A naturally occurring variant of endothelial lipase associated with elevated HDL exhibits impaired synthesis[S]

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Abstract **Human endothelial lipase (EL) is a member of a family of lipases and phospholipases that are involved in the metabolism of plasma lipoproteins. EL displays a preference to hydrolyze lipids in HDL. We report here that a naturally occurring low frequency coding variant in the EL gene (*LIPG*), glycine-26 to serine (G26S), is significantly more common in African-American individuals with elevated HDL cholesterol (HDL-C) levels. To test the hypothesis that this variant results in reduced EL function, we extensively characterized and compared the catalytic and noncatalytic functions of the G26S variant and wild-type (WT) EL. While the catalytic-specific activity of G26S EL is similar to WT EL, its secretion is markedly reduced. Consistent with this observation, we found that carriers of the G26S variant had significantly reduced plasma levels of EL protein. Thus, this N-terminal variant results in reduced secretion of EL protein, plausibly leading to increased HDL-C levels.**—Brown, R. J., A. C. Edmondson, N. Griffon, T. B. Hill, I. V. Fuki, K. O. Badellino, M. Li, M. L. Wolfe, M. P. Reilly, and D. J. Rader. **A naturally occurring variant of endothelial lipase associated with elevated HDL exhibits impaired synthesis.** ***J. Lipid Res.*** **2009.** 50: **1910–1916.**

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Endothelial lipase (EL) is a member of a family of lipases that includes LPL and hepatic lipase (HL) (1–6). Overexpression of EL in mouse models significantly reduces plasma HDL cholesterol (HDL-C) (1, 7, 8), whereas the loss of EL function in mouse models significantly elevates plasma HDL-C (7, 9, 10). In addition to its catalytic function, EL is capable of "bridging" HDL and other lipoproteins with cell surface proteoglycans (11).

The human gene encoding EL, *LIPG*, has been reported to be associated with variation in HDL-C levels in genome-wide association studies (12, 13). In addition, we have recently shown that a previously reported nonsynonymous coding variant of EL that exhibits impaired enzymatic functions in vitro and in vivo is directly associated with elevated HDL-C in multiple cohorts (14). Numerous variants have been identified within EL (14, 15); however, not all of the mechanisms by which they influence HDL-C levels, if at all, have been elucidated.

We previously sequenced a small number of subjects with extremely high levels of HDL-C and reported a low frequency coding variant that results in the substitution of a glycine at residue 26 to serine (G26S) (15). In the current study, we assessed the relationship of this variant to elevated HDL-C and studied its function to determine whether it reduces EL activity or mass. Our results indicate that this N-terminal G26S variant does not exhibit an impaired biochemical function, but rather it results in reduced secretion of EL protein, leading to increased HDL-C levels in carriers of this variant.

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Abbreviations: ALLN, *N*-acetyl-leucinyl-leucinyl-norleucinal; DPPC, dipalmitoylphosphatidyl choline; EL, endothelial lipase; ER, endoplasmic reticulum; HDL-C, HDL cholesterol; HHDL, University of Pennsylvania High HDL Cholesterol Study; HL, hepatic lipase; PennCAC, University of Pennsylvania Coronary Artery Calcification Study; PBS, phosphate-buffered saline; SIRCA, Study of Inherited Risk of Coronary Atherosclerosis; WT, wild-type.

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[S] The online version of this article available at http://www.jlr.org) contains supplementary data in the form of three figures.

EXPERIMENTAL PROCEDURES

Human subjects

Subjects from the University of Pennsylvania High HDL Cholesterol Study (HHDL; $n = 854$) and the Study of Inherited Risk of Coronary Atherosclerosis (SIRCA; $n = 885$) were assessed for the presence of either wild-type (WT) EL or the G26S variant of EL by Taqman custom genotyping (Applied Biosystems). The study designs and initial findings of subjects were previously reported from HHDL (14) and SIRCA (16). Subjects identified with the G26S variant were compared with age- and sex-matched control subjects from both SIRCA and HHDL. Subjects from the University of Pennsylvania Coronary Artery Calcification Study (PennCAC; $n = 2,616$) were assessed for the presence of either WT EL or the G26S variant of EL using the Illumina IBC Candidate Gene array, version 2 (17). The PennCAC cohort is composed of subjects from SIRCA, the Penn Diabetes Heart Study (18, 19), and the Philadelphia Area Metabolic Syndrome Network, which is an ongoing cross-sectional study of individuals with a varying number of the metabolic syndrome criteria. Age, height, mass, and histories of smoking, drinking, cardiovascular disease, type-2 diabetes, and metabolic syndrome were recorded by referring physicians. Total cholesterol, HDL-C, LDL cholesterol, and triglycerides were assessed in clinical laboratories. All studies were approved by the University of Pennsylvania Institutional Review Board and informed consent was obtained from all participants.

