20-fold; CHD risk also varies with age in FH[11, 14] and knowledge of this was not specifically sought. Taking this into account, 77% of physicians selected that the risk of CHD was increased 5-20 fold, which indicates that the majority had a good understanding that the risk of CHD is very high among patients with FH. Although only 68% correctly identified the clinical description of FH, it should also be noted that an additional 20% of physicians recognised the genetic basis of FH. Similarly, an additional 37% of physicians were able to identify that FH is genetically transmitted to first-degree relatives even though autosomal dominant inheritance of FH was not comprehended.

The prevalence of smoking in Japan and South Korea is known to be high and type 2 diabetes prevalence is increasing in Asia[15], consistent with the selections of these conditions as additional CVD risk factors in FH. South Korean physicians were more likely to recognise Lp(a) as an adjunctive risk factor. This is important since Lp(a) may be a strong risk factor for CVD in East Asian populations[16] despite lower concentrations in circulation compared with other races[17]. There is only one major international guideline on Lp(a) as a CVD risk factor[17], and whether Korean physicians were more familiar with these guidelines may be a possible explanation for the greater awareness of Lp(a).

Country differences in the choice of healthcare professional best suited for managing FH and cascade screening may reflect different healthcare systems. Only 3% of respondents in the present survey considered that there was a significant role for nurses with cardiac training in the care of FH. This differs from the Netherlands[12], UK[18] and Australia[9], where screening programs have been conducted by nursing and/or allied health staff. This may relate to cultural factors that bear on clinical practice and professional training in East Asia. Cardiologists and endocrinologists

were recognised by 31% and 30%, respectively, of respondents as a healthcare provider to detect FH. Cardiologists are well positioned to identify index cases with FH presenting with coronary events[10, 19]. Similarly, endocrinologists are positioned to identify FH in a secondary prevention setting. Lipidology is an emerging specialty grown out of endocrinology and to a less extent cardiology. PCPs are best situated to identify FH in primary prevention settings as perceived by 59% of the respondents in the present survey. Further exploration of health services and systems are warranted to optimise country-specific clinical service models. The disparate responses to roles of different specialities suggests the need for integration of care[7].

Lack of awareness of clinical services for lipid disorders may be because specialist services do not exist in their geographical area, particularly for physicians practising in suburban and rural regions, which constituted up to 44% of the surveyed physicians. The higher proportion of Japanese physicians from suburban and rural areas reported in this study may relate to different interpretation of urban and suburban settings, particularly in Japan where suburban cities are still very populous.

This study has limitations. The selection of three economically-developed countries was related to accessibility and the internet-based nature of the survey. The physicians were a self-selected group and may reflect those with more interest in FH and lipid disorders. It is possible that only better informed physicians responded so that knowledge gaps may actually be worse than reported in the present study. Knowledge deficits may be more marked in unselected physicians and in those from less developed countries.

Another limitation is that we did not inquire about obesity and hypertension, which has a high prevalence in Asia[15], as a CVD risk factor, but we expect knowledge of

these to be high. We did not gather information concerning interaction between physicians and the diagnostic laboratories that perform lipid and lipoprotein measurements. Furthermore, we did not record how many physicians practiced in the public/private sector or their awareness of guidelines. Also, we did not seek information on management practices. Of note, this is a pilot study and future studies will endeavour to improve on these limitations.

Similar questionnaires have been administered to PCPs[20] and pharmacists[21] in Australia and cardiologists in the US[10]. Knowledge shortfalls were comparable, with underestimations of hereditability, prevalence and CVD risk.

FH screening programs in Asia are presently limited to Singapore[22] and Hong Kong[13], both of which are city-states. The countries in the present survey are where detection of index cases with FH and cascade screening services may be particularly beneficial owing to high population densities.

The only guidelines and proposed diagnostic criteria in the region are by Japan[8]. The criteria are based on the detection of tendon xanthomata, which may not be present in all FH, particularly the young, and hence may have low sensitivity in screening and detecting FH. More recently, the International guidance on FH[7], endorsed by the Asian-Pacific Society of Atherosclerosis and Vascular Disease (APSAVD), has set a new benchmark and provides a foundation for developing country-specific guidelines, services and models of care in Asia. The principles are similar, but employing country-specific levels of LDL-C to diagnose FH, the integration of care and the development of regional and local FH guidelines are advocated. Country-specific challenges may include diverse healthcare systems whereby patients can bypass a community-based PCP and be assessed directly by a specialist[23], the acceptability

of statin use against the popularity of complementary and alternative medicine[24, 25], and funding strategies for national screening programs.

FH in Asia is largely undiagnosed and inadequately treated[1, 4]. Effective implementation of screening programs will be a challenge for many of the larger Asian countries, particularly those with co-existent public and private healthcare systems and limited resources and support for primary prevention strategies. Professional intervention to improve physician's awareness and knowledge in FH is the first step to improving detection, diagnosis and treatment to prevent CHD. Further audits combined with education programs can help raise awareness, particularly in countries included in the present analysis and those that are less economically-developed.

Conclusion

This survey has identified important and addressable gaps in FH knowledge and awareness in Asia. The guidance from the International FH Foundation[7], the National Lipid Association Expert Panel[2], the European Atherosclerosis Society[1] and the Japanese guidelines[8] should provide a basis to establishing country-specific models of care in Asia. Implementation of country-specific guidelines and extensive work in FH education and awareness programs are imperative to improve the care of FH. These issues will be addressed in a forthcoming investigation across several countries in the Asia-Pacific region that will examine not only physician knowledge and awareness, but also geographical differences in patient preferences and perceptions and facilities for the care of FH.

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Removal of Plasma Mature and Furin-Cleaved Proprotein Convertase Subtilisin/Kexin 9 by Low-Density Lipoprotein-Apheresis in Familial Hypercholesterolemia: Development and Application of a New Assay for PCSK9

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Context: Proprotein convertase subtilisin/kexin 9 (PCSK9) is known to be a good target to decrease LDL cholesterol (LDL-C) and two forms of PCSK9, mature and furin-cleaved PCSK9, circulate in blood. However, it has not been clarified whether and how the levels of each PCSK9 are affected by LDL-apheresis (LDL-A) treatment, a standard therapy in patients with severe forms of familial hypercholesterolemia (FH).

Objective: Our objective was to investigate the differences in LDL-A-induced reduction of mature and furin-cleaved PCSK9 between homozygous and heterozygous FH, and between dextran sulfate (DS) cellulose adsorption and double membrane (DM) columns and to clarify the mechanism of their removal.

Design: A sandwich ELISA to measure two forms of PCSK9s using monoclonal antibodies was developed. Using the ELISA, PCSK9 levels were quantified before and after LDL-A with DS columns in 7 homozygous and 11 heterozygous FH patients. A crossover study between the two column types was performed. The profiles of PCSK9s were analyzed after fractionation by gel filtration chromatography. Immunoprecipitation of apolipoprotein B (apoB) in FH plasma was performed.

