Low Density Lipoprotein Metabolism in Cultured Fibroblasts from a New Group of Patients Presenting Clinically with Homozygous Familial Hypercholesterolemia

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The metabolism of low density lipoproteins (LDL) was studied in cultured fibroblasts obtained from five local patients diagnosed, on the basis of clinical features and serum cholesterol concentrations, as having the homozygous form of familial hypercholesterolemia. LDL receptor function was assessed by measuring the binding, internalization, and degradation of $^{125}$I-labeled LDL, and by measuring the stimulation of cellular acyl-CoA cholesterol acyltransferase (ACAT) activity which followed exposure to LDL. Fibroblasts from two cases (CF and GM) showed receptor activities which were approximately 10% of the values obtained with normal cells, while ACAT stimulation by LDL was very low. These two patients were classified as homozygous for a receptor-defective abnormality. However, fibroblasts from the other three patients (JG, ES, and TT) showed greater than 25% of normal receptor activity, as assessed by $^{125}$I-LDL binding and catabolism. ACAT stimulation by LDL in cells from JG and ES was within the range of values shown by cells previously characterized as heterozygous for a receptor-negative mutation. Cells from ES behaved atypically: a low, but nonsaturable, activation by LDL was evident. ACAT stimulation by LDL was normal in cells from TT. Receptor activities of the cells from the available parents, assessed on the basis of LDL binding and degradation or of ACAT stimulation, were not clearly distinguishable from those of normal cells. These results add to the growing evidence of genetic heterogeneity underlying the clinical picture associated with familial hypercholesterolemia in different geographical distributions.

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Familial hypercholesterolemia (FH) is a monogenic, dominantly inherited disorder characterized by elevated plasma levels of low density lipoprotein (LDL) cholesterol, and is associated with the development of atherosclerosis and xanthomas. These features are especially marked in the rare homozygous state which presents with plasma cholesterol concentrations between 13 mmol/liter and 26 mmol/liter, with cutaneous and tendinous xanthomas manifesting in early childhood, and with severe aortic and coronary atherosclerosis within the first or second decades of life.¹⁻³

Despite the complexity of the LDL pathway, elucidated by Goldstein, Brown and coworkers,⁴ only three types of defect have been defined unambiguously in association with the FH phenotype: 1) complete absence of functional, high-affinity LDL receptors (receptor-negative); 2) markedly decreased activity of LDL receptors (receptor-defective); and 3) defective internalization of receptor-bound LDL (internalization-defective). It is believed that diminished clearance of LDL by peripheral tissues, arising from grossly inadequate activity of LDL receptors, is a
major determinant of the hypercholesterolemia seen in FH patients, though increased apo B synthesis and increased cholesterogenesis have also been reported and may account in part for the associated severe hypercholesterolemia.

The existence of a large number of FH homozygotes in the white Afrikaans-speaking South African population has recently been reported. The evidence suggests a heterozygote frequency of at least 1 in 100 in this group, rivaling the incidence in Lebanon. A "founder gene" effect was proposed but no evidence of the molecular defect underlying the clinical disorder was presented. We studied the metabolism of LDL in cultured fibroblasts grown from skin biopsies obtained from five patients attending the Children's Lipid Clinic at the Red Cross War Memorial Children's Hospital, Cape Town, who showed the clinical picture of homozygous familial hypercholesterolemia. Two of the patients were classified as homozygous for a receptor-defective abnormality, while receptor activities in fibroblasts from the other three patients were unexpectedly high. In one patient, atypical induction of acyl-CoA cholesterol acyltransferase (ACAT) activity in response to LDL was also noted.

Methods

Patients

The apparent homozygous state of FH was diagnosed on the basis of: 1) the presence of plasma cholesterol concentrations greater than 14.3 mmol/liter (550 mg/100 ml); 2) the appearance of xanthomas in the first decade of life, and 3) the presence in both parents of hypercholesterolemia or clinical signs indicative of the homozygous state.

