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TRABAJO ORIGINAL

Early Carotid Atherosclerosis and Hepatic Lipase-514 C/T Polymorphism: A Study in Hyperalphalipoproteinemic Individuals

Aterosclerosis carotídea subclínica y el polimorfismo HL-514 C/T de la lipasa hepática: un estudio en individuos hiperalfalipoproteinémicos

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RESUMEN

Objetivo: La Lipasa Hepática (HL) está implicada en el metabolismo de las lipoproteínas distintas y desempeña un papel en el transporte inverso del colesterol y la aterosclerosis. El objetivo de este estudio fue evaluar los efectos del polimorfismo HL-514 C/T en la aterosclerosis carotídea subclínica en los individuos e hiperalfalipoproteinémicos y controles establecidos.
Métodos: Ciento sesenta y nueve sujetos asintomáticos (edad 47 ± 16 años), 71 hiperalfalipoproteinémicos (Hyper-A, HDL-C ≥ 68mg/dL) y 98 controles (CTL, HDL-C <68mg/dL) fueron seleccionados por evaluaciones clínicas y de laboratorio. Lípidos y lipoproteínas se midieron por métodos enzimáticos. La actividad de la HL se midió en plasma después de la heparina por el método radiométrico, y los genotipos HL-514C/T se analizaron por PCR. El Grosor íntimo-medial carotídeo (cIMT) se midió mediante ecografía.
Resultados: No hubo diferencias en las frecuencias de los genotipos HL-514 C/T se observó entre los grupos. Polimorfismo HL-514 C/T no ha contribuido a los cambios en cIMT o la frecuencia de las lesiones ateroscleróticas en Hyper-A y los controles. Por otra parte, no hay interacción entre el polimorfismo HL-514 C/T y cIMT ni fueron halladas lesiones ateroscleróticas.
Conclusiones: El polimorfismo HL -514 C/T no está asociado con cambios significativos en el colesterol HDL en hiperalfalipoproteinémicos particulares y no tiene efecto en la arteriosclerosis carotídea a pesar de que la actividad de la HL ha sido reducida significativamente. **Rev Argent Endocrinol Metab 50:170-175, 2013**

Los autores declaran no poseer conflictos de interés.

Palabras clave: -514C/T polimorfismo de la Lipasa Hepática; aterosclerosis carotídea; hiperalfalipoproteinemia

ABSTRACT

Objective: Hepatic lipase (HL) is involved in the metabolism of several lipoproteins and has a key role in reverse cholesterol transport and atherosclerosis. The aim of this study was to evaluate the effects of HL -514C/T polymorphism on sub-clinical and established carotid atherosclerotic in hyperalphalipoproteinemic and control individuals.
Methods: One hundred and sixty nine asymptomatic individuals (aged 47 ± 16 years), 71 hyperalphalipoproteinemic (Hyper-A, HDL-C ≥ 68mg/dL) and 98 controls (CTL, HDL-C< 68mg/dL) were selected by clinical and laboratory evaluations. Lipids and lipoproteins were measured by enzymatic methods. HL activity was measured in post-heparin plasma by a radiometric assay and HL-514C/T genotypes were analyzed by PCR. Carotid intima-media thickness (cIMT) was measured by Doppler ultrasonography.

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Results: No differences in HL -514C/T genotype frequencies were observed between the groups. HL -514C/T polymorphism did not contribute to variations in cIMT or atherosclerotic lesion frequencies in Hyper-A and controls. Furthermore, no interactions between HL–514C/T polymorphism and cIMT or atherosclerotic lesions were found.
Conclusions: In hyperalphalipoproteinemic individuals the -514C/T polymorphism is not associated with significant variations in HDL-Cholesterol concentrations. Besides, it has no repercussions on carotid atherosclerosis, although hepatic lipase activity is significantly reduced. **Rev Argent Endocrinol Metab 50:170-175, 2013**

No financial conflicts of interest exist.

Key words: Hepatic Lipase -514C/T polymorphism; carotid atherosclerosis; hyperalphalipoproteinemia

INTRODUCTION

Hepatic lipase (HL) is a lipolytic enzyme that hydrolyzes triglycerides and phospholipids in lipoproteins, and has a separate role in lipoprotein metabolism, acting as a ligand that facilitates the uptake of lipoproteins and lipoprotein lipids by liver receptors[1-2].

