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Original Publication:

Stefan A Ljunggren, Johannes H M Levels, Kees Hovingh, Adriaan G Holleboom, Menno Vergeer, Letta Argyri, Christina Gkolfinopoulou, Angeliki Chroni, Jeroen A Sierts, John J Kastelein, Jan Albert Kuivenhoven, Mats Lindahl and Helen Karlsson, Lipoprotein profiles in human heterozygote carriers of a functional mutation P297S in scavenger receptor class B1., 2015, Biochimica et Biophysica Acta, (1851), 12, 1587-1595.
http://dx.doi.org/10.1016/j.bbalip.2015.09.006


BY NC ND

PrePrint (Unreviewed)
Full peer-reviewed manuscript: doi:10.1016/j.bbalip.2015.09.006.

Lipoprotein profiles in human heterozygote carriers of a functional mutation P297S in Scavenger Receptor class B1

Stefan A. Ljunggren[a] , Johannes H.M. Levels[b], Kees Hovingh[b], Adriaan G. Holleboom[b], Menno Vergeer[b], Letta Argyri[c], Angeliki Chroni[c], Jeroen A. Sierts[b], John J. Kastelein[b], Jan Albert Kuivenhoven[d], Mats Lindahl[e], [§]Helen Karlsson[a]

[a]Occupational and Environmental Medicine Center, and Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden, [b]Department of Vascular Medicine, Academic Medical Centre, Amsterdam, The Netherlands, [c]Institute of Biosciences and Applications, National Center for Scientific Research, "Demokritos", Athens, Greece, [d]Department of Pediatrics, sector Molecular Genetics, University of Groningen, University Medical Center Groningen, Groningen, Netherlands, [e]Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

Email addresses:
helen.m.karlsson@liu.se
stefan.ljunggren@liu.se
H.Levels@amc.uva.nl
G.K.Hovingh@amc.uva.nl
A.G.Holleboom@amc.uva.nl
j.a.kuivenhoven@umcg.nl
mats.lindahl@liu.se
mennovergeer@hotmail.com
j.j.kastelein@amc.uva.nl
achroni@bio.demokritos.gr
jasierts@yahoo.com
largyri@bio.demokritos.gr

[§]Corresponding author

Helen Karlsson
Occupational and Environmental Medicine
Heart Medical Centre
Linkoping University Hospital
SE-581 85 Linköping, SWEDEN
Phone; +46-10-1034414
Email; helen.m.karlsson@liu.se

PrePrint (Unreviewed)
Full peer-reviewed manuscript: doi:10.1016/j.bbalip.2015.09.006.

Abbreviations;
apo– apolipoprotein, CETP - cholesteryl ester transfer protein, CVD – cardiovascular disease, DCF - 2,7-dichlorofluorescein, DCFH - 2,7 dichlorofluorescin, HDL – high density lipoprotein, HDL-C – high density lipoprotein cholesterol, LDL/VLDL – low-/very low-density lipoprotein, SR-B1 - scavenger receptor class B type 1, TBS - Tris-buffered saline, WB – Western blot

Keywords; apoE, apoL-1, HDL, LDL/VLDL, SR-B1, P297S

Highlights

- SR-B1^{P297S} carriers show increased LDL apoE/free apoE compared to family controls
- SR-B1^{P297S} carriers show increased apoL-1 in HDL compared to family controls
- Carriers had equivalent HDL anti-oxidative function and PON1 activity as controls

PrePrint (Unreviewed)
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Abstract

The scavenger receptor class B type 1 (SR-B1) is an important HDL receptor involved in cholesterol efflux, but its physiological role in human lipoprotein metabolism is not fully understood. Heterozygous carriers of the SR-B1^{P297S} mutation are characterized by increased HDL cholesterol levels, impaired cholesterol efflux from macrophages and attenuated adrenal function. In the present study, the composition and function of lipoproteins was studied in relation to the SR-B1^{P297S} mutation.

Lipoproteins from six SR-B1^{P297S} carriers and six family controls were investigated by a proteomic approach. HDL and LDL/VLDL were isolated by ultracentrifugation and proteins were separated by two-dimensional gel electrophoresis and identified by mass spectrometry. In addition, antioxidant properties and paraoxonase-1 activity of HDL was assessed.

Multivariate modelling separated carriers from controls based on lipoprotein composition and plasma lipid/protein data. Specific protein analyses showed a significant enrichment of apoE in LDL/VLDL and of apoL-1 in HDL from heterozygotes compared to controls. The relative distribution of apoE in plasma was increased in LDL and in lipid-free form. There were no significant differences in paraoxonase-1 activity or antioxidant properties of HDL between carriers and controls.

These results indicate that the SR-B1^{P297S} mutation, besides raising plasma HDL cholesterol, also affects both HDL and LDL/VLDL protein composition. The increase of apoE in carriers suggests a compensatory mechanism for attenuated SR-B1 mediated cholesterol uptake by HDL. The results illustrates the complexity of the lipoprotein system that needs to be taken into account in future therapeutic strategies aiming at modulating SR-B1 mediated cholesterol efflux.