Preparation of EL expression plasmids

The cDNA for human EL (NM006033) was inserted into the pcDNA3 expression vector (Invitrogen). Mutagenesis of Gly-26 into Ser was performed using the QuikChange™ mutagenesis kit (Stratagene). The sense oligonucleotide (toward nucleotides 312–345) to generate the G26S variant is 5′-GAGCCCCGTACCT TTTAGTCCAGAGGGACGGCTG-3′; a complementary antisense oligonucleotide was also used.

Cell culture

HEK293 cells were cultured in DMEM (Invitrogen) containing 10% fetal bovine serum (Sigma) and 1% antibiotic/antimycotic (Invitrogen). Cells were grown to 90% confluency (in 12-well plates), and 0.5 μg of EL expression plasmid was transfected per well using Lipofectamine™ (Invitrogen) according to the manufacturer's instructions. For analysis of EL expression and catalytic activity, media were replaced at 32 h posttransfection with serum-free media without or with 100 U/ml heparin. At 48 h posttransfection, media were collected and centrifuged at 1,200 rpm for 10 min to remove any cell debris. The supernatant was divided into aliquots and stored at −80°C. The total extracellular EL released from transfected cells over 16 h in the absence versus presence of heparin was determined as described previously for HL (20). Cells were lysed to extract total RNA and protein, and samples were stored at −80°C. For inhibition of degradation pathways, media were replaced at 48 h posttransfection with serum-free media containing 100 U/ml heparin and either 75 μM chloroquine or 100 μM *N*-acetyl-leucinyl-leucinyl-norleucinal (ALLN; Sigma). After a 6 h incubation with chloroquine or ALLN, cells and media were collected as described above.

Pulse-chase analyses

Cells (in 60 mm dishes) were transiently transfected as described above. At 48 h posttransfection, cells were washed three times with PBS and pulse-labeled with 1 ml of 100 μCi/ml [^{35}S] methionine/cysteine (Perkin-Elmer) for 2 h in methionine/cysteine- and serum-free DMEM. Media were replaced with 2 ml serum-free media containing 100 U/ml heparin, and cells were chased for up to 2 h. The media and cells were collected at the end of the pulse and at times 15, 30, 60, 90, and 120 min of chase period. An aliquot (1 ml) of media was mixed with 10 μl of an anti-human EL polyclonal antibody, generated as previously described (21), overnight at 4°C. The cells were lysed with RIPA buffer (50 mM Tris-HCL, pH 8.0, 1 mM EDTA, 1% Triton X-100, 1% deoxycholic acid, 1 mM dithiothreitol, 150 mM NaCl, 0.015% phenylmethylsulfonyl fluoride, 0.1% SDS), and cell-associated EL was likewise immunoprecipitated from the cell lysates. The antibody-EL complexes were adsorbed to Protein A and washed six times with PBS (for medium samples) or RIPA buffer (for cell samples). The EL was eluted from Protein A with 200 μl of a lysis/gel-loading buffer (38.5 mM Tris-HCl, 0.1% EDTA, 2% SDS, 6 M urea, 0.1% dithiothreitol, 0.05% reduced glutathione, 0.001% bromophenol blue) at 100°C for 10 min and separated by electrophoresis on 10% polyacrylamide gels containing 0.1% SDS. Gels were exposed to film and bands were excised from the gel and counted for radioactivity. Data from four pulse-chase experiments were normalized based on percentage of cell ^{35}S-EL after pulse.

