Peripheral Blood Mononuclear Cell ABCA1 Transcripts and Protein Expression in Acute Myocardial Infarction

Kavita Shalia,^{1*} Dhananjaya Saranath,² and Vinod K. Shah³

¹Sir H. N. Medical Research Society, Sir H. N. Hospital and Research Centre, Mumbai, India

²School of Science, NMIMS (Deemed-to-be) University, Mumbai, India

³Sir H. N. Hospital and Research Centre, Mumbai, India

Background: ATP binding cassette transporter-A1 (ABCA1) facilitates the formation of high density lipoprotein (HDL). HDL due to its anti-atherosclerotic, anti-inflammatory and anti-thrombotic activities provides protection against atherothrombosis or myocardial infarction (MI). The aim was to investigate the role of peripheral blood mononuclear cell (PBMNC) ABCA1 expression in MI. **Methods:** The participants comprised 29 males with acute MI (AMI) and 20 healthy controls. AMI patients were normotensive, not on statins, with triglycerides < 200mg/dl and categorized into AMI with type 2 diabetes (T2DM) (N = 12) and without T2DM (N = 17). The PBMNC ABCA1 mRNA transcripts were analysed by quantitative real-time polymerase chain reaction (qRT-PCR) and protein by enzyme linked immunosorbent assay (ELISA). **Results:** PBMNC ABCA1 mRNA transcript and protein levels were not significantly differ-

ent in AMI patients or when sub-grouped into with/without T2DM, as compared to controls. ABCA1 protein correlated positively with HDL-cholesterol ($r = 0.655$, $p = 0.021$) in AMI patients with T2DM and negatively with age ($r = -0.525$, $p = 0.031$) in AMI patients without T2DM and it was more strongly associated in latter group with smoking and alcohol habit. **Conclusion:** In the present study, the effects of metabolites of diabetes and ischemia were observed on PBMNC ABCA1 protein and thus on HDL-C in AMI patients. Further influence of risk factors such as smoking and alcohol consumption observed in the present study can be evaluated in larger sample size. The control of these cardiovascular associated risk factors may increase stability of PBMNC ABCA1 protein and thus HDL-C levels. *J. Clin. Lab. Anal.* 29:242–249, 2015. © 2014 Wiley Periodicals, Inc.

Key words: ATP-binding cassette transporter A1 (ABCA1); high density lipoprotein; myocardial infarction; gene expression; mRNA transcript; peripheral blood mononuclear cells

INTRODUCTION

A defective cellular cholesterol efflux is associated with an increased risk for atherosclerosis. On the other hand, high density lipoprotein (HDL) protects against development of atherosclerosis by its role in reverse cholesterol transport (RCT) excreting excess cholesterol. ATP-binding cassette transporter A1 (ABCA1) mediates efflux of cholesterol and phospholipids to apolipoprotein A1 (Apo A1) that is the first rate limiting step in RCT process of HDL formation (1). *ABCA1* mRNA is expressed in leukocytes and macrophages, besides placenta, liver, lung, and adrenal gland (2, 3). The clinical implication of ABCA1 has been reported in Tangier's disease and familial HDL deficiency (4–6). In these diseases, defective

ABCA1 protein failed to export cholesterol and phospholipid from cells, and hence unable to form complexes with apolipoprotein to generate HDL (7). These conditions are characterized by low circulating HDL-cholesterol (HDL-C), hypercatabolism of lipid-poor nascent HDL particles,

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*Correspondence to: Dr. Kavita Shalia, Ph. D. (Biochemistry), Research Scientist, Sir H. N. Medical Research Society, Sir H. N. Hospital and Research Centre, Court House, Dhobi Talao, Mumbai 400002, India. E-mail: kavita.shalia@hnhospital.com

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lipid laden macrophages, and predisposition to coronary artery disease (CAD) (4–6). Thus ABCA1 may indirectly relate to the pathophysiology of atherosclerosis.

Studies with ABCA1 transgenic and knock-out mice have indicated association of overexpression with high HDL-C levels and deletion with atherosclerosis (8–10). Studies on ABCA1 gene and protein expression in atherosclerotic tissues have demonstrated increased mRNA transcript and decrease in ABCA1 protein showing discordance between ABCA1 gene and protein expression (11, 12). Latter study by Albrecht et al. have also analyzed ABCA1 expression from PBMNCs in atherosclerotic patients and have reported no difference from controls (12) while Demina et al. have demonstrated reduction in ABCA1 expression in patients with atherosclerosis (13).

