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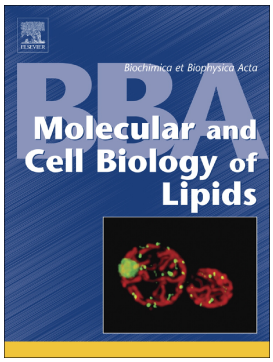

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Plasma PCSK9 levels and lipoprotein distribution are preserved in carriers of genetic HDL disorders

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Abbreviations

ApoA-I, apolipoprotein A-I
ABCA1, ATP-binding cassette transporter A1
FPLC, fast performance liquid chromatography
LCAT, lecithin:cholesterol acyltransferase
LDLR, LDL receptors
PCSK9, proprotein convertase subtilisin/kexin 9

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ABSTRACT

Proprotein convertase subtilisin/kexin 9 (PCSK9), a protein regulating the number of cell-surface LDL receptors (LDLR), circulates partially associated to plasma lipoproteins. How this interaction alters PCSK9 plasma levels is still unclear. In the present study, we took advantage of the availability of a large cohort of carriers of genetic HDL disorders to evaluate how HDL defects affect plasma PCSK9 levels and its distribution among lipoproteins. Plasma PCSK9 concentrations were determined by ELISA in carriers of mutations in *LCAT*, *ABCA1*, or *APOAI* genes, and lipoprotein distribution was analyzed by FPLC. Carriers of one or two mutations in the *LCAT* gene show plasma PCSK9 levels comparable to that of unaffected family controls (homozygotes, 159.4 ng/mL (124.9;243.3); heterozygotes, 180.3 ng/mL (127.6;251.5) and controls, 190.4 ng/mL (146.7;264.4); *P* for trend =0.33). Measurement of PCSK9 in plasma of subjects carrying mutations in *ABCA1* or *APOAI* genes confirmed normal values. When fractionated by FPLC, PCSK9 peaked in a region between LDL and HDL in control subjects. In carriers of all HDL defects, lipoprotein profile shows a strong reduction of HDL, but the distribution of PCSK9 was superimposable to that of controls. In conclusion, the present study demonstrates that in genetically determined low HDL states plasma PCSK9 concentrations and lipoprotein distribution are preserved, thus suggesting that HDL may not be involved in PCSK9 transport in plasma.

Keywords: proprotein convertase subtilisin/kexin 9, high-density lipoproteins, genetic HDL disorders, lecithin:cholesterol acyltransferase.

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1. Introduction

Proprotein convertase subtilisin/kexin 9 (PCSK9) is predominantly expressed in liver, intestine, kidney, and aortic vessels [1, 2]. PCSK9 regulates, by a post-translational mechanism, the number of cell-surface LDL receptors (LDLR) in the liver and in other tissues, *ie*, lung, adipose tissue, and kidney [3]. The *PCSK9* gene encodes a 692-amino acid glycoprotein constituted by a signal peptide, a prodomain, a subtilisin-like catalytic domain, and a variable C-terminal domain [4]. PCSK9 contains a catalytic triad (Asp^{186}, His^{226}, and Ser^{386}), in the catalytic domain, essential for its processing occurring in the secretory pathway. The autocatalytic cleavage generates a stable PCSK9 heterodimer composed of a 10-kDa prodomain fragment and a mature 64-kDa fragment containing the catalytic and C-terminal domains. The interaction of the prodomain with the catalytic domain occupies the active site inhibiting further enzymatic activity by PCSK9 that is found in an inactive 64-kDa form in plasma. A second circulating cleaved form of PCSK9 can be detected in human plasma as a result of furin-mediated processing [5].

