HDL cholesterol: atherosclerosis and beyond
Bochem, A.E.

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Increased systemic and plaque inflammation in \textit{ABCA1} mutation carriers with attenuation by statins

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Abstract

Introduction
We previously demonstrated that subjects with functional ATP-binding cassette (ABC) A1 mutations have increased atherosclerosis, which has been attributed to the role of ABCA1 in reverse cholesterol transport. More recently, a pro-inflammatory effect of Abca1 deficiency was shown in mice, contributing to a pro-atherogenic state. In the present study, we evaluated whether ABCA1 deficiency is associated with pro-inflammatory changes in humans.

Material and methods
21 heterozygous and 5 homozygous ABCA1 mutation carriers as well as 21 matched controls were included in the study. PET-CT scanning was performed in a subset of carriers and controls to assess arterial wall inflammation (target-background-ratio, TBR). Plasma cytokines and mRNA expression of inflammatory genes in monocytes were measured in ABCA1 deficient monocytes/macrophages and in control macrophages exposed to plasma from ABCA1 mutation carriers.

Results
ABCA1 mutation carriers had a 21% higher TBR compared to controls (TBR; p=0.004). In carriers using statins TBR was 20% reduced compared to non-statin users (p=0.02). In plasma from ABCA1 mutation carriers, Tumor Necrosis Factor α (TNFα) and Monocyte Chemoattractant Protein-1 (MCP-1) levels showed a gene-dose dependent increase. Macrophages incubated with lipoprotein deficient plasma isolated from ABCA1 mutation carriers showed a gene-dose dependent increase in inflammatory cytokine mRNA expression.

Conclusion
Our data confirm a pro-inflammatory state in ABCA1 mutation carriers as reflected by increased circulating cytokines, most likely secondary to a cellular effect of ABCA1 deficiency. Our findings suggest that the increased inflammation documented in ABCA1 deficient cells and animal models is also present in humans. The increased inflammation in ABCA1 mutation carriers seems to be attenuated by statins, as shown by normalization of PET-CT and plasma cytokine levels.
### Introduction

High-Density Lipoprotein Cholesterol (HDL-C) levels are inversely correlated with cardiovascular risk.\(^1\)\(^-\)\(^3\) However, recent Mendelian randomization studies have called into question whether common variation in HDL cholesterol levels is causally related to cardiovascular disease (CVD) risk.\(^4\) The atheroprotective effects of HDL have traditionally been attributed to its role in reverse cholesterol transport (RCT). The ATP-binding cassette transporter A1 (ABCA1) transporter plays a crucial role in mediating cholesterol efflux from peripheral cells, including arterial wall macrophages, to lipid-poor apolipoprotein A1 (apoAI) or pre-β HDL particles.\(^5\)\(^,\)\(^6\) Homozygous \textit{ABCA1} mutation carriers display near absent HDL-C levels, whereas heterozygous carriers are characterized by half-normal HDL-C. While single nucleotide polymorphisms (SNPs) in the \textit{ABCA1} gene have variously been reported to have no impact on CVD,\(^7\)\(^,\)\(^8\) or to be associated with increased CVD risk,\(^9\)\(^,\)\(^10\) studies in \textit{ABCA1} mutation carriers, displaying marked defects in cholesterol efflux and profound decreases in HDL levels, showed increased arterial wall thickness,\(^11\)\(^,\)\(^12\) and CVD risk\(^9\)\(^,\)\(^10\) in carriers compared to controls.

The paradigm that a macrophage-dominated inflammatory process, that is initiated by the deposition of cholesterol-rich lipoproteins in the arterial wall, is central to atherosclerosis has been widely acknowledged.\(^13\) The molecular mechanisms linking defective cholesterol homeostasis to increased inflammation are not well understood. Recent studies have implicated defective cellular cholesterol efflux pathways in increased inflammatory gene expression in monocytes and macrophages, as well as the increased production of inflammatory cells such as monocytes and neutrophils.\(^14\) Deficiency of ABCA1 and/or ABCG1 is associated with a pro-inflammatory phenotype in mouse peritoneal macrophages as well as in the macrophages of atherosclerotic plaques.\(^14\)\(^-\)\(^17\) Whether ABCA1 deficiency in humans represents a pro-inflammatory state is presently unknown.

In the present study, we assessed whether \textit{ABCA1} mutation carriers are characterized by pro-inflammatory changes of the arterial wall as assessed by PET-CT as well as a systemic pro-inflammatory state. \textit{In vitro} experiments were performed to determine whether the observed changes originate from plasma or a cellular component using plasma isolated from \textit{ABCA1} mutation carriers.