Results: Both mature and furin-cleaved PCSK9s were significantly decreased by 55–56% in FH homozygotes after a single LDL-A treatment with DS columns, and by 46–48% or 48–56% in FH heterozygotes after treatment with DS or DM columns. The reduction ratios of LDL-C were strongly correlated with that of PCSK9 in both FH homozygotes and heterozygotes. In addition, more than 80% of plasma PCSK9s were in the apoB-deficient fraction and a significant portion of mature PCSK9 was bound to apoB, as shown by immunoprecipitation.

Conclusions: Both mature and furin-cleaved PCSK9s were removed by LDL-A in homozygous and heterozygous FH either by binding to apoB or by other mechanisms. The ELISA method to measure both forms of plasma PCSK9 would be useful for investigating physiological or pathological roles of PCSK9. (*J Clin Endocrinol Metab* **100: E41–E49, 2015)**

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Abbreviations: DM, double membrane; DS, dextran sulfate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDL-A, LDL-apheresis; LDL-C, LDL cholesterol; LDLR, low-density lipoprotein receptor; PCSK9, proprotein convertase subtilisin/kexin type 9; rh, recombinant human; TG, triglyceride.

Familial hypercholesterolemia (FH) is an inherited disorder caused by mutations in the low-density lipoprotein (LDL) receptor (LDLR), apolipoprotein B (apoB) or proprotein convertase subtilisin/kexin type 9 (PCSK9) (1, 2), and is characterized by high LDL cholesterol (LDL-C) levels leading to premature coronary artery disease (CAD). PCSK9, a serine protease, regulates plasma LDL-C levels by regulating degradation of LDLR (3, 4). It has also been reported that serum PCSK9 levels were significantly higher in FH patients than in controls (5), and were correlated with serum LDL-C levels (6).

PCSK9 encodes a 692-amino-acid protein composed of a signal peptide, a prodomain, catalytic, and C-terminal domains. It undergoes autocatalytic intramolecular processing to form a ~14-kDa prodomain and a ~60-kDa moiety with catalytic and C-terminal domains. Mature PCSK9 is composed of the prodomain, which is noncovalently attached to the catalytic domain. Another proprotein convertase, furin, cleaves PCSK9 at the Arg^{218}-Gln^{219} peptide bond, and the cleaved PCSK9 includes a ~7-kDa domain, ~14-kDa prodomain, and ~53-kDa domain (furin-cleaved form) that lacks the Ser^{153}-Arg^{218} segment (7, 8). It has been reported that furin-cleaved PCSK9 has no activity (7–9) to regulate LDLR and serum LDL-C or less activity than mature PCSK9 (10). Thus, it is important to measure both forms of PCSK9 separately, in order to clarify the significance of furin-cleaved PCSK9. However, no specific method has been reported for quantifying furin-cleaved PCSK9, and thus the association of the ratio of each form of PCSK9 with various pathological or physiological conditions, such as primary hyperlipidemia, hyperlipidemia in type II diabetes, obesity, etc., has not been clarified.

LDL-apheresis (LDL-A) treatment is a standard therapy in homozygous and severe forms of heterozygous FH. In order to selectively remove LDL, LDL adsorption techniques using dextran sulfate (DS) cellulose adsorption columns and double membrane (DM) filtration methods were developed (11, 12). We and other colleagues have reported that LDL-A reduces not only atherogenic lipoproteins, but also various proteins including coagulation factors and C-reactive protein (CRP), in serum (13–15).

Recently, it has been reported that PCSK9 was eliminated by LDL-A treatment in 6 FH patients (16). However, in FH homozygotes, the serum PCSK9 levels have been reported to be unaffected by LDL-A (17). Thus, the difference in the treatment-induced reduction of PCSK9 between FH homozygotes and heterozygotes has not been clarified. In addition, the differences in the PCSK9 reduction between DS and DM columns and between the mature and furin-cleaved forms have not been clarified. In the present study, we use a novel sandwich ELISA to measure the mature and furin-cleaved forms of PCSK9 and show that both forms were removed by LDL-A treatment in FH homozygotes and heterozygotes. Furthermore, the mechanism of their removal is also discussed.

Materials and Methods

Detailed materials and methods are shown in the Supplemental Materials and Methods.

Patient characteristics

The subjects were 18 FH patients, including 7 homozygotes and 11 heterozygotes, who were receiving either regular or an initial LDL-A treatment at either the National Cerebral and Cardiovascular Center Hospital or Kenporen Osaka Central Hospital from March 2009 to October 2013. They were diagnosed with homozygous or heterozygous FH using previously described criteria (18, 19). Among the patients who had undergone genetic testing (n = 12), the majority were found to have LDLR gene mutations (n = 6; 50%), and one had mutations of both the LDLR and PCSK9 genes. One patient had homozygous forms of LDL receptor adaptor protein 1 (LDLRAP1) gene mutation, and 5 patients had no mutation on either the LDLR, PCSK9, or LDLRAP1 genes (42%) (20). The backgrounds of the patients are summarized in Supplemental Table 1. The protocol of this study was approved by the Ethics Review Committee of the National Cerebral and Cardiovascular Center (M20–26). Each patient gave written informed consent to participate in the study. All clinical investigations were conducted in accordance with the principles of the Declaration of Helsinki.

LDL-apheresis

For LDL-A treatment, an instrument (MA-03®; Kaneka) with a plasma filter (Sulflux; Kaneka) and two DS columns (Liposorber LA-15®; Kaneka) to adsorb apoB-containing lipoproteins were used. A crossover study between DS and DM columns was performed in 5 FH heterozygotes (patients No. 8, 10, 11, 12, 14 in Supplemental Table 1). LDL-A by DM columns was performed using an instrument (KPS-8800Ce; Asahi Kasei Medical Co., Ltd.) with a plasma separator (Plasmaflow OP-05W; Asahi Kasei Medical Co., Ltd.) and a plasma fractionator (Cascadeflow EC-50W; Asahi Kasei Medical Co., Ltd.).

Plasma sample collections and assays

Peripheral blood was collected from the blood removal line immediately before and after a single LDL-A procedure. Plasma levels of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL)-C were measured using enzymatic methods (Sekisui Medical Co.) and an automated analyzer (Hitachi Labospect 008, Hitachi-Hitec). Plasma Lipoprotein(a) (Lp(a)) levels were measured using a latex agglutination method (Sekisui Medical Co.). LDL-C levels were calculated by the Friedewald formula. Apolipoproteins levels were determined by turbidimetric immunoassay (LSI Medience Coorporation).

Construction, expression, and purification of recombinant PCSK9 proteins

Human PCSK9 cDNA was obtained by RT-PCR from mRNA of HepG2 cells and a C-terminal His_6 tag was added as described

(21). Briefly, PCR was carried out for mature PCSK9 (1–692 aa), and Δ218PCSK9 (219–692 aa), which corresponds to the furin-cleaved PCSK9. The respective cDNAs were subcloned into the pEF321 mammalian expression vector to yield pEF321/PCSK9 or pEF321/Δ218PCSK9 vector. CHO-K1 cells stably transfected with each vector were cultured. The mature form of recombinant human (rh) PCSK9 (rhPCSK9) from the culture medium was partially purified. For rhΔ218PCSK9, transfectant cells were collected by trypsinization, and suspended in TBS containing 1% NP-40. After centrifugation of the cell suspension, the supernatant was collected and used as a calibrator for furin-cleaved PCSK9 ELISA.

