Hyperlipoproteinemia as a Risk Factor for Ischemic Heart Disease

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We analyzed serum lipoproteins and apolipoprotein E (apo E) from 199 patients in CCU, having ischemic heart disease, and from 211 healthy subjects. It was suggested that serum lipoprotein abnormalities, especially elevated low density lipoprotein (LDL) levels, are closely related to atherogenesis in relatively young patients and subjects with severe coronary lesions. The frequency of apo E-4 was higher and that of E-2 was lower in the CCU group than in the control group. Apo E mutants, E-7 (Glu244→Lys, Glu245→Lys) and E-5 (Glu3→Lys), were also frequent in the CCU group. Apo E isoproteins have higher pl in the order E-2, E-3, E-4, and we observed that LDL-cholesterol levels increased in the same order. The apo E mutants, E-5 and E-7, are also more basic than E-4. These findings suggest that basic apo Es were associated with the development of atherosclerosis.

The prevalence of familial hypercholesterolemia (FH) in the CCU group was more than 10 times higher than the reported frequency of FH heterozygotes in normal population. The persistence of marked hypercholesterolemia from infancy probably makes FH patients susceptible to atherosclerosis. Based on the analysis of LDL-receptor protein synthesis, various types of mutations were observed even in phenotypically homozygous FH patients. FH homozygotes were divided into 2 groups, a receptor-negative group and a receptor-defective group. We found a great difference in the frequency of coronary heart disease depending on whether even a small number of receptors were present or not.

HYPERLIPOPROTEINEMIA results from an abnormality of plasma lipoprotein metabolism, and occurs on the basis of genetic factors and/or various environmental factors such as diet. The plasma lipid levels are regulated by plasma apolipoproteins, lipoprotein receptors and many other factors, and the mutation of apolipoprotein E (apo E) and the deficiency in LDL-receptors are well known genetic abnormalities.

Hyperlipoproteinemia is considered to be one of the major risk factors for arteriosclerosis. One of the causes of the recent increase in ischemic heart disease in Japan may be the increase in prevalence of hyperlipoproteinemia that has accompanied Westernization of the diet. However, it has been indicated that the prevalence of hyperlipoproteinemia may not actually be so high in Japanese patients with ischemic heart disease. Accordingly, we analyzed serum lipoproteins and apolipoproteins in patients with angina pectoris or acute myocardial infarction. In this paper, we discuss the participation of hyperlipoproteinemia as a risk factor for ischemic heart disease.

Key words:
Apolipoprotein E
Familial hypercholesterolemia
Hyperlipoproteinemia
Ischemic heart disease
Risk factor

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TABLE I THE SUBJECT OF THE PRESENT STUDY

<table>
<thead>
<tr>
<th>Subject</th>
<th>n</th>
<th>Age* (yr)</th>
<th>Sex (M/F)</th>
<th>Serum lipids*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ch</td>
</tr>
<tr>
<td>CCU</td>
<td>199</td>
<td>64.1 ± 10.2***</td>
<td>160/39**</td>
<td>201.4 ± 44.3***</td>
</tr>
<tr>
<td>Control</td>
<td>211</td>
<td>46.1 ± 9.8</td>
<td>152/59</td>
<td>187.0 ± 31.7</td>
</tr>
</tbody>
</table>

* mean ± SD ** p<0.05, *** p<0.01
Abbreviations: n = case number; Ch = cholesterol (mg/dl); TG = triglycerides (mg/dl); SD = standard deviations

TABLE II FREQUENCY OF HAYPERLIPOPROTEINEMIA IN CCU-PATIENTS AND CONTROL SUBJECTS

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>CCU (n=199)</th>
<th>Control (n=221)</th>
<th>CCU/Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ila</td>
<td>9.0% (18)</td>
<td>2.8% (6)</td>
<td>3.2</td>
</tr>
<tr>
<td>Iib</td>
<td>11.1% (22)</td>
<td>0.9% (2)</td>
<td>12.3</td>
</tr>
<tr>
<td>IV</td>
<td>21.1% (42)</td>
<td>20.9% (44)</td>
<td>1.0</td>
</tr>
<tr>
<td>total</td>
<td>41.2% (82)</td>
<td>24.6% (52)</td>
<td>1.7</td>
</tr>
</tbody>
</table>