Four patients reported here comply with all the criteria for the diagnosis of homozygous FH (table 1). A fifth subject, TT, had plasma cholesterol concentrations compatible with values in the lower range found for homozygotes or the upper range for heterozygotes, the early development of xanthomas in this case suggests homozygosity.

All the patients (except TT) have had cholesterol values generally indicative of the homozygous state. Plasma cholesterol concentrations have varied at different times, in part according to the nature and intensity of therapy, but in all cases were high, reaching levels of 20 mmol/liter or greater in four of the five patients. The increased levels of total cholesterol reflected predominantly the LDL fraction. High density lipoprotein (HDL) cholesterol and plasma triglyceride concentrations were unremarkable. All patients showed cutaneous and tendinous xanthomas before the age of 4 years except for TT, in whom xanthomas were first noted at the age of 8 years. Planar cutaneous xanthomas were found in all patients in the skin over the buttocks and, except in the case of TT, in the interdigital webs between the fingers. Such planar cutaneous xanthomas are unique to homozygotes and are not found in heterozygotes. The especially common location between the first and second fingers was found on both hands of CF and ES and on the right hand of GM. As previously reported for homozygotes, the xanthomas varied considerably in extent and distribution.

The patients have been unresponsive to conventional lipid-lowering dietary or drug therapy and none were diabetic or hyperuricemic. ES and JG received surgical portacaval shunts 6 years before this study, with subsequent spontaneous closure of the shunt in JG. GM has been treated by plasma exchange for the past 4 years, and TT had right-sided pelviureteric obstruction presumably due to renal tuberculosis, but has not manifested evidence of renal failure or significant proteinuria at any stage.

Family Studies

Full family studies have not yet been carried out, partly because of serious logistical problems, but, when possible, the plasma lipids of parents and siblings were examined and family histories elicited. None of the available parents had visible tendinous xanthomas.

F Kindred

Mrs. F is moderately hypercholesterolemic (6.2–7.2 mmol/liter) with a normal plasma triglyceride concentration (1.65 mmol/liter). There are several cases of apparent ischemic heart disease in her first degree relatives. Mrs. F died in an accident and no further family information was obtainable. CF has no brothers or sisters.

Table 1. Biochemical and Clinical Features of Homozygous FH Patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yrs), Sex</th>
<th>Cholesterol (mmol/liter)</th>
<th>Total trig*</th>
<th>XMTA †</th>
<th>IHD ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>5 M</td>
<td>17.5–22.0</td>
<td>1.3 -</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>GM</td>
<td>18 M</td>
<td>13.6–20.0§</td>
<td>1.3 +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>JG</td>
<td>10 F</td>
<td>15.5–25.0§</td>
<td>1.0 -</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ES</td>
<td>10 F</td>
<td>13.0–20.7§</td>
<td>1.9 -</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TT</td>
<td>14 M</td>
<td>10.2–15.1</td>
<td>0.8 -</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*Total triglyceride (mmol/liter).
†Planar cutaneous xanthomas.
‡ECG evidence of ischemic heart disease.
§On plasma exchange or after portacaval shunt; values obtained both after and prior to treatment.
XMTA = xanthoma; IHD = ischemic heart disease.
M Kindred

Mrs. M is hypercholesterolemic (7.8–10.2 mmol/liter), with a plasma triglyceride concentration of 1.7 mmol/liter. Mr. M had a first myocardial infarction at the age of 47 years and died of myocardial infarction at 63. GM has two siblings with plasma cholesterol concentrations in the heterozygous range (7.4–10.8 mmol/liter). Two cousins on the maternal side had xanthomas and died in their early twenties from myocardial infarction.