1. Introduction

Numerous studies have shown that plasma high-density lipoprotein cholesterol (HDL-C) levels are inversely associated with risk for cardiovascular disease (CVD) [1, 2, 3]. Since there is a need for alternative interventions alongside LDL-lowering drugs these observations have resulted in the exploration of targets, such as cholesterol ester transfer protein (CETP) inhibitors, to raise HDL-C levels [4]. In addition, a central mediator of high-density lipoprotein (HDL) metabolism is the scavenger receptor class B type 1 (SR-B1), initially identified in mice [5]. SR-B1 is a multi-functional receptor, abundantly expressed in liver and steroidogenic tissues [6], and a wide array of native and modified lipoproteins has been shown to interact with SR-B1 [7]. Given its pivotal role in HDL metabolism, SR-B1, like CETP, is considered a possible target for pharmacological modulations [8]. Regarding the role of SR-B1 in CVD, it has been shown to protect from diet-induced atherosclerosis in apoE-/- mouse models [9, 10] while SR-B1 deficient mice with high HDL-C, was associated with increased atherosclerosis and increased expression of inflammatory markers [7]. In line, it has also been shown that a coding variant for SR-B1 (I179N) caused increased atherosclerosis in LDL-receptor knockout mice on a Western-type diet [11]. In humans, variation at the *SCARB1* locus (the gene encoding for SR-B1) has been associated to CVD, albeit in a gender specific way [12, 13, 14]. These findings indicate that a functional SR-B1 receptor may be atheroprotective and that high HDL-C levels resulting from reduced hepatic clearance, by a less functional SR-B1, may not be beneficial. In accordance, a recent review concluded that therapies aimed at adjusting the HDL-C levels have so far failed and that modulation of HDL functions may be a better way to achieve therapies that reduces the risk for CVD [15]. This also raises the question whether HDL derived from carriers of *SCARB1* mutations itself has detrimental properties. In line, it has been postulated that genetic variation in *SCARB1* may give rise to changes in non-lipid pathways which affects the CVD risk, such as inflammation and endothelial function [13].

In humans, there are three described mutations of SR-B1 that causes increased HDL-C. Heterozygous carriers of the first identified mutation SR-B1^{P297S}, were characterized by increased plasma HDL-C, impaired cholesterol efflux from macrophages, altered platelet function and attenuated adrenal function but not increased atherosclerosis [16]. The other two mutations, SR-B1^{S112F} and SR-B1^{T175A}, occupying the same extracellular loop as SR-B1^{P297S}, are also suggested to be less efficient in mediating cholesterol efflux [17]. Interestingly, also

in these two mutations, clinically observed accelerated atherosclerosis was not found [18]. In summary, the alterations in SR-B1 of all three mutations caused elevated HDL-C but regarding metabolic consequences, the knowledge is still limited. Investigating the lipoprotein composition of subjects with functional mutations in the HDL metabolism may be a useful tool for improved understanding. Therefore, we have in the present study investigated the protein composition and function of HDL and LDL/VLDL from the heterozygous carriers of the SR-BI^{P297S} mutation and from family controls.

2. Materials and Methods

2.1. Plasma measurements

Plasma from SR-B1^{P297S} heterozygotes and matched family controls, previously identified by screening for individuals with high HDL-C values [16], was collected in EDTA-tubes after an overnight fast. Total cholesterol and triglycerides levels were measured using standard assays. HDL-C was measured as cholesterol remaining after precipitation of apolipoprotein (apo) B-containing lipoproteins. Informed consent was obtained from all subjects and the study was approved by the ethics committee of the Academic Medical Center in Amsterdam.

2.2. HDL and LDL/VLDL isolation

HDL and LDL/VLDL isolation of six SR-B1^{P297S} heterozygotes (2 males and 4 females, age range 21-67 years) and six family controls (3 males and 3 females, age range 39-78 years) was performed by ultracentrifugation as described [19]. Briefly, plasma was isolated from blood samples collected in EDTA by centrifugation for 10 min in 700*g* at room temperature. EDTA (1mg/mL) and sucrose (5mg/mL) were added to prevent LDL/HDL oxidation and aggregation. Five mL of EDTA-plasma was adjusted to a density of 1.24 g/mL with solid KBr. The plasma samples were layered in the bottom of a centrifuge tube and were gently overlayered with KBr/PBS with a density of 1.063 g/mL. The first ultracentrifugation step was performed at 290 000*g* for 4h at 15°C. HDL, located in the middle of the KBr/PBS solution and LDL/VLDL located at the top of the tube were then collected with a syringe. An additional centrifugation step was performed for 2h in KBr/PBS solution with a density of 1.24 g/mL. The HDL and LDL/VLDL fractions were again collected from the tube with a syringe. All fractions were desalted and protein concentrations were measured with the Bio-Rad protein assay (Bio-Rad, Hercules, CA, US). Samples were lyophilized prior to 2-DE analysis.

Full peer-reviewed manuscript: doi:10.1016/j.bbalip.2015.09.006.

2.3. 2-DE

Proteins were separated on 2-D gels as described previously [20]. Briefly, 400 µg HDL or 300 µg LDL/VLDL proteins were isoelectrically focused on immobilized pH gradient strips (pH 3–10) at 46 000 Vh (max 8 000 V), then separated overnight on homogenous (T=14, C=1.5%) gels and fixed and detected by fluorescent SYPRO Ruby protein stain (Bio-Rad). Gel images were evaluated using PDQuest 2-D gel analysis software, v. 7.1.0 (Bio-Rad). Protein abundance was expressed as percent of total 2-D gel fluorescence, multiple isoforms of proteins added together.

2.4. Mass spectrometry

Protein spots detected on 2-D gels were map-matched [19] and identified with mass spectrometry. Spots were excised manually and subjected to in-gel trypsin digestion. Peptides were analysed with MALDI-TOF MS (Voyager DE PRO, Applied Biosystems, Foster City, CA, US). Peptides were mixed 1:1 with matrix (2,5-dihydroxybenzoic acid) in 70% acetonitrile/0.3% trifluoracetic acid (20 mg/mL). Databases NCBI, Swiss-Prot, and UniProt were searched using MS-Fit as search engine (http://prospector.ucsf.edu). Search criteria were defined as; isoelectric point, molecular weight, human species, mass tolerance <50 ppm, methionine oxidation, and cysteine modification by carbamidomethylation.