Diabetes, metabolic syndrome, and hypertension are associated with increased cardiovascular risk leading to atherosclerosis (14). Besides, reduced levels of HDL-C are associated with diabetes and metabolic syndrome (15). A severe clinical manifestation of coronary atherosclerosis is atherothrombosis resulting in myocardial infarction (MI). HDL imparts anti-oxidative, anti-inflammatory, and anti-thrombotic functions and thus is protective to atherothrombosis or MI (16, 17). Low HDL-C is associated with increased risk for MI, stroke and severe premature atherosclerotic disease (18, 19). As ABCA1 is critical in HDL formation, its expression may have influence on MI. The present study was undertaken to analyze expression of *ABCA1* gene in the PBMNCs in MI, via analyzing the mRNA transcript and protein levels in Acute MI (AMI) patients.

MATERIAL AND METHODS

The study population included male AMI patients ($N = 29$) and sex and age matched healthy individuals ($N = 20$) as controls. The AMI patients demonstrated prolonged chest pain for more than 30 min and ST elevation of more than 0.5 mv on at least two adjacent leads, and were admitted in the emergency department of cardiac unit. The AMI patients had no history of hypertension but some were associated with type 2 diabetes mellitus (T2DM) and were treated for the same. The patients were categorized into two groups: AMI patients with ($N = 12$) and without ($N = 17$) T2DM. The patients were not on prior lipid lowering therapy. On admission these patients were treated with thrombolytics, antiplatelets, anti-anginals, and anti-diabetic medications. Patients with valvular heart disease, cardiomyopathy, malignancy, renal, or liver disease were excluded from the study. Controls were healthy individuals with normal ECG and carotid doppler. All groups had triglyceride levels <200 mg/dl.

Overnight fasting blood sample was collected in vacutainer tube with Na-ethylenediaminetetraacetic acid anticoagulant (9 ml) for PBMNCs separation and in vacutainer tube without anticoagulant (4 ml) for biochemical tests. Thus, only those selected AMI patients were recruited whose overnight fasting blood could be collected prior to the administration of first dose of statin, on the day of the admission in the emergency cardiac unit over a period of 5 months. Informed consent was obtained from all the study participants. Institutional Ethics Committee of Sir H. N. Hospital and Research Centre and Rajawadi Municipal Hospital approved the study protocol.

Quantitative Real-Time PCR Assay for ABCA1 Transcript

RNA isolation and cDNA preparation

PBMNCs from blood were isolated using Histopaque®-1077 (Sigma, St Louis, MO, USA). Total RNA was extracted from PBMNCs using TRI Reagent® (Ambion, Life Technologies, USA). Total RNA ($1.5 \mu\text{g}$) was reverse transcribed using High Capacity® cDNA Reverse Transcription kit as per manufacturer's instructions (Applied Biosystems, Foster City, CA, USA).

Quantitative real-time polymerase chain reaction

Quantification of *ABCA1* mRNA transcripts was carried out by quantitative real-time polymerase chain reaction (qRT-PCR) using TaqMan® Gene Expression Assay on demand, with β -Actin as internal control (Applied Biosystems, Foster City, CA, USA). The system used TaqMan® MGB probes as quencher and FAM™ dye-labeled reporter. The qRT-PCR reaction mixture consisted 1X buffer, 1X Assay on Demand (primers and probes) and 200 ng of cDNA in a final volume of $20 \mu\text{l}$. The samples were analyzed in duplicates using Step One Plus RT-PCR system (Applied Biosystems, Foster City, CA, USA). The cycling conditions constituted initial denaturation at 95°C for 10 min, followed by 40 cycles each of 15 sec at 95°C and 1 min at 60°C . The amplification efficiencies of the target and reference were approximately equal with slope of log input amount to cycle threshold (C_t) <0.1 .