G Kindred

Both parents have hypercholesterolemia with plasma cholesterol concentrations in the heterozygous range (7.7–10.3 mmol/liter). Mr. and Mrs. G have plasma triglyceride concentrations of 1.8 and 0.8 mmol/liter, respectively. On the maternal side there is a history of apparent ischemic heart disease. JG has two siblings with plasma cholesterol concentrations also in the heterozygous range (6.5–8.8 mmol/liter).

S Kindred

Both parents are hypercholesterolemic (7.8–9.9 mmol/liter). Mrs. S has a normal plasma triglyceride concentration (1.3 mmol/liter). No triglyceride concentration was obtainable for Mr. S. Both parents have family histories of apparent ischemic heart disease. A sister of ES died at the age of 18 months due to myocardial infarction associated with an apparently homozygous phenotype (cholesterol concentration, 19.6–20.5 mmol/liter). A third sibling has an apparently normal plasma cholesterol concentration (5.3–7.5 mmol/liter).

T Kindred

Mrs. T has elevated plasma cholesterol concentrations (7.2–8.4 mmol/liter) but Mr. T was borderline in this respect (6.0–6.7 mmol/liter). Plasma triglyceride concentrations of both parents are normal (0.5–0.7 mmol/liter). The T kindred family history is essentially negative for premature ischemic heart disease.

Cell Studies

Fibroblasts were obtained from the following three groups of subjects: 1) the five apparently homozygous FH patients CF, GM, JG, ES, and TT; 2) Mrs. F, Mrs. G, Mrs. T, Mr. S, and Mrs. S, the parents of the respective homozygous children (the other parents were not available for skin biopsies); and 3) six normocholesterolemic subjects (SB, male aged 3 years; AM, male aged 11 years; BT, female aged 2 months; JM, male aged 2 years; JK, male aged 3 years; and FG, male aged 12 years). In addition, fibroblast strains previously categorized as receptor-normal (GM0203), receptor-negative homozygous (GM0701, GM2000), receptor-negative heterozygous (GM0700) and receptor internalization-defective (GM2408A) were obtained from the Human Genetic Mutant Cell Repository (Camden, New Jersey) and used as controls.

Skin fibroblasts were established from skin biopsies obtained from the ventral surface of the forearms of subjects. The cells were grown in Eagle’s minimum essential medium, buffered with Earle’s salts containing 10% tryptose/phosphate broth, 10% heat-inactivated fetal calf serum, 60 μg/ml penicillin G and 100 μg/ml streptomycin sulphate. Cells were used between passages 5 to 15 in culture. Cells were always seeded at 0.1 × 10^6 cells per 60 mm Petri dish and cultures in late logarithmic growth (Day 5) were used for all measurements of receptor activity unless otherwise stated.

Human LDL (density = 1.019–1.063 g/ml) and lipoprotein-deficient serum (density > 1.25 g/ml) were isolated, and the LDL washed and labeled with ^125^I-iodine as described previously. The ability of cells grown in 60 mm dishes to bind, internalize, and degrade ^125^I-LDL protein, after an initial upregulation period in lipoprotein-deficient medium, was assayed according to Goldstein et al., except that cell layers were harvested in methanol (50%, v/v), extracted in chloroform-methanol (2:1, v/v) and ^125^I-radioactivity (intracellular content) and protein content were then determined in NaOH-solubilized protein pellets. Further details are described in the legends to the figures and tables. The rate of 1,4-C^14_1 oleic acid incorporation into cholesteryl ^14_C esters by cultured cells (ACAT activity) in response to different LDL concentrations in the medium or to 25-hydroxy-cholesterol, was measured by the method of Goldstein et al.

Lipid and Lipoprotein Assays

Blood was collected in the fasting state into tubes containing EDTA (1 mg/ml). Plasma cholesterol was measured by a single step enzymatic method (Boehringer Mannheim GmbH, Catalog No. 148393), and plasma triglyceride was also determined using a commercial kit (Boehringer Mannheim GmbH, Catalog No. 124032, Mannheim, West Germany). HDL cholesterol was determined after heparin-manganese precipitation of the apo B-containing lipoproteins, and LDL cholesterol was calculated according to the formula proposed by Friedewald et al.

