Nonstandard Abbreviations and Acronyms	
CE	cholesteryl ester
FC	free cholesterol
FED	fish-eye disease
FLD	familial lecithin:cholesterol acyltransferase deficiency
GFC	gel filtration column
HDL	high-density lipoprotein
HPLC	high-performance liquid chromatography
LCAT	lecithin:cholesterol acyltransferase
LDL	low-density lipoprotein
Lp	lipoprotein
LpX	lipoprotein-X
rLCAT	recombinant LCAT

revealed that the low-density lipoprotein (LDL) fraction contains 3 abnormal particles with different sizes, lipid composition, and associated apolipoproteins,[11,12] which were proposed to be important in the pathogenesis of renal manifestation in patients with FLD.[15–18] Of these, lipoprotein-X (LpX)[19,20] have been postulated to accumulate in glomeruli, potentially causing the renal damage observed in patients with FLD.[16–18] In 1 patient with FLD, lipid-lowering therapy led to a reduction of LpX and a concomitant reduction in proteinuria.[21] LpX is phospholipid (PL)-rich and free cholesterol (FC)–rich but triglyceride (TG)-poor particle without apolipoproteins, ranging in size between very low density lipoprotein and large LDL.[22]

To characterize the abnormal lipoproteins associated with the renal pathology of FLD, we characterized lipoprotein fractions by analyzing patients with different mutations and manifestations in comparison with another LCAT-deficiency syndrome, FED. We applied high-performance liquid chromatography with a gel filtration column (HPLC-GFC) for the first time to characterize the above abnormal lipoproteins and in fact identified lipoprotein subfractions specific to FLD. The lipid contents and particle size were biochemically determined, and the responsiveness of the lipoproteins against incubation with rLCAT was investigated in vitro.

Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

Lipoprotein Subfractions Specific to LCAT-Deficiency Syndromes

Five patients with FLD (1–5) and 4 patients with FED (6–9) were compared with 4 nonaffected normolipidemic controls. Clinical and molecular characteristics and lipid profiles of the patients are given in Tables 1 and 2, respectively. Ultracentrifugation fractionation followed by determination of lipid contents was performed in patients 1, 2, and 5 (Table I in the online-only Data Supplement). LCAT α-activities in the patients' sera were all <2% of reference. As expected in LCAT deficiency, mature HDL particles found at fraction (Fr.) 16 and 17 of unaffected controls were absent in the 9 patients (Figure 1). Although the lipid profiles of patients were heterogeneous, HPLC-GFC showed 4 lipoprotein fractions in sera of patients with FLD and FED that were not present in sera of unaffected controls: large lipoproteins (>80 nm) in Fr. 1 (Lp1), lipoproteins corresponding to large LDL in Fr. 8 (or Fr. 7–10; Lp8), lipoproteins corresponding to very small LDL and large HDL in Fr. 12 to 16 (Lp12–16), and lipoproteins corresponding to small HDL in Fr. 18 to 20 (Lp18–20). The levels of cholesterol, TG, and PL in these specific fractions varied among the 9 patients (Figure 1). Serum apolipoprotein analyses of Fr. 7 to 10, Fr. 13 to 15, and Fr. 18 to 20 in 3 patients (1, 2, and 5) showed that Fr. 13 to 15 and Fr. 18 to 20 were rich in apolipoprotein A as normolipidemic control although varied among patients (Figure I in the online-only Data Supplement). Apolipoprotein Cs were also rich in Fr. 18 to 20 but not in Fr. 13 to 15. Apolipoprotein B was mostly distributed in Fr. 8 to 10 among the 3 fraction categories. Apolipoprotein E was abundant in all 3 fraction categories when compared with that in the control.

Abnormal Lipoproteins Are Present in FLD Regardless of Degree of Proteinuria

To study the relationship between lipoproteins and the degree of proteinuria in patients with FLD, lipoproteins between 2 sibling patients with FLD homozygous for the C337Y mutation in LCAT were compared (Figure 1, patients 1 and 3). Patient 1 had proteinuria in the nephrotic range (6 g/24 h), whereas patient 3 had only mild proteinuria (0.45 g/L).[23] All 4 abnormal lipoproteins were present in both patients (Figure 2A), although 3 lipoproteins (Lp1, Lp8, and Lp18-20) were lower in the younger patient.

Table 1. Clinical and Molecular Characteristics of Patients With Lecithin:Cholesterol Acyltransferase Deficiency

Patient	Sex	Age, Y	Race	Renal Failure/Proteinuria	Corneal Opacity	Anemia	CAD	Phenotype	AA Substitution	References
1	F	17	White (Morocco)	6 g/24 h	+	11.4 g/dL	–	FLD	C337Y	23
2	F	61	Japanese	2 g/24 h	+	9.5 g/dL	–	FLD	C98Y	24
3	F	12	White (Morocco)	0.45 g/L	+	9.2 g/dL	–	FLD	C337Y	23
4	F	63	Japanese	0.23 g/24 h	+	10.3 g/dL	–	FLD	G203R	25
5	M	68	Japanese	0.5 g/L	+	6.6 g/dL	–	FLD	G54S	26
6	M	38	Japanese	–	+	–	–	FED	T147I	10
7	M	58	White (Dutch)	–	+	–	–	FED	T147I	None
8	M	36	White (Dutch)	–	+	–	–	FED	W99S/T147I	27
9	F	30	White (Dutch)	–	+	–	–	FED	T147I/V333M	28

Patients 8 and 9 are compound heterozygotes; others are homozygotes for the indicated mutations. AA indicates amino acid; CAD, coronary artery disease; F, female; FED, fish-eye disease; FLD, familial lecithin:cholesterol acyltransferase deficiency; and M, male.

