).

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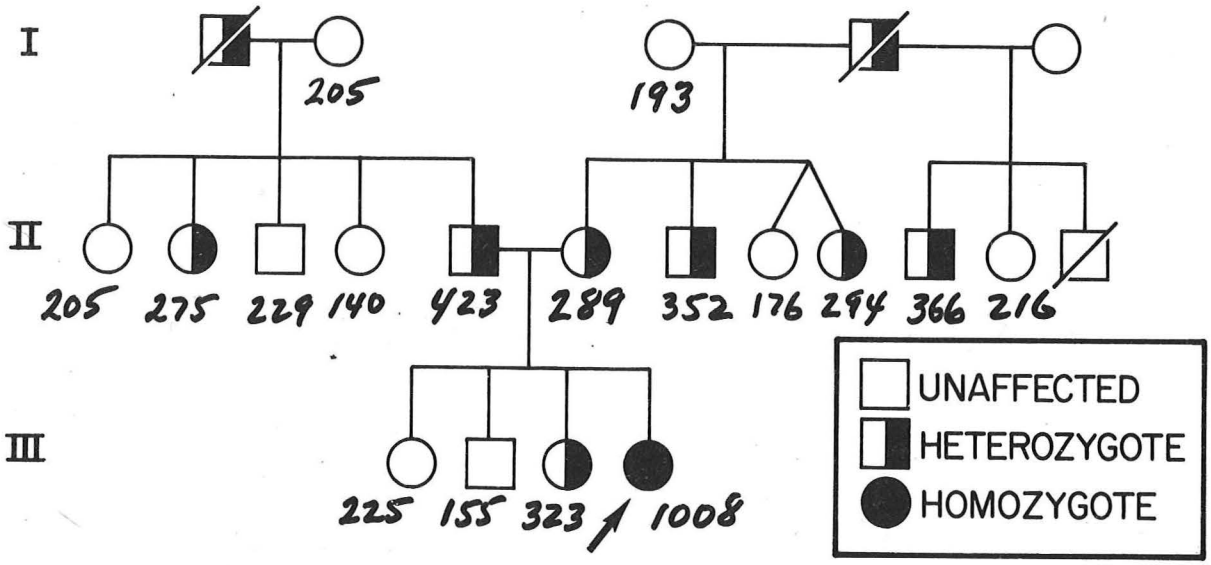
Case Report

[redacted]. is a 6 year old female who was the product of an uncomplicated pregnancy and delivery. Soon after birth, her parents noted the presence of "yellowish" skin nodules on the extensor surface of the hand, on the buttocks, and over the heels. Several dermatologists were consulted and a diagnosis of allergic dermatitis was made. However, the skin nodules progressed despite treatment with a wide variety of anti-allergic medications. At age 5 years, [redacted]. complained of chest pain on exertion and she experienced several syncopal episodes while playing at school. Cardiac evaluation at age 6 years revealed the presence of triple vessel coronary occlusive disease and hemodynamically significant aortic stenosis. The serum cholesterol level was 1008 mg % and the serum triglyceride level was 60 mg %.

Workup of [redacted] revealed the following:

Fig. 2 - Family Pedigree of [redacted] and Her [redacted]



II. CLINICAL AND GENETIC CONSIDERATIONS

Mode of Inheritance and Genetic Evidence for Heterozygous and Homozygous Phenotypes

The genetics of familial hypercholesterolemia was considered a controversial subject as recently as 10 years ago. However, it is now generally believed that the disorder is transmitted by an autosomal dominant gene (6-8). Although the gene is expressed in both heterozygotes and homozygotes, the two clinical phenotypes differ.

Heterozygotes manifest hypercholesterolemia from the time of birth but are usually asymptomatic until adult life when tendinous xanthomas, arcus corneae, and premature coronary heart disease of varying severity may develop. The total plasma cholesterol level in heterozygotes is generally in the range from 270 to 550 mg/dl and rarely exceeds 600 mg/dl. The wide variability in cholesterol levels among heterozygotes is probably a reflection of two facts: 1) the normal allele at the familial hypercholesterolemia locus may vary among affected individuals and 2) the mutant gene exerts its effect amidst a system of interacting exogenous (environmental) and endogenous (polygenic) factors that are known to modify cholesterol levels.

At least 1800 heterozygotes representing over 325 different families with well documented familial hypercholesterolemia have been described in the medical literature to date (5). In two recently reported kindreds, the number of relatives studied in each family was large enough so that a formal genetic analysis could be carried out (4,8). In both families, the demonstration of bimodality in the distribution of cholesterol values allowed a relatively unbiased classification of family members into affected or unaffected groups (Fig. 2). This permitted a segregation analysis of all mating types, the results of which were consistent with transmission of a mendelian autosomal dominant trait.