Production of monoclonal antibodies against PCSK9

Balb/c mice were immunized using a DNA-based or standard immunization method with 25 μg purified rhPCSK9 (21), and spleen cells from mice were fused with Sp2/0 myeloma cells. The supernatants of hybridoma cells were screened by ELISA using plates coated with purified rhPCSK9 (100 ng/well) and by immunoblotting. The specificities of each monoclonal antibody (Mab) obtained by standard immunization (1FB) and by DNA-based immunization (B1G, B12E, and G12D), respectively, were confirmed by ELISA and immunoblotting against purified rhPCSK9.

Measurement of plasma mature and furin-cleaved PCSK9 concentrations

Plasma mature and furin-cleaved PCSK9s were measured by an ELISA using a specific combination of Mabs as previously described (Supplemental Figure 1) (21). The absorbance was measured at 450 nm with a microplate reader.

Gel filtration chromatography

Gel filtration chromatography was performed on an AKTA purifier system (GE Healthcare). Plasma samples of 2 homozygous and 6 heterozygous FH patients were injected into two connected Superose 6 (1.0 × 30; GE Healthcare) columns (22). Cholesterol or Lp(a) was measured in the fractions using a BIO-Lis24 analyzer (Tokyo Boeki Medical System, Ltd.) or a Mercodia Lp(a) ELISA (Mercodia AB) with two Mabs against Apo(a) in accordance with the manufacturers' instructions. PCSK9s in the collected fractions were measured using the ELISA as described above.

Co-immunoprecipitation of apoB from FH plasma

A 500 μL plasma sample was adjusted to a final concentration of 50 mM HEPES [pH 7.4], 2.5 mM magnesium chloride, 1% Triton X-100, 0.5% sodium deoxycholate and protease inhibitor cocktail in a final volume of 1 mL. Samples were rotated at 4°C for 30 min, then centrifuged at 15 000 rpm for 15 min. Co-immunoprecipitation experiments were performed using a Pierce Co-Immunoprecipitation Kit (Pierce) following the manufacturer's instructions. The supernatants were applied to columns containing 20 μg of monoclonal anti-apoB antibody (Santa Cruz Biotechnology Inc.) or 20 μg of purified IgG from a nonimmunized mouse (Santa Cruz Biotechnology Inc.). The immunoprecipitates were separated by SDS-PAGE, followed by immunoblotting with monoclonal anti-apoB antibody (R&D Systems) or polyclonal PCSK9 antibody (R&D Systems). The bands were detected with ECL prime (GE Healthcare).

Statistical analysis

The statistical significance of differences between before and after LDL-A treatment was determined by the paired *t*-test. One-way ANOVA and Tukey's test were used to assess differences between groups. Spearman correlation analysis and linear regression were used to examine the relationship between PCSK9 reduction and LDL-C or HDL-C reduction. Values of $P < .05$ were considered to be statistically significant. All statistical analyses were carried out using the JMP software package (SAS Institute Inc.).

Results

Characterization of anti-PCSK9 Mabs

Purified rhPCSK9 was confirmed by SDS-PAGE followed by silver staining or by immunoblotting (Figure 1, A and B). The reactivity of Mabs to rhPCSK9 (mature form) was examined by SDS-PAGE under a nonreducing or reducing condition and by immunoblotting; Mabs 1FB, B1G, and B12E reacted with the 60-kDa mature PCSK9, while Mab G12D reacted with a 14-kDa prodomain of PCSK9 (Figure 1C). These three Mabs did not react with the mature segment of rhPCSK9 under a reducing condition. Similarly, the reactivity of all Mabs with native PCSK9 in human plasma was examined by immunoprecipitation. The 60-kDa mature PCSK9 and the 14-kDa prodomain of PCSK9 were co-immunoprecipitated with Mabs 1FB, B12E, and G12D, while the 53-kDa furin-cleaved PCSK9 alone was precipitated with Mab B1G (Figure 1, D and E).

Standardization of ELISA for the mature and furin-cleaved PCSK9s in plasma

We have established three different sandwich ELISAs specific for plasma total, mature and furin-cleaved PCSK9s (Supplemental Figure 1). Each system showed a dose-dependent response to purified rhPCSK9 or cell lysate of rhΔ218PCSK9 as well as to plasma samples, and the reactivity profiles were equivalent with both recombinant and plasma PCSK9 (Supplemental Figure 2). Calibration curves in the ELISA for total and mature PCSK9, rhPCSK9 protein, as a primary calibrator and rhPCSK9 culture medium, as a secondary calibrator were obtained (Supplemental Figure 3). Similarly, the calibration curve was made using dilutions of the cell lysate of rhΔ218PCSK9 for furin-cleaved PCSK9.

Changes of plasma lipids and apolipoproteins between before and after LDL-A with DS columns in FH homozygotes or heterozygotes

In FH homozygotes, a single procedure of LDL-A treatment with DS columns produced 57–78% reduction in

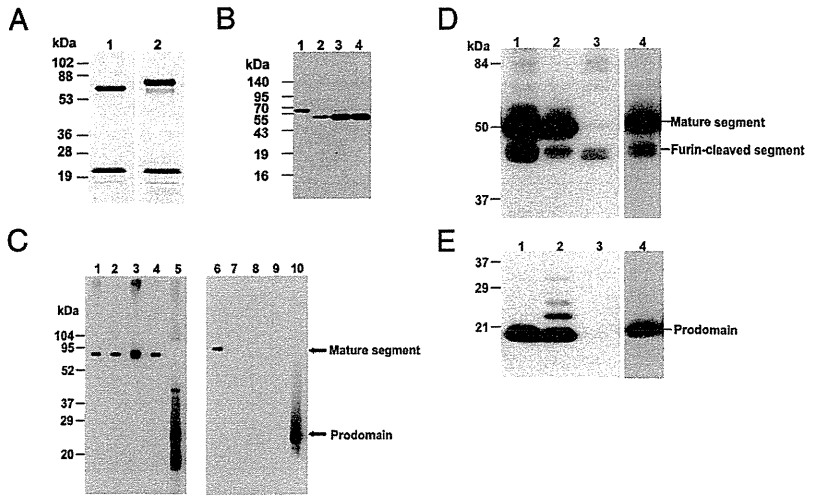

Figure 1. Characterization of recombinant PCSK9 and monoclonal antibodies to PCSK9. (A) Purified rhPCSK9 (0.75 μg) was analyzed by 5–20% SDS-PAGE under nonreducing (lane 1) and reducing (lane 2) conditions and visualized by silver staining. (B) Purified rhPCSK9 (1 μg) digested without (lane 1) or with (lane 2) recombinat furin (0.5 μg) and cell lysate of rhΔ218PCSK9 (lanes 3 and 4) was subjected to SDS-PAGE followed by immunoblotting and detection with anti-Tetra-His antibody. (C) The reactivity of each monoclonal antibody (MAb) to purified rhPCSK9 (1.0 μg) was analyzed by 5–20% SDS-PAGE under nonreducing (lanes 1–5) and reducing (lanes 6–10) condition, followed by immunoblotting as described in the Materials and Methods. Lanes 1 and 6, monoclonal PCSK9 antibody (MAB38881; R&D Systems); lanes 2 and 7, Mab 1FB; lanes 3 and 8, Mab B12E; lanes 4 and 9, Mab B1G; lanes 5 and 10, Mab G12D. (D) The immunoprecipitation of human plasma with Mabs against PCSK9 was carried out. Immunoprecipitates were separated by a 5–20% SDS-PAGE under nonreducing condition, followed by immunoblotting and detection with monoclonal PCSK9 antibody (MAB38881). Lane 1, Mab 1FB; lane 2, Mab G12D; lane 3, Mab B1G; lane 4, Mab B12E. (E) The immunoprecipitation of human plasma with Mabs against PCSK9 was carried out. Immunoprecipitates were separated by a 5–20% SDS-PAGE under a nonreducing condition, followed by immunoblotting and detection with Mab G12D. Lane 1, Mab 1FB; lane 2, Mab G12D; lane 3, Mab B1G; lane 4, Mab B12E.