The expression of *ABCA1* mRNA was quantitated using comparative C_t method as described in PE User Bulletin 2. Patients C_t data were normalized to internal control (ΔC_t) and comparative *ABCA1* mRNA expression was analyzed with respect to matched controls to give the $\Delta\Delta C_t$. The relative *ABCA1* gene expression or *ABCA1* mRNA transcript was calculated for each sample as $2^{-\Delta\Delta C_t}$. Volcano plot-based method was further used to calculate the fold change of *ABCA1* mRNA transcript in the patient group in comparison to control group.

using GeneSpring GX v 12.0 (Agilent Technologies, Santa Clara, USA). The Ct value being a numerical value, the fold change of *ABCA1* mRNA transcript is unit less and expressed as *n* fold relative to the control.

ABCA1 protein estimation

Total cell protein was extracted from PBMNCs using Mammalian Protein Extraction Reagent (MPER; Thermo Scientific, Rockford, IL, USA). Quantitation of total protein was carried out by Coomassie Bradford™ Assay (Thermo Scientific, Rockford, IL, USA).

Enzyme linked immunosorbent assay (ELISA) for estimating ABCA1 protein

ABCA1 protein was estimated by indirect ELISA. The primary antibody (0.5 mg/ml) was rabbit polyclonal to ABCA1 (ab14146, ABCAM, USA) and secondary antibody (2 mg/ml) was HRP conjugated goat polyclonal to Rabbit IgG (ab6721, ABCAM, USA). Optimal assay conditions were determined by using different protein concentrations (10–70 µg/ml), four different dilutions each of primary antibody (1:500; 1:1,000; 1:2,000; 1:5,000) and secondary antibody (1:1,000; 1:5,000; 1:10,000; 1: 50,000) in a checker board format.

Statistical Analysis

Results are expressed as mean ± SD for continuous variables and numbers with percentage for categorical variables. For continuous and categorical variables, the analysis of significance of difference between two groups was performed by student's unpaired *t*-test and by chi square test, respectively. Correlations were evaluated by Pearson correlation test. A *P* value of <0.05 was considered statistically significant. Analyses were performed using statistical software SPSS (version 21.0, Chicago, IL). The statistical significance of the fold change of ABCA1 mRNA transcript of patient group as compared to control group was calculated by applying unpaired student *t*-test and false discovery rate that was calculated using Benjamini–Hocheberg test.

RESULTS

The demographic data and risk factors of study population are depicted in Table 1. The AMI patients and controls did not differ significantly in age, BMI, and waist to hip ratio. Within AMI group there were 17 smokers while in control group there were no smokers. The percentage distribution of subjects with alcohol consumption in AMI group did not differ significantly from controls. Biochemical investigations are depicted in Table 2. There was no

significant difference in lipid profile of AMI group except in HDL-C that was significantly decreased (13.48%, *P* = 0.015) as compared to controls. Fasting sugar levels of AMI patients were significantly elevated by 16.2% (*P* = 0.015). AMI patients with T2DM demonstrated significant decrease in HDL-C (17.8%, *P* = 0.012) and significant increase in fasting blood sugar (34.4%, *P* = 0.002). AMI patients without T2DM had similar demographic and biochemical profile as controls. The decrease in HDL-C was 11.2% that did not reach statistical significance of *P* < 0.05.

ABCA1 mRNA transcript

We observed no upregulation or downregulation of ABCA1 mRNA transcript in AMI patients as depicted in the fold change of AMI group (1.06 ± 0.85 , *P* = 0.74), AMI group with T2DM (-1.15 ± 0.87 , *P* = 0.31) and AMI group without T2DM (1.19 ± 1.22 , *P* = 0.36) as compared to controls.

ABCA1 protein ELISA: Optimization of the assay

ABCA1 protein estimated by indirect ELISA, using a checker board format comprising with 10–70 µg/ml of total cell protein extract, dilutions of primary antibody (1:500 to 1:5,000) and secondary antibody (1: 1,000 to 1: 50,000), indicated optimal absorbance of 0.565 at 450 nm using 50 µg/ml total cell protein extract, primary antibody dilution of 1:1,000 (50 pg/100 µl) and secondary antibody dilution of 1:5,000 (40 ng/100 µl). Thus, 50 µg/ml total cell protein extract of each sample was subjected to ABCA1 protein quantitation with the above said primary and secondary antibody dilutions. The absorbance of control samples ranged from 0.531 to 0.951 with mean 0.79 ± 0.12 . The ABCA1 protein of all AMI patients (0.79 ± 0.14), AMI patients with T2DM (0.71 ± 0.11) and AMI patients without T2DM (0.85 ± 0.14) did not differ significantly from controls. The median of ABCA1 protein of each group was similar to their respective mean (data not shown).