Results

To determine whether defects in the function of cellular LDL receptors, similar to those previously described for FH, were present in our patients, we measured the ability of their cultured skin fibroblasts to take up and metabolize LDL. The rate of degradation of LDL by cells is known to depend on a number of steps, including binding and subsequent internalization of LDL by specific cell-surface receptors which deliver the LDL to lysosomas via receptor-mediated endocytosis. Accordingly, the cellular
binding, internalization, and degradation of LDL was measured in a series of experiments in which we followed previously described procedures which give optimal reproducibility and resolution between defined classes of receptor activity. The amounts of surface-bound 125I-LDL, intracellular 125I-LDL, and 125I-LDL degraded via the saturable high affinity receptor pathway in the different cell strains are shown in Table 2. As reported by others, considerable variation in the receptor activity of the same cell strain was found between different experiments; this is reflected in the range of values obtained for the two receptor-normal controls, GM0203 and SB. Factors known to contribute to such variability are cell density and other day-to-day variations encountered in these complex cell-biological analyses. For these reasons, cells were always assayed after the same time in culture and at least two normal control cell strains were analyzed in each experiment to permit the values of unknown cell strains to be expressed as percentages of normal activity. The receptor activity of GM0700 was found to be

![Image of Table 2](https://example.com/Table2.png)

Table 2. High-Affinity Binding, Intracellular Content, and Rates of Degradation of 125I-LDL in Cultured Fibroblasts

<table>
<thead>
<tr>
<th>Cell strain</th>
<th>Receptor-bound 125I-LDL (ng/mg)</th>
<th>Intracellular 125I-LDL (ng/mg)</th>
<th>Degradation of 125I-LDL (ng/4 hrs/mg)</th>
<th>Internalization index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normocholesteremic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM0203</td>
<td>166 (136–203)†</td>
<td>928 (657–1248)†</td>
<td>3797 (2964–4251)†</td>
<td>25–33</td>
</tr>
<tr>
<td>SB</td>
<td>220 (163–249)§</td>
<td>875 (847–908)§</td>
<td>4254 (3432–4716)§</td>
<td>19–33</td>
</tr>
<tr>
<td>AM</td>
<td>198</td>
<td>894</td>
<td>4363</td>
<td>27</td>
</tr>
<tr>
<td>BT</td>
<td>149</td>
<td>554</td>
<td>3754</td>
<td>29</td>
</tr>
<tr>
<td>JM</td>
<td>188</td>
<td>765</td>
<td>3772</td>
<td>24</td>
</tr>
<tr>
<td>JK</td>
<td>113</td>
<td>559</td>
<td>2214</td>
<td>25</td>
</tr>
<tr>
<td>FG</td>
<td>150</td>
<td>644</td>
<td>4201</td>
<td>32</td>
</tr>
<tr>
<td><strong>x = 169 ± 36</strong></td>
<td></td>
<td><strong>x = 746 ± 160</strong></td>
<td><strong>x = 3765 ± 730</strong></td>
<td></td>
</tr>
<tr>
<td>FH heterozygote</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM0700</td>
<td>63</td>
<td>331</td>
<td>1862</td>
<td>43% 35</td>
</tr>
<tr>
<td>FH homozygotes (receptor-negative)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM0701</td>
<td>&lt; 2%</td>
<td>23</td>
<td>135</td>
<td>3% -</td>
</tr>
<tr>
<td>GM2000</td>
<td>&lt; 2%</td>
<td>26</td>
<td>61</td>
<td>1% -</td>
</tr>
<tr>
<td>FH internalization-defective</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM2408A</td>
<td>68%</td>
<td>87</td>
<td>276</td>
<td>6% 2.5</td>
</tr>
<tr>
<td>FH homozygotes (clinically defined)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>9</td>
<td>52</td>
<td>338</td>
<td>8% 43</td>
</tr>
<tr>
<td>GM</td>
<td>17</td>
<td>86</td>
<td>492</td>
<td>11% 34</td>
</tr>
<tr>
<td>JG</td>
<td>62</td>
<td>356</td>
<td>1503</td>
<td>41% 30</td>
</tr>
<tr>
<td>ES</td>
<td>45</td>
<td>186</td>
<td>1206</td>
<td>28% 31</td>
</tr>
<tr>
<td>TT</td>
<td>148</td>
<td>516</td>
<td>2028</td>
<td>55% 17</td>
</tr>
<tr>
<td>FH heterozygotes (clinically defined)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mrs F</td>
<td>182</td>
<td>961</td>
<td>4170</td>
<td>113% 29</td>
</tr>
<tr>
<td>Mrs G</td>
<td>160</td>
<td>593</td>
<td>3802</td>
<td>85% 27</td>
</tr>
<tr>
<td>Mrs S</td>
<td>157</td>
<td>658</td>
<td>3898</td>
<td>90% 29</td>
</tr>
<tr>
<td>Mr S</td>
<td>111</td>
<td>609</td>
<td>3661</td>
<td>85% 38</td>
</tr>
<tr>
<td>Mrs T</td>
<td>129</td>
<td>709</td>
<td>3038</td>
<td>82% 29</td>
</tr>
</tbody>
</table>