2.5. Western blot of apoL-1

For Western blot (WB), 25 µg of HDL proteins were separated with SDS-PAGE and transferred to a PVDF membrane. After blocking 1h (5% milk in tris-buffered saline, TBS) and incubation over night with anti apoL-1 primary antibodies (Abcam, Ab79282, 1:1000 in 2% milk in Tween-TBS), the membranes were incubated for 1h with HRP-conjugated secondary antibodies 1:40 000 (2% milk in Tween-TBS) . Proteins were visualised using ECL plus WB detection system (GE Healthcare, Little Chalfont, UK) and X-ray film. The apoL-1 intensity (optical density/mm^2) was calculated with ImageLab (version 3.0.1, Bio Rad) and the relative quantity of apoL-1 was expressed as percent of the total apoL-1 intensity.

2.6. FPLC-fractionation and Western spot-blot of apoE

Lipoprotein (plasma) fractionations were carried out with equal portions of EDTA plasma from carriers for the SR-BI^{P297S} mutation (n=4) or matched controls (n=4) (total pool of 1 mL respectively). A superose-6 column combined with an ÄKTA explorer 10S system was used

for separation of the lipoprotein fractions (GE Healthcare). The buffer flow was 0.3 mL/min using TBS as flow-buffer (50 mM TRIS, 150 mM NaCl, pH 7.4). A total of 35 plasma fractions (1.0 mL each) of plasma from carriers and controls were collected and further processed for apoE Western spot-blot analysis. Spot-blot analysis was carried out using a 96 wells casting system with a mounted PVDF membrane. Of each fraction, 0.9 mL was blotted on the PVDF membrane by vacuum suction. Subsequently the PVDF membrane was blocked with PBS/0.05% tween-20/Milk 5% (pH 7.4) for 30 minutes and washed between each incubation step with PBS/0.05% Tween-20 (pH 7.4). The first incubation for 2 hours with goat anti-human apoE antibodies (1:1000, Bioconnect) as primary antibody was followed by an 1 hour incubation with anti-goat HRP labeled (1:100000, DAKO) as the secondary antibody, all at room temperature. After the final wash-step the blot was incubated with the detection agent using the ECL staining femto kit (Pierce, Rockford, IL, USA). For reading the apoE chemo luminescence intensity the blot was scanned on a ChemiDoc MP imager (Bio Rad). Values for each fraction were expressed as percent of total apoE staining in all fractions.

2.7. Measurements of anti-oxidant properties and paraoxonase-1 activity

HDL was prepared by the dextran-Mg^{2+} method [21]. The antioxidant properties of HDL were tested in the presence or absence of oxidized LDL as described [22] with some modifications [23]. In short, 2,7-dichlorofluorescin diacetate (Molecular Probes/Invitrogen, Carlsbad, CA, USA) was dissolved in fresh methanol at 2.0 mg/mL and incubated at room temperature for 20 min in the dark, resulting in the generation of 2,7-dichlorofluorescin (DCFH). Upon interaction with oxidants, DCFH is oxidized to fluorescent 2,7-dichlorofluorescein (DCF). Patient and control HDL-C (final concentration 50 μg cholesterol/mL) in the presence or absence of LDL-C (final concentration 100 μg cholesterol/mL) was added into a black 96-well plate in a final volume of 100 μl. The plate was incubated at 37ºC on a rotator for 1 h in the dark. At the end of this incubation period, 10 μl of DCFH solution (0.2 mg/mL) was added to each well, mixed, and incubated for an additional 2 h at 37°C with rotation in the dark. Fluorescence was measured with a plate reader (Fluo-Star Galaxy, BMG) at an excitation wavelength of 465 nm and an emission wavelength of 535 nm.

PON1 activity in HDL, prepared by the dextran-Mg^{2+} method, was determined using paraoxon as substrate [24]. Briefly, the assays were performed in a final volume of 250 μL

containing 5µL of dextran-Mg^{2+}-prepared HDL, 5.5 mmol/L paraoxon (paraoxon-ethyl, Sigma Aldrich), 2 mmol/L $CaCl_2$ and 100 mmol/L Tris-HCl, pH 8.0. The rate of p-nitrophenol formed by hydrolysis of paraoxon was measured by monitoring the increase in absorbance at 405nm for 25min at room temperature in a microplate spectrophotometer. PON1 activity was expressed as U per L. 1U is defined as the activity that catalyzes the formation of 1 µmol p-nitrophenol per min.

2.8. Statistical analyses

To identify alterations important for discrimination between heterozygotes and wild-types, multivariate modeling by Partial least squares (PLS) using the NIPALS algorithm was performed on 2-DE, plasma lipid/protein as well as PON1 activity data in Statistica (Statsoft, Tulsa, OK, USA). Plasma lipid/protein data, 2-DE protein staining and apoL-1 blot were investigated by comparing heterozygotes to wild-type controls with t-test in Statistica. Non-gaussian distributed proteins were log-transformed before t-test. Significant variables were also tested with age-adjusted main effects ANOVA in Statistica. Pearson correlation analysis of apoL-1 staining in 2-DE and WB was performed in Statistica. Anti-oxidant properties and paraoxonase activity were investigated by t-test in Graphpad (Graphpad Software, La Jolla, CA, USA).