( ) : case number

1. Serum lipid and lipoprotein levels in patients with ischemic heart disease

Serum lipid and lipoprotein levels were analyzed in 199 patients with unstable angina pectoris or acute myocardial infarction admitted to the coronary care unit of our hospital (CCU group), and in 211 normal healthy individuals examined for general medical checkups (control group). Very low density lipoprotein (VLDL) was separated by ultracentrifugation, and then the cholesterol (Ch) and triglyceride (TG) content was determined. High density lipoprotein (HDL)-Ch was measured by a precipitation method, and the low density lipoprotein (LDL)-Ch level was calculated as the serum Ch level minus the sum of VLDL-Ch and HDL-Ch. A classification of hyperlipoproteinemia (WHO classification) was made using our standard values for the VLDL-Ch and LDL-Ch levels (10.6 ± 12.0 mg/dl and 118.6 ± 57.8 mg/dl, respectively) obtained from 83 normolipidemic individuals. Both groups were analyzed by random sampling, except for some selections at the time of blood sampling in accordance with age and sex in the control group.

Baseline characteristics of the study groups are shown in Table I. In the CCU group there were many subjects of advanced age and males predominated. The serum Ch level was higher in the CCU group, but the TG level showed no significant difference between both groups. The frequency of subjects with hyperlipoproteinemia in the control group was 24.6% and most of them had type IV hyperlipoproteinemia, i.e., an increase of VLDL. On the other hand, in the CCU group, hyperlipoproteinemia was noted in 41.2%; 1.7 times more frequent compared with the control group (Table II). In the CCU group, type IV hyperlipoproteinemia was also the most prevalent (21.1%), and there was no significant difference between these two groups in its frequency. We found that the frequencies of type Ila and type IIb hyperlipoproteinemia (increased LDL) were 3.2 and 12.3 times higher than those of the control group, respectively (Table II).

As the age and sex distribution were different between the CCU and control
groups, we studied serum lipoproteins in both groups by 10-year age groups (data not shown). In males, the mean LDL-Ch level increased with age in the control group. It was consistent with other population studies that the Ch levels tended to increase with age in the control group. The DL-Ch levels were significantly higher in the CCU group under 60 years of age. But, in contrast to the control group, the LDL-Ch value showed a decrease with age in the CCU group. We found similar values in the 60-69 aged patients in both groups. Since the number of cases was rather small and the variation was great, we found no definite tendency in females. We could postulate that individuals with higher LDL levels had ischemic heart disease at a rather young age, while those with lower levels developed atherosclerosis at a later age from the accumulation of various risk factors other than hyperlipoproteinemia.

In 121 patients undergoing coronary angiography, a relation between serum lipoprotein levels and the number of affected vessels was studied. VLDL-Ch and HDL-Ch levels showed no definite tendency, but LDL-Ch showed an increase with an increase in the number of affected vessels, and it seemed that the severity of coronary artery disease correlated well with the LDL-Ch value (Fig. 1).

These findings suggest that serum lipoprotein abnormalities, especially ele-
Fig. 3. Concentration of serum and lipoprotein lipids in the different apo E phenotypes.

![Graph showing serum cholesterol (Ch) and triglycerides (TG) levels in different apo E phenotypes.](image)

Fig. 4. Apo E allele frequency in normolipidemia and hyperlipoproteinemia among healthy individuals.

