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Chengjiang LI, Mingzhi XU and Qing GU: ATP Binding Cassette Transporter G1 Gene Expression Is Reduced in Type 2 Diabetic Patients. Released online November 20, 2008.

The above article released online on November 20, 2008 as advance publication was withdrawn because of some errors in presenting the data.
ATP Binding Cassette Transporter G1 Gene Expression Is Reduced in Type 2 Diabetic Patients

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Received July 17, 2008; Accepted November 14, 2008; Released online November 20, 2008

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Abstract. Background: Cholesterol efflux is regulated by cellular cholesterol transporters/receptors such as ATP binding cassette transporters (ABCA1, ABCG1) and scavenger receptor class B type I (SR-BI). The aim of this study was to investigate whether the expression of these transporters/receptor is affected by type 2 diabetes and its functional effects on cholesterol efflux.

Methods: mRNA levels of ABCA1, ABCG1 and SR-BI in peripheral blood monocytes were measured in 20 diabetic patients and 20 age-, gender-matched controls. Their functional effects of cholesterol efflux from monocyte-derived macrophages to serum were also determined.

Results: ABCG1 mRNA expression was decreased in diabetic patients, whereas the levels of ABCA1 and SR-BI were similar between the two groups. Cholesterol efflux from monocyte-derived macrophages to standard serum or autologous serum was significantly reduced in diabetic patients, which were correlated with the expression of ABCG1.

Conclusion: ABCG1 expression in monocytes is reduced in type 2 diabetic patients, which results in the functional effects of the impairment in cholesterol efflux from monocyte-derived macrophages.

Key words: reverse cholesterol transport, cholesterol efflux, ATP binding cassette transporters, scavenger receptor class B type I, type 2 diabetic patients

REVERSE cholesterol transport (RCT) is a pathway that transports cholesterol from extrahepatic cells and tissues to the liver for excretion, and cholesterol efflux represents the first critical step of RCT [1]. Excess lipid accumulation within macrophages in peripheral artery wall may lead to foam cell formation, so cholesterol efflux from macrophages is particularly important with regard to atherosclerosis [1]. Macrophage ATP-binding cassette transporters (ABCA1, ABCG1) and scavenger receptor class B type I (SR-BI) are the three best characterized cellular transporters/receptor that are involved in determining macrophage cholesterol efflux in vitro and in animal models [2-3]. It is widely considered that ABCA1 mediates cholesterol efflux from macrophages to lipid-free apoAI whereas ABCG1 mediates macrophage cholesterol efflux to mature HDL in vitro. SR-BI is expressed in hepatocytes and macrophages and also mediates cholesterol efflux to mature HDL in vitro.

Diabetes is associated with the increased risk of cardiovascular diseases, and there are data suggesting that diabetes may affect these three important transporters/receptor and adversely influence cholesterol efflux. The in vitro effects of glucose remain controversial: some in vitro studies have shown that glucose can activate ABCA1, ABCG1 and SR-BI expression in HepG2 cells during overnight incubation [4-6]. In contrast, ABCG1 was...
down-regulated when macrophages were cultured in elevated glucose after longer incubation for 7 days [7], suggesting the importance of the time course in regulating ABCG1 by glucose. It is critical to explore the potential alteration of ABCA1, ABCG1 and SR-BI under hyperglycemia in vivo. In addition, oxidized LDL (oxLDL), which may also influence these regulators of cholesterol efflux, is increased diabetic state. Several in vitro studies have suggested that oxLDL can up-regulate ABCA1 and ABCG1 expression [8-9] and alter SR-BI expression [10-12]. Whether the expression of ABCA1, ABCG1 and SR-BI in vivo may be regulated oxLDL level is unclear.