plasma TC, LDL-C, TG, ApoB, ApoC-II, ApoC-III, ApoE, and Lp(a), while the plasma levels of HDL-C, ApoA-I, and ApoA-II decreased by 13–16% (Table 1 and Supplemental Table 2). In FH heterozygotes, a similar reduction was shown.

Removal of PCSK9s in FH homozygotes or heterozygotes by LDL-A with DS columns

In FH homozygotes, the plasma levels of mature and furin-cleaved PCSK9 averaged 490 ± 173 ng/mL and 74 ± 23 ng/mL, respectively, before LDL-A treatment. The two forms of PCSK9 were, respectively, reduced by 56% and 55% in FH homozygotes by a single LDL-A procedure (Figure 2A). Furin-cleaved PCSK9 constituted approximately 15% of circulating PCSK9 in the plasma of FH patients. In FH heterozygotes, the plasma levels of the two forms of PCSK9 averaged 443 ± 128 ng/mL and 55 ± 26 ng/mL, respectively, before LDL-A treatment. The two forms of PCSK9 were reduced by 46% and 48% by a single LDL-A procedure in FH heterozygotes (Figure 2B). Thus, there were no significant differences in the reduction rate of either form of plasma PCSK9 after LDL-A between FH homozygotes and heterozygotes. In addition, plasma levels of both PCSK9s in FH homozygotes before LDL-A treatment were not significantly different from those in the plasma of FH heterozygotes. As shown in Figure 3, there was a high degree of correlation between the reduction of plasma LDL-C and the reduction of mature PCSK9 in both FH homozygotes ($r = 0.79$; $P = .036$) and heterozygotes ($r = 0.79$; $P = .004$). In addition, there was a significant correlation between the reduction in plasma Lp(a) and that in mature PCSK9 in FH heterozygotes ($r = 0.74$; $P = .0098$; data not shown). On the other hand, there was no correlation between the reductions in plasma HDL-C and mature PCSK9 in FH homozygotes or heterozygotes (Supplemental Figure 4).

Crossover study of LDL-A treatment with DM columns in FH heterozygotes

A crossover study comparing the treatment efficacy between DS and DM columns was performed in 5 FH heterozygotes. A single LDL-A treatment with DM columns produced a 49–68% reduction in plasma TC, LDL-C, TG, ApoB, ApoC-II, ApoC-III, ApoE, and Lp(a), while the plasma levels of HDL-C, ApoA-I, and ApoA-II decreased by 23–26% (Supplemental Table 3). The plasma levels of mature and furin-cleaved PCSK9 before LDL-A treatment averaged 282 ± 36 ng/mL and 43 ± 28 ng/mL, respectively; the two forms were decreased by 56% and 48% by a single LDL-A treatment (Figure 2C).

Gel filtration chromatography of PCSK9 before and after a single LDL-A treatment

The plasma obtained before and after the single LDL-A treatment with DS columns was separated by gel filtration chromatography, and cholesterol and both forms of PCSK9 were measured in each fraction. Typical distribution patterns of cholesterol and PCSK9s are shown in Figure 4. Cholesterol levels were markedly reduced in the LDL fraction, while there was little change in the HDL fraction. Approximately 20% of mature PCSK9 coeluted with the apoB-containing fraction, and the rest coeluted with the apoB-deficient fraction. Meanwhile, in a portion of the FH patients, less than 5% of total PCSK9 was ob-

Table 1. Laboratory Data in FH Homozygotes and Heterozygotes Before and After a Single LDL-A Treatment With DS Columns

	FH homozygotes (n = 7)			FH heterozygotes (n = 11)		
(mg/dL)	Before	After	Reduction (%)	Before	After	Reduction (%)
TC	288 ± 63	86 ± 24[a]	70	220 ± 77	89 ± 41[a]	59
LDL-C	237 ± 49[d]	55 ± 22[a]	76	164 ± 75	51 ± 33[a]	69
HDL-C	31 ± 12	26 ± 9[a]	16	36 ± 18	32 ± 16	13
TG	99 ± 60	27 ± 21[a]	74	97 ± 43	30 ± 22[a]	70
Apo A-I	81 ± 22	71 ± 20[a]	13	99 ± 39	88 ± 35[a]	11
Apo A-II	22 ± 4	18 ± 4[a]	15	25 ± 8	21 ± 7[a]	14
Apo B	182 ± 44[d]	40 ± 21[a]	78	124 ± 46	36 ± 23[a]	71
Apo C-II	3.0 ± 2.3	1.3 ± 0.8[b]	54	3.6 ± 1.8	1.8 ± 1.3[a]	54
Apo C-III	8.5 ± 4.5	3.5 ± 1.6[a]	57	8.5 ± 2.8	4.6 ± 2.4[a]	48
Apo E	6.5 ± 2.1[c]	1.5 ± 0.8[a]	76	4.1 ± 0.8	1.1 ± 0.5[a]	73
Lp(a)	27 ± 22[d]	8.1 ± 5.8[b]	67	55 ± 27	19 ± 16[a]	68

Abbreviations: FH, familial hypercholesterolemia; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; Lp(a), lipoprotein (a); TC, total cholesterol; TG, triglyceride. All values are shown as mean ± SD.

n = 6 in Lp(a) of FH homozygotes.

[a] $P < 0.01$.

[b] $P < 0.05$ vs the respective values before LDL-A.

[c] $P < 0.01$.

[d] $P < 0.05$ vs the respective values in FH heterozygotes.

served in the apoB-fraction (data not shown). The distribution pattern of furin-cleaved PCSK9 was similar to that of mature PCSK9. Both forms of PCSK9s in the apoB-deficient fraction were reduced by 52–54%, while those in the apoB-containing fraction were reduced by 92–97%.

Coimmunoprecipitation of apoB in plasma of FH

To examine the association of apoB with PCSK9, plasma samples of FH were immunoprecipitated with monoclonal anti-apoB antibody. The control samples that were incubated in nonimmune serum instead of apoB antibody and negative control samples that were incubated in only resin showed no bands reactive to anti-apoB antibody (Figure 5A). Based on the coimmunoprecipitates of apoB, a mature PCSK9 band was detected by polyclonal PCSK9 antibody, confirming an association between mature PCSK9 and apoB in the plasma of FH. The band of furin-cleaved PCSK9 could not be detected in coimmunoprecipitation of apoB, because it overlapped that of IgG (data not shown).