A significant proportion of plasma PCSK9 (20-40%) circulates bound to lipoproteins. The observation that more than half of PCSK9 is removed from plasma together with LDL by LDL-apheresis [6] confirms a binding between LDL and PCSK9. The cleaved form of PCSK9 has also been found associated to LDL and to be cleared after apheresis [6, 7]. The association of PCSK9 with LDL particles lowers the ability of PCSK9 to bind to cell surface LDLRs thereby blunting PCSK9-mediated LDLR degradation [8]. In addition, PCSK9 also binds to Lp(a) [9], an effect that could partially explain the reduction of plasma Lp(a) levels observed in hypercholesterolemic patients treated with monoclonal antibodies against PCSK9 [10, 11], for which the molecular mechanism is still unknown, although pathways beyond the LDLR appears to be involved [12]. More controversial is the binding of PCSK9 to HDL [13]. Although Kosenko et al showed that PCSK9 binds to LDL but not to HDL [8], the incubation of human PCSK9 with mouse serum clearly demonstrated the association of PCSK9 with LDL and HDL, but not with VLDL [14]. Fast Protein Liquid Chromatography (FPLC) analysis of PCSK9 distribution within plasma lipoproteins in transgenic mice overexpressing human PCSK9 showed that the majority of PCSK9 overlapped with the distribution of purified human PCSK9, thus suggesting that PCSK9 circulates in plasma mostly as free form [15]. On the contrary, a small fraction of PCSK9 was present in a large complex that co-eluted with LDL fractions [15]. Overall, approximately 20-40% of PCSK9 associates to LDL, while the remaining part is found in the apoB free fractions [8, 15, 16].

In the present study we took advantage from the availability of a relatively large cohort of carriers of genetic HDL defects, carrying mutations in the *APOAI*, *LCAT*, and *ABCA1* genes to investigate the impact of HDL deficiency on plasma PCSK9 concentration as well as distribution among different lipoprotein particles.

2. Materials and Methods

2.1. Subjects

Seventy-nine carriers of *LCAT* gene mutations and 35 non-affected family controls, all belonging to the Italian LCAT deficient families [17-20], volunteered for the study. The carriers' group was comprised

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of 10 homozygotes and 9 compound heterozygotes, defined homozygotes throughout the paper, and 60 heterozygotes. In addition, 2 carriers of two mutations in the *ABCA1* gene, 2 carriers of one mutation in the *ABCA1* gene, 2 carriers of two mutations in the *APOAI* gene and 4 carriers of one mutation in the *APOAI* gene were included in the study [17-23]. A detailed complete overview of the LCAT, ABCA1, and ApoA-I mutations can be found in the Supplemental Table I. All subjects were fully informed of the modalities and end points of the study and signed an informed consent, and the procedures were approved by the Institutional Review Board.

Fasting blood was collected after an overnight fast; plasma was prepared by low speed centrifugation and stored in aliquots at -80°C until utilization.

2.2. Biochemical analyses

Plasma lipids (total and HDL cholesterol, unesterified cholesterol, triglycerides, and phospholipids) were measured by certified enzymatic techniques on a Roche c311 autoanalyzer. Apolipoprotein and Lp(a) levels were measured by immunoturbidimetry on a Roche c311 autoanalyzer. In addition, LDL and non-HDL cholesterol were calculated by the Friedewald's equation and as the difference between total and HDL cholesterol, respectively.

Plasma LCAT concentration was measured by an immunoenzymatic assay [19], and LCAT activity was measured as the esterification of cholesterol incorporated into an exogenous standardized substrate (reconstituted HDL) as previously described [19].

Plasma PCSK9 concentrations were measured by a commercial ELISA kit (R&D Systems, MN) able to recognize free and LDLR-bound PCSK9 [24]. Plasma samples were diluted 1:20 and incubated onto a microplate pre-coated with a monoclonal antibody specific for human PCSK9. The sample concentrations have been obtained by generating a four-parameter logistic curve-fit. The minimum detectable PCSK9 concentration was 0.219 ng/mL. Intra- and inter-assay CVs were 3.8% and 5.8%, respectively.

PCSK9 distribution within plasma lipoproteins was analyzed by FPLC in plasma samples from 6 homozygotes and 6 heterozygotes carriers of LCAT mutations, 2 homozygotes and 2 heterozygotes carriers of ABCA1 mutations, 2 homozygotes and 2 heterozygotes carriers of apoA-I mutations, and 6 controls using a Superose 6 HR10/30 column (GE Healthcare, UK) [25]. 350 µL of plasma were applied to the column, 1.2 ml-fractions were collected; total cholesterol, phospholipid, and PCSK9 concentrations were determined by enzymatic techniques.