Several studies have been carried out in animal models of diabetes. ABCA1 gene expression is severely decreased in the liver and peritoneal macrophages in streptozotocin-induced diabetic mice [5]. In type 2 diabetic db/db mice, ABCG1 expression in peritoneal macrophages is decreased and macrophage cholesterol efflux to HDL is impaired [7]. Hepatic SR-BI expression might be increased [13] or decreased [14] in diabetic rats. However, there are only limited data on these cholesterol transporters/receptor in human studies of diabetes. It has been reported that fasting glucose is negatively associated with ABCA1 level in leukocytes in healthy male subjects [15] and blood monocyte ABCA1 expression is reduced in hyperlipidemic patients with type 2 diabetes [16]. The aims of this study were, therefore, to investigate the expression of ABCG1, ABCA1 and SR-BI in peripheral blood monocytes in type 2 diabetic patients, and to determine whether the alteration of these cellular cholesterol transporters might affect cellular cholesterol efflux from monocyte-derived macrophages ex vivo.

Materials and Methods

20 type 2 diabetic patients with normal renal and liver function and urinary protein<30mg/day were recruited when the patients were admitted to our wards. The blood control of these patients failed to be adjusted to a ideal and stale level at outpatients. Patients on insulin therapy, lipid lowering drugs or thiazolidinediones, and who had currently hyperlipidemia, or had a history of cardiovascular complications were excluded. 20 age-, gender-matched healthy control subjects were recruited from the community. The study was approved by the Ethics Committee of College of Medicine, Zhejiang University, China, and informed consent was obtained from each subject.

Age, gender, weight, height, history of smoking and medications were recorded. Body mass index (BMI) was calculated from the formula of weight/height² (kg/m²). The adjustment of these potential confounding factors was made in comparison of mean difference. Blood samples were taken after an overnight fast. Plasma total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, fasting glucose and HbA1c were collected from medical records. Plasma oxLDL was measured using Mercodia oxidized LDL ELISA kit.

To isolate peripheral blood monocytes, 40 mL fasting blood was collected from each subject using 4mL 3.8% sodium citrate as anti-coagulant. Blood monocytes were isolated using Ficoll-Paque solution (Amersham Biosciences) according to manufacturer’s instruction. Briefly, blood sample was layered carefully on Ficoll-Paque solution, and centrifuged at 400g for 30 minutes at 18-20°C. Mononuclear cells at the interface were collected, washed with sterile PBS for three times and then re-suspended with RPMI 1640 culture medium (Gibco). The mononuclear cells in the medium were added to culture plates and incubated in cell culture incubator for 2-3 hours. Unattached lymphocytes were removed gently. Attached monocytes were used for cellular cholesterol efflux assay in 48-well plate, or stored at -70°C for RNA extraction.

Cellular cholesterol efflux from monocyte-derived macrophages was evaluated as the functional effects of the three cellular transporters/receptor. The attached monocytes were differentiated into macrophages ex vivo by incubating in RPMI 1640 medium containing...
10% autologous serum for 6 days according to Gianturco SH et al. [17]. Then cellular cholesterol was labeled with tracer [3H]cholesterol (1 μCi, 0.01 μg/well) for 24hrs. 5% diluted pooled standard serum or autologous serum was then used to induce cholesterol efflux from the labeled cells for 4 hours. Cholesterol efflux, expressed as percentage, was calculated as count in the medium divided by the total count of medium and cells in each well. Each sample was determined in triplicate.

Total RNA was extracted using Trizol Reagents according to the protocol provided by the manufacturer and quantified spectrophotometrically. cDNA was synthesized using TaqMan® reagents (Applied Biosystems) according to the manufacturer’s instructions. Commercial primer/probe sets for target genes ABCA1 (Hs00194045_m1), ABCG1 (Hs00245154_m1), SR-BI (Hs00194092_m1) and endogenous reference gene GAPDH were used. PCR was performed in 96-well plates using 100ng cDNA mixed with primers, probes and Taqman® Universal PCR Master Mix in a total volume of 50 μL in an ABI PRISM 7700 sequence detector. All cDNA samples were assayed in triplicate. Each target gene and GAPDH was assayed on the same samples in separate tubes. This allowed standardization of the amount of target gene to the internal reference gene to control for different amounts of cDNA used. A standard curve was obtained from a fixed reference sample, and the expression of the target gene was expressed as the folds of the reference sample.