Hyperalphalipoproteinemia is characterized as elevated high-density lipoprotein (HDL)-cholesterol levels above the 90th percentile of the general population, paired for age and sex[3]. Due to their high concentrations of plasma HDL-cholesterol, hyperalphalipoproteinemic subjects (Hyper-A) are used as a model to study various genetic polymorphisms, related mainly to HL or cholesteryl ester transfer protein (CETP) deficiencies[4-5].

A common C-to-T base substitution has been described in the nucleotide -514 in the promoter region of the hepatic lipase gene, characterizing the -514C/T polymorphism (rs1800588)[6]. The less common allele T has been associated with decreased plasma HL activity and increased HDL-C concentrations in many populations[7-10], while the C allele is associated to higher HL activity and has been associated with greater carotid intima-media thickness (cIMT)[11]. **1)** However, previous studies have shown that the -514C/T polymorphism does not alter cIMT[12-13]. Thus, the relationship between cIMT or cardiovascular risk and the -514C/T polymorphism has not yet been clearly established.

Also, no studies involving the -514C/T polymorphism and cIMT in hyperalphaliporoteinemic individuals are described in the literature.

Therefore the aim of the current study was to investigate the association of this polymorphism to carotid sub-clinical atherosclerosis in a Brazilian population-based sample of 169 adult volunteers, classified as Hyper-A or controls, verifying whether or not an atheroprotective effect could be observed in Hyper-A subjects.

METHODS

Study subjects

One hundred and sixty nine individuals (113 females and 56 males), aged from 16 to 79 years old, without clinical atherosclerotic manifestations, were selected from the Dyslipidemia outpatient clinic of the State University of Campinas (UNICAMP) Clinics Hospital in Campinas, SP, Brazil. Subjects were classified in control group (CTL, n = 98, HDL-C ≥ 32 and < 68 mg/dL) or Hyper-A (n = 71, HDL-C ≥ 68 mg/dL), according to the 90th percentile of HDL-C levels obtained from a local normolipidemic population sample (n = 1700).

The study was approved by the UNICAMP's School of Medicine's Ethics Committee, and all participants agreed to participate in the study, and gave written informed consent.

Anthropometric data was collected and dyslipidemia was defined according to the National Cholesterol Education Program[14-15].

Biochemical Analysis

Blood samples were collected after 12 hours of fasting, and serum concentrations of lipids and lipoproteins were measured by conventional enzymatic and/or homogeneous techniques, in a Hitachi 917 (Roche, Germany).

HDL_2 and HDL_3 sub-fractions were obtained by sequential micro-ultracentrifugation of the supernatants after the lipoproteins containing apolipoprotein-B100 were precipitated with dextran sulfate in a Beckman micro-ultracentrifuge (model "Airfuge"/75B, Beckman Instruments, USA)[16].

HL activity was measured in post-heparin plasma samples on the basis of fatty acids release, using a ^{3}H-triolein emulsion as the substrate[17], and the inter-assay coefficient of variation was 8 %.

Polymorphism Detection

Genomic DNA was extracted from peripheral leukocytes according to method described by Salazar in 2001[18]. HL genotyping was performed as described by Jansen et al.[6]. A C-to-T substitution 514 bp upstream of the transcription initiation site created a *NlaIII* restriction site (-CATG-); a 85-bp fragment identified the TT genotype, 215-bp the TC and 300-bp the CC genotype. The TC and TT genotypes were combined for analyses.

Measurement of carotid intima-media thickness

According to Simons et al.[19], high resolution B-mode carotid ultrassonography was carried out by a single trained sonographer, blinded to the subject´s identity, using a 4-12 MHz linear array ultrasound imaging system (ATL HDI 1500 and 3500 Ultrasound Systems, Advanced Technology Laboratories Ultrasound, USA). The cIMT individual results were expressed as right and left and as a mean of both in mm. 2) The presence of atherosclerotic lesions was considered as cIMT ≥ 1mm[20].

Statistical analysis

We used Mann-Whitney´s and Chi-Square test for comparisons and ANCOVA for correcting covariates. The linear contrast test was used to determine interactions between variables. All tests were performed using SAS software for Windows (Statistical Analysis System), version 9.1.3. (SAS Institute Inc, 2002-2003, NC, USA) and SPSS 11.5 for Windows. Statistically significant and borderline differences were detected when $p \leq 0.05$ or 3) $p < 0.10$, respectively.