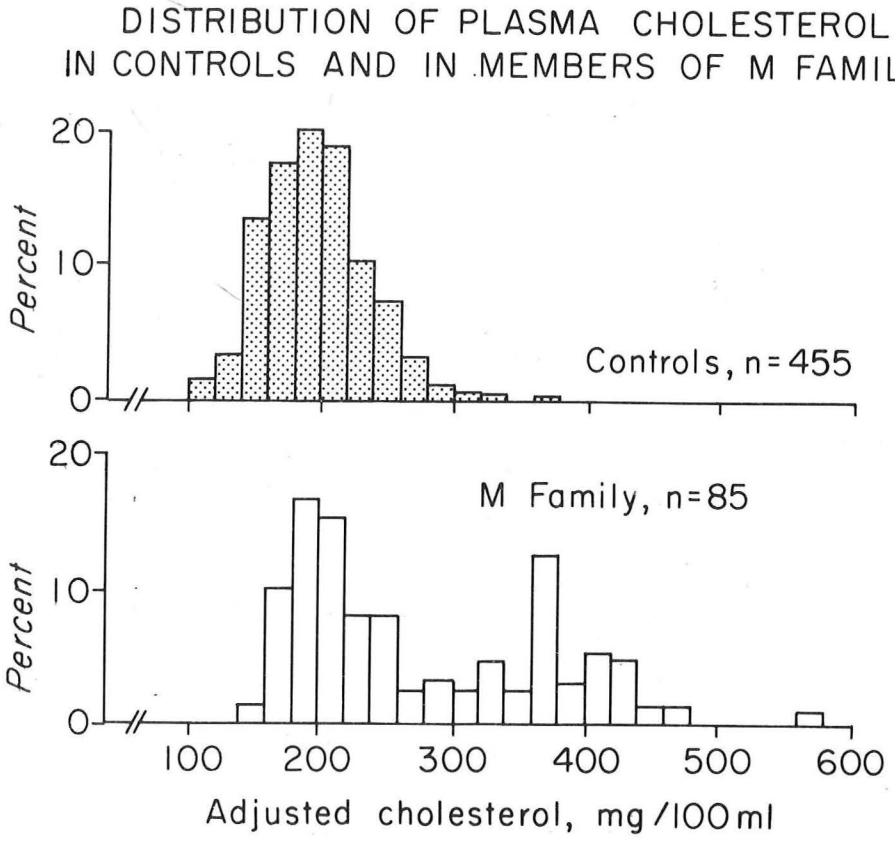
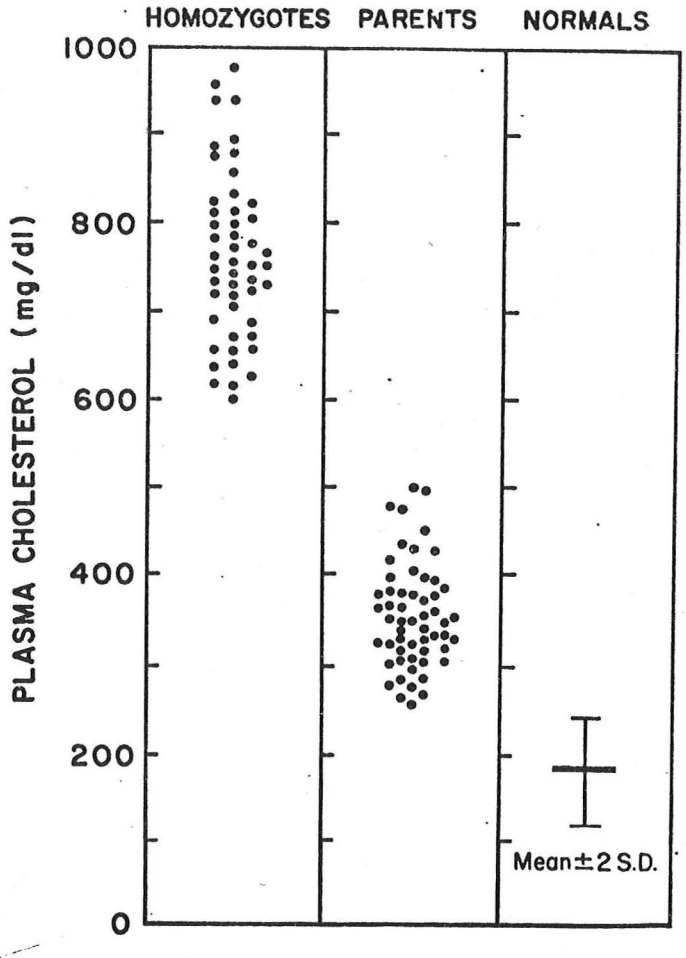


Fig. 2 - Distribution of serum cholesterol levels in controls and in members of the M family, a single large kindred with familial hypercholesterolemia. (Data of Schrott, Goldstein, Hazzard, et al. {8}).

In contrast to the heterozygote, homozygotes, who inherit a double dose of the gene and thus have no normal allele, show a unique clinical syndrome that is much more uniform, severe, and easily distinguished from normal and other hypercholesterolemic states. The syndrome in homozygotes is characterized by a profound hypercholesterolemia ranging between 650 and 1000 mg/dl, a distinct form of cutaneous xanthomas developing in the first four years of life, and signs of rapidly progressive coronary, cerebral, and peripheral vascular disease beginning in childhood and eventuating in death from myocardial infarction, often before age 20 years. (6,9,10).

The strongest genetic proof that severely affected individuals from some families with familial hypercholesterolemia are truly homozygotes has come from the studies of Khachadurian in Lebanon (6,9,10). Over the past 12 years this investigator has studied 49 young individuals presenting with juvenile xanthomatosis and rapidly developing atherosclerosis. Their mean cholesterol level (740 mg/dl) is approximately twice that of their parents (who would be obligate heterozygotes by an autosomal dominant hypothesis) and about four-fold greater than that of normal Lebanese controls. As shown in Fig. 3, these cholesterol values segregate into three nonoverlapping distributions. Such a demonstration of trimodality strongly supports the hypothesis that these presumed homozygotes do in fact possess a double dose of the familial hypercholesterolemia gene.

Fig. 3 - Distribution of total plasma cholesterol levels in 49 homozygotes, their parents (obligate heterozygotes), and normal controls. (Data of Khachadurian {9,10})



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Natural HIstory

The only available unbiased data on the natural history of the heterozygous form of familial hypercholesterolemia have come from studies in which the prevalence of various clinical findings in effected relatives of different ages from the same family has been determined at one point in time (3,8). The results of one such analysis are shown in Fig. 4. Hypercholesterolemia is the earliest detectable manifestation of the gene, being present at the time of birth; it remains the only clinical finding throughout the first decade of life. Both arcus corneae and tendinous xanthomas begin to appear in the latter part of the second decade and by the third

