cAMP induces ABCA1 phosphorylation activity and promotes cholesterol efflux from fibroblasts

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Abstract ATP-binding cassette transporter A1 (ABCA1) plays a crucial role in apoA-I lipidation, a key step in reverse cholesterol transport. cAMP induces apoA-I binding activity and promotes cellular cholesterol efflux. We investigated the role of the cAMP/protein kinase A (PKA) dependent pathway in the regulation of cellular cholesterol efflux. Treatment of normal fibroblasts with 8-bromo-cAMP (8-Br-cAMP) increased significantly apoA-I-mediated cholesterol efflux, with specificity for apoA-I, but not for cyclodextrin. Concomitantly, 8-Br-cAMP increased ABCA1 phosphorylation in a time-dependent manner. Maximum phosphorylation was reached in <10 min, representing a 260% increase compared to basal ABCA1 phosphorylation level. Forskolin, a known cAMP regulator, increased both cellular cholesterol efflux and ABCA1 phosphorylation. In contrast, H-89 PKA inhibitor reduced cellular cholesterol efflux by 70% in a dose-dependent manner and inhibited almost completely ABCA1 phosphorylation. To determine whether naturally occurring mutants of ABCA1 may affect its phosphorylation activity, fibroblasts from subjects with familial HDL deficiency (FHD) but not in TD cells were treated with 8-Br-cAMP or forskolin. Cellular cholesterol efflux and ABCA1 phosphorylation were increased in FHD but not in TD cells. Together, these findings provide evidence for a link between the cAMP/PKA-dependent pathway, ABCA1 phosphorylation, and apoA-I mediated cellular cholesterol efflux.—Haidar, B., M. Denis, L. Krimbou, M. Marcil, and J. Genest, Jr. Cyclic AMP induces ABCA1 phosphorylation activity and promotes cholesterol efflux from fibroblasts. J. Lipid Res. 2002. 43: 2087–2094.

Supplementary key words apolipoprotein A-I • high density lipoprotein • PKA • protein kinase A

The importance of ATP-binding cassette transporter A1 (ABCA1) in reverse cholesterol transport (RCT) process has been strikingly demonstrated by the identification of mutations in ABCA1 gene locus as the molecular defect of Tangier disease (TD) and familial HDL deficiency (FHD) (1, 2). Those patients were characterized by extremely low HDL levels caused by inadequate transport of cellular cholesterol and phospholipids to the extracellular space, leading to hypercatabolism of lipid-poor nascent HDL particles (3). Thus, factors affecting the structure, activity, or concentration of ABCA1 are likely to affect the homeostasis of plasma HDL cholesterol and the RCT process, one of several proposed mechanisms (4) by which HDL may protect against atherosclerosis.

ABCA1 is a 240 kDa protein belonging to a large family of conserved transmembrane proteins that transport a wide variety of substrates, including ions, drugs, peptides, and lipid across cell membranes (5). ABC transporters have been associated with many diseases such as drug-resistant cancer (6), diabetes (7), and cystic fibrosis (8), making these proteins potential targets for therapeutic intervention (9). The ABCA1 gene product is a member of the superfamily of ATP binding cassette transporters predicted to contain 12 transmembrane spanning domains and two nucleotide binding folds (NBF) responsible for the degradation of ATP to provide the energy required for the transport activity (10). The NBF domains contain the highly conserved phosphate-binding loop (11) that forms intimate contacts with the β- and γ-phosphates of bound ATP (12). In some cases, evidence has been provided for a further regulation via phosphorylation of serine/threonine residues in the NBF region (13).