Clinicopathological correlation of ABCA1 expression

In the control group, ABCA1 mRNA transcript or protein did not show significant correlation with age, BMI, Waist/Hip ratio, fasting sugar, and serum lipid levels. Whereas, the AMI group demonstrated a positive correlation between ABCA1 protein and serum HDL-C (*n* = 29, *r* = 0.478, *P* = 0.009) (Fig. 1A). This correlation was also observed in AMI patients with T2DM (*n* = 12, *r* = 0.655, *P* = 0.021) (Fig. 1B). AMI patients without T2DM demonstrated significant negative correlation (*n* = 17,

TABLE 1. Demographic and Risk Factors of AMI Patients With and Without T2DM

Parameters	AMI patients [n = 29]	AMI patients with T2DM [n = 12]	AMI patients without T2DM [n = 17]	Control group [n = 20]
Age [years]	47.24 ± 9.58 ^{NS}	45.58 ± 8.76 ^{NS}	47.78 ± 10.28 ^{NS}	45.4 ± 13.86
BMI [Kg/m ²]	24.88 ± 3.33 ^{NS}	25.24 ± 3.03 ^{NS}	24.6 ± 3.48 ^{NS}	23.63 ± 3.54
Waist/Hip	0.922 ± 0.0303 ^{NS}	0.926 ± 0.029 ^{NS}	0.919 ± 0.0310 ^{NS}	0.91 ± 0.053
SBP [mmHg]	127.03 ± 0.77 ^{NS}	126.2 ± 10.1 ^{NS}	126.56 ± 12.05 ^{NS}	121.59 ± 7.81
DBP [mmHg]	86.05 ± 7.72 ^{NS}	87.5 ± 6.21 ^{NS}	84.78 ± 8.49 ^{NS}	79.76 ± 5.20
Risk factors smokers	17 (58.62%) <i>P</i> = 0.001	5 (41.7%) <i>P</i> = 0.01	12 (70.0%) <i>P</i> = 0.001	0
Alcohol intake	11 (37.93%) ^{NS}	2 (16.7%) ^{NS}	9 (52.9%) ^{NS}	5 (25.0%)

Variables are expressed as mean ± standard deviation.

Risk factors are expressed as number of positive cases and percentage in the parenthesis; NS, nonsignificant.

$r = -0.525$, $P = 0.031$) between ABCA1 protein and age (Fig. 2).

DISCUSSION

The aim of the current study was to investigate the role of PBMNC ABCA1 in AMI patients. Our study demonstrated no significant difference in ABCA1 mRNA transcripts and protein levels in the PBMNCs of AMI patients per se or on categorizing them as with/without T2DM. However, a positive correlation between ABCA1 protein and serum HDL-C levels in AMI patients with T2DM was observed. Whereas, AMI patients without T2DM, demonstrated a negative correlation between PBMNC ABCA1 protein and age of the patients. Both the patient subgroups had decrease HDL-C levels and it was significant in the AMI patients with T2DM.

There is a dearth of information on PBMNC ABCA1 in AMI patients with/without T2DM. Demina et al. (13) have postulated that activation of PBMNCs, dur-

ing AMI could lead to altered gene expression. One of the alterations is activation of nuclear factor kappa b (NFκB); after reperfusion in AMI (20, 21). Moreover, hyperglycemia through advance glycation end product (AGEs) is also shown to activate NFκB (22). There are experimental evidences that suggest that NFκB pathway leads to the downregulation of ABCA1 expression (23, 24). However, in the present study the ABCA1 gene expression in AMI patients with as well as without T2DM did not differ from controls. AMI patients with T2DM of the present study were on anti-diabetic medications such as combination of Glimepiride and Metformin wherein Glimepiride are sulphonylureas—insulin secretagogues that target β-cell dysfunction while Metformin is a biguanide that reduces hepatic glucose production and improves insulin sensitivity. In vitro study has been shown that at lower doses Glimepiride did not have unfavorable effects on ABCA1-mediated cholesterol efflux (25) while Metformin, inhibitor of NFκB (26) has been shown to restore ABCA1 gene and protein expression (23). These