Table 2. Lipid Profiles of Patients With Lecithin:Cholesterol Acyltransferase Deficiency

Patients	TC	TG	HDL-C	LDL-C	CE/TC
1	109	179	5.8	67	0
2	123	307	9.3	52	0.13
3	47	56	10.1	26	0
4	47	89	6.3	23	0.13
5	56	59	2.0	42	0
6	85	120	4.0	57	0.57
7	133	120	4.7	104	0.54
8	144	205	3.9	99	0.57
9	98	118	4.9	70	0.39

Values for LDL-C were calculated according to Friedewald et al.[29] CE/TC indicates cholesteryl ester/total cholesterol ratio; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; and TG, triglyceride.

Next, lipoprotein profiles of a patient with FLD with homozygous for the C98Y[24] mutation before and after a fat-restricted diet, which led to a reduction of proteinuria from 2.0 g/gCr to 0.6 g/gCr, were compared (Figure 1, patient 2). All 4 lipoproteins remained present after the diet although Lp1 and Lp8 were decreased to some extent (Figure 2B).

Lp8 and Lp12 to 16 Are Specific to FLD and Not to FED

Next, composition of the 4 Lps was analyzed (Figure II in the online-only Data Supplement). In all lipoproteins, cholesteryl ester (CE) was absent in FLD and low in Lp1, Lp12 to 16, and Lp18 to 20 in FED (panel A). PL in Lp8 was significantly lower in FLD when compared with that in FED (panel D). PL and FC were increased in Lp12 to 16 in FLD when compared with that in FED (panels B and D). FC, TG, and PL in both Lp1 and Lp18 to 20 did not differ between FLD and FED.

Lp8 Is a Large LDL, Rich in FC, PL, and TG, and Different From LpX

In comparison with unaffected controls and to patients with FED, CE in the LDL fractions of FLD sera was significantly decreased, whereas TG was increased (Figure 3A). In patients with both FLD and FED, FC, TG, and PL in Fr. 8 were significantly higher than in Fr. 9, whereas in controls, FC, TG, and PL in Fr. 8 were significantly lower than in Fr. 9 (Figure 3B). As a result, average sizes of Lp8 (Fr. 7–10) in FLD were significantly increased when compared with normal, whereas averaged particle size in FLD was lower than those in FED because of the severe deficiency of CE (Figure 3C). The composition of Lp8 in our patients with FLD is consistent with the previously reported FLD-LDL, and not consistent with the lipid characteristics of LpX.

Abnormal Lipid Compositions of FLD-Specific Lps Are Ameliorated by In Vitro Incubation With rLCAT

In vitro rLCAT incubation was performed followed by HPLC-GFC analyses (Figure III in the online-only Data Supplement). Incubation of patients' sera with rLCAT increased CE, TG, and PL in Fr. 16 to 18 in both FLD and FED (Figure IV in