The interaction between apoA-I and ABCA1 is of critical importance for the active apoA-I lipidation, but the structural and functional pathways involved in this process have not been established. Of interest, the earlier attractive concept of Smith et al. (14) suggested that cAMP induces a membrane apolipoprotein receptor that does not lead to endocytosis and degradation, but instead pro-

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motes the transfer of lipids to apolipoprotein. The same group had also reported later that cAMP-mediated cholesterol efflux to apoA-I is associated with the binding, uptake, and resecretion of apoA-I in a calcium-dependent pathway in murine macrophages (15). The effect of cAMP on ABCA1 mRNA and protein appears to be specific to macrophages and is not seen with a number of other cell types (16). Moreover, it has been documented that cAMP induces apoA-I binding activity and promotes cellular cholesterol efflux via ABCA1 protein in macrophages (17). On the other hand, treatment of immortalized fibroblasts with cAMP analog also induces apoA-I-mediated lipid efflux and ABCA1 expression, but these effects are usually modest and require some cholesterol loading of cells (18, 19). We have previously shown that phospholipid breakdown products, mediated by phospholipase C and D, and the activation of protein kinase C modulate cellular cholesterol efflux (20).

The involvement of apoA-I in cellular signaling processes and its property to induce cellular cholesterol efflux via its interaction with ABCA1 led us to hypothesize that cAMP/protein kinase A (PKA)-dependent pathway may modulate cellular cholesterol efflux via activation of ABCA1 by inducing its phosphorylation. The present study aims to provide evidence for links between apoA-I-mediated cellular cholesterol efflux, cAMP, PKA, and ABCA1 phosphorylation, and to examine how these interactions could be affected by cAMP regulators, PKA inhibitors, or naturally occurring mutants of ABCA1 protein.

METHODS

Patient selection

Patients with FHD and TD were selected as previously described (1, 2, 21, 22). All these subjects carry a mutation at the ABCA1 gene locus or had clinical signs of TD. For the present study, we selected fibroblasts from two normal control subjects (CTR1-2), five patients with FHD (FHD1–5) and three patients with TD (TD1–3). Cellular studies in all TD subjects revealed a marked impairment of apoA-I-mediated cellular cholesterol efflux. Molecular analysis of the ABCA1 gene revealed a compound heterozygous state for subjects TD1 and TD3 and a homozygous missense point mutation in subject TD2. The protocol for the study was reviewed and accepted by the Research Ethics Board of the McGill University Health Center. Separate consent forms for blood sampling, DNA isolation and skin biopsy were provided.

Cell culture

Human skin fibroblasts were obtained from 3.0 mm punch biopsies of the forearm of patients and healthy control subjects and were cultured as described (21). We seeded 5 × 10⁴ cells in 35 mm cell culture wells in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 0.1% nonessential amino acids, penicillin (100 U/ml), streptomycin (100 µg/ml), and 10% FBS. At approximately 50% confluence, 0.2 or 1 µCi/ml [3H]cholesterol was added in the cell medium. When the cells reached confluence, they were washed three times in PBS containing 1 mg/ml of fatty acid free BSA and the medium was replaced by DMEM containing 2 mg/ml BSA and 20 µg/ml of free non lipoprotein cholesterol for 24 h. Cellular cholesterol pools were allowed to equilibrate for another 24 h in DMEM containing 1 mg/ml BSA. Efflux studies (0–24 h) were then performed in DMEM containing 1 mg/ml BSA and 10 µg/ml apoA-I in the presence or absence of specified efflux modulating signaling molecules.

Cellular cholesterol efflux determination

ApoA-I-mediated cholesterol efflux was carried out on the 10 cell lines; as control, BSA (1 mg/ml) only was used in the efflux medium. All experiments were performed in triplicate and efflux was determined specifically at 24 h. In some experiments, cells were treated with 8-bromo-cAMP (8-Br-cAMP) as described previously (19) with minor modifications. Briefly, the cells received 0.5 mM 8-Br-cAMP during 24 h pretreatment and during efflux time incubation. At the end time, cells were chilled on ice and the medium was collected. To precipitate any floating cells, the medium was centrifuged for 10 min at 2,500 rpm. The cell layer was dissolved overnight in 0.1 N NaOH at 4°C. Cellular protein concentration was determined by the Lowry method (23) and one aliquot was reserved for scintillation counting of cellular [3H]cholesterol content. Cellular cholesterol efflux was determined as follow: [3H]cpm in medium/[3H]cpm in medium + [3H]cpm in cells); the results were expressed as percentage of total radiolabeled cholesterol.