TABLE 2. Biochemical Data of AMI Patients With and Without T2DM

Parameters	AMI patients [n = 29]	AMI patients with T2DM [n = 12]	AMI patients without T2DM [n = 17]	Control group [n = 20]
Fasting sugar [mg/dl]	113.2 ± 28.65, <i>P</i> = 0.032	133.5 ± 31.24, <i>P</i> = 0.002	99.33 ± 15.09 ^{NS}	97.41 ± 19.44
Total cholesterol (TC) [mg/dl]	177.59 ± 36.1 ^{NS}	170.1 ± 47.46 ^{NS}	180.50 ± 26.91 ^{NS}	186.12 ± 30.64
Triglyceride [mg/dl]	120.7 ± 61.6 ^{NS}	126.8 ± 79.22 ^{NS}	114.11 ± 47.29 ^{NS}	98.5 ± 33.6
HDL-C [mg/dl]	38.93 ± 7.27, <i>P</i> = 0.015	37.0 ± 7.72, <i>P</i> = 0.012	39.55 ± 6.79 ^{NS}	45.0 ± 7.92
Low density lipoprotein-C (LDL-C) [mg/dl]	114.5 ± 30.52 ^{NS}	107.7 ± 39.7 ^{NS}	117.73 ± 22.40 ^{NS}	121.4 ± 27.1
Very low density lipoprotein-C (VLDL-C) [mg/dl]	24.14 ± 12.32 ^{NS}	25.36 ± 15.8 ^{NS}	22.82 ± 9.45 ^{NS}	19.7 ± 6.72
LDL-C/HDL-C	3.02 ± 0.78 ^{NS}	2.92 ± 0.90 ^{NS}	3.00 ± 0.76 ^{NS}	2.76 ± 0.76
TC/HDL-C	4.65 ± 0.92 ^{NS}	4.66 ± 1.23 ^{NS}	4.61 ± 0.91 ^{NS}	4.2 ± 0.86
ApoA1 [g/l]	1.05 ± 0.19 ^{NS}	0.98 ± 0.13 ^{NS}	1.03 ± 0.19 ^{NS}	1.07 ± 0.24
ApoB [g/l]	1.10 ± 0.25 ^{NS}	0.91 ± 0.30 ^{NS}	1.08 ± 0.26 ^{NS}	1.1 ± 0.25
ApoB/A1 ratio	1.10 ± 0.39 ^{NS}	0.92 ± 0.27 ^{NS}	1.09 ± 0.38 ^{NS}	1.05 ± 0.29

Variables are expressed as mean ± standard deviation; NS, nonsignificant.

Fig. 1. (A) Correlation of ABC1 protein with HDL-C in AMI patients. (B) Correlation of ABCA1 protein with HDL-C in AMI patients with T2DM.

AMI patients are also on antiplatelet medications such as Ecosprin that contains the active ingredient Aspirin (acetyl salicylic acid). In vitro studies have demonstrated that acetyl salicylic acid induced the expression of ABCA1

at both mRNA and protein levels (27, 28). Thus in the present study, an additional effect of these medications, independent of the anti-diabetic and anti-platelet role, may be responsible for returning the ABCA1 expression of the

Fig. 2. Correlation of ABCA1 protein with age in AMI patients without T2DM.

AMI patients with and without T2DM to normal levels. Supporting our findings there is a report by Hoang et al. (29) demonstrating no variation in leukocyte ABCA1 gene expression in T2DM patients who were receiving hypoglycemic medications as well as reports from Patel et al. (30), and Forcheron et al. (31) who have reported reduction in ABCA1 gene expression and/or protein concentrations in PBMNCs of T2DM patients not on any anti-diabetic medications. Patel et al. (30) have further reported reduced ABCA1 expression with increased fasting sugar levels. In vitro culture and animal studies demonstrating reduction in ABCA1 expression due to hyperglycemia have been documented (31–35). However, one in-vitro study by Senanayake et al. (36) reported no influence of high levels of glucose in the medium on ABCA1 expression in leukocytes.