2.3. Generation of human PCSK9 expression constructs and retroviral infection of HepG2 cells

To characterize the elution profile of lipoprotein-free PCSK9, the medium from HepG2 cells stably overexpressing PCSK9 was analyzed. The retroviral expression plasmid encoding PCSK9-FLAG tag was constructed using the pBM-IRES-PURO plasmid. Human PCSK9-FLAG tag cDNA was kindly provided by Prof. P. Tarugi (University of Modena) and subcloned into retroviral expression plasmid by blunt-end ligation. Retroviral infections of HepG2 were performed as previously described [26, 27]. A polyclonal population of HepG2 control and PCSK9 overexpressing cells have been then selected with 10µg/ml of

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puromycin.

2.4. Statistical analyses

Results are presented as mean±SD or as medians and interquartile range, as indicated. The trends in plasma lipid/lipoprotein and PCSK9 levels across genotypes were assessed by Spearman correlation. Univariable associations between PCSK9 and all plasma lipid/lipoprotein levels were investigated by Spearman correlation. Multivariable analyses, adjusted for age, gender,blood lipids, and statin use were performed by covariance analysis. To determine if the associations of PCSK9 with plasma variables differed between controls and carriers, the appropriate interaction terms were included in the statistical models. Variables with skewed distributions were log-transformed before analysis. P values < 0.05 were considered as statistically significant. All analyses were performed by using the SAS Software version 9.4 (SAS Inc, Cary, NC).

3. Results

3.1. Characteristics of the subjects

Three different groups of carriers of genetic HDL disorders participated in the study. The first and largest group included carriers of LCAT deficiency (19 homozygotes and 60 heterozygotes) [17]; 35 non affected family members acted as controls. As previously reported, carriers of *LCAT* gene mutations have significantly reduced HDL-C, apoA-I, apoA-II, LpA-I, LpA-I:A-II levels, and LCAT mass and activity with a gene-dose dependent effect. Plasma LDL-C and apoB levels were also significantly reduced, while triglyceride levels and preβ-HDL were significantly increased (Table 1). Two of the 19 homozygous and 7 of the 60 heterozygous carriers were taking lipid-lowering drugs (statins, n=6; fibrates, n=2; ezetimibe, n=1); none of the control subjects was taking drugs.

Two smaller groups included carriers of mutations in the *ABCA1* gene (2 homozygotes and 2 heterozygotes) [18], or in the *APOAI* gene (2 homozygotes and 4 heterozygotes) [19, 20]. None of the subjects was taking lipid-lowering drugs. The characteristics of these carriers are reported in Table 2.

3.2. Plasma PCSK9 levels in carriers of genetic HDL defects

We first measured total plasma PCSK9 levels in carriers of *LCAT* gene mutations and family controls. Plasma PCSK9 levels in controls were within the reported normal range (198.9±81.1 ng/mL) [28]. Carriers of *LCAT* gene mutations showed plasma PCSK9 levels comparable to controls (heterozygotes, 180.3 ng/mL (127.6,251.5) and homozygotes, 159.4 ng/mL (124.9,243.3); *P* for trend adjusted =0.42) (Figure 1). Notably, upon correction for age, gender, all the lipid parameters reported in Table 1, and statin use, the *P* for trend value did not vary (*P*=0.33).

As previously reported in different observational studies [28-30], a direct association between plasma PCSK9 concentration, age, total cholesterol, LDL-C, non-HDL cholesterol, and apoB levels was observed in the entire cohort of subjects (Table 3). In addition, significant positive correlations between plasma PCSK9 levels and unesterified cholesterol, phospholipid, and LpA-I levels and a significant

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negative correlation with preβ-HDL were found (Table 3). Interestingly, when carriers of LCAT deficiency and controls were analyzed separately, a significant positive correlation between PCSK9 and HDL-C levels was detected only in controls (*P* for interaction=0.017, Table 3). A positive association was also detected between PCSK9 and LpA-I levels and LCAT activity only in controls (*P* for interaction=0.052 and 0.032, respectively, Table 3).