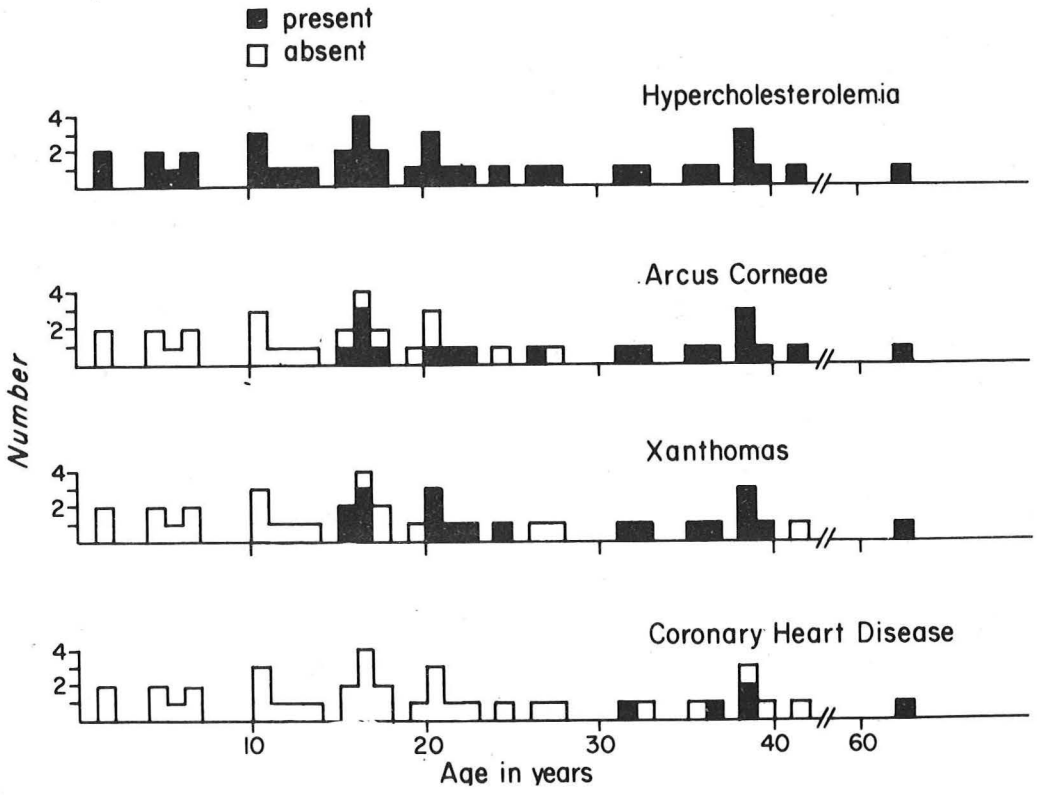

Fig. 4 - Prevalence of clinical manifestations at different ages in the affected members (heterozygotes) of a single large family with familial hypercholesterolemia. (Data of Schrott, Goldstein, Hazzard, et al. {8})

decade both are present in about half of all adult heterozygotes. As many as 80% of heterozygotes may manifest tendinous xanthomas by the time of death (11).

In a study of 104 adult heterozygotes, Slack determined that the mean age at onset of coronary heart disease was 43 years in men and 53 years in women, these figures being about 15 years below the mean age of onset of coronary disease for the general population (Table 1). For men who carry the familial hypercholesterolemia gene, the chance of a first heart attack was 5% by age 30, 51% by age 50, and 85% by age 60. For women the risks at comparable ages were 0, 12, and 58% respectively (12,13). In a 20 year follow-up of 331 members of 11 Danish families with familial hypercholesterolemia, Jensen et al. found that the frequency of coronary heart disease among the carriers of the familial hypercholesterolemia gene (32%) was 25 times greater than among the unaffected relatives (1.3%).

Table 1

Risks of Developing CHD for Heterozygotes

Sex	Mean Age of Onset of CHD	Chance of 1st Heart Attack by Age		
		30 yrs.	50 yrs.	60 yrs.
Male	43 yrs.	5.4%	51.4%	85.4%
Female	53 yrs.	0	12.2%	57.5%

Data of Slack (12,13)

In homozygotes the natural history has been defined by the long-term follow-up studies of Khachadurian (6,9,10). The clinical picture in these patients is remarkably uniform. Hypercholesterolemia is present at the time of birth and the plasma cholesterol level remains elevated throughout life. Unique yellow-orange cutaneous xanthomas on the buttocks, the creases of the hands and over the kneecaps - a finding not present in heterozygotes - develop in all homozygotes by age four years. Homozygotes inevitably develop tendinous xanthomas and arcus cornea in childhood as well as very early evidence of coronary heart disease. Death from myocardial infarction has been known to occur as early as 18 months of age (4). The mean age at death in Khachadurian's series of homozygotes was 21 years (9,10). In addition to atherosclerosis of the coronary, cerebral, and peripheral vessels, homozygotes also develop a characteristic form of xanthomatous infiltration of the aortic valve that is clinically and hemodynamically indistinguishable from rheumatic or calcific aortic valvular stenosis (14).

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A summary of the major clinical features in heterozygotes and homozygotes is tabulated in Table 2.

Table 2

Characteristic Clinical Features of the Familial Hypercholesterolemia Syndrome

Clinical Feature	Heterozygote	Homozygote
Serum Cholesterol Level	< 500 mg %	>550 mg %
Arcus Corneae	After age 20	Before age 10
Xanthomata		
Type	Tendinous Xanthelasma	Cutaneous Planar Tendinous
Onset	After age 20	1st decade of life
Joint symptoms	Acute tendinitis	Migratory polyarthritis
Heart Disease		
Type	CHD	CHD; Valvular Aortic Stenosis
Onset	After age 30	Before age 15

Frequency

The frequency of heterozygotes with familial hypercholesterolemia among patients with coronary heart disease has been determined by detailed family analysis of consecutively studied hyperlipidemic survivors of myocardial infarction. These frequency data, which have been collected by three groups of investigators, have yielded remarkably similar estimates. Three per cent of 193 survivors in London (Patterson and Slack {15}) 4 per cent of 366 survivors in Seattle (Goldstein, et al. {16}), and 6 per cent of 101 survivors in Helsinki (Nikkila and Aro {17}) appeared to have familial hypercholesterolemia. No such direct determination has yet been made amongst the general population. However, one reasonable estimate of the population frequency has been suggested. Using the data from their population genetic analysis of hyperlipidemia among consecutively studied survivors of myocardial infarction, Goldstein, et al. estimated a minimal heterozygote frequency for familial hypercholesterolemia of 1 in 500 Caucasian individuals (16).