Although in the present study, ABCA1 gene and protein expression of AMI patients did not differ from controls, AMI patients with T2DM demonstrated a positive correlation between PBMNC ABCA1 protein and serum HDL-C levels. This is in concordance with the report of Patel et al. (30) but in contradiction to the data indicating minimal contribution of PBMNC ABCA1 to plasma HDL levels (12, 37). Metabolites like free fatty acids elevated in individuals with diabetes (38) or released in patients with intermittent ischemia due to atherosclerosis (39) have been reported to destabilize ABCA1 protein by enhancing its degradation (40, 41), while AGEs have been demonstrated to strongly inhibit ABCA1-dependent transport of cholesterol from cells to Apo A1 causing decrease in HDL-C levels (42). In the present study, direct correlation between ABCA1 protein and HDL-C level was observed in the AMI patients with T2DM, and it was more pronounced in these patients without smoking and alcohol habits ($n = 8$, $r = 0.865$, $P = 0.012$). As HDL-C (35.43 ± 6.73 mg/dl) was reduced by 21.3% in these T2DM AMI patients, it suggests influence of diabetic metabolite on ABCA1 protein stability and thus HDL-C formation. In AMI patients without T2DM, a negative correlation of ABCA1 protein with age was observed in our study, suggesting aging related defect in these patients. These AMI patients although without T2DM have components such as AGEs that accumulate during aging (43) and free fatty acids (39) released due to severe ischemia. These factors affect ABCA1 protein (40–42) and cause decrease in HDL-C in AMI as compared to controls. This supports HDL-C reduction post-AMI as compared to prior AMI, via ABCA1 protein. However, accumulation of free fatty acids and AGEs is enhanced in diabetes (38) that may cause additional decrease in ABCA1 protein. This could be the reason for not observing negative relationship between ABCA1 protein and age in AMI patients with T2DM of the present study.

A similar negative correlation between ABCA1 gene expression and age ($r = -0.39$, $P = 0.043$) has been documented by Albrecht et al. in healthy normoglycemic and nonsmoking men (44). However, in the present study, correlation between ABCA1 protein and age was strongly observed in the patients who were smokers and consumed alcohol ($n = 7$, $r = -0.807$, $P = 0.028$). The effect of alcohol is however, to raise HDL-C levels that recently has been documented by increasing the transport rate of Apo A1 (45). But there is also a study that demonstrates that alcohol has no influence on leukocyte ABCA1 mRNA levels (30). On the other hand cigarette smoking has been documented to have negative impact on both HDL-C level and function (46). In the present study, the decrease in HDL-C (39.14 ± 5.01 mg/dl) in these AMI patients (without T2DM and with smoking and alcohol intake) was 13.0%, correlating with the negative influence of smoking, overshadowing positive influence of alcohol, through ABCA1 protein.

Thus from the present study it can be inferred that metabolites of diabetes and ischemia may influence stability of PBMNC ABCA1 protein and ABCA1-dependent transport of cholesterol from cells to Apo A1 and thus HDL-C levels. Further influence of risk factors such as smoking and alcohol consumption observed in the present study should be evaluated in a larger sample size. The control of these cardiovascular-associated risk factors may elevate HDL-C levels leading to a protective effect against MI.

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REFERENCES

1. Orso E, Broccardo C, Kaminski WE, et al. Transport of lipids from Golgi to plasma membrane is defective in Tangier disease patients and ABCA1-deficient mice. *Nature Genet* 2000;24:192–196.
2. Broccardo C, Luciani M-F, Chimini G. The ABCA subclass of mammalian transporters. *Biochim Biophys Acta* 1999;1461(2):395–404.
3. Langmann T, Klucken J, Reil M, et al. Molecular cloning of the human ATP-binding cassette transporter 1 (hABC1): Evidence for sterol-dependent regulation in macrophages. *Biochem Biophys Res Commun* 1999;257:29–33.
4. Bodzioch M, Orso E, Klucken J, et al. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. *Nat Gene* 1999;22:347–351.