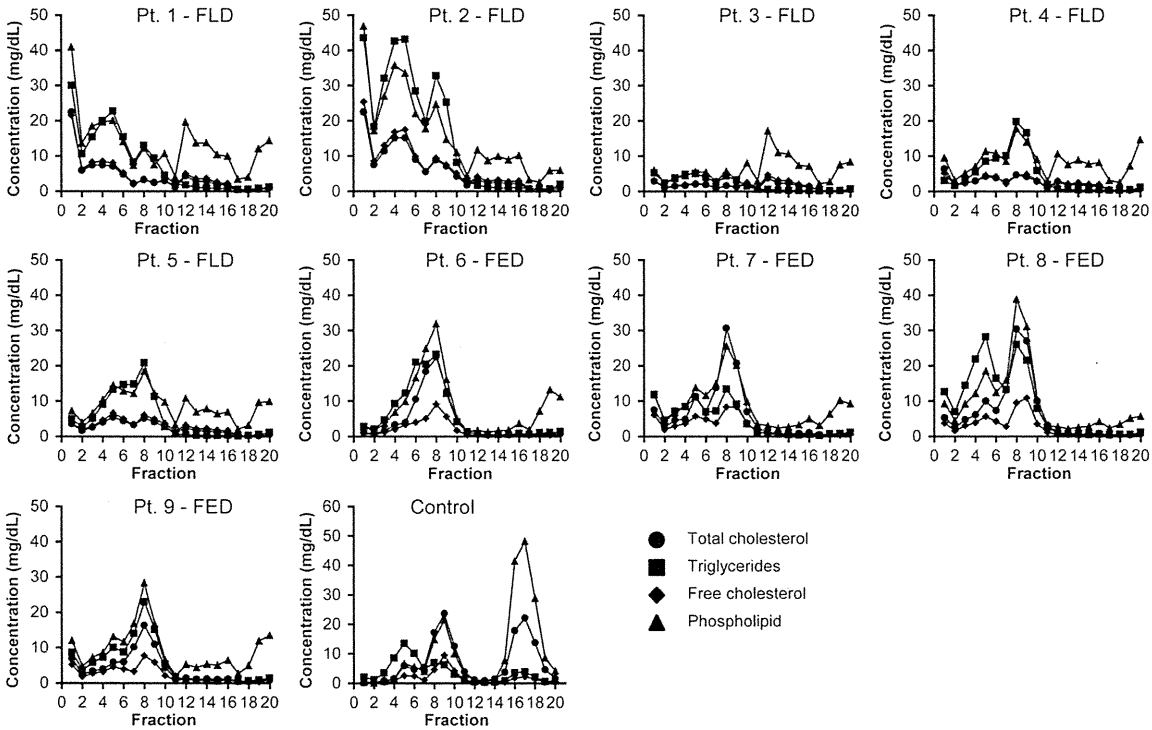

Figure 1. Lipoprotein profiles in patients with familial lecithin:cholesterol acyltransferase deficiency (FLD) by high performance liquid chromatography (HPLC) with gel filtration column (GFC). Sera from patients with 5 FLD (patients [Pts.] 1–5) and 4 Fish-eye disease (FED; Pts. 6–9) were subjected to lipoprotein size fractionation with concomitant determination of lipid concentrations in each fraction by high-performance liquid chromatography-GFC analyses. Representative result is shown for normolipidemic subjects. Concentrations of total cholesterol (●), triglyceride (■), free cholesterol (♦), and phospholipid (▲; *y* axis) in each fraction (*x* axis) are shown.

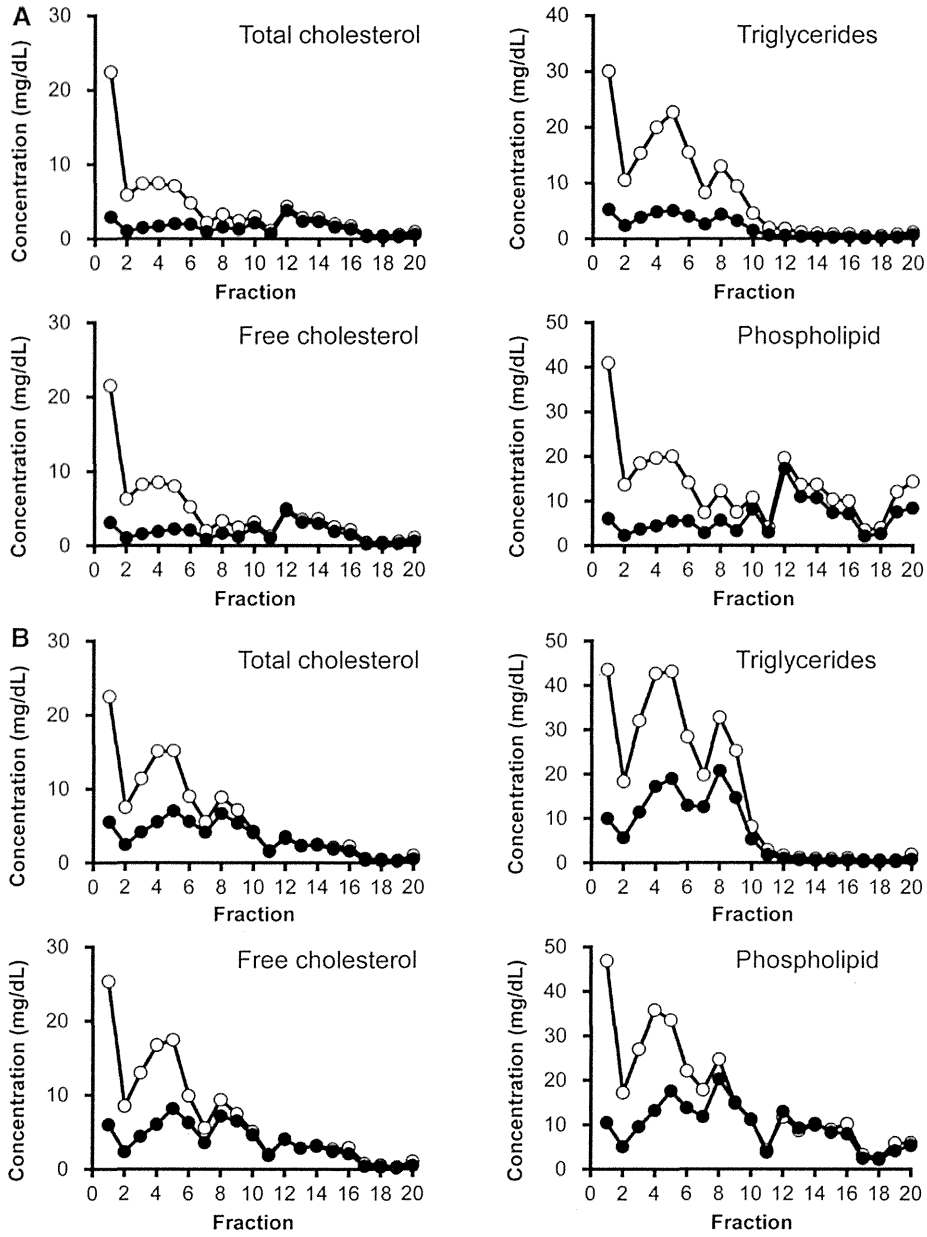

Figure 2. Differences in lipoproteins in patients with familial lecithin:cholesterol acyltransferase deficiency (FLD) with or without renal insufficiency. **A**, Lipoprotein profiles were compared between a patient with FLD with nephrotic range proteinuria (patient 1, ○) and patient 3 with mild proteinuria (●). **B**, Lipoprotein profiles were compared between before (○) and after (●) fat-restricted diet.

the online-only Data Supplement), indicating LCAT-mediated maturation of HDL. CE and PL contents of Lp8 were significantly increased and decreased, respectively, in FLD after incubation with rLCAT, whereas TG content was not significantly altered (Figure 4A and 4B). In FED, composition of Lp8 was not significantly altered by the treatment (Figure 4A and 4B). On incubation with rLCAT, Lp8 increased in size in FLD and it decreased in size in FED (Figure 4C). However, FC and PL in Lp12 to 16 decreased on incubation (Figure 4D).