Profile of Lp(a) by gel filtration chromatography

To examine the association of Lp(a) with PCSK9 in the plasma of FH, Lp(a) was measured by ELISA in the collected fractions obtained by gel filtration analysis. Lp(a) was recovered predominantly in apoB-containing fraction, and was not recovered in the apoB-deficient fraction which contains the highest levels of both PCSK9s (Figure 5B).

Discussion

In the present study, we demonstrated that the two forms of plasma PCSK9 were removed by LDL-A treatment with either DS or DM columns in both FH homozygotes and heterozygotes based on measurements using a new sandwich ELISA. The two forms of PCSK9 were significantly decreased by 55–56% in FH homozygotes after a single LDL-A treatment with DS columns, and were decreased to a similar extent in FH heterozygotes after the treatment with DS or DM columns. The removal of two forms of PCSK9 would have contributed to some extent to the control of LDL-C medi-

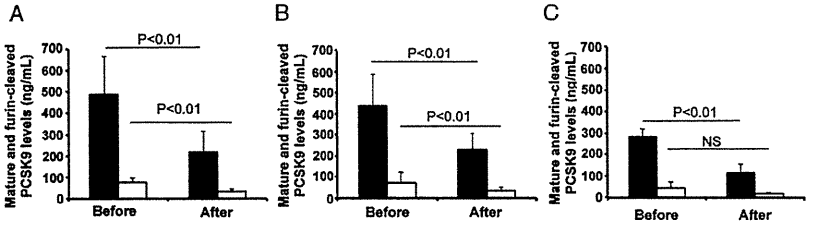

Figure 2. Change of plasma mature and furin-cleaved PCSK9 levels before and after a single LDL-A treatment. Plasma levels of mature (closed column) and furin-cleaved (open column) PCSK9s in (A) FH homozygotes (N = 7), (B) FH heterozygotes (N = 11) before and after LDL-A treatment with DS columns, and in (C) FH heterozygotes (N = 5) before and after LDL-A treatment with DM columns are shown.

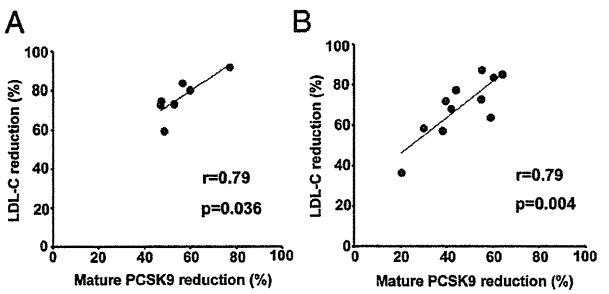

Figure 3. Correlation between plasma LDL-C reduction and mature PCSK9 reduction in FH homozygotes and FH heterozygotes. (A) Correlation between plasma LDL-C reduction (Y-axis) and mature PCSK9 reduction (X-axis) in FH homozygotes after a single LDL-A treatment with DS columns (N = 7). (B) Correlation between plasma LDL-C reduction (Y-axis) and mature PCSK9 reduction (X-axis) in FH heterozygotes after a single LDL-A treatment with DS columns (N = 11).

ated by LDLR in heterozygous FH or receptor-defective homozygous FH patients undergoing LDL-A treatment. However, it would not have contributed to the control of LDL-C in receptor-negative homozygous FH patients. In addition, DM columns whose treated volumes are limited are not usually used for patients who need a high volume of treated plasma. The use of DS columns is contraindicated for patients taking angiotensin-converting enzyme (ACE) inhibitors. Thus, we cannot decide whether DS or DM columns are superior, but we need to decide an appropriate application for each case.

Statins, the most effective commercially available medication for lowering serum LDL-C, decrease cholesterol synthesis, and increase LDLR activity in the liver. Meanwhile, they also stimulate expression of PCSK9, thereby reducing their own effects (23, 24). Thus, antisense oligonucleotides, RNA-mediated interference and Mabs that target PCSK9 have been developed as new treatment strategies for lowering LDL-C (25–29). The use of PCSK9-MAb could reduce the frequency of LDL-A and control LDL-C in heterozygous FH or homozygous FH patients with the LDLR defective type. In addition, the combination of PCSK-Mab and LDL-A treatment may improve the control of LDL-C synergistically or additively.

Recently, Dubic et al has developed an ELISA for the measurement of total PCSK9 using polyclonal antibodies (5). In the present study, we developed a new sandwich ELISA using Mabs for plasma mature and furin-cleaved PCSK9s, respectively, for the first time. This ELISA method could clarify association of the ratio of each form of PCSK9 with the effects of medication in hyperlipidemia patients with gain- or loss-of-function PCSK9 mutations and those taking cholesterol-lowering drugs (30), and with various conditions of hyperlipidemia concomitant with type II diabetes, obesity, and so on. In addition, it has been reported that furin-cleaved PCSK9 represents up to 40% of the total PCSK9 in normal subjects (9), whereas it

