Homozygous Familial Hypercholesterolemia Occurring with Apoprotein E3 Deficiency

Report of Two Cases

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This is the first report of homozygous familial hypercholesterolemia (FH) occurring together with dysbetalipoproteinemia. The former was demonstrated by deficiency of specific receptors for apoprotein B of low density lipoproteins and the latter by isoelectric focusing of the E isoapoproteins and the presence of a broad-beta band on electrophoresis. Two young boys of Lebanese extraction had extensive tuberous and tendinous xanthomata, serum cholesterol concentrations of 29.9 and 28.4 mmol/liter, respectively, and mildly raised serum triglycerides due to an accumulation of lipoprotein remnant particles. Homozygosity for FH was demonstrated in both boys by the deficiency of specific binding of low density lipoprotein to cultured skin fibroblasts (<15% and <10% of normal, respectively). The E apoprotein phenotypes showed E3/E2 in one boy and E1/E2 in the other. The treatment of both boys with cholestyramine and probucol reduced the serum cholesterol concentration to between 15 and 18 mmol/liter and dramatically lessened the severity of xanthomatosis.


Two of the more common forms of genetic hyperlipoproteinemia are familial hypercholesterolemia (FH) in its heterozygous form and Type 3 hyperlipoproteinemia or dysbetalipoproteinemia. Since the prevalence of the former is about 0.2% and that of apoprotein E3/E2 homozygosity, the basis for Type 3 hyperlipoproteinemia, about 1%, the combination of two occurring together has been described. Hazzard et al. have reported the case of a young girl with apoprotein E3 deficiency (E2/E2 phenotype) and heterozygous FH.

We report the cases of two related boys with the extremely rare combination of the homozygous form of FH together with dysbetalipoproteinemia in at least one, which we believe has not been previously described. Hypercholesterolemia was unusually severe in both.

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Figure 1. Cutaneous xanthomata in Subject TC.

Case Reports

Histories

TC and AC, cousins of Lebanese parentage, were examined first at the ages of 6 and 4 years, respectively, and showed extensive tuberous xanthomata over the knees, elbows, buttocks and heels (Figure 1), xanthomata within the Achilles tendons and extensors of the fingers, and flat xanthomata in the webs of the fingers. Serum cholesterol concentra-
tions were found to be 29.9 mmol/liter and 28.4 mmol/liter, respectively. The diagnosis of homozygous FH became highly likely when both parents of TC and the father of AC were also found to be hypercholesterolemic (Table 1). The fathers of both boys also had xanthomata in the Achilles tendons. However, only the mother of TC was consistently hypercholesterolemic (> 7.5 mmol/liter), whereas the serum cholesterol level in AC's mother has been both normal (5.0 mmol/liter) and raised (7.1 mmol/liter).

The family history (Figure 2) revealed known hypercholesterolemia and early deaths from myocardial infarction.

**Laboratory Investigation**

The initial plasma lipoprotein analyses in TC and AC (ultracentrifugal separation) are shown in Table 1 and show massively raised concentrations of low density lipoprotein (LDL) cholesterol and also of increased intermediate density lipoprotein (IDL) and very low density lipoprotein (VLDL) cholesterol with high cholesterol/triglyceride ratios. This suggested possible dysbetalipoproteinemia in addition to the obvious FH. Electrophoresis of whole plasma on agarose confirmed this by showing in both boys increased beta (LDL) and broad-beta (B-VLDL) char-

| Table 1. Plasma* and Lipoprotein† Lipids and Response to Treatment in Subjects TC and AC |
|---|---|---|---|
| Date | Treatment | Plasma lipids (mmol/liter) |
| | | CH | TG |
| **Subject TC** | | | |
| April 1980 | Pretreatment | Total | 29.9 | 2.8 |
| | | VLDL | 1.0 | 1.1 |
| | | IDL | 1.9 | 1.1 |
| | | LDL | 25.8 | 0.4 |
| | | HDL | 1.2 | 0.2 |
| November 1980 | Cholestyramine 16 g daily | Total | 26.4 | 2.35 |
| April 1981 | Cholestyramine + probucol 0.5 g/day | Total | 20.7 | 1.9 |
| June 1981 | Cholestyramine + probucol 0.75 g/day | Total | 17.1 | 1.9 |
| March 1982 | Cholestyramine + probucol 0.75 g/day | Total | 14.9 | 2.8 |
| March 1983 | Cholestyramine + probucol 0.75 g/day | Total | 17.8 | 2.4 |
| **Subject AC** | | | |
| October 1981 | Pretreatment | Total | 28.4 | 2.76 |
| | | VLDL | 1.08 | 0.84 |
| | | IDL | 1.70 | 0.65 |
| | | LDL | 24.33 | 1.05 |
| | | HDL | 1.39 | 0.22 |
| November 1982 | Cholestyramine 16 g daily + clofibrate 1.5 g/day (for 1 yr) | Total | 26.9 | 2.25 |
| December 1982 | Cholestyramine + probucol 0.75 g/day for 6 weeks | Total | 18.3 | 2.3 |
| **Parents of TC and AC** | | | |
| Father of TC | Total | 13.0 | 2.6 |
| Mother of TC | Total | 7.5 | 1.5 |
| Father of AC | Total | 12.2 | 3.2 |
| Mother of AC | Total | 7.1 | 1.4 |