![Bar chart showing the percentage of each apo E allele (ε4, ε3, ε2) in different groups.](image)

2. Heterogeneity of apolipoprotein E

Apolipoproteins play an important role in lipoprotein metabolism. In the general population, apo E shows genetic heterogeneity with three major isoproteins, E-4, E-3, and E-2. Apo E can bind to LDL-receptors and to apo E specific remnant receptors, and regulates serum cholesterol metabolism. Type III hyperlipoproteinemia is characterized by a high prevalence of atherosclerosis, and is caused by genetic abnormalities of apo E, e.g., homozygosity for apo E-2. Apo E is a polypeptide composed of 299 amino acids. Interchanges of Arg and Cys in the 112th and 158th amino acid residues of the wild type of apo E-3 give us the E-4 (Cys112 → Arg) and E-2 (Arg158 → Cys) isoproteins, respectively. The electrical charge of the whole apo E molecule is respectively more positive or negative by 1 unit of charge in apo E-4 and apo E-2 than in the wild type E-3 (Fig. 2). These apo E isoproteins can be identified by isoelectric focusing due to the difference
TABLE III  APO E PHENOTYPE FREQUENCY IN HEALTHY SUBJECTS AND CCU-PATIENTS

<table>
<thead>
<tr>
<th>Apo E phenotype</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th>CCU-Patients</th>
<th></th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Male &lt;60 y.o.</td>
<td>Male ≥60 y.o.</td>
<td>Female Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% (n)</td>
<td>% (n)</td>
<td>% (n)</td>
<td>% (n)</td>
<td>% (n)</td>
<td>% (n)</td>
<td></td>
</tr>
<tr>
<td>E4/4</td>
<td>1.0 (2)</td>
<td>4.4 (7)</td>
<td>5.3 (3)</td>
<td>7.7 (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E4/3</td>
<td>17.5 (37)</td>
<td>15.0 (24)</td>
<td>19.3 (11)</td>
<td>12.6 (13)</td>
<td>25.6 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E4/2</td>
<td>1.0 (2)</td>
<td>0.6 (1)</td>
<td>1.8 (1)</td>
<td>- (0)</td>
<td>2.6 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E3/3</td>
<td>67.2 (142)</td>
<td>68.7 (110)</td>
<td>64.8 (37)</td>
<td>70.9 (73)</td>
<td>61.5 (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E3/2</td>
<td>11.3 (24)</td>
<td>8.1 (12)</td>
<td>3.5 (2)</td>
<td>10.7 (11)</td>
<td>- (0)</td>
<td></td>
<td>2.6 (1)</td>
</tr>
<tr>
<td>E2/2</td>
<td>0.5 (1)</td>
<td>1.3 (2)</td>
<td>- (0)</td>
<td>1.9 (2)</td>
<td>- (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E7/3</td>
<td>1.0 (2)</td>
<td>1.3 (2)</td>
<td>3.5 (2)</td>
<td>- (0)</td>
<td>2.6 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E5/3</td>
<td>0.5 (1)</td>
<td>0.6 (1)</td>
<td>1.8 (1)</td>
<td>- (0)</td>
<td>- (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100% (211)</td>
<td>100% (160)</td>
<td>100% (57)</td>
<td>100% (103)</td>
<td>100% (39)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

( ) : case number

of their isoelectric points.

By analyzing apo E isoproteins in patients with hyperlipidemia and atherosclerosis, we demonstrated two types of new apo E mutants, apo E-5 and E-7, showing isoelectric points more basic than all other known apo E isoproteins. The mutation points in these apo E mutants were determined by gene analysis. In E-5, guanine (G) coding for Glu (GAG) on the 3rd position from the N-terminal of the apo E polypeptide was replaced by adenine (A) to change Glu into Lys (AAG). In E-7, two G → A substitutions, as was the case with E-5, had taken place at 2 sites coding —Glu—Glu— on the 244th and 245th residues to produce —Lys—Lys— (Fig. 2).

3. Apo E isoproteins and serum lipoprotein in the control group

We studied serum lipoprotein levels according to apo E phenotype in male control subjects who were under 60 years of age and within ±15% of ideal body weight. Patients with severe hyperlipoproteinemia (serum Ch and/or TG values more than 300 mg/dl) were excluded.