3. Results

The SR-B1^{P297S} mutation was found in a family during a study involving screening of individuals with HDL-C above the 95th percentile [16]. The carriers of the novel mutation showed increased plasma HDL-C and apoA-I as well as slightly decreased apoB compared to their family controls. In the present study, a subgroup of heterozygous SR-B1^{P297S} carriers and controls were analyzed showing similar differences in plasma lipid and protein levels (Table 1). For HDL and LDL/VLDL proteomics, 2-DE and mass spectrometry was used and representative protein patterns are illustrated in Figure 1 and protein identities are presented in Table 2.

To study differences in SR-B1^{P297S} heterozygotes compared to wild-type controls, 2-DE protein intensities, plasma lipid/protein data and PON1 activity data were investigated by multivariate modelling with PLS. Two components were used and the score plot showed a clear separation of the individuals in the two groups along the predictive x-axis (Figure 2A,

x-axis; R^2=0.26, Q^2=0.51). The variables with the greatest score contribution (defined as the average sample contributions in each group), and thereby the most important values for separating the carriers from the controls, were apoE in LDL/VLDL and apoL-1 in HDL (score contribution of 1.10 and 1.24 for the heterozygotes, respectively). As illustrated in figure 2B, these two variables were located closest to the heterozygote node in the loading plot. In addition, univariate statistics of the 2-DE data showed a significant increase of apoE in the LDL/VLDL and a significant increase of apoL-1 in the HDL fraction (Table 2, $p<0.05$). Confirming the 2-DE analysis, western blot of apoL-1 in HDL showed a significant increase in the heterozygotes compared to the wild-type controls (Figure 3A-B) and further supporting the data, western blot intensities were positively correlated to the 2-DE intensities (Figure 3C, $r=0.75$, $p<0.05$). Moreover, plasma was fractionated using FPLC and the relative levels of apoE in different lipoprotein fractions were analyzed by western spot-blot. The analysis showed an increase of apoE in LDL and a decrease in small HDL of the heterozygotes as compared to the wild-type controls (Figure 4). Interestingly, an increase in lipid-free apoE was also found.

The in vitro anti-oxidative effect of HDL in SR-B1^{P297S} carriers and controls were measured by incubating HDL with oxidized LDL and the ability of HDL to inhibit oxidized LDL from forming DCF was measured. There were no significant changes in the anti-oxidative effect of HDL in SR-B1^{P297S} carriers compared to the controls (Supplemental figure 1A). The paraoxonase activity of PON1 was measured, and in line with unchanged anti-oxidative capacity, no significant difference between carriers and controls was observed (Supplemental figure 1B).

4. Discussion

Multivariate modeling in the form of PLS was utilized to investigate differences between controls and SR-B1^{P297S} heterozygotes regarding LDL/VLDL and HDL proteomics, plasma lipid/protein data as well as PON1 activity data. The modelling investigates which factors are responsible for separating the groups. This showed that increased levels of apoE in LDL as well as increased levels of apoL-1 in HDL were important for discriminating between heterozygote carriers for SR-B1^{P297S} and wild-type controls. The model also contained plasma lipid/protein data which reflected the apparent increase of apoA-I and HDL-C as well as the decrease of apoB and LDL-C in carriers compared to controls.

ApoE, being an LDL receptor ligand, mediates the cellular uptake of triglyceride-rich lipoproteins [25] and is regarded to have anti-atherosclerotic and anti-oxidative properties [26]. In this study we found an enrichment of apoE in LDL/VLDL from SR-B1^{P297S} heterozygotes, which is in line with higher plasma apoE levels in heterozygotes from the same family [16] and with SR-B1 mutant mice having elevated levels of apoE in LDL [27]. To further study the apoE distribution in the carriers, different plasma fractions was assessed after FPLC size-exclusion chromatography. More apoE was found in the LDL fraction while less was present in the HDL3 fraction (smaller/denser HDL particles) in carriers compared to controls (Fig 3). Interestingly, the FPLC-separation also revealed an increase of lipid-free apoE in the heterozygotes compared to the controls. Free circulating apoE is normally found at very low concentrations in the circulation and it has been proposed that tissues with increased need for cholesterol such as liver and adrenal gland may secrete free apoE to facilitate the SR-B1 mediated cholesterol uptake [28]. Moreover, overexpression of apoE in mice has been shown to increase the uptake of cholesterol through SR-B1 [29]. Enrichment of apoE in LDL/VLDL combined with increased free apoE, as found in the present study, may therefore reflect compensatory mechanisms for attenuated cholesterol uptake in the SR-B1^{P297S} carriers. Notably, in the multivariate model apoC-I in HDL were the lipoprotein with the most negative score contribution (-0.33), which indicates a lower abundance associated to carriers. ApoC-1 is a known inhibitor of SR-B1 and CETP action [30, 31] and a depletion of apoC-I may therefore be a consequence of reduced need for inhibition of SR-B1 pathways in carriers.