The frequency of homozygotes in this country is not known. However, at least 25 homozygotes from North America have been reported in the literature since 1960. If the heterozygote frequency is assumed to be 1 in 500, then the homozygote frequency should theoretically be 1 in 1 million (1/500 x 1/500 x 1/4). Thus, 1 in 250,000 couples in the general population would be at genetic risk for producing a homozygote.

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Diagnosis of Familial Hypercholesterolemia and Its Relation to Familial Combined Hyperlipidemia

The clinical diagnosis of the homozygote with familial hypercholesterolemia causes no difficulty. Not only is the clinical picture distinct but the finding of a plasma cholesterol level exceeding 600 mg/dl in a nonjaundiced child is virtually pathognomonic. The heterozygote is also easy to identify clinically, provided either that he manifests hypercholesterolemia in association with tendinous xanthomas or that he has a family pedigree in which there is evidence

for vertical transmission of hypercholesterolemia in association with tendinous xanthomas (18). However, many heterozygotes, including most below age 20 and approximately 25 per cent of those above age 20, have only hypercholesterolemia without tendinous xanthomas (3-5). Without a complete family analysis the latter individuals are especially difficult to diagnose since familial hypercholesterolemia is only one of several causes of an elevated level of LDL-cholesterol.

In terms of the Fredrickson system of lipoprotein typing (19), most heterozygotes and homozygotes with familial hypercholesterolemia are classified as having a type IIa pattern (increase in LDL-cholesterol with normal plasma triglyceride level). If for some reason, the plasma triglyceride concentration is also elevated, they are then classified as having a type IIb pattern. The term familial hypercholesterolemia, however, should not be considered genetically equivalent or synonymous with the term familial type II hyperlipoproteinemia. In fact, most hypercholesterolemic individuals with a type IIa or type IIb lipoprotein pattern due to a familial form of hyperlipidemia do not have familial hypercholesterolemia (16,18,20,21). In addition to familial hypercholesterolemia, there are at least two other genetic causes of a primary form of type II hyperlipoproteinemia - familial combined hyperlipidemia and a polygenic form of hypercholesterolemia (16,18,21,22).

Table 3

Causes of a Type II Lipoprotein Pattern

I Primary Disorders

1. Familial Hypercholesterolemia
2. Familial Combined Hyperlipidemia
3. Polygenic Hypercholesterolemia

II Secondary Disorders

The most common cause of a familial elevation in the LDL-cholesterol level is familial combined hyperlipidemia, a disorder that has until recently been confused with familial hypercholesterolemia (16). In contrast to familial hypercholesterolemia, its distinct feature is a variability in expression of lipid levels among affected relatives of the same family (16,18,21,22). In families with combined hyperlipidemia affected relatives characteristically show elevated levels of both LDL-cholesterol and very low density (VLDL)-triglyceride and hence have a type IIb lipoprotein pattern, but increased levels of cholesterol alone (type IIa) or of triglyceride alone (type IV or type V) are also

observed. Thus, in individual patients with a primary type II lipoprotein pattern and no tendinous xanthomas, it is often difficult to decide between a diagnosis of familial hypercholesterolemia and familial combined hyperlipidemia. Indeed, in patients with coronary heart disease familial combined hyperlipidemia appears to be about three times more common than familial hypercholesterolemia (16). There are, however, clinical and genetic differences which may be helpful in providing a basis for their separation. First, heterozygotes with familial hypercholesterolemia tend to have on the average higher cholesterol levels. The typical total cholesterol level for a heterozygote with familial hypercholesterolemia is 350 $\pm$ 40 mg/dl as compared to a typical value of 300 $\pm$ 40 mg/dl in combined hyperlipidemia (16,18). Second, heterozygotes with familial hypercholesterolemia do not usually have relatives with lipoprotein abnormalities of multiple types as is the usual finding in combined hyperlipidemia (16,18,21,22). Third, hypercholesterolemic individuals with combined hyperlipidemia do not appear to have tendinous xanthomas (16). Finally, hypercholesterolemia does not fully express itself in children at risk for familial combined hyperlipidemia (16,18).

In addition to these two dominantly inherited forms of primary hypercholesterolemia, elevation in the LDL-cholesterol (type IIa or IIb lipoprotein patterns) also occurs in a polygenic form. This polygenic form of hypercholesterolemia is distinguished from familial hypercholesterolemia and familial combined hyperlipidemia by the fact that unlike the latter two conditions the frequency of lipid abnormalities among first-degree relatives is less than 50% and is usually no higher than 10% (16,18).

Although family analysis provides a reasonably accurate way to distinguish the heterozygotes with familial hypercholesterolemia from individuals with familial combined hyperlipidemia and polygenic hypercholesterolemia, this approach is a laborious one that is not always clinically feasible. The earlier expectation that lipoprotein typing might provide a means of genetic classification of hyperlipidemic individuals has not been realized. What is needed is a marker enzyme or protein that can specifically and accurately identify patients with the familial hypercholesterolemia gene from among the total population of hypercholesterolemic individuals.

By measuring the level of LDL-cholesterol in the cord blood of babies born to a parent who is already known to carry the familial hypercholesterolemia gene, it is possible to detect heterozygotes at the time of birth (23). However, cord blood screening does not appear to be a reliable means for identification of heterozygotes among the general population since the vast majority of newborns with hypercholesterolemia do not have familial hypercholesterolemia (24). The earliest age that heterozygotes can be accurately identified from among the general population is probably one year and even then a complete family analysis is required to confirm that the elevated cholesterol level is due to familial hypercholesterolemia.