*Percentages of the mean of the values obtained for GM0203 and SB, both analyzed in parallel to the unknown on the same day.

†Internalization index: amounts 125I-LDL intracellular + degraded/bound.

‡Mean and range of 6 experiments performed over a 2-month period.

§Mean and range of 4 experiments performed over a 2-month period.

I-Mean and standard deviation of normal controls.

High-affinity receptor binding of 2 ng/mg corresponded to approximately 80 cpm per dish.

Cells were seeded in medium containing 10% fetal calf serum at a density of 10^5 cells/60 mm dish. Medium was changed on Day 3. After 5 days (Day 5), the medium was changed to medium containing LPDS (5 mg protein/ml) in the place of fetal calf serum. On Day 6, the medium was replaced with a fresh aliquot of the same medium. On Day 7, each cell layer received 2 ml of medium containing LPDS plus 10 μg protein/ml 125I-LDL (150–250 cpm/ng), either in the absence or presence of 300 μg protein/ml unlabeled LDL. After incubation for 4 hours at 37°C, the medium was removed, and its content of non-iodide trichloroacetic acid-soluble radioactivity measured. The receptor bound and intracellular radioactive content of the cells were measured after washing and release of surface-bound LDL using dextran sulfate (10 mg/ml) as described in Methods. High affinity values, which represent the difference between the values obtained in the absence and presence of unlabeled LDL, are presented. All values are the average of triplicate incubations carried out at each LDL concentration.
approximately 40% of the normal control value, as was predicted from the heterozygous state of this cell strain. The cell strains, GM0701 and GM2000, both previously characterized as homozygous FH of the receptor-negative type, each displayed the very low values (≤ 3%) expected for this type of defect. A reasonably close correlation between the measured parameters of LDL receptor function was obtained with fibroblasts from all cell strains. The relative degradation rates and intracellular concentrations of LDL in the different cell strains always reflected their receptor-binding activities.

There was a wide variation between the receptor activities of fibroblasts from the five patients (table 2). Cells derived from CF and GM manifested values approximately 10% of normal (LDL binding 4% to 8% of normal) indicating a typical receptor-defective abnormality (homozygous form) in each case. Cells from ES, JG, and TT manifested receptor binding activity significantly greater than that of either CF or GM (JG was 29%; ES, 27%; and TT, 69% of normal). The corresponding degradation values were 41% for JG, 28% for ES, and 55% for TT. These values were, therefore, similar to or even greater than those for the control FH heterozygous cell type (GM0700).

