Original Article

Multicenter Study to Determine the Diagnosis Criteria of Heterozygous Familial Hypercholesterolemia in Japan

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**Aim:** Heterozygous patients of familial hypercholesterolemia (FH) are known to have a high risk of coronary artery disease (CAD). Early diagnosis and prompt treatment are necessary to prevent their CAD. In this study we tried to amend the Japanese diagnostic criteria of FH for general practitioners by examining each component of the current criteria.

**Methods:** A multicenter study was performed, which included 1356 dyslipidemic patients at 6 centers. Pretreatment demographic information including LDL-cholesterol (LDL-C), Achilles tendon thickness (ATT), family history of FH and premature CAD and the result of genetic analysis were analyzed.

**Results:** Of 1356 patients, 419 were diagnosed with FH by criteria in 1988, which were used as a golden standard. We tried to define FH according to 3 conventional major items, i.e., 1) LDL-C, 2) ATT and/or cutaneous nodular xanthomas (CX), 3) family history of FH and/or family history of premature CAD. We then determined the cutoff of LDL-C using the new criteria. When we used 180 mg/dL as the cutoff of LDL-C, 94.3% of FH patients and 0.85% of non-FH satisfied 2 or more criteria. When we used 190 mg/dL, 92.1% of FH and 0.85% of non-FH satisfied 2 or more criteria; therefore, we chose 180 mg/dL for the cutoff of LDL-C in the new criteria and proposed that the diagnosis of definite FH can be made if 2 or more criteria are satisfied.

**Conclusions:** We examined each component for the diagnosis of heterozygous FH in a multicenter study in Japan.


**Key words:** Diagnosis criteria, Familial hypercholesterolemia, LDL cholesterol, Achilles tendon thickness, LDL receptor

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**Introduction**

Familial hypercholesterolemia (FH) is a genetic disease caused by a mutation in genes related to low-density lipoprotein (LDL) metabolism. Heterozygous FH patients manifest high LDL cholesterol (LDL-C)
levels, skin and/or tendon xanthomas, and increased risk of premature coronary artery disease (CAD)\(^1\). High LDL-C levels are the first symptom that appears even from birth, while xanthomas on the Achilles tendon usually appear during or after the late 10s and CAD that determines the prognosis of FH patients appears during or after the third decade of life in men and the fifth decade in women\(^2\)\(^-\)\(^4\). Because morbidity and mortality of CAD in heterozygous FH are much higher than in the general population\(^1\),\(^5\)\(^-\)\(^7\), special attention should be paid to screen these patients and to prevent their atherosclerotic complications. For the diagnosis of FH, several criteria have been published throughout the world, including ours, reported in 1988\(^8\); however, appropriate diagnosis of FH by primary care physicians is not performed in general practice in Japan\(^9\). Therefore, it is very important to establish useful diagnostic criteria for primary care physicians to diagnose FH with high specificity and sensitivity.

Because FH patients are estimated to be more than 250,000, primary care physicians need to take care of most of them; therefore, the criteria should be as simple as possible for clinical usefulness and have high sensitivity and specificity. We have used diagnosis criteria for FH published in 1988 in Japan\(^8\), which include hypercholesterolemia, presence of skin/tendon xanthoma and reduced LDL receptor activity as major items; however, it is difficult to measure LDL receptor activity in routine clinical practice and even lipid specialists do not measure its activity. Furthermore, it is not covered by Japan’s health insurance system; therefore, it is necessary to make the current diagnostic criteria easy to use for general practitioners. Toward this end, we performed a multicenter collaborative study of 1397 patients with dyslipidemia.