Discussion

In this study, 4 lipoprotein fractions specific to LCAT-deficiency syndromes were identified by the HPLC-GFC analysis of samples from genetically diagnosed patients with different mutations and manifestations. Two of these had lipid compositions that were specific to FLD and thus may be involved in causing the renal damage that characterizes FLD. In vitro incubation with rLCAT corrected the abnormal fractions.

Lp1, one of the abnormal lipoproteins characteristic to LCAT-deficiency syndrome, was rich in TG and PL, and associated with the degree of proteinuria in 2 siblings with FLD, and was decreased on fat restriction in another patient with FLD (Figure 2). Indeed, abnormal lipoproteins with size of ≈100 nm corresponding to Lp1 have been identified in patients with LCAT deficiency with renal failure.[2,11,12,15] The lipid composition of Lp1 did not change on incubation with rLCAT (data not shown). Together, this suggests that Lp1 is most likely secondary to renal failure rather than directly caused by LCAT deficiency.

As opposed to controls, Fr. 8 was richer in total cholesterol, TG, FC, and PL than Fr. 9 in the patients with LCAT

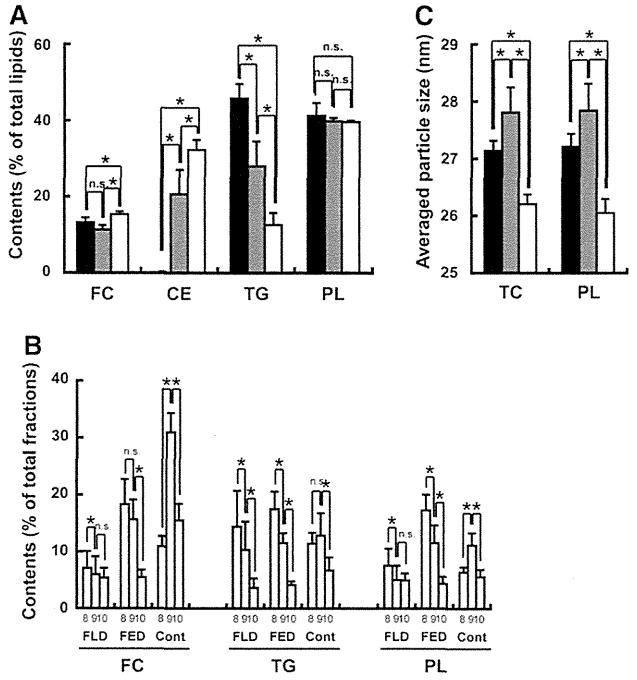

Figure 3. Characterization of lipid profiles in Lp8 of familial lecithin:cholesterol acyltransferase deficiency (FLD) and Fish-eye disease (FED). **A**, Lipid compositions of Fr. 7 to 10 fractions (Lp8) were compared among FLD (closed column), FED (gray column), and normal (open column). *P<0.05. **B**, Lipid concentrations of fractions 8, 9, and 10 were compared in FLD (n=5), FED (n=4), and controls (n=4). *P<0.05. Cholesteryl ester (CE) concentrations in FLD are not shown because levels were undetectable. **C**, Size distribution of lipoproteins in Lp8 (Fr. 7–10) was compared among FLD (closed column), FED (gray column), and normal (open column) based on total cholesterol (TC) and phospholipid (PL) concentrations. *P<0.05. FC indicates free cholesterol; and TG, triglyceride.

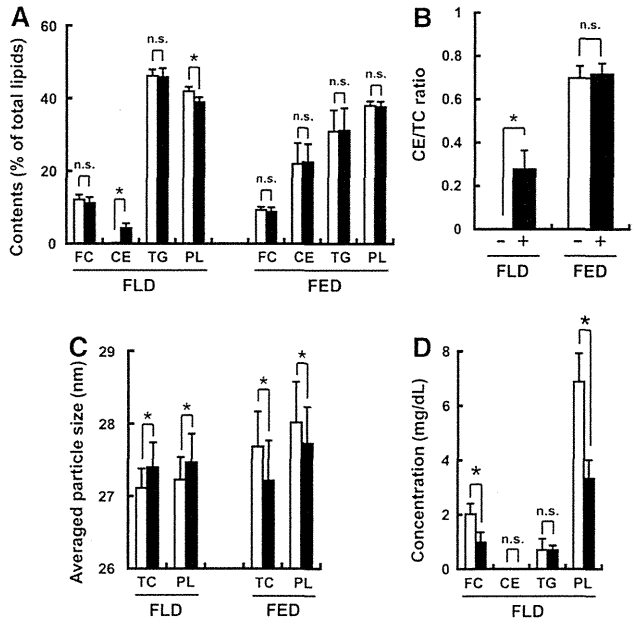

Figure 4. Effects of in vitro familial lecithin:cholesterol acyltransferase (LCAT) supplementation on the lipid profiles of abnormal lipoproteins in LCAT-deficiency syndrome. After analyses described in Figure II in the online-only Data Supplement, lipid composition (**A**), cholesteryl ester (CE)/TC ratio (**B**), averaged particle size based on total cholesterol (TC) and phospholipid (PL) concentrations (**C**), in Lp8, and lipid concentrations in Lp12 to 16 (**D**), were compared between culture media containing recombinant LCAT (rLCAT; closed column) and media without rLCAT (open column). *P<0.05. FC indicates free cholesterol; FED, Fish-eye disease; and TG, triglyceride.