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III. BIOCHEMICAL AND PATHOPHYSIOLOGICAL CONSIDERATIONS

Lipid Abnormalities in Blood and Tissues

The elevated cholesterol content in the plasma of heterozygotes and homozygotes is localized to the lipoprotein fraction of density 1.006 to 1.063 g/ml, the low density lipoproteins (LDL) (3,25). A number of studies have indicated that although the protein component of this family of lipoproteins is increased in amount, it is of normal composition and structure with regard to lipid and protein content, amino acid composition, and antigenic and physical properties (26,27).

Accompanying the increase of LDL-cholesterol concentration in plasma, there is a widespread deposition of cholesterol in many tissues of the body. Histologic studies in one homozygote performed at post-mortem showed an accumulation of cholesterol-laden foam cells in many body organs in addition to that occurring in tendons and in arterial plaques (28). Although there is a paucity of data describing direct quantitative measurement of cholesterol content of tissues from patients with this disorder, Samuel, et al. showed that in one homozygote there was a two to five-fold increase in the total cholesterol content of several tissues including liver, skin, adipose tissue, and skeletal muscle, when compared with previously published values of control post-mortem specimens (29).

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Metabolic Studies in Patients

The accumulation of cholesterol in the blood and tissues of patients with familial hypercholesterolemia could theoretically occur in any of three ways: (1) by an increased rate of absorption from the diet; (2) by a decreased rate of excretion of cholesterol itself or its only known metabolic breakdown product, the bile acids; or (3) by an inappropriately high rate of synthesis. Since a genetically and metabolically valid animal model for familial hypercholesterolemia has not yet been described, the possibilities for exploration of the site of the biochemical lesion in this disorder have been limited and indirect. Most clinical metabolic studies to date, particularly those in which techniques of sterol balance and isotopic cholesterol turnover have been employed, have been inconclusive for several reasons. First, the type of hypercholesterolemia of the patient being studied has not been genetically defined. In many studies, patients are simply designated as having "type II hyperlipoproteinemia." As noted above, the type II lipoprotein phenotype embraces patients in whom hypercholesterolemia is due to several different genetic and nongenetic causes, each of which presumably has a different underlying metabolic defect. Thus, when such patients are grouped together, it is difficult to make any generalization concerning the pathogenesis of the specific disorder, familial hypercholesterolemia. Second, since most metabolic studies require the prolonged cooperation of the subject and involve the administration of radioisotopes, it has been difficult to obtain

adequate numbers of normolipidemic control subjects. Third, despite the fact that the genetic defect in familial hypercholesterolemia is expressed most completely in homozygotes, most studies to date have concentrated on heterozygotes whose metabolic behavior might be expected to deviate only slightly from that of normal since they possess one normal allele at the familial hypercholesterolemia locus.

Cholesterol Absorption

Direct studies have given no indication that a primary increase in cholesterol absorption occurs in patients with familial hypercholesterolemia (30). That such an increase is not responsible for the disorder is also suggested indirectly by the fact that when heterozygotes and homozygotes are placed on low cholesterol diets, the plasma cholesterol level almost never returns to normal (31).

Cholesterol Excretion

Although it has been claimed that the excretion of bile acids is decreased in patients with familial hypercholesterolemia (32), most studies of cholesterol metabolism have failed to reveal a consistent defect in bile acid metabolism or neutral sterol excretion in affected patients (33, 34).

Evidence or a derangement in the catabolism of circulating LDL has been claimed in one study in which the rate of disappearance of radioiodinated LDL protein was measured in heterozygotes with familial hypercholesterolemia (35). Although both the synthetic rate and the absolute catabolic rate of LDL protein were normal, the calculated value of the fractional catabolic rate for LDL was lower in familial hypercholesterolemia heterozygotes than in control subjects. In direct contrast, another study of LDL turnover in patients with this disorder has shown a normal half-life, an elevated catabolic rate, and by inference an elevated synthesis rate (36).

Cholesterol Synthesis

Studies of the endogenous rate of cholesterol synthesis in intact humans require complex combinations of sterol balance and isotopic decay techniques (37). In order to interpret these data, it is first necessary to define what is meant by a "normal" rate of synthesis. Thus, if one measures the total body synthesis rate in patients with familial hypercholesterolemia and finds that it is the same as or only slightly lower than that of normal controls, this does not necessarily mean that synthesis in the patients is "normal". In the face of an increase in the total body stores of cholesterol (as is the case in familial hypercholesterolemia) such a "normal" rate of synthesis may be inappropriate and thus excessive. This follows from the observation that in every vertebrate species so far studied, including birds, rodents, dogs, and non-human primates, the hepatic synthesis of

cholesterol markedly decreases when the total body pool of cholesterol is expanded (38). More specifically, the rate of cholesterol synthesis in rat liver has been shown to be inversely proportional to the cholesterol content of this organ (38). In man, such a relationship between hepatic cholesterol content and its rate of synthesis has been difficult to demonstrate consistently because of the technical problem of expanding the total body pool of cholesterol by cholesterol feeding (reviewed in reference {39}). Hence, it seems appropriate to consider the absolute rate of cholesterol synthesis in man in relation to the total body store of cholesterol.

To summarize all the metabolic studies of cholesterol metabolism performed in man to date, the only abnormality that has been consistently observed in patients with familial hypercholesterolemia is the presence of a "normal" rate of cholesterol synthesis despite abnormal accumulation of this sterol (30, 33, 34, 40-42). The finding of an apparently "normal" rate of cholesterol synthesis in the presence of greatly elevated levels of cholesterol in plasma, peripheral tissues, and liver itself (29) strongly suggests that the feedback mechanism regulating cholesterol synthesis in patients with familial hypercholesterolemia is abnormal.