Analyses of EL expression

Proteins in conditioned media samples from transfected cells were separated on Nupage™ 10% Bis-Tris gels (Invitrogen), and gels were transferred to nitrocellulose membranes. Nitrocellulose membranes were subjected to chemiluminescent immunoblot analyses for EL (using a 1:5,000 dilution of the anti-human EL polyclonal antibody and a 1:5,000 dilution of horseradish peroxidase-conjugated anti-rabbit IgG). Total RNA from cells transfected with EL was subjected to real-time PCR analyses for human EL and β-actin using commercially available primers (Applied Biosystems). The mass of all EL proteins used in lipase activity assays and lipoprotein binding assays, semiquantified as arbitrary units, was determined using an ELISA in the same assay (21, 22). The mass of EL in preheparin plasma from human subjects was quantified by ELISA as ng/ml using a human EL protein standard (kindly provided by Dr. Karen Badellino, University of Pennsylvania).

Lipase assays

Triglyceride lipase and phospholipase assays using glycerol-stabilized substrates of triolein and dipalmitoylphosphatidyl choline (DPPC), respectively, were performed as described previously (23). LDL and HDL_3 were isolated by potassium bromide density gradient ultracentrifugation (24). Assays of the kinetics of lipoprotein lipid hydrolysis by EL were performed as described previously (25). The free fatty acids generated by the hydrolysis of lipoproteins were measured using a commercial kit (Waco Pure Chemical Industries) according to the manufacturer's instructions. All activity data were corrected for protein mass (determined as described above) and were normalized to the percentage of WT EL.

Lipoprotein bridging assays

HEK293 cells in 12-well plates were transfected with EL as described above. At 32 h posttransfection, media were changed to 0.5 ml of serum-free medium containing 0.2% BSA. At 48 h, the serum-free media with BSA were replaced with fresh serum-free media with BSA containing either 5 μg/ml [^{125}I]LDL or 5 μg/ml [^{125}I]HDL_3 ± 100 U/ml heparin. LDL and HDL_3 were radiolabeled using the iodine monochloride method (26). Cells were incubated at 4°C for 1 h, and cell-associated lipoproteins were measured. Additional wells were transfected to assess cell surface-bound

EL within experiments. To assess cell surface-bound EL, at 48 h, the serum-free media with BSA was replaced with serum-free media containing only 100 U/ml heparin. Cells were incubated at 4°C for 1 h, and conditioned media were assessed for EL by immunoblot analyses as described above. EL-mediated binding of lipoproteins was calculated as the amount of lipoprotein bound per cell protein above mock-transfected background. Multiple experiments were normalized based on a percentage of WT.

In vitro translation

The expression plasmids for EL and empty vector (which also contain the T7 promoter) were used to express EL by in vitro transcription/translation using a rabbit reticulocyte system (Promega) in the presence of [^{35}S]methionine according to manufacturer's instructions. Reactions were halted at various time points for up to 60 min, and proteins were separated on Nupage™ 10% Bis-Tris gels. Gels were exposed to film, and protein bands were excised from the gel and counted for radioactivity.

Statistical analyses and equations used

Error bars indicate ±SD. A nonparametric version of the *t*-test (Wilcoxon's Rank-Sum) was used for comparisons of plasma lipid levels among African-American probands in the HHDL cohort. Plasma lipid levels among subjects from the PennCAC cohort were analyzed using multivariable linear regression after adjustment for age, gender, diabetes, body mass index, and alcohol use. Plasma EL levels were compared using a two-tailed *t*-test for unequal variance. Rate constants for pulse-chase analyses and in vitro translation were calculated using GraphPad Prism software [assuming one-phased kinetics with the formula $Y = (Y_0\text{-}Y_\infty) \times \exp(-k \times t) + Y_\infty$, where Y represents amount of radiolabeled protein at time t, Y_0 represents amount of radiolabeled protein at time zero, Y_∞ represents maximal or minimal amount of radiolabeled protein at infinite time, and k represents the rate constant in reciprocal units of time]. All biochemical studies were analyzed using a two-tailed paired *t*-test.

RESULTS

Carriers of G26S EL were exclusively identified in African-Americans

We genotyped 854 unrelated subjects from the HHDL cohort for the G26S variant of EL. Of the 68 African-Americans in the cohort who were genotyped, 8 (11.8%) were identified as carriers for the G26S variant. In contrast, of the 767 Caucasians in the HHDL cohort who were genotyped, none were found to be carriers of the G26S variant. The G26S probands within the HHDL cohort had significantly higher levels of HDL-C versus noncarriers within the same cohort (**Table 1**). Genotyping of family members from 3 of the G26S probands within the HHDL cohort has revealed 6 additional subjects with the G26S variant.