Numerical data were expressed as mean ± SD. Data that were not normally distributed were logarithmically transformed before analyses were made. Mean differences of continuous variables between two independent groups were evaluated using student t-test. Chi-square test was used to compare differences in proportions between groups. Pearson’s correlations were used to test the relationship between variables. P-value<0.05 was considered as statistically significant.

Results

The general characteristics and metabolic parameters of the subjects were shown in Table 1. Age, gender and the proportion of smokers were not significantly different between controls and diabetic patients. BMI tended to be higher in diabetic patients but did not reach statistical significance. The mean duration of diabetes was 10 ± 6.7 years. The diabetic patients had significantly lower levels of plasma HDL cholesterol and apoAI. No hyperlipidemia was found in our diabetic patients: plasma total cholesterol, LDL cholesterol and triglycerides were not significantly different between the two groups. As expected, fasting glucose and HbA1c were significantly higher in diabetic patients. Diabetic subjects had significantly higher concentration of plasma oxLDL compared with healthy controls, despite the similar levels of LDL cholesterol between the two groups.

Peripheral blood monocytes mainly expressed ABCA1 and ABCG1, and the expression of SR-BI was relative low (Fig. 1). There was a significant reduction in the mRNA level of ABCG1 in diabetic patients when compared with healthy controls, whereas the levels of ABCA1 and SR-BI were comparable between the two groups. The reduction in ABCG1 in diabetic patients remained significant after adjusting for age, gender, BMI and smoking status. We performed correlation analyses to see the potential factors in diabetes might contribute to the alteration of these regulators, however, no significant correlations between fasting glucose, HbA1c, plasma lipids or oxLDL and the expression of ABCG1, ABCA1 and SR-BI expression were found (Table 2).

To determine whether the reduction of ABCG1 expression in diabetic subjects might have a functional effect, cholesterol efflux from monocyte-derived macrophages was also measured. Cholesterol efflux from macrophages was measured by incubating [3H]cholesterol labeled cells with a pooled standard serum for 4 hours. Cholesterol efflux from macrophages to the standard serum was decreased in diabetic patients compared with
healthy controls (Fig. 2a). Experiments were also repeated using autologous serum and cholesterol efflux from macrophages to autologous serum was also significantly decreased in diabetic patients (Fig. 2b). The reduction in cholesterol efflux to autologous serum was more marked compared with that to the standard serum in diabetic patients. Cholesterol efflux from macrophages to standard serum or autologous serum was significantly correlated with the expression of ABCG1 (Fig. 3a, Fig. 3b respectively), but not with that of ABCA1 and SR-BI.

**Discussion**

Cellular cholesterol efflux, being the first step in RCT, plays an important role in reducing the accumulation of lipids within macrophages in arterial wall and preventing the development of atherosclerosis. This process is regulated by several cellular cholesterol transporters/receptor, and the best-characterized three of which are ABCA1, ABCG1 and SR-BI. This is the first study to examine the expression of these three major cholesterol transporters in type 2 diabetic patients in vivo and their functional effects in cholesterol efflux from human peripheral blood monocyte-derived macrophages.

We have found that among the three cellular cholesterol transporters/receptor in the human subjects recruited in this study, ABCG1 expression was the most abundant, followed by ABCA1 whereas the level of SR-BI expression was the lowest. In type 2 diabetic patients, ABCG1 expression was significantly reduced whereas the levels of ABCA1 or SR-BI were similar to that in healthy controls. This is consistent with the findings from animal studies showing that peritoneal macrophages isolated from type 2 diabetic db/db mice have decreased ABCG1 expression [7]. Our findings were also consistent with the previous report by Mauldin JP et al. [18] that ABCG1 expression in macrophages differentiated from monocytes in vitro is reduced in patients with type 2 diabetes mellitus. Since the expression of ABCA1, ABCG1 and SR-BI might be altered during the differentiation of monocytes to macrophages in vitro [12, 19-20], we determined mRNA levels of ABCA1, ABCG1 and SR-BI in freshly isolated blood monocytes in order to determine the basal level of gene expression in vivo. Our study also showed that the expression of ABCG1, but not that of ABCA1 and SR-BI, was significantly correlated with cholesterol efflux from macrophages, suggesting the most important role of ABCG1 in the initial step of RCT among the three transporters/receptor.