5. Brooks-Wilson A, Marcil M, Clee SM, et al. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat Genet* 1999;22:336–345.
6. Rust S, Rosier M, Funke H, et al. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. *Nat Gene* 1999;22:352–355.
7. Oram JF, Heinecke JW. ATP-binding cassette transporter A1: A cell cholesterol exporter that protects against cardiovascular disease. *Physiol Rev* 2005;85:1343–1372.
8. Singaraja RR, Bocher V, James ER, et al. Human ABCA1 BAC transgenic mice show increased high density lipoprotein cholesterol and ApoAI-dependent efflux stimulated by an internal promoter containing liver X receptor response elements in intron 1. *J Biol Chem* 2001;276:33969–33979.
9. Aiello RJ, Brees D, Bourassa PA, et al. Increased atherosclerosis in hyperlipidemic mice with inactivation of ABCA1 in macrophages. *Arterioscler Thromb Vasc Biol* 2002;22:630–637.
10. van Eck M, Bos IS, Kaminski WE, et al. Leukocyte ABCA1 controls susceptibility to atherosclerosis and macrophage recruitment into tissues. *Proc Natl Acad Sci USA* 2002;99:6298–6303.
11. Wellington CL, Walker EK, Suarez A, et al. ABCA1 mRNA and protein distribution patterns predict multiple different roles and levels of regulation. *Lab Invest* 2002;82:2732–2783.
12. Albrecht C, Soumian S, Amey JS, et al. ABCA1 expression in carotid atherosclerotic plaques. *Stroke* 2004;35:2801–2806.
13. Demina EP, Miroshnikova VV, Rodygina TI, et al. ABCA1 gene expression in peripheral blood lymphocytes and macrophages in patients with atherosclerosis. *Mol Biol (Mosk)* 2011;45:289–293.
14. Grossman E, Messerli FH. Diabetic and hypertensive heart disease. *Ann Intern Med* 1996;125:304–310.
15. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: Epidemiology, pathophysiology, and management. *JAMA* 2002;287:2570–2581.
16. Mineo C, Deguchi H, Griffin JH, Shaul PW. Endothelial and antithrombotic actions of HDL. *Circ Res* 2006;98:1352–1364.
17. Barter PJ. Cardioprotective effects of high-density lipoproteins—the evidence strengthens. *Arterioscler Thromb Vasc Biol* 2005;25:1305–1306.
18. Goldbourt U, Yaari S, Medalie JH. Isolated low HDL cholesterol as a risk factor for coronary heart disease mortality: A 21-year follow-up of 8000 men. *Arterioscler Thromb Vasc Biol* 1997;17:107–113.
19. Weverling-Rijnsburger AWE, Jonkers IJA, van Exel E, et al. High-density vs. low-density lipoprotein cholesterol as the risk factor for coronary artery disease and stroke in old age. *Arch Intern Med* 2003;163:1549–1554.
20. Liao XX, Li X, Ma ZF, et al. Role of nuclear factor- κ B in endothelial injury in acute myocardial infarction. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue* 2008;20:413–415.
21. Moss NC, Stansfield WE, Willis MS, Tang RH, Selzman CH. IKK β inhibition attenuates myocardial injury and dysfunction following acute ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 2007;293:H2248–H2253.
22. Schmidt, A.-M., O. Hori, J.X. Chen, et al. AGEs interacting with their endothelial receptor induce expression of VCAM-1 in cultured human endothelial cells and in mice. *J Clin Invest* 1995;96:1395–1403.
23. Jeffrey Field F, Watt Kim, Mathur Satya N. TNF- α decreases ABCA1 expression and attenuates HDL cholesterol efflux in the human intestinal cell line Caco-2. *J Lipid Res* 2010;51:1407–1415.
24. Baranova I, Vishnyakova T, Bocharov A, et al. Lipopolysaccharide down regulates both scavenger receptor B1 and ATP binding cassette transporter A1 in RAW cells. *Infect Immun* 2002;70:2995–3003.
25. Terao Y, Ayaori M, Ogura M, et al. Effect of sulfonylurea agents on reverse cholesterol transport in vitro and vivo. *J Atheroscler Thromb* 2011;18:513–530.
26. Hattori Y, Suzuki K, Hattori S, Kasai K. Metformin inhibits cytokine-induced nuclear factor κ B activation via AMP-activated protein kinase activation in vascular endothelial cells. *Hypertension* 2006;47:1183–1188.