The multivariate analysis pointed out apoL-I in HDL as a key component to discriminate heterozygotes from controls regarding lipoprotein patterns. Accordingly, apoL-1 was significantly enriched in HDL from carriers vs controls, which was verified by western blots. As illustrated in figure 4, two 37 kDa bands of apoL-1 were detected by immunoblotting, corresponding to glycosylated and non-glycosylated apoL-1 [32]. In addition a 25 kDa band was detected only in heterozygotes. This may represent a truncated form of apoL-1 but was not included in the quantitative analysis. ApoL-1 is a HDL-associated apolipoprotein which has been reported to exert several extra- and intracellular functions in host defence and hemostatic mechanisms [33, 34]. Although previously mostly studied for its role in trypanosomal lysis [35], recent research has shown polymorphisms in apoL-1 associated to an increased risk for CVD [36]. Thus, indicating a possible, but yet to be identified, function for apoL-1 in CVD development. Notably, apoL-1 plasma levels have been found to be significantly increased in patients with primary cholesteryl ester transfer protein (CETP) deficiency [37], a condition that, like the SR-B1 mutation, results in increased HDL-C levels. The apoL-1 containing HDL subpopulation has previously been shown to be about 10 % of total HDL [34] and apoL-1 is preferentially associated with the more dense HDL3 fraction [19, 38]. In contrast, the SR-B1^{P297S} heterozygotes in the present study have a shift towards more large HDL2 particles [16], and more apoL-1 in HDL is therefore not explained by an increased proportion of HDL3 vs HDL2. Further studies are warranted to clarify the role of apoL-1 in HDL and how enrichment in HDL may affect HDL functionality in SR-BI^{P297S} carriers.

Regarding functional parameters in SR-B1^{P297S} carriers, there were no differences in anti-oxidative or PON1 activity of HDL from SR-B1^{P297S} carriers compared to controls. Similar, heterozygous carriers of a CETP deficiency mutation, also resulting in increased levels of HDL-C, did not show any change in anti-oxidative activity and PON1 activity as compared to family matched controls [39].

5. Concluding remarks

The present study indicates how the functional mutation SR-B1^{P297S} affects lipoprotein composition but not anti-oxidative properties. The increase of apoE in carriers may indicate a compensatory mechanism for attenuated SR-B1 mediated cholesterol uptake by HDL. Investigating lipoprotein composition alterations in subjects with functional mutations may

be a useful tool for improved understanding of metabolic consequences and in the development of future therapeutic strategies.

6. Acknowledgements

This work was funded by EU's Sixth Framework Program: FP6-2005 No. 037631 (HDLomics), European Union (TransCard: FP7-603091-2), CardioVascular Research Initiative (CVON2011-19; Genius), the Research Council of South East Sweden (FORSS-3755), County Council of Östergötland (C-ALF) and Faculty of Health Sciences in Linköping.
Drs Hovingh and Holleboom are supported by Veni grants (project numbers 91612122 and 91613031, respectively) from NWO. Dr. Kuivenhoven is supported by grants of the Leducq Foundation and CVON (GENIUS; #2011-19).

7. Conflicts of interest statement

All authors have filled in ICMJE disclosure form for Potential Conflicts of Interest.
Dr. Kastelein reports personal fees from Cerenis, The Medicines Company, CSL Behring, Amgen, Regeneron, Eli Lilly, Genzyme, Aegerion, Esperion, AstraZeneca, Omthera, Pronova, Vascular Biogenics, Boehringer Ingelheim, Catabasis, AtheroNova, UniQure, Novartis, Merck, Isis Pharmaceuticals, Kowa, Dezima Pharmaceuticals and Sanofi, not connected to the submitted work.

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PrePrint (Unreviewed)
Full peer-reviewed manuscript: doi:10.1016/j.bbalip.2015.09.006.

Table 1. Plasma lipid and protein data.

	Controls (n=6)	SR-B1^{P297S} Heterozygotes (n=6)
Total Cholesterol (mmol/L)	4.7 ± 1.0	4.2 ± 1.3
HDL-C (mmol/L)	1.1 ± 0.3	1.6 ± 0.6
LDL-C (mmol/L)	3.0 ± 0.7	2.5 ± 1.1
Triglycerides (mmol/L)	1.5 ± 0.6	1.0 ± 0.3
ApoA-I (mg/dL)	146 ± 22	177 ± 36
ApoB (mg/dL)	91± 20	66 ± 20

Lipid and lipoprotein levels in SR-B1^{P297S} heterozygotes (n=6) and wild-type family controls (n=6). Values are mean ± SD.

Table 2: Data from protein identification by 2-DE/ MALDI TOF MS and from relative quantification of proteins on 2-D gels.

Protein	Seq. Cov. (%)	No. of pept.	kDa/pI	Controls	SR-BI^{P297S} Heterozygotes
			HDL		
ApoA-I	31.5	14	29/5.4	46.15 ± 7.05	44.16 ± 3.98
ApoA-II	21.0	3	9/6.5	1.08 ± 0.87	0.53 ± 0.84
ApoA-IV	49.7	20	43/5.2	0.27 ± 0.12	0.23 ± 0.11
ApoC-I	65	7	7/7.9	0.13 ± 0.19	0.02 ± 0.03
ApoC-II	50.5	5	9/4.7	0.22 ± 0.29	0.28 ± 0.38
ApoC-III	37.4	4	9/4.7	3.34 ± 2.69	2.82 ± 1.70
ApoE	64	23	34/5.5	1.52 ± 0.95	2.30 ± 1.23
ApoL-1	32	16	41/5.7	0.03 ± 0.04	0.15 ± 0.10*
ApoM	18.1	4	21/5.7	0.17 ± 0.1	0.23 ± 0.17
SAA1	25.4	3	12/5.9	0.13 ± 0.17	0.16 ± 0.18
SAA4	50	10	13/9.2	5.37 ± 2.68	4.26 ± 3.52
TTR	25.2	3	14/5.3	0.20 ± 0.26	0.06 ± 0.07
			LDL/VLDL		
ApoA-I	59.2	20	29/5.4	2.84 ± 2.38	2.88 ± 0.88
ApoC-II	34.7	2	9/4.7	0.06 ± 0.04	0.40 ± 0.52
ApoC-III	37.4	4	9/4.7	2.21 ± 0.54	3.13 ± 0.94
ApoE	64	22	34/5.5	1.59 ± 0.45	8.62 ± 2.91*
ApoJ	18.3	9	50/5.9	0.13 ± 0.06	0.21 ± 0.17
ApoM	23.4	5	21/5.7	1.85 ± 1.39	3.04 ± 0.85
SAA4	32.3	4	13/9.2	1.56 ± 0.43	1.07 ± 0.78