Cholesteryl ester hydrolysis

The rate of cholesteryl ester hydrolysis was determined by radioisotopic labeling using [3H]cholesterol. At 50% confluence, fibroblasts were radiolabeled with [3H]cholesterol for 48 h then loaded with free cholesterol for an additional 24 h at 37°C as described above. An acyl-coenzyme A-cholesterol acyltransferase (ACAT) inhibitor, avasimibe 10 µM (provided by Pfizer Pharmaceutical, Ann Arbor, MI), and 0.5 mM 8-Br-cAMP (Sigma Chemical CO, St. Louis, MO) were added to the equilibration medium supplemented with 1 mg/ml of BSA at 0 h. At subsequent indicated times the cells were washed with PBS and extracted for both lipids and proteins. Cell lipids were extracted with hexane-isopropanol (3:2, v/v) at room temperature for 30 min, followed by a wash with another 0.5 ml of solvent. Protein in the lipid-free wells was extracted with 0.5 ml of 0.1 N NaOH for 30 min at room temperature and measured by Lowry method. Lipid extracts were dried under N₂ stream then resuspended in 25 µl of chloroform and plated onto LiChrosil silica gel 60 TLC plates. Free cholesterol (FC) and cholesteryl ester (CE) were separated from other lipids using heptane-ethyl ether-methanol-acetic acid, 80:30:3:1.5 (v/v/v/v) as the solvent system and visualized with I₂. The FC and CE bands were scraped, and radioactivity was measured by liquid scintillation counting.

ApoA-I preparation

HDL was isolated from fresh plasma of normalplinemic donors using a standard sequential ultracentrifugation with density (1.125–1.210 g/ml) adjusted with KBr. The HDL preparation was delipidated in acetone-ethanol (1:1, v/v) and total proteins were fractionated at 4°C on two Sephacryl S-200 (Amersham Pharmacia Biotech AB, Uppsala, Sweden) columns (2.6 × 100 cm). Fractions contained in the apoA-I peak were extensively dialyzed in 0.01 M NH₄HCO₃, then lyophilized and resuspended in PBS at concentration of 1 mg/ml. Protein purity was assayed by polyacrylamide gradient gel electrophoresis (PAGGE) on each apoA-I fraction and appropriate fractions were pooled, dialyzed in PBS, and lyophilized before being stored at −70°C as stock.

Probes and Northern blots

A 517 bp probe for human ABCA1 and mouse ABCA1 were prepared by reverse transcription performed on total RNA ex-
tracted from normal human skin fibroblasts and from J774 mouse macrophages. This was followed by a PCR step using the forward primer 5’-CTTGGTTCAGGGATAT-3’ and the reverse primer 5’-AGGATGCTTCAGGATGTCG-3’. The amplified fragment was subcloned into pGEM-T (Promega, Madison, WI) and used to transform JM109 cells, then sequenced to ensure the right sequence. After digestion of the subcloned product with SalI and SacII, the insert was extracted from agarose gel and 32P-labeled using the Amersham/Pharmacia Oligolabeling kit. The probe was used at concentration of 10^6 cpm/ml in Northern blots. Ten to fifteen micrograms/lane of the total cell RNA were loaded on a 1% formaldehyde-containing agarose gel and transferred to a Hybond N+ (Amersham Biosciences, Piscataway, NJ) membrane for probing. Bands were revealed on a Typhoon phosphorimager (Amersham Biosciences, Sunnyvale, CA).