### Methods

#### Study participants

Subjects with low HDL-C levels, defined as HDL-C < 5\(^{th}\) percentile, were selected from a cohort of hypoalphalipoproteinemia patients\(^18\) and screened for \textit{ABCA1} (GenBank No. AF275948) mutations. Family members of \textit{ABCA1} mutation carriers were recruited. Carriers of functional \textit{ABCA1} gene mutations and controls matched for age and gender were enrolled in this study. Body mass index (BMI) was calculated from weight and length. Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg or use of antihypertensive medication. Blood was obtained after an overnight fast and stored at -80 °C. All participants provided written informed consent. The study protocol was approved by the Institutional Review Board at the AMC, The Netherlands.
Genotyping
Mutation detection was performed as published previously. In short, the sequence reactions were performed using a BigDye terminator ABI prism kit (Applied Biosystems, Foster City, CA, USA). Sequences were analysed with the Sequencher package (Gene Codes Co, Ann Arbor, MI, USA).

Plasma processing
Blood samples were collected from all subjects after 12-hour fasting. Plasma cholesterol, low-density lipoprotein cholesterol (LDL-C), HDL-C and triglycerides (TG) were measured using commercially available kits (Randox, Antrim, United Kingdom and Wako, Neuss, Germany). Plasma apolipoprotein AI and apolipoprotein B were measured using a commercially available turbidometric assay (Randox, Antrim, United Kingdom). All analyses were performed using the Cobas Mira autoanalyzer (Roche, Basel, Switzerland). For experiments with apoB depleted plasma, apoB-containing particles were precipitated from plasma by adding 100μl of plasma to 40μl of 20% polyethylene glycol (PEG, Sigma P-2139 in 200mM glycine, pH10) solution. This mixture was incubated at room temperature for 15 min after which it was centrifuged at 4,000rpm for 20 min. The supernatant was removed for use in experiments. This is referred to as PEG-plasma. For experiments with lipoprotein deficient plasma (referred to as LPDS), plasma from 10 controls or ABCA1 mutation carriers, or from 4 TD patients, was pooled, and LPDS was isolated by ultracentrifugation according to Havel and co workers. LPDS was then dialyzed against PBS for use in experiments.

Carotid 18F-FDG PET-CT
Carotid 18F-FDG PET-CT scanning was performed in 16 ABCA1 mutation carriers and 15 controls. Seven of the ABCA1 mutation carriers were using statin therapy. Scans were performed on a Gemini time-of-flight multidetector helical PET/CT scanner (4 min/bed position) (Philips, Best, the Netherlands) as previously published. Subjects fasted for at least 6 h before i.v. injection of 18F-FDG (200 MBq, 5.5 mCi). After 90 minutes of 18F-FDG circulation time subjects underwent PET/CT imaging according to a previously validated acquisition and reconstruction protocol. Mean and maximum standardized uptake values (SUV) were measured in both carotids at 5 mm intervals on axial slices. SUV is a widely used PET quantifier, calculated as a time-corrected and dose-corrected ratio of tissue radioactivity divided by body weight. Then, target to background ratio (TBR) was calculated from the ratio of arterial SUV of right and left common carotid artery compared with the background activity in the jugular veins. Both TBR\text{max}, the mean of maximum SUV values and TBR\text{mean}, the average of mean TBR values derived from every axial section of the vessel were calculated. Images were analysed by two experienced readers, blinded for patient data.

In vitro glucose uptake
Monocytes from controls and ABCA1 mutation carriers were treated with 100nM phorbol myristate acetate (PMA) to facilitate differentiation into macrophages. Cells were cultured
with 40 μM 2-NBDG (a fluorescently-labeled deoxyglucose analog) and washed. Cellular uptake 2-NBDG uptake was measured by means of quantitative flow cytometry.

**Plasma cytokine measurements**

Plasma levels of tumor necrosis factor α (TNFα), and monocyte chemoattractant protein-1 (MCP-1) were measured using ELISA (R&D systems, Minneapolis, USA).