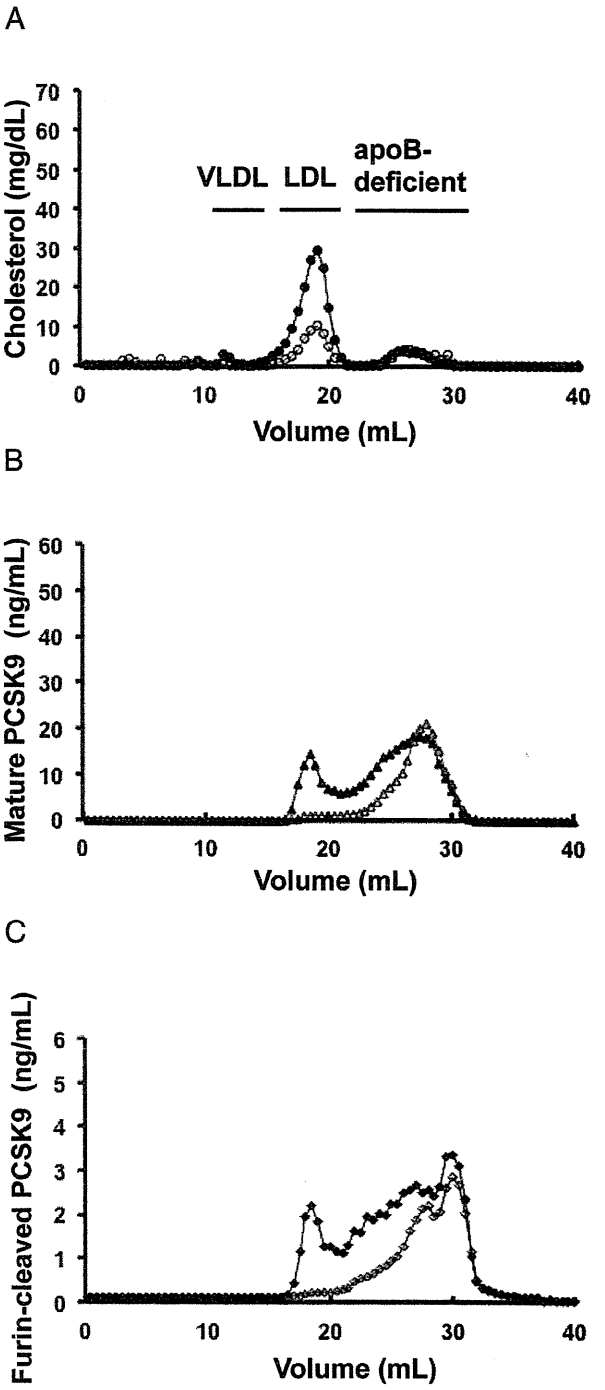

Figure 4. Typical gel filtration chromatography of mature and furin-cleaved PCSK9s and cholesterol in FH plasma before and after LDL-A treatment. Profiles of cholesterol (A, closed circles: before; open circles: after), mature (B, closed triangles: before; open triangles: after), and furin-cleaved PCSK9s (C, closed diamonds: before; open diamonds: after) were analyzed in FH plasma before and after a single LDL-A treatment with DS columns after fractionation by gel filtration chromatography as described in Materials and Methods.

represented 15% of the total PCSK9 in FH patients in the present study. We thus formed a hypothesis that FH shows high LDL-C levels due to not only LDLR mutations but also higher activity of LDLR degradation. The association

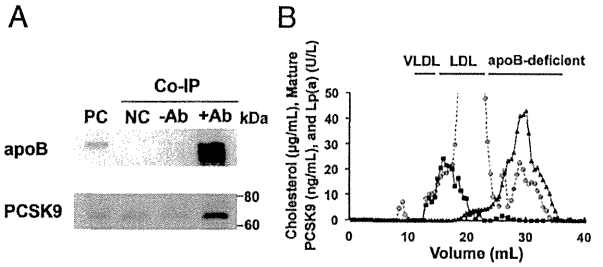

Figure 5. Coimmunoprecipitation of apoB and profile of Lp(a) by gel filtration chromatography in FH plasma. (A) Immunoprecipitation (IP) was performed with 500 μL of plasma from an FH patient treated by 1% triton X-100 and 0.5% sodium deoxycholate (final concentration). An equal volume of supernatants was also applied to columns without IgG and processed in the same way as the antibody coupling resin [negative control (NC)]. The immunoprecipitates were separated by 8–16% SDS-PAGE under denaturing and reducing conditions followed by immunoblotting with monoclonal anti-apoB antibody or polyclonal anti-PCSK9 antibody, and mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP (eBioscience) or HRP-linked anti sheep IgG antibody (Santa Cruz Biotechnology Inc.). Control samples that were incubated in nonimmune serum were also analyzed (-Ab). The bands of apoB and PCSK9 were examined in an apoB-IP sample (+Ab). PC; positive control, -Ab; control-IP, +Ab; apoB-IP. (B) Profiles of Lp(a), cholesterol, and mature PCSK9 levels in the plasma from an FH patient as determined by gel filtration chromatography. Their levels in the collected fractions were measured as described in Materials and Methods. Closed squares: Lp(a); open circles: cholesterol; closed triangles: mature PCSK9 in plasma before LDL-A.

of the ratio of each form of PCSK9 with the regulation of LDL-C metabolism would be validated by the present method.

Gel filtration chromatography analysis showed that 20% of the total plasma PCSK9s existed in the apoB-containing fraction, which is a typical profile for plasma PCSK9 in FH patients (Figure 4). It has previously been reported that 35–39% or > 40% of PCSK9 was associated with the LDL fraction in normolipidemic subjects by size exclusion chromatography or natural density gradient (31, 32). Thus, it was suggested that the amount of PCSK9 contained in the apoB-containing fraction in the plasma of FH patients was lower than that in normolipidemic subjects. Two forms of PCSK9 were reduced by 92–97% in the LDL fraction on gel filtration chromatography and the reduction in mature PCSK9 was strongly correlated with that in LDL-C after a single LDL-A treatment (Figure 3). By immunoprecipitation, plasma mature PCSK9 was confirmed to be bound to apoB (Figure 5A). Thus, it was suggested that a portion of plasma PCSK9 was removed in association with apoB by LDL-A. The distribution of Lp(a) was not overlapped like that of mature PCSK9, suggesting that mature PCSK9 was not associated with Lp(a) (Figure 5B). In addition, the reason why PCSK9 associated with LDL decreases more than LDL-C has not been clarified. Further studies are necessary to clarify the mechanism underlying the removal of the proportion of PCSK9 associated with LDL by LDL-A.

Recently, it has been reported that LDL-bound PCSK9 in human plasma exhibits diminished binding activity toward cell surface LDLR (31). However, it has not been clarified whether PCSK9-associated LDL is incorporated by LDLR, and further studies will be needed to examine the question. In addition, the interaction between apoB and PCSK9 has been reported to inhibit intracellular degradation of apoB and to result in increased secretion of apoB-containing lipoproteins (33). This secreted PCSK9-associated apoB may be derived from LDL associated with PCSK9 in plasma. A portion of PCSK9 may be bound to LDL extracellularly, thereby promoting cellular degradation of LDLR in the endosome.

In the present study, we found that the two forms of PCSK9 were reduced by 52–54% in the apoB-deficient fraction from gel filtration chromatography analysis. Circulating mature and furin-cleaved PCSK9s were mainly present in the apoB-deficient fraction. PCSK9s were removed by apoB-independent pathways based on the electric charge or nonspecific binding to the DS columns while they were removed based on particle size or nonspecific binding to the DM columns. The apoB-deficient fraction has been reported to contain PCSK9 that is mostly of a higher molecular weight, likely dimers and trimers (16, 32, 34). In addition, a previous study has shown that various proteins are present in this fraction, including albumin, globulin, serum amyloid-A, and more (35). LDL-A is thus suggested to remove circulating PCSK9 that is of high molecular weight, likely a dimmer or trimer, in association with these proteins in the apoB-deficient fraction. Meanwhile, other lipoproteins such as HDL have been reported to affect the self-association of PCSK9 (35). It has been calculated that two forms of PCSK9 are negatively charged (mature PCSK9: pI = 6.6; furin-cleaved PCSK9: pI = 7.02) (16), so they are not likely to bind directly to the DS column which is also negatively charged. A future study will be required to investigate the forms of PCSK9 in the apoB-deficient fraction.

The result that PCSK9 was removed by LDL-A in homozygous FH is not consistent with the report by Cameron et al (17). Because the columns used in their study were not described, they may have been different from those used in our present study or the study by Tavori et al (16). Thus, the differences in columns, race, life-style, forms of PCSK9 in plasma and proteins associated with PCSK9 may affect the removal of PCSK9 by LDL-A.