**Methods**

**Subjects**

A total of 1397 patients with dyslipidemia, referred to the outpatient clinic of 6 hospitals (Kyoto University Hospital, Osaka University Hospital, Nippon Medical School Hospital, Chiba University Hospital, Kanazawa University Hospital, and National Cerebral and Cardiovascular Center Hospital), were the subjects of this study. Among these patients, 41 were excluded due to missing data. Consequently, 1356 patients with dyslipidemia were eligible for the present study. Most had been diagnosed with or without FH by lipid specialists at each hospital according to the criteria for FH reported in 1988, and genetic analysis was performed in 223 patients, some of whom were diagnosed with FH based only on mutations of the LDL receptor or PCSK9. The criteria were as follows: Major items included 3 items, (1) IIa or IIb phenotype at serum cholesterol level of 260 mg/dL or above; (2) Tendinous xanthoma or xanthoma tuberosum is present; (3) Reduced or abnormal receptor activity. Minor items included 3 items: (1) Xanthoma palpebratum; (2) Arcus juvenalis (<50 years); (3) Juvenile (<50 years) ischemic heart disease.

**Determination of Conventional Criteria for FH**

In this study we tried to amend the current criteria. For the primary care setting, three major items, i.e., serum level of LDL-C, family history and specific physical findings of FH, were chosen as diagnostic items because all are easily assessed by general practitioners. Family history and specific physical findings were also separated in more detail. Finally, we set 5 items, (1) LDL-C, (2) specific physical findings: a) ATT, b) cutaneous nodular xanthomas (CX), (3) family history: a) family history of FH in 1st or 2nd degree relatives, b) family history of premature CAD in 1st or 2nd degree relatives. A family history of premature CAD was defined as having CAD before the age of 55 in males and 65 in females. First, we assessed the prediction for FH by the combination of physical findings and family history, and then we determined the cutoff point of LDL-C with the combination of the above-mentioned two items. LDL-C levels were calculated by the Friedewald formula. ATT levels were measured by X-ray according to the method previously described and determined as positive with 9 mm or more\(^10\).

The data in the medical records of the patients were sent to the National Cerebral and Cardiovascular Center and examined. The study protocol was approved by the ethics committee of the National Cerebral and Cardiovascular Center (D#M20-25-2 for the multicenter trial and ID#M17-56-4 for the genetic analysis). The ethics committee of each hospital also approved the study protocol.

### Table 1. Clinical characteristics of non-FH and FH patients in this cohort

<table>
<thead>
<tr>
<th></th>
<th>non-FH</th>
<th>FH</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>937</td>
<td>419</td>
<td></td>
</tr>
<tr>
<td>Male (n, %)</td>
<td>453 (48.3%)</td>
<td>170 (42.7%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age (y.o.)</td>
<td>58.3 ± 16.3</td>
<td>52.9 ± 18.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>236 ± 53</td>
<td>339 ± 72</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>146 ± 46</td>
<td>257 ± 67</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Among 1356 patients, 419 had been diagnosed with FH, while 937 with non-FH. Patient demo-
graphic data are shown in Table 1. FH patients were younger than non-FH patients. TC and LDL-C levels were 339 and 257 mg/dL in FH patients, respectively, and were significantly higher than in non-FH patients. The distribution of LDL-C levels in both groups is shown in Fig. 1. FH patients were divided into 3

**Statistical Analyses**

Continuous variables are presented as the means ± SD. Categorical data are presented as numbers and percentages. Unpaired Student’s t-test and one-way analysis of variance (ANOVA) were used to assess differences between groups in continuous variables. Differences in categorical variables were assessed by the $\chi^2$ test.