27. Lu L, Liu H, Peng J, et al. Regulations of the key mediators in inflammation and atherosclerosis by aspirin in human macrophages. *Lipids Health Dis* 2010;9:16.
28. Wang YH, Chen YF, Chen SR, et al. Aspirin increases apolipoprotein-A-I-mediated cholesterol efflux via enhancing expression of ATP-binding cassette transporter A1. *Pharmacology* 2010;86:320–326.
29. Hoang A, Tefft C, Duffy SJ, et al. ABCA1 expression in humans is associated with physical activity and alcohol consumption. *Atherosclerosis* 2008;197:197–203.
30. Patel DC, Albrecht C, Pavitt D, et al. Type 2 diabetes is associated with reduced ATP-binding cassette transporter A1 gene expression, protein and function. *PLoS One* 2011;6:e22142.
31. Forcheron F, Cachefo A, Thevenon S, Pinteur C, Beylot M. Mechanisms of the triglyceride- and cholesterol-lowering effect of fenofibrate in hyperlipidemic type 2 diabetic patients. *Diabetes* 2002;51:3486–3491.
32. Uehara Y, Engel T, Li Z, et al. Polyunsaturated fatty acids and acetoacetate downregulate the expression of the ATP-binding cassette transporter A1. *Diabetes* 2002;51:2922–2928.
33. Tang C, Kanter JE, Bornfeldt KE, Leboeuf RC, Oram JF. Diabetes reduces the cholesterol exporter ABCA1 in mouse macrophages and kidneys. *J Lipid Res* 2010;51:1719–1728.
34. Maurer R, Ebert S, Langmann T. High glucose, unsaturated and saturated fatty acids differentially regulate expression of ATP-binding cassette transporters ABCA1 and ABCG1 in human macrophages. *Exp Mol Med* 2009;41:126–132.
35. Gao F, Yan T, Zhao Y, Yin F, Hu C. A possible mechanism linking hyperglycemia and reduced high-density lipoprotein cholesterol levels in diabetes. *J Huazhong Univ Sci Technolog Med Sci* 2010;30:318–321.
36. Senanayake S, Brownrigg LM, Panicker V, et al. Monocyte-derived macrophages from men and women with type 2 diabetes mellitus differ in fatty acid composition compared with non-diabetic controls. *Diabetes Res Clin Pract* 2007;75:292–300.
37. Haghighpassand M, Bourassa PA, Francone OL, Aiello RJ. Monocyte/macrophage expression of ABCA1 has contribution to plasma HDL levels. *J Clin Invest* 2001;108:1315–1320.
38. Foley JE. Rationale and application of fatty acid oxidation inhibitors in treatment of diabetes mellitus. *Diabetes Care* 1992;15:773–784.
39. Kleinfeld AM, Prothro D, Brown DL, Davis RC, Richieri GV, DeMaria A. Increases in serum unbound free fatty acid levels following coronary angioplasty. *Am J Cardiol* 1996;78:1350–1354.
40. Wang Y, Kurdi-Haidar B, Oram JF. LXR-mediated activation of macrophage stearoyl-CoA desaturase generates unsaturated fatty acids that destabilize ABCA1. *J Lipid Res* 2004;45:972–980.
41. Wang Y, Oram JF. Unsaturated fatty acids inhibit cholesterol efflux from macrophages by increasing degradation of ATP-binding cassette transporter A1. *J Biol Chem* 2002;277:5692–5697.
42. Passarelli M, Tang C, McDonald TO, O'Brien KD, Gerrity RG. Advanced glycation end product precursors impair ABCA1-dependent cholesterol removal from cells. *Diabetes* 2005;54:2198–2205.

43. Oram F, Heinecke JW. ATP binding cassette transporter A1: A cell cholesterol exporter that protects against cardiovascular disease. *Physiol Rev* 2005;85:1343–1372.
44. Albrecht C, Simon-Vermot I, Elliott JI, Higgins CF, Johnston DG, Valabhji J. Leukocyte ABCA1 gene expression is associated with fasting glucose concentration in normoglycemic men. *Metabolism* 2004;53:17–21.
45. De Oliveira E, Silva ER, Foster D, et al. Alcohol consumption raises HDL cholesterol levels by increasing the transport rate of apolipoproteins A-I and A-II. *Circulation* 2000;102:2347–2352.
46. He BM, Zhao SP, Peng ZY. Effects of cigarette smoking on HDL quantity and function: Implications for atherosclerosis. *J Cell Biochem* 2013;114:2431–2436.