**ABCA1 phosphorylation and immunoprecipitation procedures**

Confluent fibroblasts were loaded with free cholesterol (20 μg/ml) for 24 h, washed twice in MEM-free phosphate solution (Invitrogen, Burlington, Canada), and incubated with 0.5 μCi/ml of 32P-labeled orthophosphate for 2 h at 37°C. The cells were washed twice in MEM-free phosphate solution and then treated or not with 1 mM 8-Br-cAMP for varying periods of time as indicated in figures. Cells were also treated with forskolin (FRK) as specified for each experiment. At the indicated time, the cells were washed twice with ice-cold PBS and scraped into 0.5 ml of immunoprecipitation (IP) buffer containing 20 mM Tris (pH 7.5), 0.5 mM EDTA, and 0.5 mM EGTA, 1% Triton-X 100 (Invitrogen, Burlington, Canada) and the suspension was allowed to stand for 30 min at 4°C in presence of a protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany). Cellular debris were removed by centrifugation at 100 g for 10 min at 4°C. Protein was estimated by BCA protein assay reagent (Pierce, Canada) according to the protocol from manufacturer using BSA as standard. Cell lysate (150 μg) was cleared of cellular debris by in-

![Fig. 1](image1.png)  **Fig. 1.** Effect 8-bromo-cAMP (8-Br-cAMP) or cAMP regulator on apoA-I and cyclodextrin-mediated cellular cholesterol efflux. A: Normal control fibroblasts were cholesterol-loaded and [3H]cholesterol-labeled, as described in Methods. Cells were incubated with 10 μg/ml apoA-I alone or in the presence of 0.5 mM 8-Br-cAMP, 5 μM forskolin (FRK), or 1 mM IBMX. After 2, 4, and 6 h incubations, radioactivity in the medium and in the cells was determined. Cholesterol efflux is presented as the percent of total radioactivity recovered from the cells and the medium, and represents the mean ± SD from triplicate wells. B: Normal control fibroblasts were cholesterol-loaded and [3H]cholesterol-labeled. Cells were incubated with 1 mM cyclodextrin (CDX) or 10 μg/ml apoA-I in the presence or absence of 0.5 mM 8-Br-cAMP, 5 μM FRK, and 1 mM IBMX. After 24 h incubation, cholesterol efflux was determined as in A. One experiment, representative of three, is shown.

![Fig. 2](image2.png)  **Fig. 2.** Effect of 8-Br-cAMP on ATP-binding cassette transporter A1 (ABCA1) mRNA expression in normal control fibroblasts and J774 cells. A: Normal fibroblasts were incubated in presence or absence of 1 mM 8-Br-cAMP for 24 h and then harvested. Total RNA was isolated and 15 μg were loaded per lane on agarose gel. After transfer, the blot was probed with human ABCA1 cDNA, as described in Methods. B: J774 cells were incubated in presence or absence of 1 mM 8-Br-cAMP for 24 h and then harvested. Northern blots were incubated with a murine ABCA1 cDNA probe. J744 cells were used as control in this experiment.

![Fig. 3](image3.png)  **Fig. 3.** Effect of 8-Br-cAMP on cholesteryl ester (CE) hydrolysis. Normal control fibroblasts were cholesterol-loaded and [3H]cholesterol-labeled, as described in Methods. 0.5 mM 8-Br-cAMP and 10 μM avasimibe, an ACAT inhibitor, were added at 0 h as indicated by vertical arrow in panel. Cellular radiolabeled CE content was determined in each time point by TLC. Results were expressed as percentage of total radioactivity at 0 h (100%), and represent the mean ± SD from triplicate wells.