The measurement of plasma PCSK9 levels in carriers of mutations in the *ABCA1* and *APOAI* genes confirmed the observation that the severe HDL defect was associated with plasma PCSK9 levels similar to those of controls (Figure 1).

3.3. PCSK9 distribution within plasma lipoproteins in carriers of genetic HDL disorders

We next determined the distribution of PCSK9 within plasma lipoproteins by FPLC analysis. Lipoprotein profiles of 4 control subjects, with different plasma lipids, are reported in Figure 2. As expected, and independently from the lipid profile, the majority of cholesterol was found in LDL (fractions 14-18), and a lower amount in HDL (fractions 19-25); phospholipids were almost equally distributed between LDL and HDL. In all the subjects, PCSK9 peaked in a region between LDL and HDL (Figure 2). Interestingly, the elution peak of cell derived PCSK9 was superimposable to that of controls (Figure 2 - Control 1).

The lipoprotein profile of homozygous carriers of LCAT deficiency remarkably differed from that of controls, with a dramatic reduction of HDL, almost no detectable, and a prevalence of large, phospholipid-enriched LDL-VLDL particles (Figure 3). Notably, the lipoprotein distribution of PCSK9 in LCAT deficient carriers was superimposable to that of controls, despite the severe HDL deficiency (Figure 2 and 3), and indeed only minimally overlapped the cholesterol profile (Figure 3). The lipoprotein profiles of carriers of ABCA1 and apoA-I mutations also revealed the complete defect of lipids in the HDL region, and confirmed a PCSK9 lipoprotein distribution similar to that of controls (Figure 2 and 3).

4. Discussion and conclusion

The present study shows, for the first time, that PCSK9 levels are within the normal range in carriers of genetic HDL disorders, and that the lipoprotein distribution of the circulating PCSK9 is also maintained despite the severe HDL defect.

Plasma concentrations of PCSK9 vary over approximately a 100-fold range (33–2988 ng/ml) in the population [28], and PCSK9 half-life time in circulation is very short, approximately 5-15 minutes [3, 31]. The clearance of PCSK9, at least in mice, occurs in two phases; the first, rapid and dependent upon LDLR, while the second largely independent from the LDLR [31]. The degradation of LDLR in mouse liver occurs at 30 min post-injection of recombinant PCSK9, thus with a significantly slower kinetic than the clearance of PCSK9 [3, 31]. These data indicate that the mechanisms behind PCSK9-driven LDLR degradation are still largely unknown. PCSK9 is also able to self-associate in order to form dimers and trimers, which showed an improved LDLR degrading activity compared to monomeric PCSK9 [14]. HDL

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partially inhibit the PCSK9 self-association and thus may reduce the efficacy of PCSK9 to degrade the LDLR [14].

Several observational and clinical trials have documented the correlation of PCSK9 plasma levels with several metabolic parameters including HDL-C [28-30]. These studies were performed starting from the assumption that PCSK9 was present in a free-form in plasma. However, an important breakthrough in this field was the discovery that PCSK9 is detected in different lipoprotein particles including LDL [8, 14], Lp(a) [9], and HDL [14, 15].

In the present study, we took advantage of the availability of a large series of Italian carriers of genetic HDL defects to study the impact of partial or complete HDL deficiency on PCSK9 metabolism. Carriers of genetic HDL disorders are characterized by a significant reduction in plasma HDL levels, associated with profound changes in HDL subclasses [32, 33], and thus represent a unique tool to study PCSK9 distribution within plasma lipoproteins. The results of this study show that in spite of the dramatic changes in lipoprotein profile, total plasma levels of PCSK9 are within the normal range in carriers of HDL deficiency, suggesting that the HDL defect does not alter PCSK9 homeostasis. The analysis of PCSK9 lipoprotein distribution by FPLC confirmed the observation that in control plasma PCSK9 is detected in fractions corresponding to LDL and HDL, as previously reported [15], and independently from plasma lipids and the presence or not of Lp(a). Surprisingly, the same lipoprotein distribution of PCSK9 was detected in plasma from carriers of different genetic HDL defects. Moreover, the elution profile of cell derived, lipoprotein-free PCSK9 was superimposable to the PCSK9 profile observed in controls and carriers, supporting the concept that the majority of plasma PCSK9 is likely in a lipoprotein-free fraction, as previously reported [8, 9, 34].