deficiency (Figures 1 and 4B). Lp8 also differed in composition between FLD and FED: in FLD, it contained increased TG and decreased CE in comparison with FED (Figure 3A). Importantly, although the levels varied with the severity of renal damage as did those in Lp1, the buoyance of the peak at Fr. 8 did not vary with severity of renal damage (Figure 2), strongly suggesting that Lp8 directly results from a lack of LCAT and not from metabolic disturbances that occur during proteinuria and progressive renal failure.

In addition to the above-mentioned characteristics for Lp8 in LCAT-deficiency syndrome, HPLC-GFC analyses clarified novel unique lipid properties of Lp8 in FLD in comparison with that in FED; the averaged sizes of Lp8 are smaller in FLD than those in FED (Figure 3C). The lipid compositions of Lp8 in FLD were, in part, ameliorated by rLCAT incubation (Figure 4A). The averaged sizes of the Lp8 increased in FLD, whereas those in FED decreased (Figure 4C). rLCAT increased the CE formation in both LDL and HDL fractions in FLD sera. Thus, these findings indicated that the abnormal compositions were most likely caused primarily by the dysfunction of LCAT in the patients, and that the abnormal characteristics of Lp8 were not because of metabolic disturbances that occur during proteinuria and progressive loss of kidney function.

Previous extensive analyses using electron microscopy have identified 3 abnormal lipoproteins in the LDL fraction of FLD[12]: TG-rich and CE-poor particles of sizes similar to normal LDL (FLD-LDL); FC- and PL-containing particles of sizes distributing from 40 to 60 nm (LpX-like particle)[2]; particles with a diameter of 100 nm (designated as LM-LDL)[17,30] that were later reported to be identical to LpX.[15] LpX is FC- and PL-rich but TG-poor lipid particles (30%, 60%, and 2%, respectively)[22] without apolipoproteins, which range from very low density lipoprotein to large LDL fractions in fast performance liquid chromatography analysis.[31] The abnormal particles have been shown to be decreased by lipid-lowering therapy in a patient with FLD.[21] Lipoproteins in Lp8 were different from LpX in the lipid contents; the fractions were rich in FC and PL and also rich in TG (13.2±1.3%, 41.4±3.3%, and 45.8±3.8%, respectively). The composition analyses suggested that Lp8 corresponds to FLD-LDL, but the calculated sizes of Lp8 were larger than normal LDL using the data obtained by size fractionation with HPLC-GPC in the present study. Thus, the identified Lp8 in LCAT-deficiency syndrome was most likely not identical to LpX in the characteristics.

There is a limitation for the interpretation of the quantitative measurement of LpX in the frozen samples collected in our study because the abnormal lipoproteins were known to be labile to freezing-and-thawing treatment. In this context, fresh sera were collected from patients 2 and 4 and analyzed by agarose gel electrophoresis. The lipid staining of lipoproteins electrophoresed in agarose gel detected the abnormally slowly migrating TG-poor lipoproteins, LpX, at the expectedly migrating position, as well as TG-rich abnormal β-lipoproteins (LDL) in the once-frozen sample, as well as the fresh sample

in patient 4, although the staining intensity tended to decrease in comparison with the fresh counterpart. However, LpX was not detected in either sample with or without freeze-and-thaw treatment from patient 2. Thus, LpX was indeed labile to freeze/thawing, and the frozen samples were not adequate for the quantitative measurement. However, the presence was still able to be evaluated after once-freezing treatment. On the basis of background data, HPLC-GFC analysis showed that lipid contents in Lp8 were not largely affected by once-freezing treatment in both patients 2 and 4: in contrast, the contents of TG and PL were slightly decreased in lipoproteins with peak of Fr. 5 (data not shown). Additional studies using fresh samples of patients with distinct mutations and manifestations are needed to interpret the significance of novel lipoproteins in comparison with LpX for the development of renal insufficiency in LCAT0deficiency syndrome quantitatively.

In FLD but not in FED, Lp12 to 16 were heterogeneous in size and rich in PL. rLCAT decreased PL in these fractions specifically (Figure 5D; Figure II in the online-only Data Supplement). This may suggest that the heterogeneous-sized PL-rich particles in Fr. 12 to 16 converge to normal-sized HDL (Fr. 16–18) on incubation with rLCAT, with concomitant esterification of FC.

In conclusion, 4 lipoprotein fractions specific to LCAT-deficiency syndromes were identified by the HPLC-GFC analysis of samples from genetically diagnosed patients with different mutations and manifestations. The composition of 2 of these was unique to only FLD; these were not likely compatible with the previously reported LpX. These abnormal lipoproteins may be causal to the renal pathology in FLD, the main cause of increased morbidity and mortality in this condition. The regular evaluation of these specific lipid fractions during LCAT enzyme replacement therapy in patients with LCAT deficiency may provide guidance for success of the intervention. The value of these lipid fractions for risk of future renal disease needs to be addressed in prospective follow-up studies in patients with FLD with various mutations in the *LCAT* gene before the onset of proteinuria.