In contrast to our findings, Forcheron et al. have reported that the expression of ABCA1 in monocytes was decreased in hyperlipidemic type 2 diabetic patients and the level of SR-BI is unchanged, but ABCG1 was not measured in that study [16]. Such conflict findings of ABCA1 expression might be, at least partially, due to the differences in the selection of patients as none of our patients had hyperlipidemia. In a recent study on type 2 diabetic patients without hyperlipidemia, monocytes-derived macrophages ABCA1 mRNA level was similar to that in controls [21]. Cholesterol efflux is a complex process, which includes at least two aspects: one is cellular cholesterol transporters/receptor, another is extracellular cholesterol acceptors. In this study, we mainly focused on cellular aspect, to investigate the possible alteration of ABCA1, ABCG1 and SR-BI in diabetic condition and their functional effects in cholesterol efflux. We chose diabetic patients whose blood glucose was not well controlled, and they were not hyperlipidemic, to reduce the potential confounding factors from extracellular lipoprotein aspect. It has been well known that type 2 diabetic patients are associated with high triglycerides and low HDL. However, plasma triglycerides are easily altered by diet, and clinically we also find that triglycerides decreased quickly when blood glucose is better controlled even without any lipid lowering drugs. This might, at least partially express why the diabetic patients recruited in this study were normolipidemic, although their HbA1c had not been decreased to an ideal level yet.
Due to the limited amount of blood monocytes isolated from each of the subjects, the protein level of ABCG1, ABCA1 and SR-BI in monocytes was not measured in our study. Instead, we have performed functional studies measuring cholesterol efflux from monocyte-derived macrophages to serum to see whether the alteration of these regulators may result in the impact of cholesterol efflux. We have chosen to use serum rather than purified acceptor particles such as apoAI or HDL in these experiments in order to mimic the in vivo situation. The lower ABCG1 expression in patients with type 2 diabetes was correlated with a reduction in cellular cholesterol efflux to serum and the magnitude of reduction in cholesterol efflux was most marked when autologous serum was used. This would suggest that in addition to the reduction in ABCG1 expression in type 2 diabetic patients, changes in the cholesterol acceptors in serum like low HDL levels in these patients might further contribute to the impairment in cholesterol efflux.

We have measured the expression of ABCG1, ABCA1 and SR-BI in peripheral blood monocytes because these cells are easily accessible. However, the expression of these transporters/receptor in peripheral blood monocytes may not directly reflect their levels in macrophages in the arterial wall. Although we did not measure the protein levels of ABCG1, ABCA1 and SR-BI in monocytes, we have performed functional studies to determine whether the alterations in mRNA level were accompanied by the changes in cholesterol efflux.

Although the present data suggested that ABCG1 expression in our diabetic patients reduced and it might lead to the decreased cholesterol efflux from macrophages. Further studies are also required to explore the underlying mechanisms via which ABCG1 expression is reduced in type 2 diabetic patients. It has been shown that macrophage ABCA1 and ABCG1 expression can be induced via the activation of the nuclear receptors liver X receptor (LXR) in vitro [2]. However, the reduction of ABCG1 expression in our diabetic patients might not be mediated by this LXR-dependent pathway because we did not find any association between the expression of ABCG1, ABCA1 and glucose or oxLDL, which are known activators of LXR [6, 8]. This was in contrast to the finding of Mauldin JP et al. [18] that upregulation of LXR dramatically reduced foam cell formation in macrophages from type 2 diabetic patients. TO-901317 is a very strong LXT agonist, whether its in vitro effect in upregulating ABCG1 might implicate LXR pathway in modulating ABCG1 expression in diabetic patients in vivo remains to be determined.