We also genotyped 2,616 unrelated subjects from the PennCAC cohort for the G26S variant of EL. Of the 521 African-Americans who were genotyped, we identified 55 (10.6%) subjects as carriers for the variant. None of the 2,095 Caucasians were found to be carriers of the G26S variant. Furthermore, we failed to identify any carriers for the G26S variant in Caucasians (n=851/885) from the SIRCA cohort, thus strengthening the likelihood that the G26S variant is specific to African-Americans. The G26S probands within the PennCAC cohort exhibited a small but significant increase of HDL-C versus noncarriers within the same cohort (**Table 2**).

TABLE 1. Clinical characteristics of African-American noncarriers and carriers of the G26S variant of EL from the High HDL cohort

	Noncarriers	G26S Carriers
n	60	8
Age (y)	58.4 ± 13.8	58.1 ± 15.4
Height (cm)	164.7 ± 9.5	165.1 ± 11.7
Mass (kg)	73.7 ± 18.4	68.8 ± 15.7
BMI (kg/m^2)	26.9 ± 5.3	25.1 ± 4.2
TC (mg/dl)	224.8 ± 45.1	240.3 ± 66.9
TG (mg/dl)	69.5 ± 23.6	69.0 ± 23.7
LDL-C (mg/dl)	124.5 ± 38.4	121.7 ± 41.0
HDL-C (mg/dl)	86.4 ± 16.2	104.8 ± 27.7*
% smoke	11.7	0
% drink	51.7	25.0
% family Hx CAD	5.0	12.5

BMI, Body mass index; TC, total cholesterol; TG, triglyceride; LDL-C, LDL cholesterol; Hx CAD, history of coronary artery disease. Data represent the mean ±SD. *, p=0.03 versus noncarriers.

Analysis of G26S EL catalytic function

We suspected that the G26S variant of EL may have an impaired function leading to elevated HDL-C levels. In transient transfections of the G26S EL and WT cDNAs, we consistently observed a profoundly reduced level of both cell-associated and secreted G26S EL protein (full-length 68 kDa protein, plus the 40 kDa and 28 kDa cleavage products of full-length EL) versus WT EL, despite identical levels of mRNA (**Fig. 1**). We confirmed that an epitope recognized by our antibody was not disrupted with the G26S variant of EL by comparing in vitro translated G26S EL and WT EL through immunoblot analyses (Supplemental Fig. I). The specific hydrolytic activity of recombinant G26S EL toward synthetic substrates triolein and DPPC was comparable to WT EL (**Fig. 2**). We also tested the kinetics of catalytic activity by the G26S EL variant using HDL_3 as substrate, and we found that both the apparent K_M and V_{max} values between WT and G26S EL were similar (appK_M: WT, 464 ± 51 μM HDL_3 phospholipid vs. G26S, 363 ± 119 μM HDL_3 phosoholipid; appV_{max}: WT, 272 ± 18 nmol free fatty acid/EL mass/h vs. G26S, 349 ± 61 nmol free fatty acid/EL mass/h).

TABLE 2. Clinical characteristics of African-American noncarriers and carriers of the G26S variant of EL from the PennCAC cohort

	Noncarriers	G26S Carriers
n	466	55
Age (y)	55.4 ± 10.1	55.9 ± 10.7
Height (cm)	169.2 ± 10.7	169.9 ± 8.1
Mass (kg)	93.0 ± 19.1	94.7 ± 16.8
BMI (kg/m^2)	32.6 ± 6.5	32.9 ± 6.3
TC (mg/dl)	187.9 ± 42.1	178.4 ± 34.0
TG (mg/dl)	109.6 ± 75.2	101.7 ± 59.9
LDL-C (mg/dl)	110.7 ± 34.4	99.5 ± 26.0
HDL-C (mg/dl)	52.0 ± 15.2	55.3 ± 15.1*
% smoke	14.4	16.7
% drink EtOH	41.2	44.4
% Type-2 diabetes	75.5	88.8
% Metabolic syndrome	59.6	61.1

BMI, Body mass index; EtOH, alcohol; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol. Data represent the mean ±SD. *, P = 0.04 versus noncarriers.