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Disclosures

None.

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Significance

Lecithin:cholesterol acyltransferase-deficiency syndromes are classified into 2 forms: familial lecithin:cholesterol acyltransferase deficiency and fish-eye disease. Patients with familial lecithin:cholesterol acyltransferase deficiency develop renal failure, whereas fish-eye disease patients do not. This study was performed to identify abnormal lipoproteins associated with the renal damage of patients with different mutations and manifestations. Size fractionation with gel filtration of patients' sera and in vitro incubation experiments with recombinant lecithin:cholesterol acyltransferase showed abnormal lipoproteins associated with the renal damage. Thus, our novel analytic approach identified large low-density lipoprotein and high-density lipoprotein with a composition specific to familial lecithin:cholesterol acyltransferase deficiency but not to fish-eye disease. The identification of abnormal lipoproteins may shed light on the clarification of renal pathology and the development of treatment for the patients with familial lecithin:cholesterol acyltransferase deficiency.

Ⅴ．資　　料

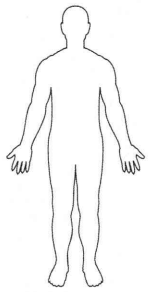
Crow-Fukase症候群に対するサリドマイドの試験（寛解導入試験）

プロジェクト責任者／桑原 聡　千葉大学医学部附属病院　神経内科　教授

疾患概要

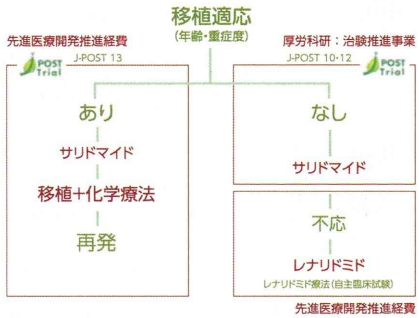
Crow-Fukase症候群（POEMS症候群）は、形質細胞腫に関連して上昇するVEGF（血管内皮増殖因子）により多彩な症状を呈する全身性疾患です。

Polyneuropathy（多発性神経炎）
Organomegaly（臓器腫大）
Endocrinopathy（内分泌異常）
M-protein（M蛋白）
Skin changes（皮膚症状）

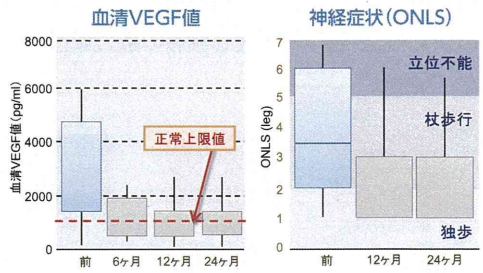


全国患者数　推定340名

■ 治療方針



■ 先行臨床試験成績（自験データ）



試験概要

開発試験物	サリドマイド（サレド® カプセル　藤本製薬（株））
対象疾患	Crow-Fukase症候群
開発の最終目標	製造販売承認（適応拡大）
プロジェクトの出口	医師主導治験の終了（千葉大学） 製造販売承認事項一部変更承認申請（藤本製薬（株））
現時点での到達点	症例登録中　9例/5-10例
作用機序	サリドマイドの形質細胞への直接効果、VEGF産生抑制効果
市場での位置づけ	高齢者に対する治療、自己末梢血幹細胞移植療法前の寛解導入療法

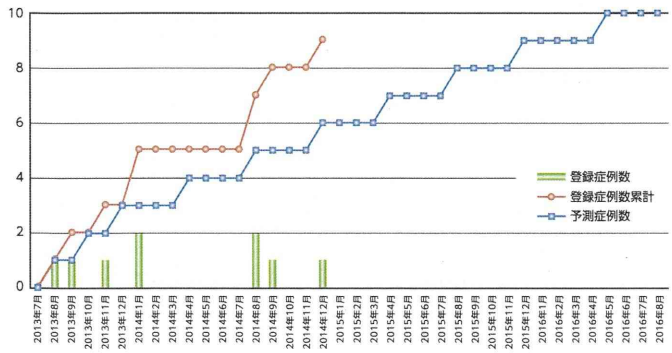
■ 試験デザイン

** サリドマイド投与終了後、2～4週以内に行う。
G-CSFを6日間（day1-6）連日投与し、day5、6に幹細胞採取を行う。

- 研究期間：2013/7/4 ～ 2016/8/31（登録期間：2013/7/4 ～ 2016/1/31）
- 目標症例数：5 ～ 10 例
- 実施医療機関：千葉大学医学部附属病院