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In Vitro Studies of Cultured Human Fibroblasts

In light of the difficulties involved in interpretation of *in vivo* human experiments, we have recently focused attention on the use of cultured skin fibroblasts as an experimental system for studying the metabolic defect in familial hypercholesterolemia (43-47). The indication that human fibroblasts would provide a useful system for studying the regulation of cellular cholesterol metabolism by extracellular lipoproteins was provided by the earlier and extensive studies of Bailey (reviewed in reference {48}), Rothblat and Kritchevsky (49), and Avigan (50), who have shown that a variety of mammalian cell types cultured *in vitro* synthesize sterols at a rate inversely related to the cholesterol content of the growth medium. This regulatory system in cell culture resembled in some respects that described for intact mammalian liver (38,39). As demonstrated by Siperstein and others, regulation of cholesterol synthesis in liver is mediated by dietary cholesterol, which suppresses the activity of the rate-limiting enzyme in the cholesterol biosynthetic pathway, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) (reviewed in reference {38}). As shown in Fig. 5, this microsomal enzyme catalyzes the irreversible conversion of 3-hydroxy-3-methylglutaryl coenzyme A

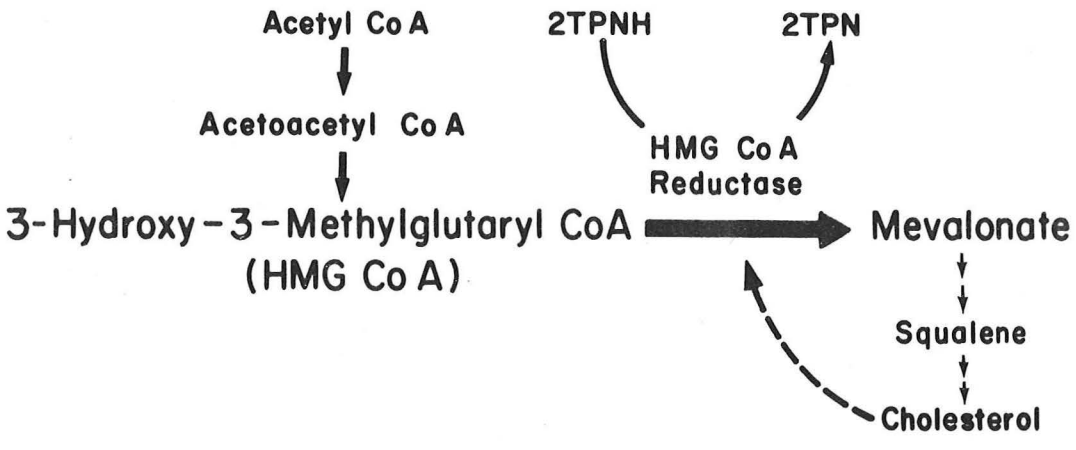


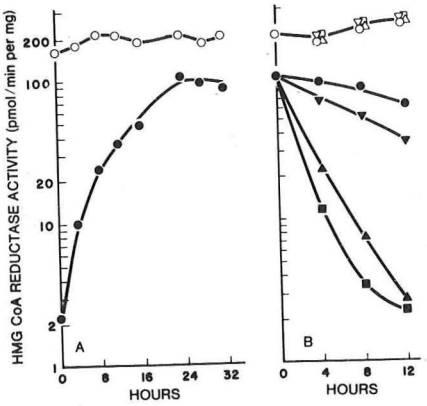
Fig. 5 - Pathway of cholesterol synthesis from acetyl CoA in mammalian tissues, showing the site of feedback regulation by cholesterol.

(HMG CoA) to mevalonate, which then is ultimately converted to cholesterol.

Recent studies in our laboratory have demonstrated that the activity of HMG CoA reductase is measurable in cultured human fibroblasts and that this activity is regulated by the content of specific lipoproteins in the culture medium (43). When fibroblasts have been cultured in medium containing no lipoproteins, the addition to the medium of LDL suppresses the activity of HMG CoA reductase by more than 95% (43).

When the regulation of HMG CoA reductase activity was examined in fibroblasts from homozygotes with familial hypercholesterolemia, a striking abnormality was observed (44-46). A typical experiment is shown in Fig. 6. When grown in medium containing serum lipoproteins, cells from a homozygote had a specific activity of this enzyme that was 50-fold greater than that observed in normal cells (Fig. 6A, 0 time). When lipoproteins were removed from the medium, HMG CoA reductase activity of the normal cells progressively increased by more than 40-fold, whereas the enzyme activity of the cells from the homozygote did not significantly change (Fig. 6A). Moreover, although the subsequent addition of human LDL to the normal cells resulted in a decrease in enzyme activity, the activity of the cells from the homozygote showed no such decline (Fig. 6B). We have made similar observations in cells from four other unrelated homozygotes (45).

Fig. 6 - HMG CoA reductase activity in cultured fibroblasts of a normal control subject (●—●) and a patient with homozygous familial hypercholesterolemia (○—○).



A. Cells were grown to confluence in monolayer culture in petri dishes containing 10% whole serum. At 0 time, the medium was replaced with fresh medium containing 5% lipoprotein-deficient serum. At the indicated time, cells were assayed for HMG CoA reductase activity. B. 24 hrs after the addition of lipoprotein-deficient serum, LDL at the indicated cholesterol concentrations was added and cells were assayed for HMG CoA reductase activity at the indicated time (Data of Goldstein and Brown, (44).

The enhanced level of HMG CoA reductase activity in the homozygotes' cells is associated with a parallel rise in the rate of cholesterol synthesis from acetate (44). Thus, we have concluded that these mutant cells overproduce cholesterol because of a genetically determined inability to respond to feedback suppression of HMG CoA reductase activity by LDL (44-46).

43. Brown, M.S., Dana, S.E., and Goldstein, J.L.: Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts by lipoproteins, Proc. Nat. Acad. Sci. USA. 70: 2162, 1973.