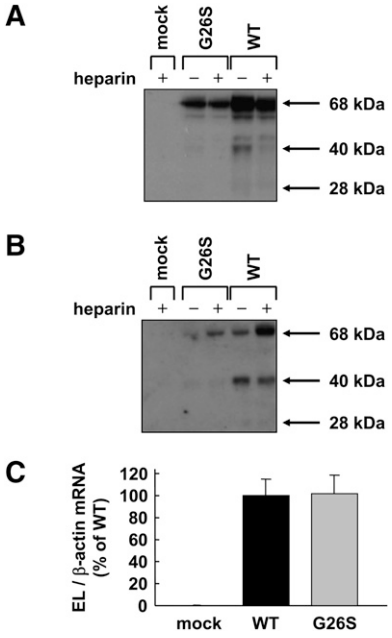

Fig. 1. Transient expression of WT and G26S EL. WT EL and the G26S variant of EL were transiently expressed using HEK293 cells in the absence or presence of heparin. A, Cell samples, and B, media samples were collected and EL proteins were visualized by immunoblotting using the anti-human EL antibody. The 68 kDa mature WT EL protein and the 40 kDa and 28 kDa proteolytic cleavage products of EL were observed as indicated. C: From at least 6 individual experiments, total RNA was isolated from cells and subjected to real-time PCR analysis to quantify EL mRNA relative to β-actin. Data are expressed as a percentage of WT. Error bars indicate ±SD.

Analysis of G26S EL bridging function

To assess the bridging function of G26S EL, we first addressed the cell surface association of the variant. As shown in Fig. 1B, immunoblot analyses of the media from transfected cells in the presence of heparin show that the protein mass of both WT and G26S EL was greater than the WT and G26S EL protein mass of the media from transfected cells in the absence of heparin during a 16 h incubation period. We determined that the release of uncleaved full-length (68 kDa) G26S EL into heparin-free media was 22 ± 11% (calculated from densitometry data of immunoblots), which was comparable to the 14 ± 7% release of uncleaved WT EL (**Fig. 3A**). Having ascertained that the cell surface association of G26S EL and WT EL are comparable, we determined the ability of cells expressing each EL to bridge ^{125}I-labeled LDL and HDL_3 to the cell surface at 4°C. We show that transfected cells expressing WT and G26S EL can equally bind LDL (Fig. 3B) and HDL_3 (Fig. 3C) to the cell surface. However, the amount of uncleaved full-length (68 kDa) G26S EL on the cell surface in our bridging assays is 50% lower (calculated from densitometry data of immunoblot) than WT EL (Fig. 3D); thus, normalizing the bridging data to EL expression would suggest that G26S EL has a 2-fold greater ability to bind lipoproteins to the cell surface.

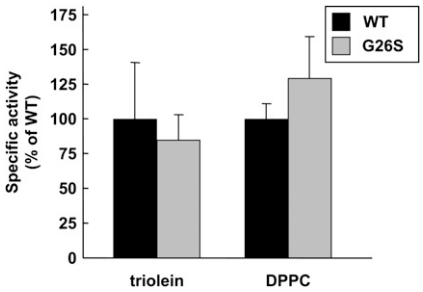

Fig. 2. Activities of WT and G26S EL. Media from cells transiently expressing WT or G26S EL in the presence of heparin were collected and assayed for hydrolysis of triolein and DPPC. Free fatty acids released were normalized for EL protein and quantified as described under "Experimental Procedures." The specific activity data for the EL variants were normalized to 100% of WT EL. Assays were performed at least in triplicate. Error bars indicate ±SD.