**Results**

Among 1356 patients, 419 had been diagnosed with FH, while 937 with non-FH. Patient demo-
graphic data are shown in Table 1. FH patients were younger than non-FH patients. TC and LDL-C levels were 339 and 257 mg/dL in FH patients, respectively, and were significantly higher than in non-FH patients. The distribution of LDL-C levels in both groups is shown in Fig. 1. FH patients were divided into 3
Table 2. LDL-C levels in FH patients with or without genetic data

<table>
<thead>
<tr>
<th>LDL-C (mg/dL)</th>
<th>FH (Total)</th>
<th>FH (Mut +)</th>
<th>FH (Mut −)</th>
<th>FH (no genetic data)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>419</td>
<td>224</td>
<td>41</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>257.4</td>
<td>266.2*</td>
<td>229.0*</td>
<td>252.9</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>67.39</td>
<td>69.85</td>
<td>60.14</td>
<td>63.70</td>
<td>0.003</td>
</tr>
<tr>
<td>MEDIAN</td>
<td>244</td>
<td>253</td>
<td>216</td>
<td>241</td>
<td></td>
</tr>
<tr>
<td>IQ 25%</td>
<td>205</td>
<td>213</td>
<td>189</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>IQ 75%</td>
<td>300</td>
<td>308</td>
<td>244</td>
<td>295</td>
<td></td>
</tr>
</tbody>
</table>

FH (Mut +): mutations in the LDL receptor or PCSK9, FH (Mut −): no mutations found, FH (no genetic data): no genetic analysis
* \( p < 0.005 \) by Bonferroni

Table 3. Sensitivity and specificity in screening FH by physical findings and family history

<table>
<thead>
<tr>
<th>Physical findings</th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATT (+) (%)</td>
<td>98.6</td>
<td>64.1</td>
</tr>
<tr>
<td>CX (+) (%)</td>
<td>99.6</td>
<td>9.4</td>
</tr>
<tr>
<td>ATT (+) or CX (+) (%)</td>
<td>98.6</td>
<td>64.6</td>
</tr>
<tr>
<td>ATT(+) and CX(−)</td>
<td>99.6</td>
<td>11.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Family history</th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history of FH (+) (%)</td>
<td>93.6</td>
<td>98.2</td>
</tr>
<tr>
<td>Family history of CAD (+) (%)</td>
<td>96.3</td>
<td>28.3</td>
</tr>
<tr>
<td>Family history of FH (+) or CAD (+) (%)</td>
<td>91.7</td>
<td>98.7</td>
</tr>
<tr>
<td>Family history of FH (+) and CAD (+) (%)</td>
<td>98.2</td>
<td>27.4</td>
</tr>
</tbody>
</table>

ATT: Achilles tendon thickness, CX: Cutaneous nodular xanthomas
FH (\( n = 224 \)) was diagnosed by mutations in the LDL receptor and/or PCSK9. Non-FH (\( n = 937 \)) was diagnosed by specialists.

groups depending on their genetic data: FH with mutation(s) in LDL receptor or PCSK9, FH with no mutation(s) and FH with no genetic data. The mean and median of LDL-C along with SD and interquartile range of each group are shown in Table 2. LDL-C levels in FH with mutations were higher than those in FH without mutations.

We tried to define FH according to the screening standards as 3 major items, i.e., 1) LDL-C, 2) ATT and/or cutaneous nodular xanthomas (CX), 3) family history of FH and/or family history of premature CAD. We used LDL-C instead of total cholesterol, because LDL-C should better reflect the activity of the LDL receptor and is used for the goal of lipid management in the current Japanese guideline\(^8\). We incorporated “family history” as a major item because general practitioners were able to find FH by a family history of FH and/or premature CAD instead of LDL receptor activity. Sensitivity and specificity in screening FH by physical findings and family history are listed in Table 3. Based on these data, we decided to use 1) ATT or CX, and 2) family history of FH or CAD as 2 major items in addition to high LDL-C levels.