**ABCA1 phosphorylation and immunoprecipitation procedures**

Confluent fibroblasts were loaded with free cholesterol (20 μg/ml) for 24 h, washed twice in MEM-free phosphate solution (Invitrogen, Burlington, Canada), and incubated with 0.5 μCi/ml of 32P-labeled orthophosphate for 2 h at 37°C. The cells were washed twice in MEM-free phosphate solution and then treated or not with 1 mM 8-Br-cAMP for varying periods of time as indicated in figures. Cells were also treated with forskolin (FRK) as specified for each experiment. At the indicated time, the cells were washed twice with ice-cold PBS and scraped into 0.5 ml of immunoprecipitation (IP) buffer containing 20 mM Tris (pH 7.5), 0.5 mM EDTA, and 0.5 mM EGTA, 1% Triton-X 100 (Invitrogen, Burlington, Canada) and the suspension was allowed to stand for 30 min at 4°C in presence of a protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany). Cellular debris were removed by centrifugation at 100 g for 10 min at 4°C. Protein was estimated by BCA protein assay reagent (Pierce, Canada) according to the protocol from manufacturer using BSA as standard. Cell lysate (150 μg) was cleared of cellular debris by in-
Fig. 4. Time-dependent phosphorylation of ABCA1 in the presence or absence of 8-Br-cAMP. Normal intact fibroblasts were incubated with 32P-labeled orthophosphate for 2 h, and then treated or not with 1 mM 8-Br-cAMP for varying periods of time (0, 10, 30, 60, 120 min; lanes a to e). 32P-labeled ABCA1 was immunoprecipitated and separated by electrophoresis, and then transferred to PVDF-membrane as described in Methods. 32P-labeled ABCA1 was revealed and quantified by phosphorimager. Percent increase of 32P-ABCA1 from three different experiments from the same cells was presented. Plotted values are mean ± SD of triplicate values (lower panel). ABCA1 protein were detected in the same membrane by anti-ABCA1 antibody and used as control for protein loading. 32P-ABCA1 was not normalized to ABCA1 mass protein. One experiment, representative of three, is shown in the upper panel.
to be a cAMP regulator, were as effective as 8-Br-cAMP (0.5 mM) in promoting apoA-I-mediated cellular cholesterol efflux in normal cells (Fig. 1A, B). The effect of 8-Br-cAMP on cellular cholesterol efflux was shown to be stable and was essentially unchanged over 24 h. Based on these experiments all cellular cholesterol efflux analyses were made at 24 h.

We tested in normal cells whether the increase in apoA-I-mediated cholesterol efflux induced by 8-Br-cAMP was associated with changes in cellular ABCA1 mRNA. Human skin fibroblasts were incubated for 24 h with 8-Br-cAMP (0.5 mM). There was no effect of cAMP on ABCA1 mRNA levels in fibroblasts (Fig. 2A). However, 8-Br-cAMP increased dramatically ABCA1 mRNA in J774 mouse peritoneal macrophages used as control in this experiment (Fig. 2B).

Previous studies showed that cAMP could stimulate neutral cholesteryl ester hydrolase activity in some cell types (24). In order to test the hypothesis that apoA-I-mediated cellular cholesterol efflux stimulation by 8-Br-cAMP was not dependent on an increased CE turnover and free cholesterol availability, normal control fibroblasts were cholesterol-loaded and [3H]cholesterol-labeled, as described in Methods. 0.5 mM 8-Br-cAMP and 10 μM avasimibe, an ACAT inhibitor, were added at 0 h as indicated by vertical arrow (Fig. 3). At subsequent times the rate of CE was decreased due to the presence of the ACAT inhibitor. Cellular radiolabeled CE content was determined at each time point by TLC. The addition of 8-Br-cAMP had no effect on the hydrolysis of radiolabeled esterified cholesterol (Fig. 3).