Fig. 5. Concentration of lipoprotein lipids in CCU-patients and control subjects, according to apo E phenotype.
mean values + 1SD in addition to the mean values + 2SD. The frequency of the E4 gene (ε4) was higher in both type IIa and IIb hyperlipoproteinemia, and this became clearer when the criteria of the mean + 2SD was used rather than the mean + 1SD. Furthermore, in type IIa hyperlipoproteinemia no individuals with the E-2 gene (ε2) were noted, and also in type IIb hyperlipoproteinemia the frequency of ε2 was low. On the other hand, a high frequency of ε2 was observed in type IV hyperlipoproteinemia, and this tendency was made stronger by using the criteria of mean + 2SD (Fig. 4).

4. Ischemic heart disease and apo E isoproteins

Apo E phenotypes were determined in both the CCU and control subjects to investigate the relationship between the development of atherosclerosis and the apo E isoproteins. More of the E4/4 and less of the E3/2 phenotypes were observed in the CCU group compared with the control group, but these differences were not marked. When the CCU group was divided into male and female groups, the E4/4 and E4/3 phenotypes were obviously more frequent among the females, and subjects with the E3/2 phenotype were not present (Table III). The apo E-7 mutant was noted in one case (2.6%). However, in males we found a distribution of apo E phenotypes similar to

![Image](image-url)

Fig. 6. The frequency of the subjects with apo E-4 or apo E-2 isoproteins in ischemic heart disease. Subjects with apo E-7 and apo E-5 were excluded.

![Image](image-url)

Fig. 7. Distribution of LDL- and VLDL-cholesterol levels in CCU-patients and control subjects with hyperlipoproteinemia. Arrows indicate patients with probable familial hypercholesterolemia.

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that of the control group.

The males in the CCU group were then divided into 2 groups, one above* and one below 60 years of age, and the apo E phenotypes were investigated (Table III). In younger males, E4/4 and E4/3 were frequent and E3/2 was rare, as observed in females. Mutant apo E was also noted at a high frequency, and was suggested to be a risk factor for ischemic heart disease. It is considered that serum lipoprotein abnormalities related to the development of ischemic heart disease are more prominent in younger than in older individuals, as mentioned above.

We have shown previously that, in normal subjects, the E-4 isoprotein raises LDL levels, while the E-2 isoprotein raises VLDL levels but tends to lower LDL levels (Fig. 3, Fig. 4). In the males of the CCU and control groups, VLDL-TG and LDL-Ch levels were related to the phenotype of apo E. Individuals with the E4/4 or E4/3 phenotypes were combined into one group as the subjects with apo E-4 because of the small number of cases. Both CCU and control groups well demonstrated the effects of E-4 and E-2 increasing or decreasing VLDL and LDL levels, as described above (Fig. 5). The VLDL level in each apo E-group was slightly higher in the control group, but LDL levels were higher in all CCU apo E-groups. The mean LDL-Ch value in the E3/2 CCU group (the lowest among the CCU groups) was higher than that in the E4/4 and E4/3 control group, which was the highest among the control groups (Fig. 5). A similar tendency was also observed in the females (data not shown).

5. Apo E as a genetic risk factor for ischemic heart disease

The relationship between apo E and ischemic heart disease is summarized in Fig. 6 regarding the frequency of individuals with apo E-4 or E-2 isoprotein (subjects with mutant apo E are excluded). In ischemic heart disease, both a high frequency of E-4 and a low frequency of E-2 were obtained, and this was most prominent in females of the CCU group. Compared with males, females are less influenced by risk factors such as stress and smoking, so that abnormalities of lipoprotein metabolism seem to affect the development of atherosclerosis more directly in females than in males. A similar finding was observed in younger male subjects in whom lipoprotein abnormalities were considered to be important. In older males, however, the distribution of apo E isoproteins was similar to that of the control group, suggesting that risk factors other than serum lipoprotein abnormalities may be more important in this group. These results suggested that apo E-4 and apo E-2 were positive and negative risk factors for the development of atherosclerosis, respectively. The action of apo E isoproteins to increase or decrease serum LDL levels may be one of the mechanisms of atherosclerosis.