**Carotid magnetic resonance imaging**

Scans were performed as described previously. In short, scans were obtained in a 3.0 Tesla Philips whole-body scanner (Philips, Best, the Netherlands), using a single-element microcoil (Philips, Hamburg, Germany). Ten slices were scanned of the distal 3.0 cm of the left and right common carotid artery. A total of 20 images were obtained per scan. Images were saved in DICOM format using standardized protocols. Quantitative image analysis was performed using semi-automated measurement software (VesselMass, Leiden University Medical Center, the Netherlands). One trained reader, with excellent scan-rescan and intraobserver variability analyzed all the images using standardized protocols for reading and rating images combined with dedicated semi-automated software, blinded for all data of the participants. Mean wall thickness (MWT), lumen area (LA) and outer wall area (OWA) were measured. Normalized wall index (NWI) was calculated as: NWI= MWA / OWA.

**mRNA expression levels**

Total RNA was extracted from human monocytes or macrophages using an RNeasy Micro or Mini kit (Qiagen), respectively. For monocytes, RNA was synthesized using SuperScript VILO and for macrophages using M-MLV (Invitrogen). mRNA levels of TNFα, IL-1β, MCP-1, IL-6, IL-8, and iNOS were assessed using qPCR on on a Stratagene Mx3000P (Agilent Technologies), and initial differences in RNA quantity were corrected for using the housekeeping gene cyclophilin.

**Experiments in THP-1 cells**

THP-1 monocytes were cultured in RPMI 1640 medium supplemented with 10% FBS and 1% pen-strep at 37°C in 5% CO₂. For experiments, cells were plated at a concentration of 5x10⁴ cells/ml and incubated with 100nM PMA for 72 h to facilitate differentiation into macrophages. Macrophages were then washed, and incubated o/n with RPMI 1640 medium containing 2.5% PEG-plasma or LPDS from controls, ABCA1 mutation carriers, or TD patients. Cells were then lysed and RNA was extracted, cDNA synthesized, and mRNA expression assessed as described above.

**Statistics**

All data are presented as means ± SEM. Student’s t-test was used to test for differences between two datasets. To define differences between more datasets, One-way Analysis of Variance (ANOVA) was used with a Bonferroni multiple comparison post-test. The criterion for significance was set at P<0.05. Statistical analyses were performed using GraphPad Prism version 5.01 (San Diego, CA, USA) and PASW statistics 18 (Chicago, IL, USA).
Results

Baseline characteristics

Baseline characteristics of study participants are listed in Table 1. 25 ABCA1 mutation carriers from 14 separate families were included, comprising 3 homozygous 2 compound heterozygous and 21 heterozygous patients. Homozygous and compound heterozygous subjects suffer from Tangier Disease (TD). Subjects were carriers of the following mutations: p.Leu1056Pro, c.3535+1G>C, c.6401+2T>C, p.Asn1800his, p.Ser930Phe, p.Phe1760Valfs*21, p.Ser824Leu, p.Gln1038Ter, p.Thr929Ile, p.Arg587Trp, p.Asn935Ser and p.Arg579Gln. Heterozygosity for these mutations has been shown to impair cholesterol efflux by 40 to 85 %,12,18,26-28

Twenty one controls from the general population were matched for age and sex; statin users were excluded from the control cohort (Table 1). Total cholesterol levels were 21% lower in ABCA1 mutation carriers (p=0.005), largely due to a 50% reduction in HDL-C (p<0.001). ApoAI was correspondingly decreased by 40% (p<0.001). Normalized wall index and mean wall thickness of the carotid arteries were increased in both statin using ABCA1 mutation carriers (p<0.001 and 0.006 respectively), as well as non-statin using ABCA1 mutation carriers (p<0.001 and 0.002 respectively, Supplementary figure 1), consistent with earlier reports.12 Other parameters were not significantly different (table 1).

Vascular PET-CT

PET-CT scanning was performed in a random subset of ABCA1 mutation carriers (n=16) and controls (n=15). In the whole group, TBR was not significantly different in ABCA1 mutation carriers compared to controls (data not shown). However, the average mean TBR of the left and right carotid was significantly higher in non-statin using ABCA1 mutation carriers compared to statin using ABCA1 mutation carriers (p=0.02 for left mean TBR; p=0.008 for right mean TBR, figure 1). After excluding the ABCA1 mutation carriers using statins, the right mean TBR was significantly higher in ABCA1 mutation carriers compared to controls (p=0.01, figure 1), whereas the left mean TBR showed a trend towards an increase (p=0.06, figure 1). Max TBR was significantly higher for the right carotid (p=0.004, figure 1), but not in the left carotid (p=0.12, figure 1). The inverse association between statin use and TBR signal was not observed on 3T-MRI (supplementary Figure 1).