Protein differences in HDL and LDL/VLDL from a family with SR-B1^{P297S} mutation (heterozygotes) compared to matched family wild-type controls. Values are mean ± SD expressed as percent of total 2-D gel fluorescence. * $p < 0.05$ as compared to controls.

Figure texts

Figure 1. 2-DE of HDL and LDL/VLDL in SR-B1^{P297S} carriers and controls.
The 2-DE patterns illustrate increased abundance of apoL-1 in HDL (A) and increased abundance of apoE in LDL/VLDL (B) in heterozygous carriers of SR-BI^{P297S} mutation compared to family controls. 400 µg and 300 µg of proteins were loaded on HDL and LDL/VLDL gels respectively. Proteins were visualized by Sypro Ruby staining and identified by MALDI-TOF MS.

Figure 2. Partial least squares model of HDL and LDL/VLDL 2-DE, plasma lipid/protein data and PON1 activity in SR-B1^{P297S} carriers and controls.
A. Score plot showing the separation between the two groups in the model. X-axis represents the separation between the two groups and the Y-axis individual variation in the two groups. B. Loading plot of the variables showing an association between heterozygotes and proximal variables apoL-1 in HDL as well as apoE in LDL/VLDL. WT-wildtype controls, Het – SR-B1^{P297S} heterozygotes. Apo – apolipoprotein, SAA – serum amyloid A, TTR – transthyretin.

Figure 3. ApoL-1 levels in HDL from SR-B1^{P297S} carriers and family controls.
A. Western blot of apoL-1, 25 µg of HDL loaded in each well. B. Graph representing results from of ApoL-1 Western blot. The values are mean ± SD percent of total apoL-1 intensity, * $p < 0.05$ as compared to controls. C. Correlation between apoL-1 levels determined by 2-DE/Sypro Ruby staining and the Western blot.

Figure 4. ApoE plasma distribution in the main lipoprotein classes.
Fractionation was performed by FPLC of plasma from heterozygous carriers of the SR-BI^{P297S} mutation compared to controls. After spot blot of all fractions (0.9 ml) of carriers (Het) and controls (WT) on PVDF membrane the relative abundance (%) of each apoE fraction of the SR-B1^{P297S} carriers (■) and controls (▲) was determined. The main fractions (VLDL, LDL, HDL and lipid-free) have been indicated in the graph.