Interestingly, when carriers and controls were analyzed separately, a positive correlation between PCSK9 and HDL-C levels was observed in controls but not in carriers Moreover, again only in controls, PCSK9 significantly and positively correlated with LpA-I levels and LCAT activity, and negatively with preβ-HDL, thus suggesting that mature, cholesteryl esters enriched, apoA-I-containing HDL particles could be involved in PCSK9 binding. Notably, these specific HDL subclasses are very reduced or even absent in carriers of LCAT deficiency [17, 35], which should lead to reduced PCSK9 levels if they were involved in PCSK9 transport in plasma.

LDL contribute to transport of PCSK9 in plasma [6, 8], and indeed in our entire cohort we confirmed a positive association of PCSK9 to LDL-C and apoB as previously reported [30, 36-38]. In support to the role of LDL in PCSK9 transport, using an approach similar to the one used in the present study it has been shown that carriers of familial hypobetalipoproteinemia due to mutations in apoB (FHBL1) or in ANGPTL3 (FHBL2), characterized by very low circulating LDL, have significantly reduced plasma PCSK9 levels [34, 36]. Interestingly, Lambert et al have shown that in carriers of FHBL1 plasma PCSK9 levels do not correlate with non-HDL cholesterol, in contrast to what observed in control subjects [36]. Lp(a) has also been shown to bind PCSK9, specifically in subjects with high levels of Lp(a) (39 to 320 mg/dl) [9]. In our cohort of subjects Lp(a) does not seem to play a major role in PCSK9 transport, since the cohort includes many subjects with undetectable Lp(a) levels. Moreover, PCSK9 lipoprotein

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distribution was not affected by Lp(a) levels in control subjects, as shown in Figure 2. Finally, we could detect a weak association between PCSK9 and Lp(a) levels only in controls and not in carriers, thus excluding that in genetic HDL defects the binding of PCSK9 to Lp(a) may play an important role in PCSK9 homeostasis.

In conclusion, the present study demonstrates that conditions characterized by genetically determined very low HDL levels are associated with normal PCSK9 plasma concentrations and lipoprotein distribution, thus suggesting that HDL may not be involved in PCSK9 transport in plasma.

Conflict of interest

The authors declare no conflict of interest.

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Table 1
Demographic and lipid/lipoprotein profile of carriers of LCAT deficiency

	Carriers of LCAT deficiency			*P* trend
	Controls	HEZ	HOZ	
N	35	60	19	
Sex (m/f)	13/22	38/22	16/3	0.0004
Age (years)	45.1 ± 22.3	47.7 ± 21.3	40.4 ± 18.2	0.52
Total Cholesterol (mg/dL)	190.4 ± 41.0	166.5 ± 42.9	154.2 ± 84.7	0.002
Unesterified Cholesterol (mg/dL)	52.1 ± 12.3	51.3 ± 13.3	117.0 ± 72.4	<0.0001
LDL-Cholesterol (mg/dL)	111.2 ± 33.0	97.1 ± 39.4	92.1 ± 62.8	0.01
HDL-Cholesterol (mg/dL)	59.6 ± 13.7	42.2 ± 16.2	10.6 ± 7.1	<0.0001
Non HDL-Cholesterol (mg/dL)	132.6 ± 39.5	124.2 ± 45.4	143.5 ± 86.4	0.66
Triglycerides (mg/dL)	76.0 (58,108)	110.0 (80,154.5)	180.0 (114,387)	<0.0001
Phospholipids (mg/dL)	205.3 ± 42.7	205.2 ± 65.5	268.6 ± 122.7	0.01
Apolipoprotein A-I (mg/dL)	128.9 ± 25.0	106.5 ± 26.6	48.4 ± 20.6	<0.0001
Apolipoprotein A-II (mg/dL)	31.6 ± 6.4	30.0 ± 5.8	9.1 ± 5.7	<0.0001
Apolipoprotein B (mg/dL)	87.8 ± 24.1	91.8 ± 27.3	67.0 ± 47.1	0.12
Lp(a) (mg/dL)	12.8 ± 17.0	7.6 ± 10.7	3.6 ± 4.0	<.0001
LpA-I (mg/dL)	59.0 ± 15.9	44.6 ± 13.9	28.3 ± 15.2	<0.0001
LpA-I:A-II (mg/dL)	69.8 ± 20.6	61.4 ± 22.8	19.0 ± 16.8	<0.0001
preβ-HDL (% of apoA-I)	14.1 ± 4.6	16.8 ± 6.4	35.7 ± 16.0	<0.0001
LCAT concentration (µg/mL)	4.92 ± 0.93	4.05 ± 1.37	1.83 ± 0.95	<0.0001
LCAT activity (nmol/mL/h)	38.5 ± 12.5	24.5 ± 12.7	nd	<0.0001