RESULTS

The -514C/T polymorphism was highly prevalent in Hyper-A individuals, as well as in controls, and no differences in the frequencies of the genotypes were observed between both groups ($p \leq 0.454$). The genotypic frequencies were: CC 41 %, TC 37 % and TT 22 % (TC + TT 59 %) in Hyper-A individuals; and CC 42 %, TC 43 % and TT 15 % (TC + TT 61 %). The allele frequencies were consistent with Hardy–Weinberg equilibrium in controls ($p = 0.440$) but not in Hyper-A ($p = 0.041$).

Table 1 shows the anthropometric data and the lipids and lipoproteins measurements. The Hyper-A individuals were older ($p \leq 0.001$), and had a higher female frequency (66 %, $p \leq 0.001$). Hyper-A, as expected, and due to the classification criteria, 4) presented higher total cholesterol levels

TABLE I. Clinical and biochemical characterization of hyperalphalipoproteinemic and control subjects by -514c/t polymorphism genotypes

GROUPS	Hyper-A			CTL		
PARAMETERS	Total (n=71)	CC (n= 29)	TC+TT (n=42)	Total (n=98)	CC (n=29)	TC+TT (n= 57)
Age (years)	55 ± 12[a]	54 ± 11[b]	51 ± 13	40 ± 16	42 ± 16	42 ± 15
Sex F/M (n)	60/11[a]	27/2	33/9	53/45	18/23	35/22
Cholesterol (mg/dL)	228 ± 35[a]	233 ± 35[c]	225 ± 35	179 ± 41	177 ± 37d	179 ± 44
Triglycerides (mg/dL)	92 ± 38	91 ± 38	93 ± 38	91 ± 45	89 ± 40	92 ± 48
HDL-Cholesterol (mg/dL)	80 ± 9	79 ± 9	81 ± 8	52 ± 10	49 ± 10e	54 ± 10
HDL2-Cholesterol (mg/dL)	18 ± 4a	13 ± 4	18 ± 5	12 ± 7	12 ± 9f	18 ± 4
HDL2- Triglycerides (mg/dL)	9 ± 5	7 ± 5	9 ± 5	7 ± 6	7 ± 6	8 ± 4
HDL3-Cholesterol (mg/dL)	60 ± 7a	40 ± 10	60 ± 7	38 ± 10	35 ± 9g	60 ± 7
HDL3- Triglycerides (mg/dL)	23 ± 10	19 ± 10	24 ± 10	18 ± 10	17 ± 11	23 ± 9
LDL-Cholesterol (mg/dL)	130 ± 33	135 ± 34	125 ± 32	110 ± 32	112 ± 31	109 ± 33
VLDL-Cholesterol (mg/dL)	18 ± 8	18 ± 8	18 ± 7	18 ± 9	18 ± 8	19 ± 9
HL (nmolFFA/mL/h)	1441 ± 1021[a]	1681 ± 989[h]	1269 ± 1022	2531 ± 1517	2974 ± 1511[i]	2187 ± 1445[i]

F/M: female/male; Cholesterol, Triglycerides, HDL-Cholesterol, LDL-cholesterol, HL= hepatic lipase; FFA= free fatty acids values expressed as mean ±SD; n= sample number;. level of significance: at p ≤ 0.05 and borderline at p> 0.05 and ≤ 0.10; corrections of age and sex ANCOVA ; 10) Total Hyper-A vs Total CTL: a p ≤0.001; CC vs TC+TT: b p≤0.020; c p≤0.001;d ≤0.033, e ≤ 0.010, f≤ 0.009, g ≤0.006, h ≤ 0.025; ip≤0.010

(35 %) and HDL-C sub-fractions (33 %). They also presented lower HL activity (43 %) which explains their HDL-C phenotype.

Hyper-A CC and TC + TT subjects were older and had higher total cholesterol levels when compared to CTL CC and TC + TT. Also, 5) Hyper-A carriers of the T allele presented reduced HL activity when compared to CTL T carriers by 25 %, without HDL-C concentration changes. In CTL carriers of the T allele, increases of HDL-C (9 %), HDL_2C (33 %) and HDL_3C (8 %) were observed and HL activity was reduced by 26 %.