Since the TBR signal depends on glucose uptake in macrophages in the arterial wall29 and ABCA1 has been reported to have a role in glucose uptake,30 we evaluated a potential direct effect of ABCA1 expression on macrophage glucose uptake. However, macrophage-glucose uptake did not differ between ACBA1 mutation carriers and controls (supplementary Figure 2), indicating that the differences in TBR signal cannot be explained by a direct effect of ABCA1 on glucose uptake.

Systemic inflammatory phenotype in ABCA1 mutation carriers

To assess whether the apparent inflammatory phenotype in the arterial wall of ABCA1 mutation carriers also manifested itself systemically, plasma cytokines were measured. Plasma levels of TNFα were significantly higher in homozygous ABCA1 mutation carriers
Increased inflammation in *ABCA1* mutation carriers

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (n=21)</th>
<th>ABCA1 mutation carriers (n=25)</th>
<th>P</th>
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<tr>
<td><strong>Age (years)</strong></td>
<td>51.0±11.3</td>
<td>49.8±14.6</td>
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<td><strong>Male sex, n (%)</strong></td>
<td>9 (43)</td>
<td>11 (44)</td>
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<td><strong>Body Mass Index (kg/m2)</strong></td>
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<td><strong>Smokers, n (%)</strong></td>
<td>2 (10)</td>
<td>4 (16)</td>
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<td><strong>Diabetes, n (%)</strong></td>
<td>1 (5)</td>
<td>2 (8)</td>
<td>0.66</td>
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<tr>
<td><strong>Statin use (%)</strong></td>
<td>0</td>
<td>9 (36)</td>
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Blood pressure

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<td><strong>Systolic (mmHg)</strong></td>
<td>129 (122-138)</td>
<td>139 (132-149)</td>
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<td><strong>Diastolic (mmHg)</strong></td>
<td>80 (74-85)</td>
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<td><strong>Hypertension, n (%)</strong></td>
<td>3 (14)</td>
<td>1 (6)</td>
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Lipid metabolism

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<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td>5.39±0.92</td>
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<td><strong>LDL-cholesterol (mmol/L)</strong></td>
<td>3.49±0.79</td>
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<td><strong>HDL-cholesterol (mmol/L)</strong></td>
<td>1.53±0.40</td>
<td>0.76±0.46</td>
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<td><strong>Triglycerides (mmol/L)</strong></td>
<td>1.01 (0.64-1.42)</td>
<td>1.10 (0.88-1.52)</td>
<td>0.38</td>
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<tr>
<td><strong>Apolipoprotein B (mg/dL)</strong></td>
<td>110.65±21.44</td>
<td>124.89±45.15</td>
<td>0.23</td>
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<tr>
<td><strong>Apolipoprotein A-I (mg/dL)</strong></td>
<td>161.99±19.87</td>
<td>97.13±53.34</td>
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Vessel wall thickness

<table>
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<th>ABCA1 mutation carriers</th>
<th>P</th>
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<tbody>
<tr>
<td><strong>NWI</strong></td>
<td>0.32±0.03</td>
<td>0.38±0.07</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>MWT (mm)</strong></td>
<td>0.66±0.11</td>
<td>0.85±0.21</td>
<td>0.001</td>
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</table>

Values are indicated as mean ± SD unless otherwise indicated. P-value for student’s T-test, compared to control, unless otherwise specified. * p for X2 test. #: median and interquartile range; P for Mann Whitney U test. NWI is mean wall thickness; MWT is mean wall thickness.

versus controls (*figure 2A*). In line with the PET-CT data, TNFα levels were significantly higher in non-statin using *ABCA1* heterozygous mutation carriers compared to statin using heterozygous carriers (*figure 2A*). TNFα levels also appeared to be higher in the non-statin using homozygous carriers compared to statin using homozygous carriers. Plasma MCP-1 levels were dose dependently increased in heterozygous *ABCA1* mutation carriers and homozygous *ABCA1* mutation carriers compared to controls (*figure 2B*). In contrast to TNFα, no effect of statin use was observed (*figure 2B*). IL-1β levels in controls and heterozygotes were too low to detect, even using a high sensitivity IL-1β ELISA. Furthermore, circulating Tangier disease patients’ monocytes showed increased inflammatory gene expression (data not shown).
ABCA1 deficiency and macrophage inflammation