進捗状況

■ 登録状況



■ 開発スケジュール（ロードマップ）

CHIBA UNIVERSITY　千葉大学医学部附属病院

問い合わせ先　担当PL　片山　TEL.043-226-2737　FAX.043-226-2735
E-mail　jpost.k@gmail.com

CCRC

厚生労働省　臨床研究中核病院整備事業

医師主導治験

難病の新しい治療法を千葉から世界へ――

Wing of Innovation and Science for the Development of new Medical care

WISDOM PROJECT

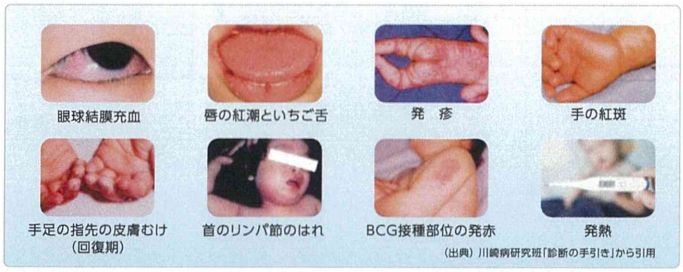
重症川崎病に対するシクロスポリンの医師主導治験（適応拡大）

プロジェクト責任者／羽田　明　千葉大学医学部附属病院　公衆衛生学　教授

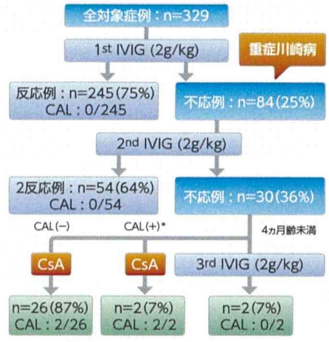
疾患概要

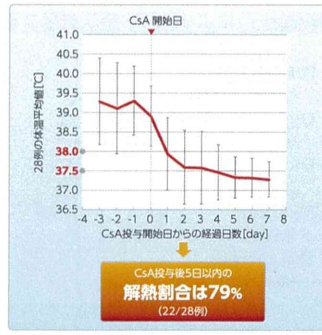
川崎病は主に乳幼児に冠動脈病変を引き起こす可能性のある疾患で、日本では年間1万人以上発症している。罹患率の低い欧米諸国においても、小児の後天性心疾患の最大原因となっている。

■ 主要症状



■ 先行臨床試験成績（自験データ）

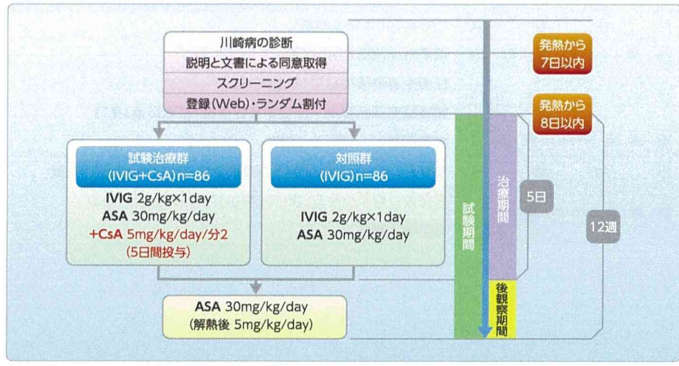




Suzuki H, Terai M, Hamada H, et al: Cyclosporin A treatment for Kawasaki disease refractory to initial and additional intravenous immunoglobulin. Pediatr Infect Dis J. 2011;30:871-876.

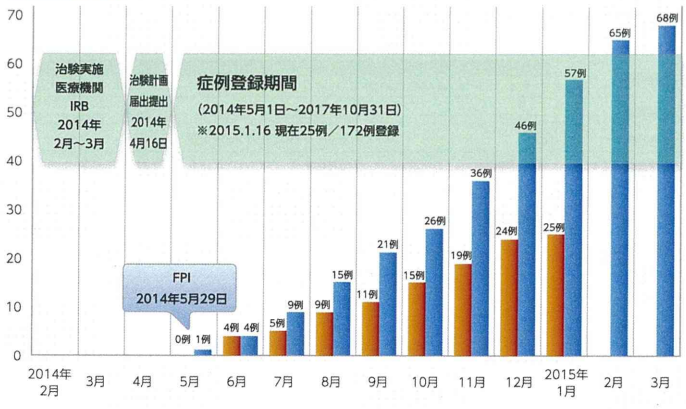
試験概要

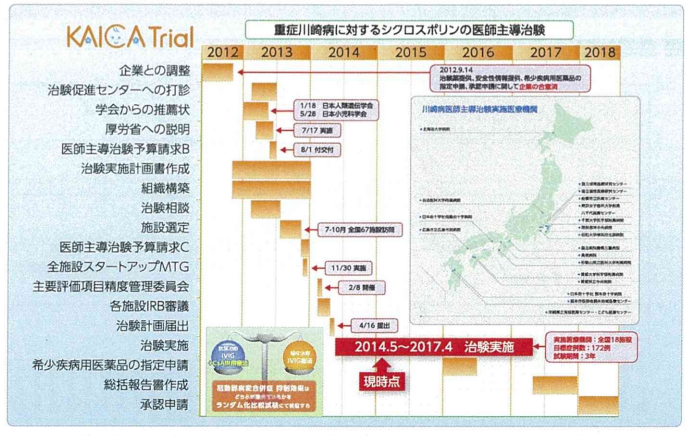
開発試験物	シクロスポリン（ネオーラル®ノバルティスファーマ（株））
対象疾患	重症川崎病
開発の最終目標	製造販売承認（適応拡大）
プロジェクトの出口	医師主導治験の終了（千葉大学） 製造販売承認事項一部変更承認申請（ノバルティスファーマ（株））
出口に至る主なハードル	症例リクルート
出口に至る現時点での到達点	医師主導治験　実施中 ・スタートアップミーティング…… 2013年11月30日 ・心エコー講習会…… 2014年　2月　8日 ・各施設のIRB…… 2014年　2月～3月 ・治験届提出…… 2014年　4月16日 ・治験開始…… 2014年　5月　1日