CH = cholesterol; TG = triglyceride; VLDL = very low density lipoprotein (d < 1.006 g/ml); IDL = intermediate density lipoprotein (d 1.006-1.019 g/ml); LDL = low density lipoprotein (d 1.019-1.063 g/ml); HDL = high density lipoprotein.

*Plasma cholesterol and triglyceride concentrations were measured in a Technicon II autoanalyzer using enzymic techniques. Conversion factors for cholesterol, mmol/liter to mg/dl, multiply by 38.8; for triglyceride, mmol/liter to mg/dl, multiply by 88.5.

†Lipoproteins were isolated by the method of Havel et al (see reference 4).

‡3 months postpartum.
Characteristics of Type 2 and Type 3 hyperlipoproteinemic phenotypes, respectively.23

The definitive diagnosis for FH is the demonstration of deficient or defective interaction of LDL with the specific cellular receptor for apoprotein B of LDL.2 Fibroblasts were cultured from skin biopsies of both boys. After five passages in culture, the cells were tested for their capacity to bind and degrade human LDL. Binding studies were carried out in triplicate at 4° and 37°C. Cells were seeded at about 10^5 cells per 35-mm dish and studied on the fifth day. The cells had been preincubated for 12 hours in lipoprotein-deficient medium and then exposed to increasing concentrations of radioiodinated (125I) LDL, in the absence or presence of excess unlabelled LDL (500 μg/ml), as shown in Figure 3 for Subject TC. After 3 hours of incubation at 37°C, the medium was removed and degradation of LDL was calculated from the radioactivity in the noniodide trichloracetic acid-soluble fraction.7 Surface-bound LDL was calculated from the heparin-releasable radioactivity obtained after the cells had been washed. Binding of LDL at 4°C was assayed by measuring total cell-associated radioactivity after the medium had been removed.

The results of the binding studies carried out at 37°C are shown in Figure 3 for Subject TC, and revealed a deficiency rather than a total absence of LDL receptors. At an LDL concentration of 10 μg/ml medium, 160 ng/mg cell protein bound specifically to normal skin fibroblasts in contrast to 22 ng/mg with the mutant cells (14% of normal). At 4°C the mutant cells bound 8% of normal. Similar results are shown for degradation: after subtracting the values for non-specific degradation (in the presence of excess LDL) and for "background no-cell" proteolysis, the mutant fibroblasts degraded 18% of that found with normal fibroblasts.

Similar data were obtained with fibroblasts from Subject AC. At an LDL concentration of 10 μg/ml medium, the mutant cells bound and degraded >5% and <10% of that obtained with normal cells at 37°C.

The two boys and their parents were tested for apoprotein E phenotype. As shown in Figure 4, Subject AC was homozygous for E2, whereas TC and his father were heterozygous (E2/E3), showing diminished E2/E3 ratios.19,10 Remarkably, both of AC's parents were also homozygous for apoprotein E2. Both boys therefore had proven homozygous FH, with deficiency rather than absence of LDL receptors, and were either heterozygous for the alleles that specify synthesis of apoprotein E2 (TC) or homozygous for apoprotein E2 (AC).

Total plasma apoprotein E concentrations were measured by electroimmunoassay. The values for Subject AC (E2 homozygote) and Subject TC (E2 heterozygote) were 22 mg/dl and 21 mg/dl, respectively, about fourfold higher than for normal subjects in our laboratory. Apoprotein E was also clearly visible in LDL (d = 1.019–1.050 g/ml) as well as in VLDL and in IDL, electrophoresed in 0.1% SDS, 15% polyacrylamide gels; 2.6 mg/dl apoprotein E was recovered in the LDL fraction from Subject AC.

Figure 2. Family tree of known relatives of Subjects TC and AC. Relatives known to have had a myocardial infarction or raised serum cholesterol are shown; information is incomplete about the others.
HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

NORMAL

MUTANT

Figure 3. Binding of ¹²⁵I low density lipoprotein (heparin-releasable radioactivity) at 37°C in skin fibroblasts from a normal subject (A) and from mutant Subject TC (B), expressed in ng LDL protein per mg cell protein. o = total protein bound; • = protein bound in presence of excess unlabelled LDL (nonspecific binding).