We then investigated whether the decreased HDL levels and the increased inflammatory cytokines in the plasma from ABCA1 mutation carriers and TD patients could contribute to enhanced macrophage inflammation. We thus added LPDS from controls, heterozygous ABCA1 mutation carriers, and TD patients to THP-1 macrophages. LPDS from heterozygous ABCA1 mutation carriers increased TNFα, IL-1β, and MCP-1 mRNA expression, whereas LPDS from TD patients increased mRNA expression of almost all pro-inflammatory cytokines (TNFα, IL-1β, MCP-1, IL-8, and iNOS) (figure 3). These experiments suggest that the increased macrophage inflammation is caused by pro-inflammatory cytokines (e.g. TNFα) present in the LPDS fraction from ABCA1 mutation carriers or TD patients. Subsequently, we studied whether the lower HDL levels in ABCA1 mutation carriers could also affect the inflammatory response in macrophages. Incubation of THP-1 macrophages with apoB depleted plasma from TD patients led to a major increase in mRNA expression of all inflammatory cytokines measured (TNFα, IL-1β, MCP-1, IL-8, iNOS, and IL-6), compared to both controls and heterozygous ABCA1 mutation carriers. PEG-plasma from heterozygous ABCA1 mutation carriers did not affect macrophage inflammation compared to controls (supplementary figure 3). This suggests that the decreased HDL levels in TD patients also have a major contribution to the increased macrophage inflammation and that the ~50% lower HDL-C levels in ABCA1 mutation carriers could be sufficient to reverse the increased inflammatory response induced by the cytokines present in the LPDS fraction.

Figure 1. Increased vessel wall inflammation in ABCA1 mutation carriers. Vessel wall inflammation was assessed by PET-CT in controls (n=15), and ABCA1 mutation carriers without (n=7) and with statin (n=7) treatment. TBR denotes target to background ratio. Data are presented as mean ± SEM. P-values are indicated.
**Discussion**

*ABCA1* mutation carriers displayed both increased vessel wall inflammation as assessed by PET-CT as well as increased systemic inflammation, as reflected by a pro-inflammatory plasma cytokine profile and increased inflammatory gene expression in circulating monocytes. *In vitro* experiments revealed a pro-inflammatory effect of plasma from *ABCA1* mutation carriers, most likely secondary to increased levels of plasma cytokines. Both cellular ABCA1 deficiency and reduced levels of plasma HDL likely contributed to increased monocyte and macrophage inflammatory responses. These data show for the first time that ABCA1 deficiency in humans is associated with increased systemic and plaque inflammation, probably contributing to the increased atherosclerotic plaque volume that has been observed in *ABCA1* mutation carriers.\(^{11,12}\)

\(^{18}\)F-FDG PET/CT has emerged as a reliable technique to non-invasively visualize metabolic activity in the arterial wall in humans.\(^{31}\) Metabolic activity likely reflects the inflammatory state of the arterial wall, since the arterial uptake of FDG has been shown to correlate with circulating inflammatory biomarkers,\(^{32}\) inflammatory gene expression,\(^{33}\)
CVD risk factors, as well as the number of plaque macrophages. We showed that the PET-CT signal in the arterial wall of non-statin using ABCA1 mutation carriers was increased compared to matched controls, paralleling an increase in vessel wall thickness. Although suspected based on studies in macrophages from Abca1−/− mice, this is the first confirmation of an in vivo role for ABCA1 in the suppression of inflammation in humans. The finding of increased vessel wall inflammation in ABCA1 mutation carriers is likely to contribute to their increased cardiovascular risk, since carotid arterial wall FDG uptake has been associated with increased cardiovascular risk, independent of the degree of stenosis.

Interestingly, the increased inflammatory status in the vessel wall of ABCA1 mutation carriers was also manifested systemically, as plasma levels of TNFα and MCP-1 were increased in a gene-dose dependent manner. This is consistent with previous reports, showing that ABCA1 suppresses secretion of IL-1β, IL-6 and TNF-α. Furthermore, mRNA expression of inflammatory markers was increased in Tangier patients’ circulating monocytes, consistent with a systemic pro-inflammatory state. These findings are also consistent with reports that plasma C-reactive protein levels are negatively associated with

Figure 3. Effect of the lipoprotein deficient serum fraction isolated from controls, heterozygous and homozygous ABCA1 mutation carriers on macrophage inflammation. THP-1 macrophages were incubated o/n with polyethylene glycol supernatants from controls (n=13), ABCA1 mutation carriers (n=13), or TD patients (n=4). Samples were pooled, ultracentrifuged, and n=3 samples per condition were used. RNA was isolated and the mRNA expression of TNFα, IL-1β, MCP-1, IL-8, inducible nitric oxide synthase (iNOS), and IL-6 were assessed, corrected for the housekeeping gene cyclophilin. Data are presented as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001.
Increased inflammation in ABCA1 mutation carriers

ABCA1 mRNA levels in human peripheral monocytes. The cross-sectional design of this study precludes us from answering whether plaque inflammation is causal or secondary to the atherosclerosis. However, since the increases in inflammatory mediators in our study are secondary to genetic changes in ABCA1, this suggests the excessive plaque inflammation contributes to increased atherosclerotic burden.