It should be noted that the receptor values in these experiments represent maximum expression of receptor activities brought about by a 48-hour upregulation in lipoprotein-deficient serum (LPDS). In normal control cell strains, a 7- to 15-fold increase in receptor binding was achieved in this way. Maximum upregulation in JG, ES, and TT was also obtained after 48 hours in LPDS; JG showed an increase of 4.5-fold; ES, an increase of 5.7-fold; and TT, an increase of 12.3-fold.

Fibroblasts from the available parents were not, as a group, clearly distinct from receptor-normal cells on the basis of these three parameters, which showed overlapping ranges. This is evident from the high values relative to the two normal controls analyzed simultaneously, and from the mean absolute values of 125I-LDL binding for normal controls and for this group, which were 169 ± 36 and 148 ± 29 ng/mg cell protein, respectively (p > 0.3). Corresponding degradation rates in these cells were 3765 ± 730 and 3712 ± 420 ng/4 hrs/mg cell protein, respectively (p > 0.8). The receptor activities of Mr. S and Mrs. T were the lowest of the parent cell strains and both exhibited values which were lower than six of the seven normal strains. This would be in line with a possible heterozygous FH status of these cells.

The "internalization index," defined as the ratio of the amount of LDL internalized and degraded to that bound, has been used to characterize a unique cell strain (GM2408A) in which LDL receptors were able to bind, but not internalize, LDL particles.14,21 Such cells have a very low internalization index: 2 compared with the normal range of 17–48. The internalization indices for the cell strains analyzed in this study are also given in table 2; the range of values obtained indicates a relatively normal rate of internalization of bound LDL for every cell strain examined, except GM2408A which gave an expectedly low internalization index of 2.5.

The degradation of 125I-labeled LDL was also measured at varying LDL concentrations in order to determine the affinity of binding between LDL and receptor (figure 1). Normal fibroblasts showed a typical response to increasing LDL concentrations, with receptor-dependent degradation approaching a maximum at approximately 40 μg/ml, whereas the receptor-negative cells (GM0701) showed virtually no receptor-dependent degradation. The half-maximal degradation rate in all the cell strains examined was reached at LDL concentrations of approximately 10 μg protein/ml, except in the case of CF which showed half-maximal values at 25 μg protein/ml. It should be noted that the curves shown in figure 1, which represent affinity analyses, were not obtained under conditions necessary for a valid comparison of the relative surface receptor concentrations of the various cell strains; these values were determined as shown in table 2. The results thus indicate that the relatively high receptor-dependent activities found in JG, ES, and TT (corresponding to an LDL-concentration of 10 μg protein/ml, table 2) reflect the num-

![Figure 1](https://example.com/figure1.png)

**FIGURE 1.** Degradation of 125I-LDL by human skin fibroblasts. Confluent cell cultures (grown for 7–14 days) were upregulated for 24 hours in 2 ml medium containing lipoprotein-deficient serum (LPDS-medium), and then incubated in 2 ml fresh LPDS-medium containing the specified concentrations of 125I-LDL for 4 hours at 37°C. At the end of the incubation, the medium was removed, and analyzed for non-iodide trichloroacetic acid-soluble radioactivity (degradation). All values are the means of duplicate dishes which did not vary by more than 10% from the mean. The data obtained were analyzed for the saturable component of the curve by subtracting from the total degradation values at each LDL concentration, the corresponding nonsaturable component value. The nonsaturable component was derived from the linear portion of the curve at higher LDL concentrations. Half maximal degradation occurred at the following LDL concentrations: SB, 8.5 μg/ml; ES, 9.0 μg/ml; TT, 10.0 μg/ml; JG, 13.0 μg/ml; CM, 9.0 μg/ml; CF, 25.0 μg/ml.
All determinations were done in duplicate and ratios were changed after 3 days. After 5 days, the medium was the same experiment.