Next we tried to determine the cutoff levels of LDL-C. The percentage of the patients who satisfied each criterion according to LDL-C levels is listed in Table 4. Levels of 180 or 190 mg/dL are suggested as candidate cutoff levels. Therefore, the criteria for model 1 were set as those who satisfy 2 or more of the 3 criteria: 1) LDL-C 180 mg/dL or higher, 2) ATT (+) or CX (+), 3) Family history of FH or CAD, and for model 2, for which the cutoff point of LDL-C was changed to 190 mg/dL or higher, their sensitivity, specificity, and false positive and false negative rates were compared (Table 5). When we compared model 1 with model 2, higher sensitivity in model 1 than model 2 was obtained without any change in specificity, suggesting that 180 mg/dL is a better cutoff for LDL-C. The percentages were quite similar in FH with mutation(s) in LDL receptor or PCSK9, FH with no mutation(s) and FH with no genetic data. The diagnostic criteria of FH were then determined
have no symptoms. The reason for undiagnosed FH patients to go to a clinic may be mainly divided into the following 4 situations: 1) a chance visit to a primary care physician due to flu or gastritis, etc., 2) recommendation of further medical examination due to high cholesterol at a health checkup, 3) transportation to the emergency room due to the development of acute coronary syndrome, 4) recommendation of medical consultation due to the presence of FH in his/

Table 4. Percent satisfying each LDL-C level in non-FH and FH patients

<table>
<thead>
<tr>
<th>N</th>
<th>non FH</th>
<th>FH (All)</th>
<th>FH (Mut +)</th>
<th>FH (Mut −)</th>
<th>FH (No genetic data)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLD-C ≥170 mg/dL (%)</td>
<td>30.5</td>
<td>94.5</td>
<td>96.0</td>
<td>85.4</td>
<td>94.8</td>
</tr>
<tr>
<td>NLD-C ≥180 mg/dL (%)</td>
<td>24.3</td>
<td>94.3</td>
<td>94.6</td>
<td>82.9</td>
<td>92.9</td>
</tr>
<tr>
<td>NLD-C ≥190 mg/dL (%)</td>
<td>16.6</td>
<td>92.1</td>
<td>93.7</td>
<td>75.6</td>
<td>89.7</td>
</tr>
<tr>
<td>NLD-C ≥200 mg/dL (%)</td>
<td>11.6</td>
<td>80.0</td>
<td>84.3</td>
<td>63.4</td>
<td>78.1</td>
</tr>
</tbody>
</table>

FH(Mut +): mutations in the LDL receptor or PCSK9, FH(−): no mutations found, FH (no genetic data): no genetic analysis

Table 5. Accuracy metrics of FH criteria using LDL-C cutoff levels of 180 or 190 mg/dL

<table>
<thead>
<tr>
<th>Model 1: Satisfying 2 or more of the following criteria: 1) LDL-C ≥180 mg/dL, 2) ATT(+) or CX(+), 3) Family history of FH or CAD</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>False positive (%)</th>
<th>False negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>94.5</td>
<td>99.1</td>
<td>0.85</td>
<td>5.5</td>
</tr>
<tr>
<td>Model 2: Satisfying 2 or more of the following criteria: 1) LDL-C ≥190 mg/dL, 2) ATT(+) or CX(+), 3) Family history of FH or CAD</td>
<td>92.1</td>
<td>99.1</td>
<td>0.85</td>
<td>7.9</td>
</tr>
</tbody>
</table>

Table 6. Diagnostic criteria for adult (15 years or older) heterozygous FH

1. Hyper-LDL-cholesterolemia (LDL-C level before treatment: 180 mg/dL or more)

2. Tendon xanthoma (tendon xanthoma of the dorsal hands, elbows, and knees, or Achilles tendon thickening) or nodular xanthoma of the skin