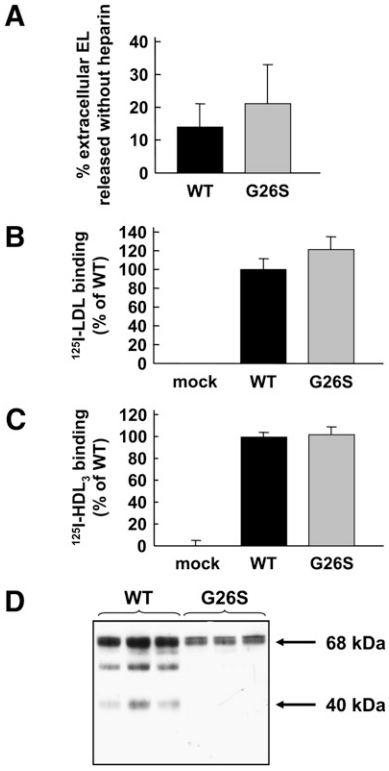

Fig. 3. Cell surface association and lipoprotein bridging by WT and G26S EL. WT and G26S EL were transiently expressed in HEK293 cells. A: Cells were incubated with or without 100 U/ml heparin for 16 h. Media EL was analyzed by immunoblot analyses and the intensities of uncleaved media EL protein secreted in the absence or presence of heparin from triplicate transfections were quantified by scanning densitometry of the immunoblots. Data are presented as total extracellular EL released into heparin-free medium as a percentage of that released into medium containing heparin. Error bars indicate ±SD. At 48 h posttransfection, transfected cells were incubated with 5 μg/ml [^{125}I]LDL (panel B) or [^{125}I]HDL_3 (panel C) for 1 h at 4°C. EL-mediated binding was calculated as the amount of lipoprotein bound per cell protein above untransfected background. Data are from at least triplicate experiments and normalized as a percentage of WT. Error bars indicate ±SD. D: During the bridging experiments, parallel wells with transfected cells were incubated with 100 U/ml heparin for 1 h at 4°C to release EL associated with the cell surfaces. Media EL from three separate experiments were analyzed by immunoblot analyses.

G26S EL expression in vitro and in vivo

We next focused our attention on the markedly reduced G26S EL protein mass in transfected cells by addressing the possibility that G26S EL may be subjected to intracellular degradation. The lysosomal degradation inhibitor chloroquine failed to raise the cell-associated (**Fig. 4A**) or media (Fig. 4B) G26S EL mass to levels comparable to WT EL. We also assessed whether G26S EL was degraded via the ubiquitin-proteosomal pathway by incubating cells in the presence of ALLN. Like the lysosomal inhibition, ubiquitin-proteosomal inhibition failed to raise both the cell-associated G26S EL (Fig. 4C) and media G26S EL (Fig. 4D) to levels comparable to WT EL. We confirmed the effectiveness of our chloroquine and ALLN treatments by assessing the lysosomal degradation of LDL apolipoprotein B and the accumulation of polyubiquitinated proteins, respectively (Supplemental Fig. II).

To address whether newly synthesized G26S EL was being degraded through an alternate mechanism, we assessed the trafficking of newly synthesized EL using pulse-chase analyses. From quadruplicate experiments with cells transiently transfected with G26S or WT EL, following a 2 h pulse with [^{35}S]methionine/cysteine, we consistently observed a ~20% reduction of total (cell and media) immunoprecipitated newly synthesized ^{35}S-G26S EL versus newly synthesized ^{35}S-WT EL at all time points throughout a 2 h chase (Supplemental Fig. III). Despite the reduced mass of ^{35}S-G26S EL versus ^{35}S-WT EL throughout the chase, the rate of disappearance from cells (**Fig. 5A**), the rate of appearance into media (Fig. 5B), and the stability throughout the chase (Fig. 5C) of both ^{35}S-G26S EL and ^{35}S-WT EL were comparable. The rate constants for the disappearance of EL from cells (WT: 0.013 ± 0.003 min^{-1}; G26S: 0.020 ± 0.017 min^{-1}, errors represent ±SD) and the appearance of EL into media (WT, 0.031 ± 0.015 min^{-1}; G26S, 0.019 ± 0.010 min^{-1}) were not significantly different. These data show that there was no difference in trafficking between G26S EL and WT EL, but it suggests that a defect exists in the translation of G26S EL. Using an in vitro transcription/translation rabbit reticulocyte system in the presence of [^{35}S]methionine, we compared the rates of translation between G26S EL and WT EL. Under these conditions, we failed to observe any difference in the rate of protein production between G26S EL (with a rate constant of 0.035 ± 0.005 min^{-1}) and WT EL (with a rate constant of 0.040 ± 0.008 min^{-1}) (**Fig. 6**).

If our in vitro observation that less G26S EL protein is made and secreted from cells is physiologically relevant, carriers of the variant should have reduced levels of EL protein in vivo. Using an ELISA developed in our laboratory, we measured preheparin plasma EL mass by ELISA from eight G26S probands from the HHDL cohort plus six family members with G26S EL and compared them to both Caucasian and African-American noncarrier controls. Carriers of the G26S EL variant had a significant 40% reduction of EL mass compared with controls (**Table 3**).