This study has some limitations. The major limitation is that a small sample size of 5 may be insufficient to test subtle differences between the two methods of apheresis on PCSK9. However, we could not get enough subjects for a crossover study comparing the treatment efficacy between DS and DM columns. A second limitation is that we

did not perform a time-dependent study of rebound trajectories in LDL-C, apoB, Lp(a), and PCSK9 in the interval between apheresis. That could provide greater insight into the mechanisms of the coordinated regulation of apoB, Lp(a), and PCSK9. A third limitation is that the number of gel filtration analyses was limited because there were not enough plasma residues for analysis.

In conclusion, our present study has shown that plasma mature and furin-cleaved PCSK9s were removed in FH homozygotes and heterozygotes by binding to apoB or other mechanisms. This report is also the first to demonstrate for an ELISA method to measure both forms of plasma PCSK9–mature and furin-cleaved form–and this technique is expected to be useful for investigating the effects of medications or the physiological or pathological roles of PCSK9.

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Committee Report 9

Familial Hypercholesterolemia

Executive Summary of the Japan Atherosclerosis Society (JAS) Guidelines for the Diagnosis and Prevention of Atherosclerotic Cardiovascular Diseases in Japan—2012 Version

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This is a collaborative work to describe the guidelines for familial hypercholesterolemia issued by the Committee for Epidemiology and the Clinical Management of Atherosclerosis, the Committee for the Diagnosis and Treatment of Familial Hypercholesterolemia and the Research Committee for Primary Hyperlipidemia, Research on Measures against Intractable Diseases.

Heterozygous Familial Hypercholesterolemia

1. Condition and Clinical Picture of FH

Familial hypercholesterolemia (FH) is an autosomal dominant disease caused by abnormal LDL receptors or LDL receptor-related genes, characterized by the triad of (1) hyper-LDL cholesterolemia, (2) premature coronary artery disease (CAD) and (3) tendon/cutaneous xanthoma. Arcus corneae is also characteristic of FH; however, the rate is approximately 30%.

FH by itself is a very high-risk condition for CAD. Untreated men 30 to 50 years of age and women 50 to 70 years of age are likely to develop CAD, such as myocardial infarction and angina pectoris[1]. Early diagnosis and appropriate treatment result in the prevention of premature death. Heterozygous FH exists in approximately one in 500 people, and it is estimated that there are approximately 300,000 patients in Japan. Therefore, heterozygous FH is one of the genetic diseases most frequently encountered by general practitioners.

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2. Diagnosis of Heterozygous FH

1) LDL-C Cutoff Value

Table 1 shows the diagnostic criteria. Using data obtained from a total of 1,397 untreated dyslipidemic patients, including 439 patients with FH and 958 patients without FH, an analysis was performed of

Table 1. Diagnostic Criteria for Heterozygous FH in Adults (15 Years of Age or Older)

1. Hyper-LDL cholesterolemia (an untreated LDL-C level of ≥ 180 mg/dL)
2. Tendon xanthoma (tendon xanthoma on the backs of the hands, elbows, knees, etc. or Achilles tendon hypertrophy) or xanthoma tuberosum
3. Family history of FH or premature CAD (within the patient's second-degree relatives)

- The diagnosis should be made after excluding secondary hyperlipidemia
- If a patient meets two or more of the above-mentioned criteria, the condition should be diagnosed as FH. In cases of suspected FH, obtaining a diagnosis using genetic testing is desirable.
- Xanthoma palpebrarum is not included in xanthoma tuberosum.
- Achilles tendon hypertrophy is diagnosed if the Achilles tendon thickness is ≥ 9 mm on soft X-ray imaging.
- An LDL-C level of ≥ 250 mg/dL strongly suggests FH.
- If a patient is already receiving drug therapy, the lipid level that led to treatment should be used as the reference for diagnosis.
- Premature CAD is defined as the occurrence of CAD in men < 55 years of age or women < 65 years of age.
- If FH is diagnosed, it is preferable to also examine the patient's family members.

major items, including an LDL-C level of ≥ 180 mg/dL, the presence of Achilles tendon hypertrophy or cutaneous xanthoma and a history of FH or premature CAD in relatives within the second degree. The results showed a sensitivity of 94.3% and a specificity of 99.1%. In cases involving an LDL-C level of ≥ 190 mg/dL, the sensitivity was 92.1% and the specificity was 99.1%. Therefore, 180 mg/dL, the level at which the specificity was the same and the sensitivity was higher than that observed at 190 mg/dL, was adopted as the LDL-C cutoff value[2]. Because this analysis showed that 5% of patients with an LDL-C level of ≥ 250 mg/dL do not have FH, a diagnosis of FH is thus strongly suspected in the presence of an LDL-C level of ≥ 250 mg/dL alone[3].

2) Soft X-Ray Radiography of the Achilles Tendon

Achilles tendon hypertrophy should be evaluated using soft X-ray radiography. Positioning is performed so that the lower leg bones and sole of the foot form a 90-degree angle, and radiation is administered so that the X-ray enters the center of the lateral malleolus from the side of the foot. The imaging distance should be 120 cm, and the imaging conditions should be 50 kV and 5.0 mA. When the greatest dimension is ≥ 9 mm, hypertrophy is diagnosed. Conducting the evaluations using ultrasonography is possible, although it has not yet been standardized.

3) Differential Diagnosis

Diseases that must be distinguished from FH include conditions that cause secondary hyperlipidemia (e.g., diabetes mellitus, hypothyroidism and nephrotic syndrome) and a similar disease, familial combined hyperlipidemia (FCHL). FCHL is distinguished by the absence of tendon xanthoma, the presence of small, dense LDL, the presence of other types of dyslipidemia (types IIa, IIb and IV) in the patient's family and, in children, a lower degree of increase in the LDL-C level compared with that observed in FH.

3. Management Targets for LDL-C in Heterozygous FH

Because FH is a disease associated with a very high risk of CAD, FH should be considered to correspond to secondary prevention, and it is desirable to set a management target for the LDL-C level at < 100 mg/dL. However, in many cases, it is difficult to achieve a management target for an LDL-C level of < 100 mg/dL in FH patients in clinical practice. Therefore, it is acceptable to aim for < 50% of the pretreatment level if the management target for LDL-C is not achieved. The achievement of the management target does not always assure the absence of future cardiovascular events. In the treatment of FH, risk assessment cannot be applied using the risk charts provided in these guidelines. This management target should be applied to patients with FH ≥ 30 years of age, and it is desirable to administer the treatment under the direction of a specialist, in principle. Treatment for FH in patients 15-29 years of age must be administered under the direction of a specialist.

4. Treatment of Heterozygous FH

1) Lifestyle Modification

Lifestyle modification should be performed in FH patients after diagnosis and continued as described in committee report 7A[4]. However, due to the high risk of cardiovascular disease (CVD), screening for CVD before administering exercise therapy is essential. CVD should be evaluated using patient interviews to determine the presence or absence of effort angina, and exercise electrocardiography and echocardiography should be performed. If the existence of ischemic heart disease is suspected, administering treatment for ischemic heart disease before initiating exercise therapy is thus preferred. Smoking cessation and obesity management are also important.