Unknown cell strains were always assayed in parallel with at least two normal controls determined using the means.Unknown cell strains were after separation of lipids by thin layer chromatography.

Fibroblasts from GM, TT, and the cell strains from the indicated number of separate experiments. Fibroblasts from ES were atypical in their or, in some cases, undetectable activities of these determined for JG and GM0700 (FH heterozygous) were indistinguishable by either assay. The receptor activity displayed by JG was, therefore, significantly greater than the activities of the two receptor-defective cells, CF and GM, and was within the range expected for FH heterozygous cells. In the case of cells from ES, the activity induced with 25-OH-cholesterol was, on average, about double that induced by LDL at a concentration of 200 µg protein/ml (ratio = 0.56). ES, therefore, apparently is within the heterozygous range for this assay. However, the response of ES to increasing LDL concentrations was atypical.

ACAT ratios obtained in the cells of the parents of our patients were similar to values obtained in normal cell strains, and in some cases (Mrs. F and Mr. S), notably higher. Therefore, these results indicate that none of the cells in this group have a significantly reduced receptor activity compared to normal cells. To classify cells, controlling for variations in the esterification capacity of different cell strains and between different cell preparations, the ACAT activities induced by LDL were compared with those induced by 25-OH-cholesterol; the latter effect does not depend on receptor number (table 3). Normal and heterozygous controls showed ratios close to the expected published ranges; cells from CF and GM had very low, but detectable, activities induced by LDL. From these and the data presented in table 2, the fibroblasts from CF and GM are, therefore, classified as homozygous for the receptor-defective mutation.

On the other hand, fibroblasts from TT yielded ratios well within the normal range. The values obtained for JG were in keeping with the LD binding data, in that JG and GM0700 (FH heterozygous) were indistinguishable by either assay. The receptor activity displayed by JG was, therefore, significantly greater than the activities of the two receptor-defective cells, CF and GM, and was within the range expected for FH heterozygous cells. In the case of cells from ES, the activity induced with 25-OH-cholesterol was, on average, about double that induced by LDL at a concentration of 200 µg protein/ml (ratio = 0.56). ES, therefore, apparently is within the heterozygous range for this assay. However, the response of ES to increasing LDL concentrations was atypical.

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In a series of experiments measuring LDL binding, internalization, and degradation, the values obtained in cells from JG, ES, and TT were between 20% to 69% of normal values (table 2). These patients, therefore, do not comply with the criteria for classification as either receptor-negative or receptor-defective, and manifest values which are more consistent with the reputedly intermediate status of FH heterozygous patients. On the basis of clinical pictures and family histories, ES and JG are unequivocally “homozygous” for FH. Both have markedly elevated plasma total cholesterol and LDL-cholesterol concentrations well within the homozygous range, both developed cutaneous and tendinous xanthomas in early childhood, and both have hypercholesterolemic parents with strong family histories of premature ischemic heart disease. The evidence in favor of homozygosity for TT is less unequivocal in view of the ambiguous paternal cholesterol levels and the absence of a convincing family history. Nevertheless, his markedly elevated plasma cholesterol concentration and the development of xanthomas by the age of 8 years probably provide sufficient clinical evidence to classify this patient as homozygous FH, possibly of a milder form resembling patients with the variant forms 1 and 2 described by Khachadurian and Uthman.

As pointed out by others, the true genetic status with respect to FH can only be defined at the receptor level. On the basis of the receptor studies reported here, JG, ES, and TT might be designated as FH heterozygotes. The severe hypercholesterolemia and clinical picture shown by these patients and their parents, however, suggest the possibility that these patients may be doubly heterozygous, having inherited another as yet undefined form of hypercholesterolemia that is not due to FH. Alternatively, these patients might be true homozygotes with respect to a milder receptor defect, as opposed to the receptor-defective or receptor-negative types. Because of the natural variability in receptor activity among cells, the genetic inheritance pattern of such a defect(s) would be extremely difficult to characterize. Studies using greater numbers of siblings will be needed to exactly define such defect(s), and to clarify the inheritance pattern, especially in terms of the number of genes involved in the clinically observed pathophysiology. Our findings clearly indicate that the clinical picture of homozygous familial hypercholesterolemia is not always associated with a classic LDL receptor defect.