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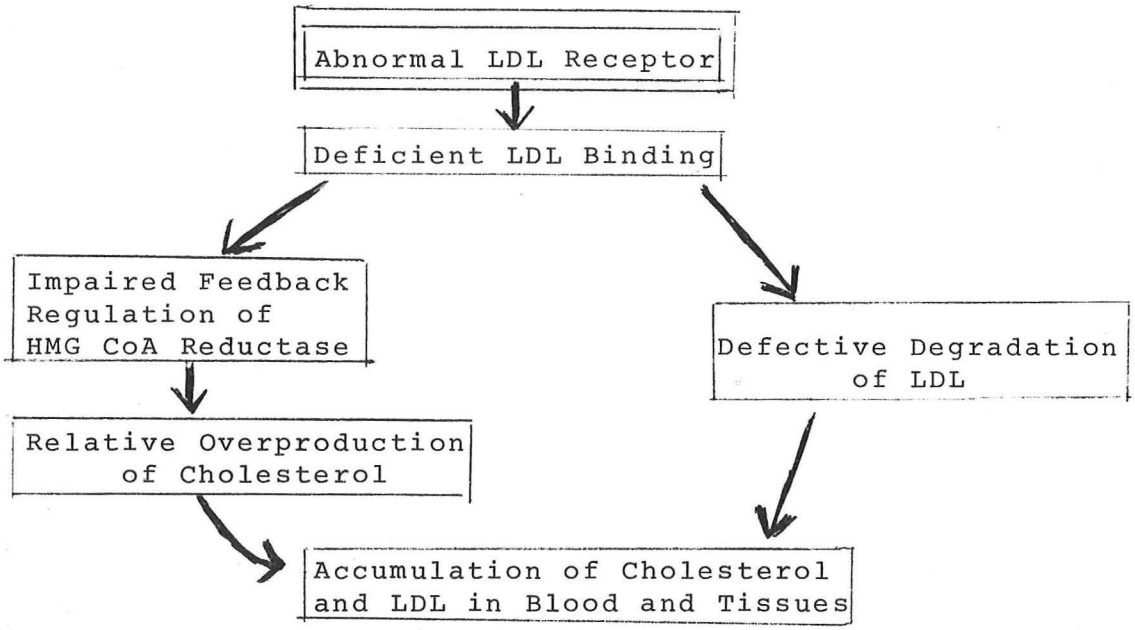
Nature of the Genetic Defect

The primary genetic defect in familial hypercholesterolemia does not involve the structural gene that codes for the enzyme HMG CoA

reductase, but rather the mutation appears to occur in a gene whose normal product functions to regulate the activity of this enzyme. In normal cells, HMG CoA reductase activity is regulated by LDL. This regulation is effected through the binding of LDL to a cell surface membrane receptor. It is this LDL membrane receptor which is abnormal in the cells from patients with familial hypercholesterolemia (47): (Table 4)

Table 4

CONSEQUENCES OF AN ABNORMAL LDL RECEPTOR IN CELLS OF PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA



Assuming that the familial hypercholesterolemic gene is expressed *in vivo* in a manner similar to its action *in vitro*, then the presence of an abnormal LDL receptor would lead 1) to increased cholesterol synthesis by cells and 2) to decreased breakdown of circulating LDL, both of which would produce an enhanced cholesterol accumulation in the body.

Although our studies of fibroblasts indicate that subjects with familial hypercholesterolemia carry in all cells of the body an abnormal gene that has the potential to induce excessive cholesterol production and to retard the degradation of LDL, the tissues in which this abnormal gene is expressed *in vivo* and the conditions governing its expression cannot be determined from the cell culture studies described here. Since human cells of a given type in culture may

express genes *in vitro* that they do not express *in vivo*, the demonstration of excessive cholesterol synthesis in cultured fibroblasts derived from skin does not necessarily imply that such cells of patients with familial hypercholesterolemia overproduce cholesterol *in situ*. Thus, the precise mechanism by which the genetic defect in the LDL receptor leads in patients to an elevation of plasma LDL cholesterol as well as to cholesterol deposition in tendon xanthomas, arterial plaques, and in other tissues remains to be determined.

IV THERAPEUTIC CONSIDERATIONS

Objectives and Indications

Although the predictive association between serum cholesterol levels and coronary heart disease seems well established in an epidemiologic sense, there is still no rigorous proof that lowering the cholesterol level in people with familial hypercholesterolemia delays or reverses the atherosclerotic lesions that they develop. Nevertheless, in the light of current knowledge, the only rational basis for treatment in this disorder is the assumption that therapy aimed at reversing the abnormal lipid pattern is beneficial.

As to who should be treated, our own current view is that it is not practicable to treat every individual who carries the familial hypercholesterolemia gene. Table 5 presents guidelines for selection of subjects for long-term treatment.

Table 5

WHICH SUBJECTS WITH FAMILIAL HYPERCHOLESTEROLEMIA SHOULD BE GIVEN LONG-TERM TREATMENT?

1. All homozygotes, male or female
2. Adult male heterozygotes who have not yet had a major coronary occlusion
3. Adult female heterozygotes in whom xanthomas, ischemic EKG changes, or angina pectoris are present
4. All pediatric male heterozygotes

In our view and in the opinion of Myant and Slack (51), the need to treat all heterozygous females is considerably less compelling than that for heterozygous males since many hypercholesterolemic females live a normal life span without symptoms (12,13). Therefore,

a woman or young girl discovered to have symptomless familial hypercholesterolemia in the heterozygous form (i.e., no xanthomas and no EKG changes of ischemia) should not necessarily be given life-long treatment to lower her serum cholesterol level. On the other hand, if other members of her own family are affected and require treatment, it would be reasonable to consider therapy.

51. Myant, N.B. and Slack, J.: Type II hyperlipoproteinemia, Clinics in Endocrinology and Metabolism 2: 81, 1973.

Diet

Modification of the diet is the basis of all current methods for treatment of subjects with familial hypercholesterolemia. In general, the diet should be modified in a reasonable manner such that cholesterol intake is limited to no more than 300 mg per day for adults and not more than 150 mg per day for children and that saturated fat intake is reduced and polyunsaturated fat is increased. Table 6 lists those foods which should be avoided.