Data are reported as mean±SD or median (interquartile range). nd=not detectable

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Table 2
Demographic and lipid/lipoprotein profile of carriers of ABCA1 and ApoA-I mutations

	ABCA1 mutations		ApoA-I mutations	
	HEZ	HOZ	HEZ	HOZ
n	2	2	4	2
Sex (m/f)	1/1	1/1	2/2	2/0
Age (years)	55.0 ± 1.4	41.0 ± 21.2	52.8 ± 3.2	34.5 ± 3.5
Total Cholesterol (mg/dL)	159.5 ± 3.5	117.5 ± 103.9	153.3 ± 21.0	126.0 ± 91.9
LDL-Cholesterol (mg/dL)	85.5 ± 24.7	79.5 ± 98.3	100.3 ± 15.0	84.0 ± 84.9
HDL-Cholesterol (mg/dL)	29.5 ± 16.3	2.5 ± 2.1	26.8 ± 17.1	11.5 ± 2.1
non HDL-Cholesterol (mg/dL)	130.0 ± 12.7	115.0 ± 101.8	126.5 ± 10.1	114.5 ± 94.0
Triglycerides (mg/dL)	222.5 ± 187.4	177.5 ± 20.5	131.0 ± 81.3	154.5 ± 46.0
Apolipoprotein A-I (mg/dL)	84.0 ± 17.0	10.5 ± 12.0	70.5 ± 31.8	nd
Apolipoprotein B (mg/dL)	97.0 ± 12.7	113.0 ± 73.5	91.5 ± 9.6	75.5 ± 30.4
Lp(a) (mg/dL)	61.0 ± 82.0	39.0 ± 53.7	16.5 ± 21.3	4.5 ± 4.9

Data are reported as mean±SD. nd=not detectable.

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Table 3
Variables associated with circulating PCSK9 levels in the studied population (LCAT deficient carriers and controls)

	PCSK9 (ng/mL)						
	Entire cohort (n=114)		Carriers (n=79)		Controls (n=35)		*P* Interaction
	R	*P*	R	*P*	R	*P*	
Age (y)	0.19	0.047	0.08	0.530	0.17	0.370	
Total Cholesterol (mg/dL)	0.24	0.011	0.20	0.071	0.29	0.090	0.793
Unesterified Cholesterol (mg/dL)	0.23	0.013	0.22	0.049	0.35	0.044	0.304
LDL-Cholesterol (mg/dL)	0.24	0.011	0.21	0.057	0.25	0.150	0.855
HDL-Cholesterol (mg/dL)	0.11	0.246	-0.04	0.733	0.54	0.001	0.017
Non-HDL Cholesterol (mg/dL)	0.19	0.047	0.20	0.07	0.16	0.36	0.34
Triglycerides (mg/dL)	0.02	0.856	0.14	0.224	-0.06	0.729	0.077
Phospholipids (mg/dL)	0.20	0.037	0.24	0.034	0.14	0.440	0.293
Lp(a) (mg/dL)	0.18	0.069	0.09	0.416	0.32	0.080	0.546
Apolipoprotein B (mg/dL)	0.24	0.010	0.26	0.024	0.21	0.237	0.972
Apolipoprotein A-I (mg/dL)	0.05	0.599	-0.01	0.954	0.17	0.324	0.585
Apolipoprotein A-II (mg/dL)	-0.03	0.765	0.01	0.941	-0.27	0.258	0.010
LpA-I (mg/dL)	0.23	0.016	0.11	0.322	0.50	0.002	0.052
LpA-I:A-II (mg/dL)	-0.04	0.669	-0.06	0.611	-0.02	0.905	0.433
preβ-HDL (% of apoA-I)	-0.21	0.027	-0.15	0.204	-0.31	0.074	0.553
LCAT mass (µg/mL)	0.07	0.448	0.03	0.805	0.07	0.702	0.737
LCAT activity (nmol/mL/h)	0.05	0.588	-0.14	0.233	0.46	0.007	0.032