3. Family history (relatives in the second degree): FH or premature CAD

Discussion

FH has the highest prevalence in genetic metabolic diseases, being heterozygous in one in 500 of the general population. Most young heterozygous FH patients have no symptoms other than high LDL-C levels, and those who have Achilles tendon thickness and are shown in Table 6.
Heterozygous FH patients show high levels of LDL-C, cutaneous and tendon xanthomas, and are complicated with myocardial infarction at young age by atherosclerosis due to intravascular exposure to high levels of LDL-C for many years. Because early diagnosis and treatment are recommended for these patients, the diagnostic criteria for FH have been reported in many countries including Japan\(^8\), \(^12\)-\(^17\). While some criteria give a satisfactory diagnosis of FH using specific items, others are adopting a scoring system. The Japanese criteria reported in 1988\(^8\) were as follows. Major items included the following 3 items: (1) the patient shows the IIa or IIb phenotype at a serum cholesterol level of 260 mg/dL or above, in principle; (2) Tendinous xanthoma or xanthoma tuberosum is present; (3) Reduced or abnormal receptor activity is noted by LDL receptor analysis; however, for LDL receptor activity, even lipid specialists do not routinely measure activity. It would be even more difficult for primary care physicians to measure activity for the diagnosis of FH.

The cutoff level of serum cholesterol used in the first criterion in the criteria published in 1988 was 260 mg/dL; however, LDL-C is directly affected by dysfunction of the LDL receptor and is routinely measured in clinics by the direct method or Friedewald formula; therefore, we tried to use LDL-C as a cutoff level instead of total cholesterol. The presence of tendon and/or cutaneous nodular xanthomas was also used because of its convenience, high sensitivity and specificity. A family history of FH or premature CAD in 1st or 2nd degree relatives was proposed for the third criterion instead of measuring LDL receptor activity in the new diagnostic criteria. A family history of FH showed high sensitivity and specificity; however, primary care physicians may have difficulty obtaining this because it was not easy for them to reach a diagnosis of FH with the previous criteria. In the present study, accurate diagnosis of a family history of FH seemed to have been given because lipid specialists made the diagnosis at all the hospitals; however, the same result may not be applied to primary care physicians. Therefore, a family history of CAD, which may be easier to obtain, was added to the criteria. It should be noted that the sensitivity of a “family history of FH or CAD” was slightly higher than that of a “family history of FH”. Accordingly, we chose a “family history of FH or premature CAD in 1st or 2nd degree relatives” as the third criterion.

The cutoff level of LDL-C for the diagnosis of FH should be set by its sensitivity and specificity in different cutoff points. The cutoff level of LDL-C for the diagnosis of FH was reported to be 190 mg/dL in Simon Broome\(^17\), NICE\(^15\) and 205 mg/dL in MEDPED\(^16\). In this study, 180 mg/dL was selected as the cutoff level together with the presence of xanthoma and the family history as the criteria for the diagnosis of FH because of its high sensitivity and specificity.

Reduced LDL receptor activity is direct evidence of FH and was used as one of the criteria in the previous version. Usually, LDL receptor activity is determined by the binding of fluorescent-labeled LDL to lymphocytes. The procedure of measuring LDLR activity is cumbersome and it is difficult to measure in routine clinical settings. Further, few companies can measure LDLR activity. Indeed, the specialists involved in this study measured LDLR activity only in 7 of 419 patients of FH, showing the sensitivity of the previous criteria as 60.9%. Therefore, in order to determine criteria sensitive enough to give a diagnosis of FH, the third item was changed from LDLR activity to family history.

There are some limitations in the present study. First, the patients analyzed in this study may have different characteristics from those followed by primary care physicians, because the physicians in this study are taking care of many FH patients and information about family history can be obtained more easily than by primary care physicians. Second, it is sometimes difficult for primary care physicians to take a complete family history, especially FH, and to diagnose ATT and/or the presence of CX, about which information can be missed in the primary care setting. Third, FH has been reported to have mutations in LDL receptor, PCSK9 and apolipoprotein B. Because mutations in PCSK9 may cause milder forms of FH, the sensitivity of the criteria may be reduced in these patients. Further study is required to address the applicability of the criteria for the primary care setting.

In conclusion, we have determined the cutoff of LDL-C for the diagnosis of FH by a multicenter study and proposed conventional diagnostic criteria by using high LDL-C, ATT and/or the presence of CX, and a family history of FH and/or CAD for primary care settings.

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Diagnosis Criteria of FH

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