In conclusion, the expression of ABCG1 in peripheral blood monocytes is reduced in patients with type 2 diabetes, which appears to be via an LXR-independent mechanism. The reduction in ABCG1 expression is associated with the impairment in cholesterol efflux from macrophages and these alterations may potentially contribute to the accelerated foam cell formation and atherogenesis in diabetic patients.

References


Table 1. The general characteristics, lipid profile and metabolic parameters of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>Type 2 diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male / female, %)</td>
<td>40 / 60</td>
<td>45 / 55</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47.9 ± 4.6</td>
<td>48.1 ± 5.3</td>
</tr>
<tr>
<td>BMI (kg / m²)</td>
<td>23.7 ± 2.7</td>
<td>25.1 ± 2.9</td>
</tr>
<tr>
<td>Smoking (no / yes, %)</td>
<td>85 / 15</td>
<td>90 / 10</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>-</td>
<td>10.0 ± 6.7</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.07 ± 0.77</td>
<td>4.92 ± 0.63</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.92 ± 0.64</td>
<td>2.97 ± 0.71</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.57 ± 0.38</td>
<td>1.31 ± 0.46*</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.18 ± 0.53</td>
<td>1.30 ± 0.68</td>
</tr>
<tr>
<td>ApoAI (g/L)</td>
<td>1.59 ± 0.25</td>
<td>1.34 ± 0.24†</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>0.87 ± 0.24</td>
<td>0.84 ± 0.27</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>4.78 ± 0.28</td>
<td>8.07 ± 1.05‡</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.41 ± 0.39</td>
<td>7.99 ± 0.93‡</td>
</tr>
<tr>
<td>Plasma oxLDL (mU/L)</td>
<td>9.17 ± 1.26</td>
<td>12.64 ± 1.92*</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD. * P<0.05 vs. healthy controls, † P<0.01 vs. healthy controls, ‡ P<0.001 vs. healthy controls.
Table 2. Simple correlations between fasting glucose, HbA1c, plasma oxLDL, plasma lipids and mRNA expression of ABCG1, ABCA1, SR-BI in monocytes in all the subjects.

<table>
<thead>
<tr>
<th></th>
<th>log (ABCG1)</th>
<th>log (ABCA1)</th>
<th>log (SR-BI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose</td>
<td>-0.02</td>
<td>0.09</td>
<td>0.16</td>
</tr>
<tr>
<td>HbA1c</td>
<td>-0.16</td>
<td>0.09</td>
<td>-0.04</td>
</tr>
<tr>
<td>Plasma oxLDL</td>
<td>-0.03</td>
<td>0.09</td>
<td>0.11</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.02</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Total triglycerides</td>
<td>-0.15</td>
<td>-0.01</td>
<td>-0.1</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.08</td>
<td>0.09</td>
<td>0.07</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.18</td>
<td>-0.03</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Pearson correlation coefficients are shown. All P-value>0.05. ABCG1, ABCA1 and SR-BI mRNA were logarithmically transformed before analysis.
Fig. 1. mRNA expression of ABCA1, ABCG1 and SR-BI in peripheral blood monocytes isolated from healthy controls and type 2 diabetic patients. Data were expressed as mean ± SD. *P<0.05 compared with healthy controls.
Fig. 2. Cholesterol efflux from monocyte-derived macrophages to a standard serum (a) and to autologous serum (b) respectively. Data were expressed as mean ± SD. *P<0.05 compared with healthy controls.
Fig. 3. The association between monocyte ABCG1 mRNA expression and cholesterol efflux from monocyte-derived macrophages to standard serum (a) and autologous serum (b) respectively in all the subjects. ABCG1 expression was logarithmically transformed before analysis.