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Figure 1

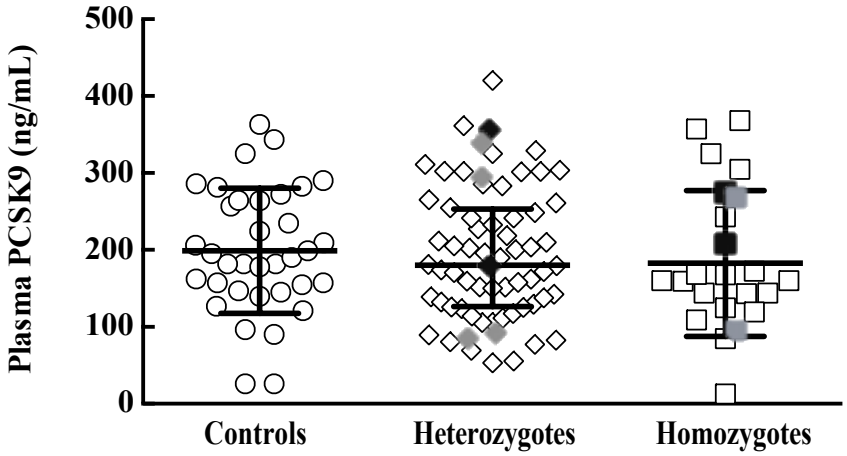

Fig. 1. Plasma PCSK9 levels (ng/mL; median and interquartile range) were measured by ELISA in carriers of *LCAT*, *ABCA1*, and *apoA-I* gene mutations and in non-affected family members (Controls). Empty circles are the controls (n=35); empty diamonds are carriers of one mutation in the *LCAT* gene (n=60); black diamonds are carriers of one mutation in the *ABCA1* gene (n=2); gray diamonds are carriers of one mutation in the *APOAI* gene (n=4); empty squares are carriers of two mutations in the *LCAT* gene (n=19); black squares are carriers of two mutations in the *ABCA1* gene (n=2); gray squares are carriers of two mutations in the *APOAI* gene (n=2).