The pro-inflammatory properties of plasma isolated from ABCA1 mutation carriers (figure 3) are likely due to increased circulating cytokines such as TNFα and MCP-1. In addition, the lower levels of plasma HDL-C could contribute to the pro-inflammatory changes. However, as in mouse macrophages, knockdown of ABCA1 resulted in enhanced inflammatory gene expression in either the basal condition or after LPS stimulation. These effects have been attributed to increased plasma membrane lipid raft formation and increased cell surface levels and signalling of TLR4-MD2 complexes.

Impact of statin therapy
Our findings suggest a marked anti-inflammatory effect of statin treatment in humans as determined both by reduced PET-CT signals and decreased circulating levels of cytokines. Although effect of statins on PET-CT signal in atherosclerotic subjects is in line with previous publications, the ~16% decrease in TBR in statin users in our study is larger than the ~10% and ~9% decreases in other reports. This may be explained by the short treatment period of 3-6 months in these intervention trials versus long-term use in our patients or the increased inflammatory status of ABCA1 mutation carriers. The finding of a statin effect on PET-CT, but not on MRI underlines the capacity of PET-CT to visualize inflammation. Libby and colleagues have suggested a specific effect of statins to decrease the uptake of FDG-glucose by macrophages in a hypoxic plaque environment; a contribution of such an effect to our results certainly cannot be excluded. Notably, however, statins also decreased some systemic markers of inflammation strongly suggesting that the decreased PET-CT signal was indicative at least in part of decreased inflammation in plaque.

Conclusion
Our data confirm a pro-inflammatory state in ABCA1 mutation carriers as reflected by increased circulating cytokines, most likely secondary to a cellular effect of ABCA1 deficiency. Our findings suggest that the increased inflammation documented in ABCA1 deficient cells and animal models is also present in humans. The increased inflammation in ABCA1 mutation carriers seems to be attenuated by statins, as shown by normalization of PET-CT and plasma cytokine levels.

Acknowledgements
The research was supported by a grant from Fondation LeDucq. Part of the research was supported by a grant from the Netherlands Heart Foundation (2011-B019: generating the best evidenced based pharmaceutical targets for atherosclerosis (GENIUS)). M. Westerterp has received funding from The Netherlands Organization of Scientific Research (NWO VENI-grant 916.11.072).
Reference List


**Supplementary Data**

**Supplementary Figure 1.** Vessel wall thickness in controls and \(ABCA1\) mutation carriers. Vessel wall thickness was assessed by MRI in controls \((n=21)\), \(ABCA1\) mutation carriers without \((n=16)\) and with \((n=8)\) statin treatment. TBR denotes target to background ratio; NWI normalized wall index, and MWT mean wall thickness. Data are presented as mean ± SEM and p-values for student’s t-test are indicated.

**Supplementary Figure 2.** 2-NBD Glucose uptake in macrophages from controls and \(ABCA1\) mutation carriers. Peripheral blood mononuclear cells were isolated from controls \((n=9)\) and \(ABCA1\) mutation carriers \((n=9)\) and differentiated into macrophages. Uptake of the glucose analogue 2-NBDG was measured using flow cytometry. Each datapoint represents one condition. N.s. is non significant.
Supplementary figure 3. Effect of the apolipoprotein B depleted serum fraction isolated from controls, heterozygous and homozygous ABCA1 mutation carriers on macrophage inflammation. THP-1 macrophages were incubated o/n with polyethylene glycol supernatants from controls (n=13), heterozygous ABCA1 mutation carriers (n=13), or homozygous ABCA1 mutation carriers (n=4). RNA was isolated and the mRNA expression of TNFα, IL-1β, MCP-1, IL-8, inducible nitric oxide synthase (iNOS), and IL-6 were assessed, corrected for the housekeeping gene cyclophilin and normalized to control values. Data are presented as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001.