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Figures

Fig 1

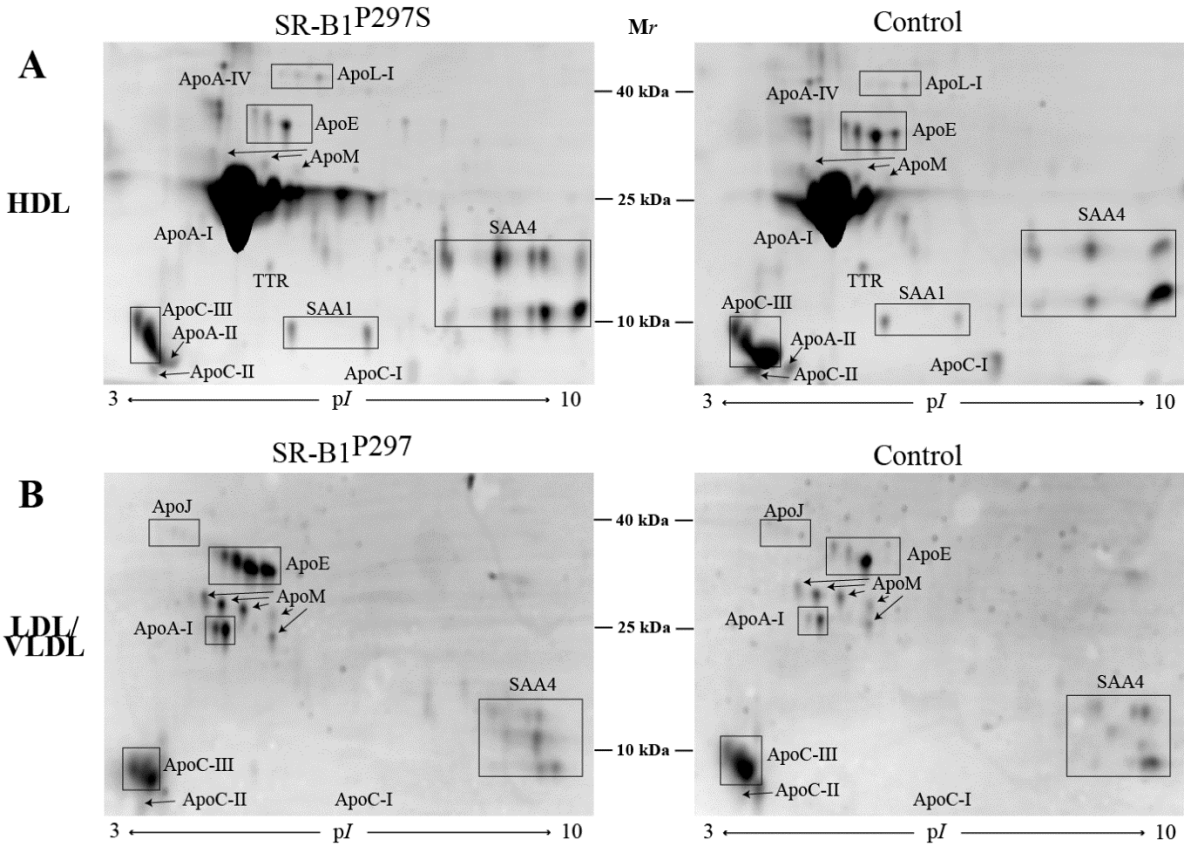

Fig 2

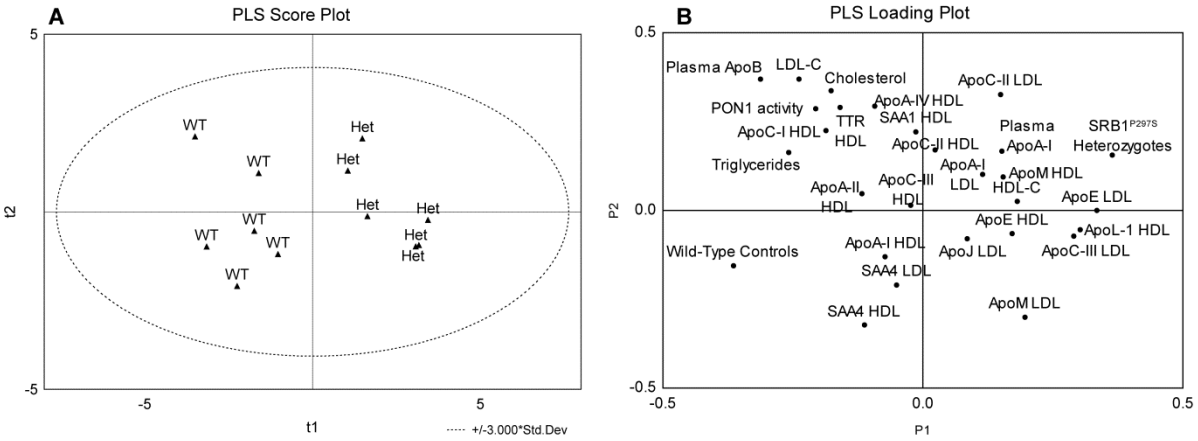

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Fig 3

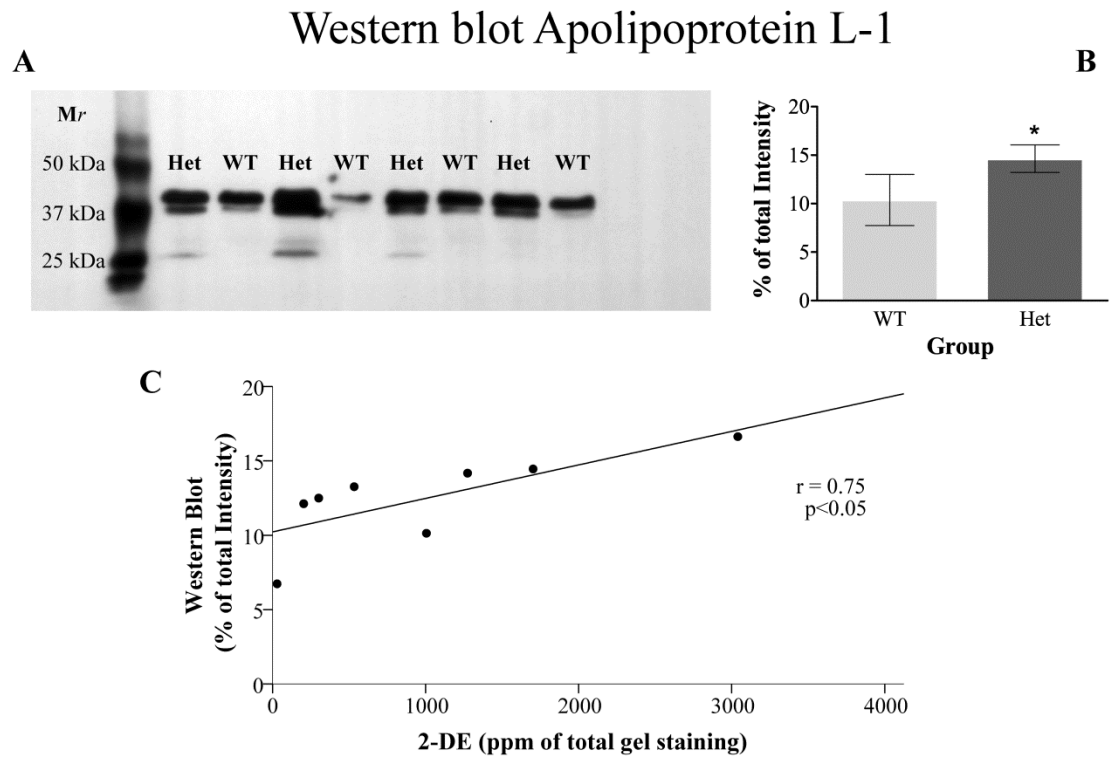
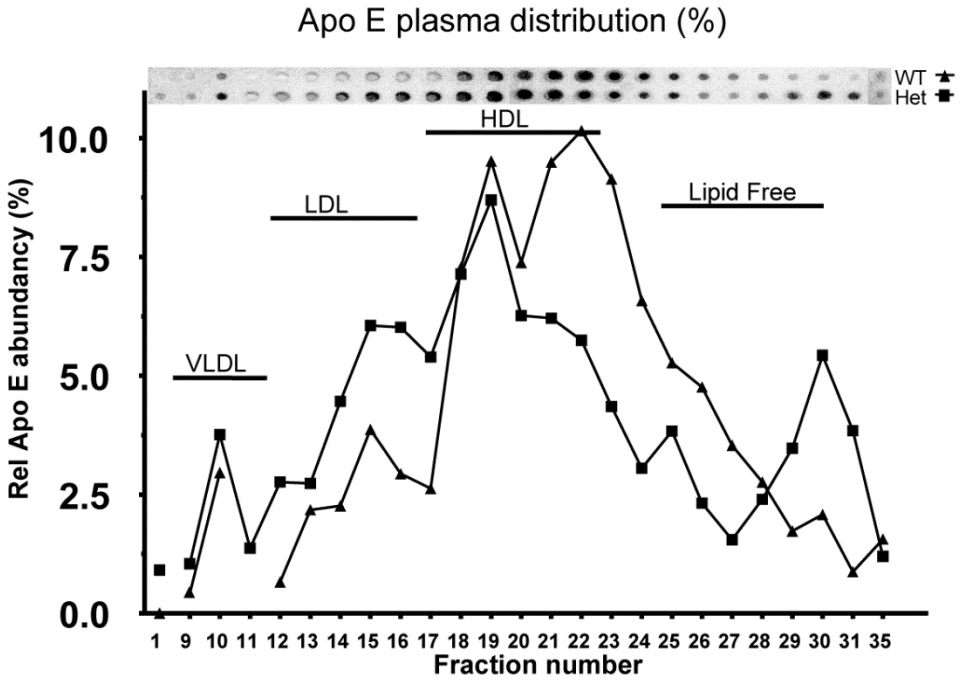