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Figure 2

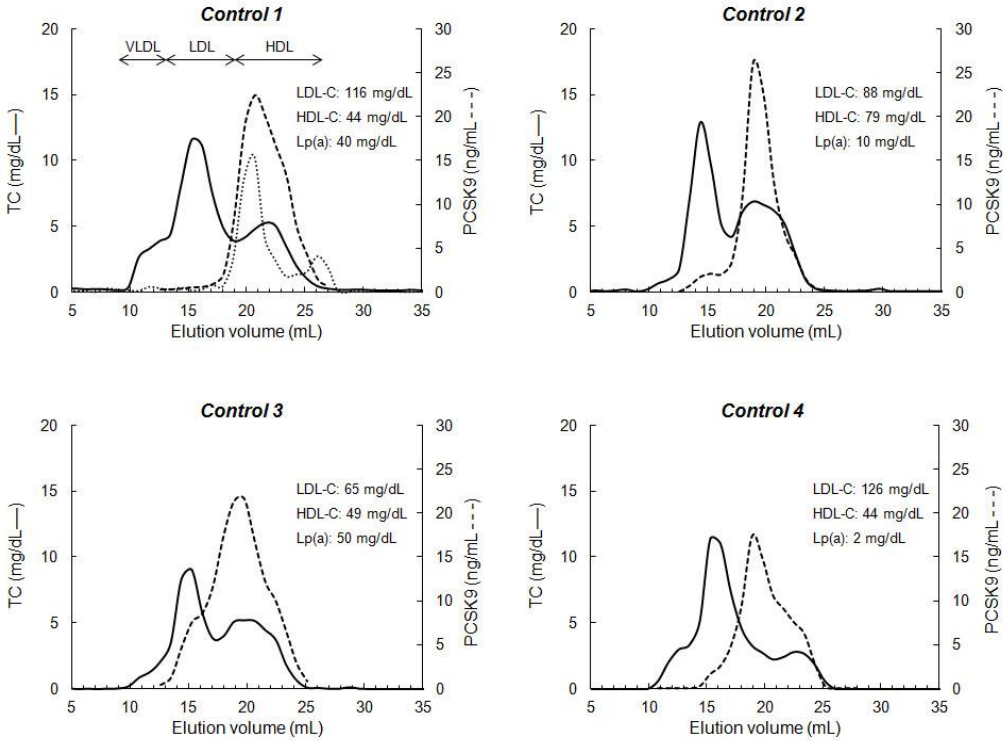

Fig. 2. PCSK9 distribution within plasma lipoproteins in control subjects. FPLC fractions from control plasma were analyzed for total cholesterol (TC) and PCSK9. Profiles of four representative controls subjects, and their plasma lipids, are reported. Medium released from HepG2 cells overexpressing PCSK9 ($HepG2^{PCSK9}$) was fractionated by FPLC (upper left panel, dotted line).

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Figure 3

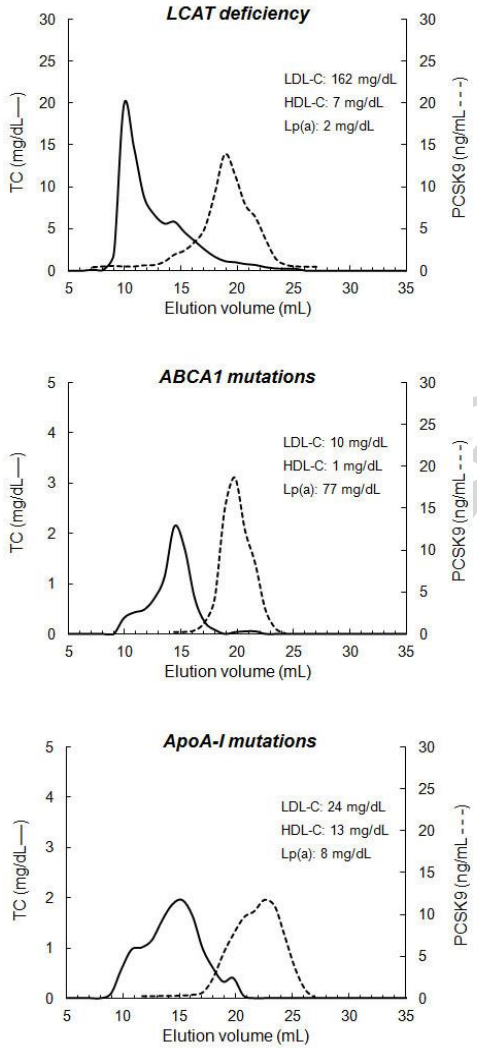

Fig. 3. Lipoprotein profile of carriers of *LCAT*, *ABCA1* and *apoA-I* gene mutations. FPLC fractions from plasma from homozygous carriers of *LCAT*, *ABCA1* and *apoA-I* gene mutations were analyzed for total cholesterol (TC) and PCSK9. Profiles of three representative homozygous carriers, and their plasma lipids, are reported.

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Highlights

- *PCSK9 circulates partially associated to plasma lipoproteins*
- Carriers of genetic HDL defects have significantly reduced plasma HDL levels
- Genetic HDL defects represent a unique tool to study PCSK9 distribution within lipoproteins
- Carriers of genetic HDL defects have normal PCSK9 plasma concentrations
- HDL particles may not contribute to PCSK9 transport in plasma

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