2) Drug Therapy

Statins are the first-line drugs for FH treatment. A retrospective analysis of 329 patients with heterozy-

gous FH conducted in Japan revealed that the use of statins delayed the onset of CAD[5]. If the patient does not respond to monotherapy with statins, other lipid-lowering drugs should be concomitantly used. Such concomitant drugs include ezetimibe, bile acid-binding resins (cholestyramine and colestimide), probucol, fibrates and nicotinic acid derivatives. Although there is no evidence that these combination therapies inhibit cardiovascular events in patients with FH more effectively than statin monotherapy, strict management of the LDL-C level is recommended in patients with FH. A retrospective investigation suggested that probucol delays the recurrence of CAD in patients with heterozygous FH[6].

3) Indications for LDL Apheresis

In heterozygous FH patients, LDL apheresis should be considered if the total cholesterol (TC) level does not decrease to ≤250 mg/dL following intensive drug treatment in the presence of CAD. If LDL apheresis is indicated, it is desirable to consult a specialist.

5. FH in Children

1) Diagnosis of Heterozygous FH in Children

The initial finding of heterozygous FH is hyper-LDL cholesterolemia. In childhood, many patients do not develop physical signs associated with hyper-LDL cholesterolemia, such as Achilles tendon xanthoma and arcus corneae. Therefore, FH in children is primarily diagnosed based on the presence of hyper-LDL cholesterolemia and family history. In the diagnosis of FH in children, if the parent(s) has/have hyper-LDL cholesterolemia, a diagnosis of FH in the parent(s) should be established. The diagnostic criteria for heterozygous FH in children are shown in **Table 2**. Because 95% of healthy children have an LDL-C level of ≤140 mg/dL[7], the cutoff value for screening is defined as 140 mg/dL.

2) Treatment for Heterozygous FH in Children

• Nutritional Guidance and Lifestyle Modification

If heterozygous FH is diagnosed, the affected child and their guardians should be directed to modify their lifestyle as soon as possible. Affected children with a smoking habit should be directed to stop smoking. In addition, they should be directed to avoid smoking throughout their life and receive an explanation of the risk of passive smoking; their family members should also be directed to stop smoking.

• Drug Therapy

Evidence pertaining to the age from which treatment should be administered in patients with heterozygous FH has not yet been established in Japan. Because atherosclerotic changes in the coronary arteries are observed from an earlier age in heterozygous FH patients, appropriate LDL-C management is recommended at an earlier age. According to the proposal of the American Academy of Pediatrics, if a patient has an "LDL-C level of ≥190 mg/dL" or an "LDL-C level of ≥160 mg/dL and a family history of premature CAD or at least two risk factors," lipid-lowering treatment should be initiated, even in children, and if lifestyle modification is inadequate, drug therapy should also be considered in boys aged 8 to 10 years or older and in girls after menarche[8]. Among patients who are at a very high risk, such as patients with tendon xanthoma or aortic stenosis or those with a family history of remarkable atherosclerosis, a differential diagnosis of heterozygous FH should be performed. With respect to drug therapy, in terms of safety for growth and development, bile acid-binding resins, which are not absorbed from the gastrointestinal tract, are typically used and are the first-line drugs. Drug therapy for children should be administered under the direction of a specialist.

Table 2. Diagnostic Criteria for Heterozygous FH in Children

1. Hypercholesterolemia: an untreated LDL-C level of ≥140 mg/dL (measure the LDL-C level if the TC level is ≥220 mg/dL)
2. Family history of FH or premature CAD within the patient's second-degree relatives

• Pediatric patients exhibit few symptoms, such as tendon xanthoma. Therefore, diagnosing FH in the patient's family members is important.
• The LDL-C level may vary during development. Providing careful follow-up is necessary.
• Premature CAD is defined as the occurrence of CAD in men <55 years of age or women <65 years of age.

6. Heterozygous FH in Women

Drug therapy, other than bile acid-binding resins, during pregnancy should be carefully considered due to concerns regarding the risk of fetal malformations. According to the National Institute for Health and Clinical Excellence[9], if pregnancy is diagnosed during drug therapy, lipid-lowering drugs other than bile acid-binding resins should be immediately discontinued, and, if there is a possibility of pregnancy, pregnancy after the discontinuation of drug treatment for three months should be recommended.

Homozygous Familial Hypercholesterolemia

1. Diagnosis of Homozygous FH

Homozygous FH is characterized by the presence of a TC level of ≥600 mg/dL, xanthoma and CVD from childhood, with both parents being heterozygous for FH. Therefore, making a clinical diagnosis is possible. If homozygous FH is suspected even when the TC level is <600 mg/dL, obtaining the diagnosis and therapeutic decisions from a specialist is essential.

2. Drug Therapy for Homozygous FH

Similar to that recommended for patients with heterozygous FH, lifestyle modification, including diet therapy, exercise therapy, smoking cessation and obesity management, provides the basis for treatment in patients with homozygous FH, although intensive LDL-C-lowering treatment is required at an earlier age because patients with homozygous FH face a considerable risk with respect to the development and progression of CAD. However, homozygous FH is much less responsive to drug treatment than heterozygous FH. Therefore, the administration of LDL apheresis once every one to two weeks is necessary. Probucol exerts LDL-C-lowering effects on homozygous FH and may cause the regression or disappearance of xanthoma in the skin or Achilles tendon. For patients with homozygous FH who wish to have children, screening for CAD and the presence of aortic stenosis and supravalvular stenosis should be performed, and appropriate measures should be taken as required to ensure the safe continuation of pregnancy and delivery[10].

3. LDL Apheresis for Homozygous FH

In patients with homozygous FH, it is difficult to decrease the LDL-C level sufficiently using existing drug therapies, and many patients require continued LDL apheresis with extracorporeal circulation starting in childhood. Considering the inhibition of the progression of CVD, the earlier LDL apheresis is initiated, the better; however, it is difficult to perform LDL apheresis until the affected child can be kept in bed during apheresis. Realistically, the timing of treatment initiation is 4 to 6 years of age, when children can lie in bed and extracorporeal circulation can be performed; however, it is recommended that the treatment be initiated as early as possible.

4. Pregnancy and Delivery of Patients with Homozygous FH

It is important to permit patients with homozygous FH to become pregnant as planned. Before pregnancy, screening for atherosclerosis should be performed using carotid ultrasonography, echocardiography and exercise tolerance tests to assess the status of atherosclerosis. By three months before the planned pregnancy, treatment with lipid-lowering drugs other than bile acid-binding resins should be discontinued. Because the cardiovascular system is greatly stressed during late pregnancy, particularly at delivery, performing LDL apheresis during pregnancy is desirable. LDL apheresis can also be safely administered during pregnancy.

5. Homozygous FH Designated as a Specified Disease

In October 2009, homozygous FH was designated as a specified disease in the Specified Disease Treatment Research Program. The criteria for designation are as follows: patients with homozygous FH definitively diagnosed using a genetic analysis of genes involved in the LDL metabolic pathway or measurement of the LDL receptor activity are definitively designated, and patients with remarkable hypercholesterolemia and those with cutaneous xanthoma starting in childhood who are refractory to drug treatment should be designated.

Footnotes

This is an English version of the guidelines of the Japan Atherosclerosis Society (chapter 9) published in Japanese in June, 2012. The details of this Committee Report 9 on Familial Hypercholesterolemia have been previously published as an original manuscript[11]; this is a brief summary.

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