In order to establish the relationship of cAMP/PKA-dependent pathway with ABCA1 phosphorylation activity, ABCA1 was phosphorylated in intact cells using [32P]orthophosphate, and then immunoprecipitated with anti-ABCA1 antibody, as described in Methods. The specificity of ABCA1-antibody was assessed by immunoprecipitation of [35S]methionine labeled-ABCA1, which show a molecular mass of 250 kDa, and was induced by both hydroxycholesterol and 9-cis-retinoic acid (data not shown). The phosphorylation of ABCA1 was investigated by treatment of normal cells with 8-Br-cAMP for varying periods of time and untreated cells were used as control for each period of time. Phosphorylated ABCA1 was immunoprecipitated, and then separated by SDS-PAGE (4–12.5%) gel. Gels were transferred to PVDF-ImmobilonP membrane. [32P]-labeled ABCA1 was detected by phosphorimagier, and then the same membrane was revealed for ABCA1 protein by anti-ABCA1 antibody, as described in Methods. As shown in Fig. 4, treatment of normal intact fibroblasts with 8-Br-cAMP increased ABCA1 phosphorylation in a time-dependent manner. Maximum phosphorylation was reached in less than 10 min, representing a 260% increase compared to basal phosphorylation level of ABCA1 in untreated cells, and remained constant for the remaining 120 min of the experiment. [32P]-labeled ABCA1 was quantified by phosphorimagier. Percent increase of [32P]-ABCA1 from three different experiments from the same cell lines is presented (Fig. 4, lower panel). One experiment, representative of three different experiments, is shown in the upper panel of Fig. 4.

In order to provide evidence for a specific role of PKA in apoA-I-mediated cellular cholesterol efflux process and ABCA1 phosphorylation activity, we examined the effect of H-89 PKA inhibitor on cellular cholesterol efflux and ABCA1 phosphorylation. Treatment of normal intact cells with 20 μM H-89 decreased significantly basal apoA-I-mediated cholesterol efflux and ABCA1 phosphorylation (0, 5, 10, 20 μM; lanes a to d). Inhibition of PKA by H-89 PKA inhibitor had no effect on 8-Br-cAMP-mediated cholesterol efflux and ABCA1 phosphorylation (Fig. 5A, B). However, 8-Br-cAMP increased ABCA1 phosphorylation in a time-dependent manner. Maximum phosphorylation was reached in less than 10 min, representing a 260% increase compared to basal phosphorylation level of ABCA1 in untreated cells, and remained constant for the remaining 120 min of the experiment. [32P]-labeled ABCA1 was quantified by phosphorimagier. Percent increase of [32P]-ABCA1 from three different experiments from the same cell lines is presented (Fig. 4, lower panel). One experiment, representative of three different experiments, is shown in the upper panel of Fig. 4.
we observed that inhibition of the serine/threonine phosphatases by okadaic acid increased the phosphorylation of ABCA1 in normal cells (data not shown).

To determine whether naturally occurring mutants of ABCA1 may affect cellular cholesterol efflux and ABCA1 phosphorylation activity, fibroblasts from subjects with FHD and TD (Table 1) were treated with 8-Br-cAMP or FRK. Cellular cholesterol efflux was increased in control, FHD, but not in TD cells (Fig. 6A) in response to 8-Br-cAMP treatment. Furthermore, cells from a normal control (CTR1), FHD (FHD4), and TD (TD1) subjects (Table 1) were incubated with 32P-orthophosphate in the presence or absence of 8-Br-cAMP (1 mM). As shown in Fig. 6B (upper panel), ABCA1 phosphorylation was increased in control and FHD cells compared to basal phosphorylation levels in the absence of 8-Br-cAMP. However, under the same condition, ABCA1 from TD1 cells shows no further phosphorylation over baseline. The control experiment was performed to verify the 8-Br-cAMP response. We demonstrated that FRK (5 μM) was as effective as 8-Br-cAMP (0.5 mM) in inducing ABCA1 phosphorylation in control and FHD, but not in TD cells (Fig. 6B, lower panel).