Table 6

FOODS TO BE AVOIDED BY PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

Egg Yolk

Organ Meats (brain, liver, sweetbreads)

Shellfish

Butter

Milk

Cream & Cream Cheeses

Visible fat on meats

Chocolate

Coconut

Whenever dietary treatment is prescribed or suggested to patients, it is essential that the patient and the appropriate family members be referred to a knowledgeable dietician.

Drugs

In most patients with familial hypercholesterolemia, adherence

to a modified diet of the type outlined in Table 6 does not restore the cholesterol level to normal, although it does effect a modest (10-15%) reduction in serum levels. Thus, dietary therapy usually must be combined with drugs. Most experts in the field generally agree that clofibrate (Atromid-S) has little effect on the serum cholesterol level in familial hypercholesterolemia and that the most effective and least toxic agent is cholestyramine (51-54). Cholestyramine is a nonabsorable anionic exchange resin that binds bile salts in the intestinal lumen and thus prevents their absorption from the ileum. This leads to increased fecal excretion of bile salts and depletion of the bile salt pool in the enterohepatic circulation (41). To compensate for the loss of bile salts, hepatic synthesis of bile acids from cholesterol increases and this leads to a fall in serum cholesterol in normal subjects and in most patients with the heterozygous form of familial hypercholesterolemia.

Treatment of affected adults with 12 to 30 g of cholestyramine daily in 3 or 4 doses has resulted in reductions in serum cholesterol varying between 15 to 30% in different series (55-57). In the NIH's recent double-blind study, cholestyramine significantly ($p < 0.005$) lowered the serum levels of cholesterol from a mean of 333 $\pm$ 54 mg % to 264 $\pm$ 48 mg %. The drug had its greatest lipid-lowering effect during the first week of therapy. This study represents the first double-blind comparison of cholestyramine and placebo therapies in an outpatient population of heterozygotes. However, since the treatment periods in this study were short-term (14 weeks), the critical question, "Is the resin an effective cholesterol-lowering agent over long periods of time?", remains unanswered.

That some heterozygotes on the resin show a tendency for the control of serum cholesterol to "escape" after several years of therapy is indicated by the observations of Moutafis and Myant, who noted that the increased catabolism of cholesterol to bile acids in their subjects is balanced by an increased synthesis of cholesterol in liver (34). In their severely affected patients, a combination of cholestyramine with another drug that counteracts the increase in hepatic cholesterol synthesis, such as nicotinic acid, brought about a substantial fall in serum cholesterol level and, eventually, the disappearance of the skin xanthomas (51).

The newly available orange-flavored preparation of cholestyramine is palatable and in the NIH study did not meet with any objectionable patient responses except for mild constipation in some but not all subjects (57). However, it should be noted that since cholestyramine is an anionic resin, it is therefore capable of binding other drugs such as digoxin and related compounds, coumadin, thyroxine, phenylbutazone, and folic acid.

Other cholesterol-lowering drugs such as estrogens, D-thyroxine, clofibrate (Atromid-S), and neomycin do not appear to be effective in this disorder (51,53).

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Ileal By-Pass and Portacaval Shunt

Since most homozygotes are resistent to conventional therapy with diet and drugs (cholestyramine + nicotinic acid), two different surgical procedures have been introduced. Ileal by-pass, which was performed for the first time 10 years ago by Buchwald and Varco (58), lowers the serum cholesterol level by preventing the reabsorption of bile acids and is therefore the surgical equivalent of treatment with cholestyramine (51). Ileal by-pass does not appear to be any more effective than cholestyramine in the treatment of either heterozygotes or homozygotes (59,60).

Portacaval shunt for the treatment of familial hypercholesterolemia was performed for the first time 10 months ago in one homozygote by Starzl (61). The results in this single case have been no less than dramatic. Within several months after the end-to-side shunt, the serum cholesterol fell from a pre-operative level of > 1000 mg % to 200 mg %, the skin xanthomas have regressed, the angina pectoris has disappeared, and the hemodynamically significant valvular aortic stenosis has objectively improved (62). The physiologic basis for the apparently miraculous effect of the shunt on cholesterol metabolism in this one patient is not yet known.

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61. Starzl, T.E., Chase, H.P., Putram, C.W., and Porter, K.A.: Portacaval shunt in hyperlipoproteinemia. The Lancet ii: 940, 1973.

62. Chase, H.P.: Personal communication, December, 1973.

Management of the Heterozygous Child

Heterozygous children present a special problem to the parents and to the pediatrician and the internist. In the future a large number of these children will probably be detected through routine population screening, but at present they are usually discovered because the familial hypercholesterolemia syndrome has been discovered in a parent or a close relative during a work-up for premature heart disease. The vigor and enthusiasm with which long-term treatment is imposed on a child by the physicians and parents should depend mainly on the risks inherent in familial hypercholesterolemia for that child.

As mentioned earlier, the available evidence is not very compelling in the case of heterozygous girls. Since Slacks's data indicate that 85% of female heterozygotes have a normal expectation of life (12,13), it would seem unjustifiable at the present time to impose on these girls a life-time of rigid dietary and drug control and constant reminders of the presence of a disease in which there is no significant certainty of improvement in the prognosis. On the other hand, the risk to heterozygous boys is appreciable - there is a 50% risk of death between 40 and 60 years of age with a more substantial risk of developing clinical signs of coronary heart disease at an even earlier age (12,13). Thus, lowering serum cholesterol levels in these boys is considered obligatory by many workers in the field.

The recent studies of Lloyd (63,64) and Glueck (65) indicate that cholestyramine in a daily dosage of 8 to 24 g is tolerated well by children and is very effective. In Lloyd's series, the serum cholesterol concentration was reduced by a mean of 30% (range 27 to 47%) in 19 heterozygous children and the effect was maintained for periods up to 20 months without any dietary modifications (64). The only long-term side effect of the resin in children was a lowering of the serum folate in all patients; this was due to binding of the folate anion by the resin in the intestine.

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64. West, R.J., and Lloyd, J.K.: Use of cholestyramine in treatment of children with familial hypercholesterolemia, Arch. Dis. Childhood 48: 370, 1973.

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