Goldstein and Brown have briefly reviewed three other clinically homozygous patients in whom fibroblast LDL-receptor function was 35% to 55% of normal; two of these have been further investigated with respect to cholesterol and LDL metabolism. In contrast to previous reports on homozygous FH patients, both patients showed reduced rates of whole body cholesterol synthesis and low bile acid and neutral sterol excretion. It is possible that this intermediate range of receptor defects may include both

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**Figure 2.** ACAT activities of human skin fibroblasts. Confluent cultures of the indicated cell strains were upregulated and assayed for ACAT activities as described in the legend of table 3.
heterozygous and mildly receptor-defective homozygous patients.

We conclude from our results with cells from the parents of CF, JG, ES, and TT, that in many cases FH heterozygotes cannot be distinguished from normal cells on the basis of LDL receptor assays. This conclusion is supported by the findings of Slack et al.,25 and Andersen and Johansen26 who reported that in a large group of subjects studied, individuals cannot be designated as FH heterozygous or as normal on the basis of receptor-mediated LDL suppression of cholesterol synthesis.

A further interesting observation concerns the ACAT-inducibility of this group of patients. As expected, fibroblasts from CF and GM showed minimal inducibility in keeping with their receptor-defective status. On the other hand, TT, mildly deficient in receptor activity, manifested ACAT inducibility similar to that displayed by normal cell strains and considerably greater than that of the control heterozygous cell strain GM0700. Cells from the other two receptor-deficient patients, JG and ES, showed low ACAT induction at 200 µg LDL protein/ml approximated in keeping with their receptor activity and similar to the inducibility displayed by the heterozygous cell line GM0700. However, in the case of ES, ACAT induction did not reach a plateau with increasing LDL concentrations in the incubation medium whereas, in the other cell strains tested, maximum induction was approached at 50–100 µg LDL protein/ml consistent with the known saturability of LDL receptors at this LDL concentration. Goldstein et al.,15 previously recorded that cell strains from certain FH homozygotes of the defective type showed non-saturability of ACAT induction up to 400 µg/ml added LDL protein. This, however, was found in cell strains with very low ACAT induction levels and was not observed in the case of cells, such as FH heterozygotes, with relatively higher ACAT activities. The observation that in cells from ES, the stimulation of ACAT continued to increase at LDL concentrations up to 800 µg protein/ml, would be consistent with the possibility that the LDL receptor in these cells has reduced binding affinity. However, the results indicating normal receptor affinity in ES (figure 1) show that this is not the case.

The exact relation between the amount of LDL metabolized and the stimulation of esterification is not known and depends on a number of steps in addition to LDL binding and degradation. Detailed analyses of these steps in cells from ES is needed to resolve the unusual response in this cell strain. It should be noted that this phenomenon occurred only in cells from ES and that ACAT inducibility in the fibroblasts from the two parents displayed normal kinetics. Further investigation is also required to establish whether the apparent defect in ACAT-inducibility is primary, resulting in a receptor deficiency secondary to an intracellular accumulation of free cholesterol, or whether it is a coincidental, but separate, defect.

The group of patients studied in this report presented with severe hypercholesterolemia and live in a region geographically distinct from the previously reported large group of South African FH hypercholesterolemics from the Transvaal province.6 It is possible, therefore, that our patients are not representative of this group. We are presently undertaking cellular studies of a number of Transvaal FH families.

Our group of patients add to the growing evidence of genetic heterogeneity underlying the clinical FH phenotype in different geographical distributions. A further implication of these data is that the host of clinical homozygotes in South Africa may include more than one type of molecular defect.

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