Table 1 lists the changes in serum cholesterol in the two boys who were treated initially with up to 4 sachets (36 g) of cholestyramine daily, a strict cholesterol-lowering diet, and added vitamins (with clofibrate also added for AC). This treatment produced little benefit, so probucol was added to the cholestyramine for TC and substituted for clofibrate for Subject AC, (0.5 g/day or one-half the adult dose for 3 months, and since then 0.75 g/day). The serum cholesterol in TC was halved in 5 months, and more than 1 year later, despite a small rise in serum cholesterol, there has been a complete regression of the small interdigital cutaneous lesions and marked reduction in other cutaneous xanthomata. Subject AC, who has been treated for less than 1 year, is showing rapid regression of interdigital xanthomata. Clofibrate was apparently ineffective.

Discussion

This is the first case report of the extremely unusual combination of homozygous FH, which has a prevalence of about one person per million,² with dyslipoproteinemia in at least Subject AC. In the homozygous state, E₃/E₃ phenotype is present in about 1% of the population.¹, ¹⁰ Whereas E₂/E₂ heterozygotes such as TC rarely show significant hyperlipidemia, their VLDL are enriched with cholesterol suggestive of VLDL remnant accumulation. The combination of this phenotype, however, especially
in the E\textsubscript{2}E\textsubscript{2} homozygous state, together with a second genetic disorder of lipoprotein metabolism may lead to an exacerbation of hyperlipoproteinemia.\textsuperscript{1, 10} A well-recognized presentation of Type 3 hyperlipoproteinemia reflects the genetic pairing of familial combined hyperlipoproteinemia with the E\textsubscript{2}E\textsubscript{2} phenotype. The overproduction of VLDL apoprotein B in familial combined hyperlipoproteinemia\textsuperscript{11, 12} heightens the retention of VLDL remnants which depend partly for their removal on the recognition by hepatic receptors for apoprotein E\textsubscript{2}.\textsuperscript{10, 13} When apoprotein E\textsubscript{3} is absent or reduced, the clearance of triglyceride-transporting particles is retarded.\textsuperscript{13, 14} Receptors for apoprotein E have recently been demonstrated in human liver.\textsuperscript{15}

The mechanism for the presence of increased amounts of VLDL remnants in TC (note the high concentrations of cholesterol-rich VLDL and IDL) is less apparent partly because he is heterozygous for the apoprotein E\textsubscript{2} phenotype and partly because VLDL production is not raised in FH.\textsuperscript{16} On the other hand, IDL concentrations are reportedly significantly raised in subjects with familial FH and the fractional removal rate may be reduced, suggesting dependence of IDL removal on normal B receptor activity.\textsuperscript{16} The latter has been shown clearly in the WHHL rabbit.\textsuperscript{17, 18} which is deficient in LDL receptors and shows an accumulation of IDL.

Homozgyous FH is most commonly due to the absence or deficiency of receptors that recognize apoprotein B so that regulated removal of LDL through this route ceases.\textsuperscript{2} In the case of TC, receptor binding was less than 14\% of normal, and in the case of AC, less than 10\% of normal. Goldstein and Brown,\textsuperscript{19} in discussing the heterogeneity in the degree of LDL receptor deficiency in clinically homozygous subjects, have defined the receptor-defective state as one in which receptor activity is expressed at between 2\% and 25\% of normal. Coetzee et al.\textsuperscript{20} have described in their large group of apparent homozygotes in South Africa even smaller deficiencies of receptor activity. They have therefore raised the possibility of additional genetic factor(s) producing hypercholesterolemia. Indeed, Oslund et al.\textsuperscript{21} have recently reported a further mutant for FH characterized by a low receptor capacity for LDL but a heightened affinity.

A further metabolic abnormality in homozygous FH is the overproduction of LDL apoprotein B,\textsuperscript{22, 23} which may be a consequence of reduced hepatic uptake of LDL. These two defects may have exaggerated the severity of the hyperlipoproteinemia in these children. A serum cholesterol concentration of about 30 mmol/liter (1160 mg/dl) is high even for homozygous FH.\textsuperscript{24-27} The unusually high concentration of LDL may indicate that the clearance of LDL in homozygous FH, albeit retarded, occurs partly through the apoprotein E receptor (which is not impaired) possibly due to the presence of a small but significant amount of apoprotein E in LDL.\textsuperscript{28} Havekes et al.\textsuperscript{29} have reported that LDL from a subject with homozygous FH contained more apoprotein E than occurs normally.

A final point of interest is the relatively satisfactory improvement when probucol was added to cholestyramine treatment. A reduction in the serum cholesterol of more than 12 mmol/liter is unusual in this disorder. It is all the more surprising because probucol by itself is of limited therapeutic value in severe hypercholesterolemia.\textsuperscript{30} However, we have since observed a strong synergistic response with the use of the two drugs in patients with heterozygous FH.\textsuperscript{31} We have also found that probucol increases the fractional catabolic rate of LDL, although not to the degree reported for cholestyramine.\textsuperscript{32}

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