Table 2 presents the cIMT results according to the genotypes. There were no differences in cIMT (mm) and 6) frequency of cIMT ≥ 1mm between allele T carriers and non carriers in both groups. 7) A small trend (2 %) to higher cIMT in the CC genotype was found in Hyper-A when compared to CC CTL individuals.

As observed in Table 3, no significant associations of the -514C/T polymorphism, cIMT and frequency of cIMT ≥ 1mm in both groups were found.

TABLE III. interactions between -514c/t polymorphism t carriers and carotid atherosclerosis in hyperalphalipoproteinemic and controls

Variables	Interactions	
	Hyper-A	CTL
Mean cIMT (mm)	p≤0.554	p≤0.192
cIMT >1mm	p≤0.220	p≤0.385

Interactions in Hyper-A and CTL: Linear contrast test

DISCUSSION

Unpublished data from our laboratory, in a larger Brazilian population sample (n = 291) indicate that the –514C/T polymorphism frequency in this population is similar to that found in previous studies[21-28].

As well as seen in our present study, various previous studies have reported low HL activity associated to increased HDL-C levels[6,29-30], even though other studies failed to see this association[31-33].

One of the roles of the HL in lipoprotein metabolism is the promotion of the conversion of buoyant HDL_2 particles to small, dense, HDL_3 particles, through the remodeling of triglycerides and phospholipids[34]. Our findings of increased HDL_2 levels in carriers of the T allele corroborate to previous studies that have reported lower HL activity associated to higher HDL_2 levels[29,31], even though in these studies no differences in HDL_3 levels were found.

To our knowledge, this is the first study in the literature that investigates the effects of the -514C/T polymorphism on cIMT in Hyper-A subjects. Few reports have investigated the cIMT and this polymorphism, and all in different clinical situations than this study. Rundek et al[11] found higher frequency of the CC genotype and increased cIMT in stroke-free individuals, but Burdon et al., in subjects with type 2 diabetes mellitus[12], and Isaacs et al.[13], in a large prospective cohort study, found no associations.

In other studies, the CC genotype was positively associated with an abundance of macrophages in patients with severe carotid artery stenosis[35], while the C allele was associated with decreased neointimal formation[36]. Also, the T allele was found to be beneficial in atherosclerosis progression in hormone replacement therapy users[37].

TABLE II. Clinical and biochemical characterization of hyperalphalipoproteinemic and control subjects by -514c/t polymorphism genotypes

GROUPS		Hyper-A			CTL	
PARAMETERS	Total (n=71)	CC (n= 29)	TC+TT (n=42)	Total (n=98)	CC (n=29)	TC+TT (n= 57)
right cIMT (mm)	0.87 ± 0.25	0.86 ± 0.21a	0.87 ± 0.26	0.69 ± 0.18	0.68 ± 0.15a	0.70 ± 0.19
left cIMT (mm)	0.85 ± 0.24	0.83 ± 0.17	0.86 ± 0.27	0.68 ± 0.18	0.66 ± 0.17	0.68 ± 0.18
mean cIMT (mm)	0.85 ± 0.24	0.87 ± 0.17 b	0.86 ± 0.24	0.69 ± 0.17	0.59 ± 0.17b	0.57 ± 0.18
right cIMT ≥ 1mm*	50/21	23/6	27/12	91/7	39/2	52/5
left cIMT ≥ 1mm*	55/16	24/5	31/11	92/6	39/2	53/4
mean cIMT ≥ 1mm*	54/17	24/5	30/12	91/7	40/2	52/5

*cIMT= carotid intima-media thickness; values expressed as mean ± SD; n= sample number; level of significance: at p ≤ 0.05 and borderline at p> 0.05 and ≤ 0.10; corrections of age and sex ANCOVA; Mann-Whitney and Chi-Square for CC vs TC+TT; a≤0.083, b≤0.096; *=No/Yes*

More recently, in younger healthy adults, no significant association between cIMT, carotid artery compliance (CAC) or brachial artery flow-mediated vasodilatation (FMD) and -514C/T polymorphism was found; only serum lipids were associated with the -514C/T polymorphism, which did not seem to be a determinant of cIMT, brachial artery FMD or CAC[38].

Concluding, in hyperalphalipoproteinemic individuals the -514C/T polymorphism 8) is not associated with significant variations in HDL-Cholesterol concentrations. Besides that it has no repercussions on carotid atherosclerosis, although hepatic lipase activity is significantly reduced.

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