進捗状況

■ 登録状況





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E-mail　chi-csa-004@ML.chiba-u.jp
CCRC

医師主導治験

難病の新しい治療法を千葉から世界へ――

Wing of Innovation and Science for the Development of new Medical care

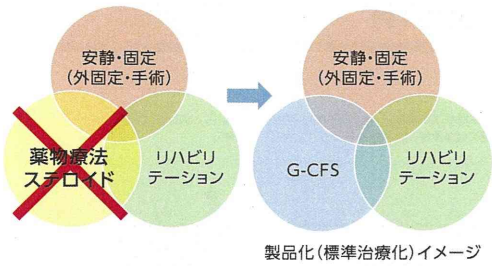
WISDOM PROJECT

急性脊髄損傷に対するG-CSFを用いた試験

プロジェクト責任者／高橋和久　千葉大学医学部附属病院　整形外科　教授

疾患概要

■ 脊髄損傷の治療法

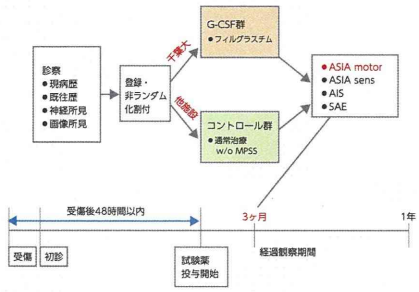


製品化(標準治療化)イメージ

第２相試験

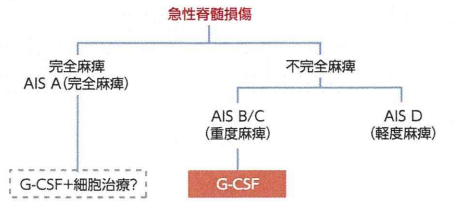
■ 先行臨床試験成績(自験データ)

早期フェーズⅡ: 多施設前向き・非ランダム化・非盲検比較試験



第３相試験

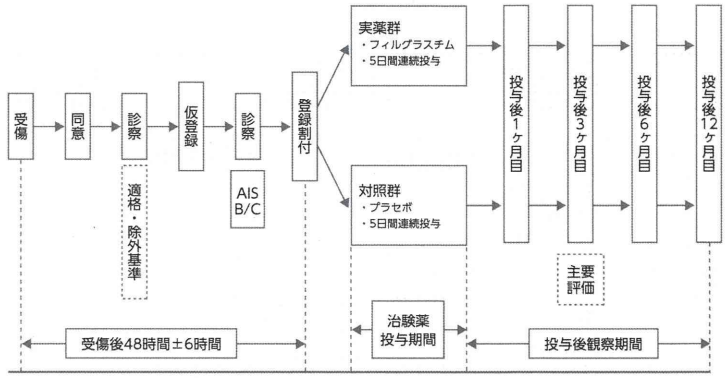
■ 医師主導治験



効能効果：急性脊髄損傷
用途用量：点滴静注 (400μg/m²/day 5 日間投与)

試験概要

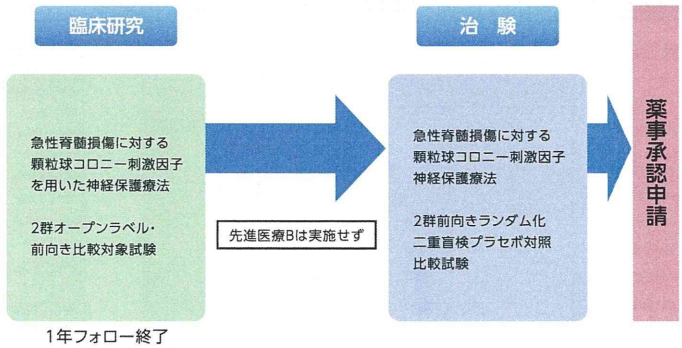
目　　的	急性脊髄損傷に対するG-SCFを用いた神経保護療法を確立する
試験デザイン	ランダム化、プラセボ対照、二重盲検並行群間比較試験
対　　象	頚髄損傷患者(重度不全麻痺)
主要エンドポイント	投与後3ヶ月のASIA運動score変化量
副次エンドポイント	①投与後6ヶ月間および12ヶ月時点でのASIA 運動scoreの変化量 ②投与後3ヶ月、6ヶ月および12ヶ月時点でのASIA 痛覚scoreの変化量 ③投与後3ヶ月、6ヶ月および12ヶ月時点の機能障害をAIS分類5段階で評価 ④レスポンダーの割合(AIS 1段階以上改善した患者の割合) ⑤神経学的損傷高位 (neurological level of injury: NLI) ⑥Spinal cord independence measure (SCIM) ⑦EQ-5D ⑧有害事象(副作用)



施設選定

治験施設候補リスト作成　20施設選定	
千葉大学ネットワーク	千葉県内の施設(君津中央病院、済生会習志野病院、千葉労災病院、船橋市立医療センター等)
研究者のネットワーク	研究者関連(金沢医科大学、独協医科大学、中部ろうさい病院、長崎労災病院、日本医科大学千葉北総病院、東邦大学医療センター佐倉病院等)
関東甲信越アライアンス	国立大学8大学アライアンス(新潟大学等)
国立大学臨床試験推進協議会TG2(ネットワーク)	TG2(トピックグループ2)を利用 (三重大学、浜松医科大学、広島大学等)
DPC情報を利用	小郡第一総合病院、埼玉医科大学、東海大学等

最終ゴール



1年フォロー終了

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