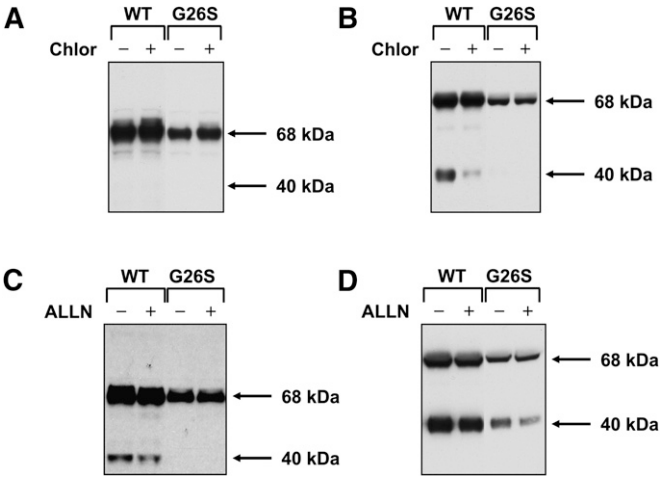

Fig. 4. Effects of chloroquine and ALLN on EL expression. WT and the G26S variant of EL were transiently expressed using HEK293 cells. At 48 h posttransfection, cells were incubated with ± 75 μM chloroquine (Chlor, to inhibit lysosomal degradation) or ± 100 μM ALLN (to inhibit proteosomal degradation) for 6 h in the presence of heparin. Cell samples (panels A and C) and media samples (panels B and D) were collected and EL proteins were visualized by immunoblotting using the anti-human EL antibody. The 68 kDa mature WT EL protein and the 40 kDa cleavage product of EL were observed as indicated. Data are representative of six experiments.

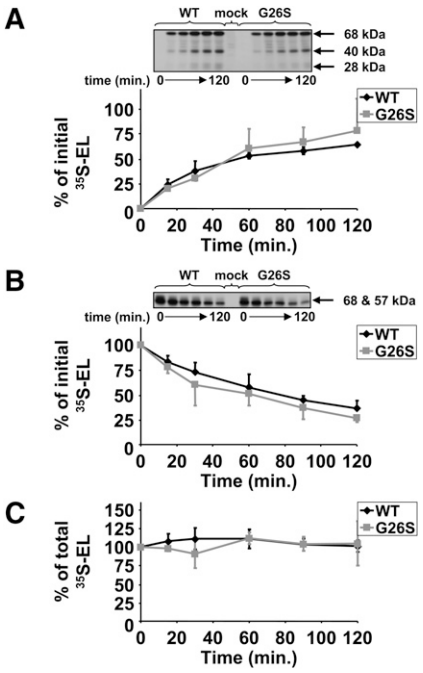

Fig. 5. Pulse-chase analysis of EL secretion. WT and the G26S variant of EL were transiently expressed using HEK293 cells. At 48 h posttransfection, cells were pulse-labeled with [^{35}S]methionine/cysteine for 2 h and chased for 2 h in the presence of 100 U/ml heparin. The EL proteins from A, media, and B, cells were immunoprecipitated using the polyclonal anti-human EL antibody, resolved by SDS-PAGE and analyzed by fluorography (insets). The radioactivity associated with the medium EL was excised from the gel and quantified. Data from four experiments were normalized as a percentage to the [^{35}S]EL following pulse. C: The total of all radiolabeled cell and media EL represents the stability of the newly synthesized EL during the pulse-chase experiments. Error bars indicate ±SD.

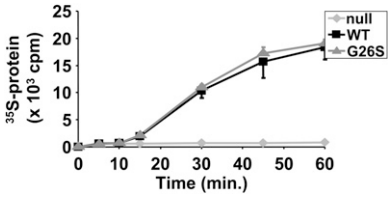

Fig. 6. In vitro translation of WT and G26S EL. WT and the G26S variant of EL were expressed in a rabbit reticulocyte in vitro transcription/translation system through the T7 promoter. Completely and incompletelysynthesized EL proteins from three separate experiments were separated by SDS-PAGE and counted for radioactivity. *Null*, empty vector. Error bars indicate ±SD.

Of note, no difference was observed in noncarriers between Caucasian and African-Americans.