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TABLE IV COMPARISON OF CLINICAL FEATURES BETWEEN RECEPTOR-NEGATIVE AND RECEPTOR-DEFECTIVE TYPES OF FAMILIAL HYPERCHOLESTEROLEMIA HOMOZYGOATES

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Type of FH</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Receptor-negative</td>
<td>Receptor-defective</td>
<td></td>
</tr>
<tr>
<td>Serum Ch*</td>
<td>687±130</td>
<td>637±139</td>
<td></td>
</tr>
<tr>
<td>Serum TG*</td>
<td>136±108</td>
<td>94±39</td>
<td></td>
</tr>
<tr>
<td>HDL-Ch*</td>
<td>27±7</td>
<td>39±12</td>
<td></td>
</tr>
<tr>
<td>Xanthoma</td>
<td>13/13</td>
<td>6/6</td>
<td></td>
</tr>
<tr>
<td>CHD</td>
<td>10/13</td>
<td>1/6</td>
<td></td>
</tr>
</tbody>
</table>

* mean ± SD

Abbreviations: FH = familial hypercholesterolemia; Ch = cholesterol (mg/dl); TG = triglycerides (mg/dl);
CHD = coronary heart disease; SD = standard deviations

6. Ischemic heart disease and familial hypercholesterolemia

Familial hypercholesterolemia (FH) is a genetic disorder characterized by a deficiency of LDL-receptors. Most parenchymal cells have a specific mechanism to recognize apo B-100 in LDL and take up LDL through the LDL-receptors on their surfaces, so that they can collect cholesterol efficiently for their proliferation and maintenance even in a low cholesterol environment. FH heterozygotes, having only one half the number of LDL-receptors, develop hypercholesterolemia with serum cholesterol levels similar to adult patients by the age of 1 year. Homozygous FH, in which there is a lack of detectable LDL-receptors on the cell surface, shows more severe hypercholesterolemia from infancy. This characteristic of FH, the severe and early onset of hypercholesterolemia, is one of the reasons for the very high risk of ischemic heart disease in FH.

Figure 7 shows the distribution of VLDL-Ch and LDL-Ch levels in the hyperlipoproteinemic subjects from the CCU and control groups. The increas of VLDL-Ch was similar in both groups, but LDL-Ch levels were markedly high in the CCU group, and heterozygous FH was suspected to exist in many cases with LDL-Ch levels above 220 mg/dl. Patients diagnosed to have probable FH (shown by arrows) comprised at least 3% of the CCU group (6 out of 199 subjects) (Fig. 7). The prevalence of FH in this group was more than 10 times higher than the reported frequency of FH heterozygotes in a normal population (one in 500 persons, 0.2%) and seems to imply a high risk of ischemic heart disease in FH patients. The persistence of marked hypercholesterolemia from infancy might make FH patients susceptible to atherosclerosis.

7. Heterogeneity of LDL-receptor abnormalities in FH

We examined LDL-receptor protein synthesis in skin fibroblasts from FH homozygotes. Fig. 8 shows an autoradiogram of 35S-methionine labelled LDL-receptors separated by SDS-electrophoresis from three homozygous patients with receptor-negative FH. The LDL-receptor is synthesized as a 120-kilodalton (kd) precursor and is processed to a 160-kd mature form by the addition of sugar chains. The receptor protein was completely absent in patient SS, and the mature form of the receptor was synthesized in small amounts in patient SH. In patient KN, post translational processing of the receptor was markedly impaired, and only a little of the mature protein was noted (Fig. 8).

Thus, various types of disturbances in receptor synthesis were observed even in phenotypically homozygous FH patients. We investigated 19 homozygotes from 16 kindreds, and detected few identical mutations of LDL-receptor synthesis. From the results of the assay of LDL-receptors in cul-
tured skin fibroblasts, 19 FH homozygotes were divided into 2 groups, a receptor-negative group showing no detectable receptor activity, and a receptor-defective group with about 10–30% of the normal activity. In both groups, there was no great difference in the mean values of serum cholesterol. However, HDL-Ch was significantly higher in the receptor-defective group. Coronary heart disease was observed in 10 of 13 subjects in the negative group, whereas it was detected in only 1 of 6 subjects in the defective group (Table IV). It was very impressive that we found a great difference in the frequency of coronary heart disease depending on whether only a small number of receptors were present or not.

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