**DISCUSSION**

cAMP has been recognized to promote apoA-I-mediated cellular lipid efflux (14). Here we have shown that 8-Br-cAMP increased apoA-I-mediated cellular cholesterol efflux in normal cells, but did not affect diffusion-mediated non-specific cholesterol efflux (Fig. 1B). At the same time, treatment of intact normal cells with 8-Br-cAMP increased ABCA1 phosphorylation in a time-dependent manner. At 10 min, a 2.5-fold increase in ABCA1 phosphorylation was observed compared to the basal phosphorylation level in untreated cells (Fig. 4). The effect of 8-Br-cAMP on cellular cholesterol efflux and ABCA1 phosphorylation was not due to increased ABCA1 mRNA and increased protein levels (Fig. 2A). However, previous studies have documented that both ABCA1 mRNA and protein levels are induced by cAMP treatment in macrophages (16, 17). Furthermore, 8-Br-cAMP did not affect the activity of cholesterol ester hydrolase (Fig. 3). It is more likely that a cAMP-dependent signaling pathway directly induces or activates key proteins involved in the apolipoprotein-mediated lipid removal pathway.

The effects of 8-Br-cAMP on cellular cholesterol efflux and ABCA1 phosphorylation were supported by experiments showing that FRK, known as an activator of adenylate cyclase, induces both increased cellular cholesterol efflux and ABCA1 phosphorylation in normal cells (Fig. 1A, B and Fig. 6B, lower panel). These are consistent with the concept that a cAMP/PKA-dependent pathway may regulate cellular cholesterol efflux. This concept is further

### TABLE 1. Molecular characterization of ABCA1 gene in study subjects

| Cell Lines | HDL-C | Nucleotide Change | Predicted Protein Alteration |
|------------|-------|-------------------|-----------------------------|
| CTR1       | 1.63  | —                 | —                           |
| CTR2       | 1.20  | —                 | —                           |
| FHD1       | 0.27  | Exon 14 Δ2917-9   | ΔΔL693                      |
| FHD2       | 0.18  | Exon 18 C2665T    | R909X                       |
| FHD3       | 0.39  | Exon 41 Δ5618-23  | ΔΔED1893.4                  |
| FHD4       | 0.18  | Exon 48 G6370T    | R2144X                      |
| FHD5       | 0.09  | Exon 36 G6527,78C | f1628G, Q1636X              |
| TD1        | <0.1  | Exon 30 T4696C,   | C1477R; Part of the         |
|            |       | exon 24 splice site G→C | transcript deleted          |
| TD2        | <0.1  | Exon 13 A1790G    | Q957R                       |
| TD3        | <0.1  | Exon 48 ΔG6370;   | 2145X; nd                   |

FHD1–5 are compound heterozygous for the reported mutation; TD1,3 are compound heterozygous and TD2 is homozygous. nd, not determined.
Cellular cholesterol efflux appears to be an important determinant of plasma HDL-C levels. This is based on the observation that ABCA1 defects lead to a marked deficiency of plasma HDL-C, and cellular cholesterol efflux correlates with plasma HDL-C level (33). The regulation of ABCA1-mediated cellular cholesterol efflux is complex and is regulated both at the transcriptional and post-transcriptional levels in cells. It might be insufficient, therefore, to increase ABCA1 mRNA levels in peripheral cells such as fibroblasts in order to increase cellular cholesterol efflux. Strategies targeting the modulation of both the amount of ABCA1 protein and its activation by phosphorylation have potential as therapeutic interventions aimed at raising HDL-C levels in plasma of patients at increased risk for developing atherosclerotic vascular disease.

The results presented in this study provide evidence supporting that apoA-I interacts with cAMP/PKA-dependent pathway leading to ABCA1 phosphorylation. This process might play in vivo a key functional role in the activation of ABCA1 by increasing its phosphorylation, allowing cholesterol to bind free apoA-I.

Further study of the role of apoA-I in mediating cellular signaling and its interaction with ABCA1 protein may provide new insights into the mechanism of reverse cholesterol transport, plasma factors affecting HDL metabolism, and the therapeutic potential of ABCA1 in preventing or treating atherosclerotic vascular disease.

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