DISCUSSION

This study demonstrates that a G26S substitution in the N-terminal region of the EL protein results in markedly reduced synthesis and secretion of EL, leading to reduced levels of EL in plasma and plausibly explaining the association of this variant with elevated HDL-C levels. Our data point toward a very different mechanism for raising plasma HDL-C versus our recent study demonstrating that the asparagine-396 to serine variant of EL has normal protein secretion but impaired enzymatic activity (14). The G26S variant of EL also appears to display an enhanced bridging of lipoproteins, but the enhanced association does not translate into improved catalytic activity, as the hydrolysis of lipoprotein lipids and lipid emulsions is comparable to WT EL.

In attempting to define the mechanism behind why we observe reduced G26S EL protein both in vitro and in vivo, we tested and ruled out intracellular degradation via a lysosomal or ubiquitin-proteosomal pathway, as well as any other unknown degradation mechanism of newly synthesized EL protein through pulse-chase analyses. We were unable to determine an impairment in translation of the G26S transcript through in vitro translation. We are currently limited by our lack of knowledge about what intracellular interactions EL may have; however, our data suggest that this N-terminal substitution of a serine for a glycine results in a cotranslational/translocational disruption of EL protein production. Two candidate proteases that may be responsible for reducing the amount of newly synthesized G26S EL are the endoplasmic reticulum (ER) chaperones ER-60 and ERp72. Both ER-60 and ERp72 are members of the protein disulfide isomerase family and they exhibit a cysteine protease activity that is unaffected by lysosomal or proteosomal inhibition toward the ER proteins protein disulfide-isomerase and calreticulin (27–29). ER-60 has been shown to interact with the secretory protein lysozyme when only in a misfolded form (30). In addition to proteosomal and lysosomal degradation, apolipoprotein B100 is directly degraded by ER-60 (31). It is possible that during translocation into the ER, a significant proportion of G26S EL is misfolded, perhaps due to a poor interaction with chaperones that may normally interact with EL; thus, the G26S EL peptide may be degraded by ER-60 and/or ERp72 prior to complete translation and translocation.

Some naturally occurring coding variants of HL and LPL have been shown to have impaired protein secretion. Unlike the G26S variant of EL, which has a reduction of newly synthesized protein but normal secretion of the protein that is synthesized, cell culture studies of the serine-267 to phenylalanine and threonine-383 to methionine variants of human HL showed that these variants had impaired activity and secretion, but intracellular HL protein was comparable to WT (32). The glycine-142 to glutamate variant of LPL also has impaired secretion, but newly synthesized protein is rapidly degraded due to targeting to lysosomes (33), which we ruled out for the G26S variant of EL. It is clear that these lipase variants undergo different fates that lead to impaired secretion, and it is likely due to changes in protein structure.

The G26S variant of EL has an allele frequency of about 5% in persons of African descent, but it is rare in persons of European descent. It is well established that persons of African descent have significantly higher HDL-C levels than those of European descent (34). It was previously suggested that a variant in HL that is more common in Africans might contribute to the higher HDL-C levels (35). Our studies suggest that this G26S variant of EL might also help to explain the higher HDL-C levels in persons of African descent.

In summary, very little is known about structural variation in EL and how this might affect EL function and the clinically important phenotype of HDL-C. Our studies here indicate that the G26S variant found in persons of African descent is associated with elevated HDL-C, and the cellular expression of the variant results in markedly reduced protein production, which is associated with reduced plasma levels of EL in vivo. Our results emphasize that genetic variation of EL is a contributor to variation in HDL-C.

TABLE 3. Preheparin plasma EL in WT and G26S carriers

Subjects	*n*	HDL-C (range) (mg/dl)	EL Mass (range) (ng/ml)
Caucasian noncarriers	40	71.1 ± 28.1 (35-164)	599 ± 295 (147-1338)
African-American noncarriers	25	62.5 ± 24.7 (32-132)	607 ± 545 (123-2272)
G26S carriers	14	89.6 ± 28.7* (40-166)	359 ± 194** (83-671)

Subjects were matched for age and sex. G26S carriers include eight probands and six family members. Data represent the mean ±SD. *, $P < 0.02$ versus Caucasian noncarriers and African-American noncarriers. **, $P = 0.001$ versus Caucasian noncarriers, and $P = 0.04$ versus African-American noncarriers.

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