Fig 4



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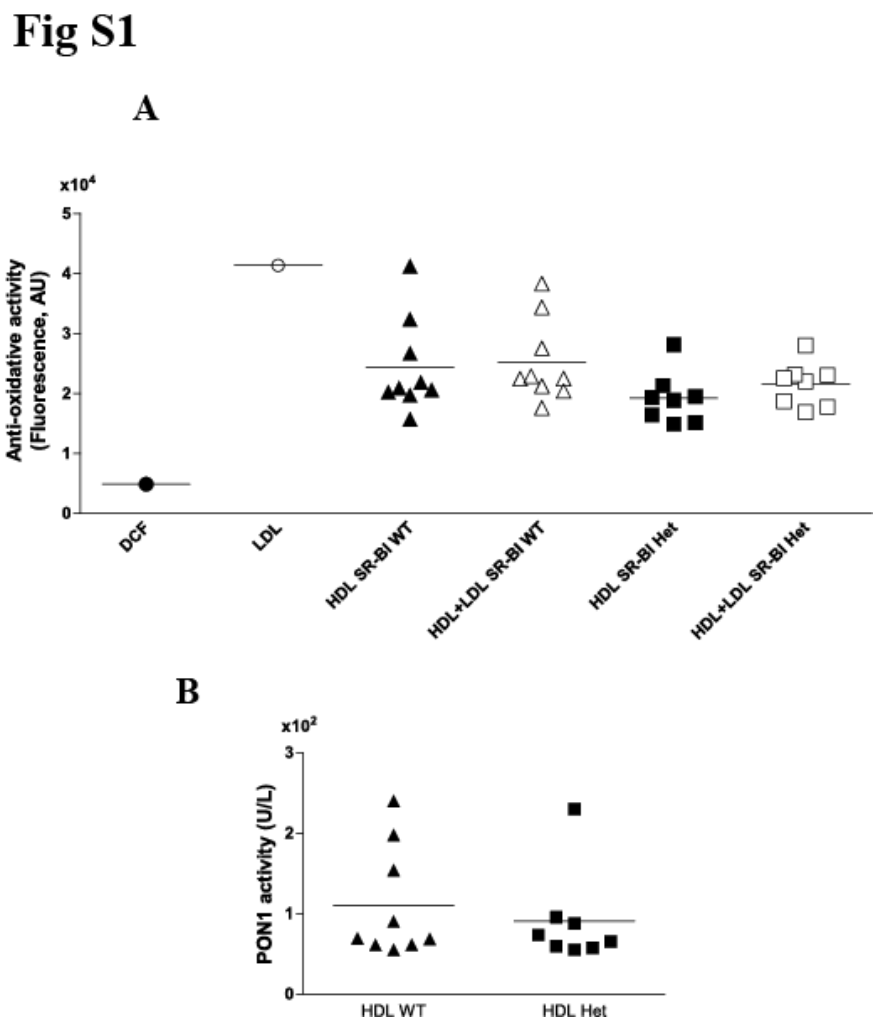
Supplemental Figure 1



Supplemental figure 1. Anti-oxidant properties and PON1 activity of HDL from SR-B1^{P297S} mutation heterozygotes and family controls.

A: Antioxidant properties of HDL analyzed by the DCF assay. The fluorescence intensity resulting from oxidation of DCFH by HDL in the presence or absence of LDL was measured in a spectrofluorometer and expressed in arbitrary units (AU). B: HDL-associated PON1 paraoxonase activity, expressed as units/L (U/L). WT= wild-type control; Het = heterozygote.