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Cholesteryl Ester Transfer Protein Gene Polymorphisms and Longevity Syndrome

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Abstract: *Purpose*: High levels of high density lipoprotein (HDL) cholesterol are associated with a decreased risk of coronary heart disease (CHD). Subjects with high levels of HDL cholesterol (>70 mg/dl; 1.79 mmol/l) as well as high levels of low density lipoprotein (LDL) cholesterol, could represent a group with longevity syndrome (LS). Since HDL particles are influenced by cholesteryl ester transfer protein (CETP) activity, it is worth studying the CETP polymorphism. The aim of the study was to detect whether 2 genetic variants of the CETP are associated with the LS.

Subjects and Methods: The study population consisted of 136 unrelated men and women with no personal and family history of CHD; 69 met the criteria for LS and 67 did not meet these criteria and had "normal" HDL cholesterol (>40 and <70 mg/dl; >1.03 and <1.79 mmol/l). All patients were genotyped for the TaqIB and I405V polymorphisms.

Results: The B2 allele frequency of TaqIB polymorphism was higher in the LS in comparison with the non-LS group (p=0.03) whereas B1 allele frequency was higher in the non-LS group (p=0.03).

Conclusions: Gene polymorphisms could help decide whether individuals who have increased levels of both LDL cholesterol and HDL cholesterol require treatment. Some of the prerequisites could include that subjects with LS should not only have very high levels of HDL cholesterol but also favorable gene polymorphisms. However, further investigations with a larger sample and including other gene polymorphisms, are needed.

Keywords: Coronary heart disease, high density lipoprotein-cholesterol, longevity syndrome, TaqIB and I405V polymorphisms.

INTRODUCTION

The hypothesis that low high density lipoprotein (HDL) cholesterol concentrations are associated with a greater risk of coronary heart disease (CHD) has been raised since the 1950s [1]. More than 50 years on, following a number of prospective studies [2-7], HDL cholesterol is now established as an independent risk factor for CHD [8]. However, there may also be a positive association of HDL cholesterol levels with increased CHD risk [9].

Based on clinical evidence that elevated HDL cholesterol levels reduce CHD risk it is plausible that this characteristic could also be associated with longer life expectancy [10]. This hypothesis is further supported by the fact that high HDL cholesterol levels are often observed in healthy elderly persons aged >85 years [10, 11]. The levels of HDL cholesterol are influenced by various environmental and genetic factors. Family and twin studies have estimated that the heritability of HDL cholesterol levels varies from 35 to 66% [10, 12, 13]. A degree of heritability has been also reported in longevity. Siblings of centenarians have an 8- to 17-fold higher probability of living past the age of 100 years, accounting for only approximately 1 of 10000 individuals in the general population [14]. Several genes can affect HDL metabolism; among these are those related to cholesteryl ester transfer protein (CETP). A number of polymorphisms and rare variants in the human CETP gene have been identified. Two of the common polymorphisms, TaqIB and I405V, are associated with plasma HDL cholesterol levels [15-17].

In the current study, the prevalence of very high serum HDL cholesterol (>70 mg/dl), in at least 2 related members of a family plus a history of longevity (any living or deceased relative >90 years old) in the family are referred to as longevity syndrome (LS). The occurrence of LS is very rare. It is very likely that subjects with LS, besides high HDL cholesterol levels, have additional protective metabolic traits. Taking into account the National Cholesterol Education Pro-

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gram Adult Treatment Panel III (NCEP ATP III) guidelines for low-density lipoprotein (LDL) cholesterol levels [18, 19], the dilemma arises whether subjects with "raised" levels of LDL cholesterol but also with very high HDL concentration are still candidates for hypolipidaemic treatment. Gene polymorphisms could help decide whether individuals who have increased levels of both LDL cholesterol and HDL cholesterol require treatment. In line with previous work from our group [20], we tested the hypothesis that subjects with LS may carry the favorable variants of the CETP TaqIB or/and I405V polymorphisms compared with subjects not meeting the criteria for LS.

MATERIALS AND METHODOLOGY

Study Population

The study population consisted of 136 unrelated men and women without history of CHD. They were recruited from the Outpatient Lipid Clinic of our hospital (Athens, Greece) or self-referred (hospital personnel). All subjects were referred for moderate hypercholesterolaemia. In order to exclude underlying CHD the study population underwent a physical and a stress testing examination. A detailed family history was also obtained.

LS Group

Sixty nine probands of long-lived subjects met the following criteria for LS (LS group). The characterization of LS was based on ALL of the following criteria: 1) plasma fasting HDL cholesterol concentration >70 mg/dl (1.79 mmol/l), 2) at least 1 relative with plasma HDL cholesterol concentration >70 mg/dl (1.79 mmol/l), 3) at least 1 relative (deceased or alive) over the age of 90 years old, and, 4) no personal or family history of cardiovascular disease.

Non-LS Group

Sixty seven subjects served as controls (non-LS group). They had desirable HDL cholesterol (>40 mg/dl and <70 mg/dl; >1.03 and <1.79 mmol/l), according to the NCEP ATP III guidelines [18, 19]. They did not have any relative (deceased or alive) with plasma HDL cholesterol >70 mg/dl (1.79 mmol/l) nor any relative (deceased or alive) over the age of 90 years. This information was derived from the analysis of 3 generations.

Both LS and non-LS groups had no obesity, diabetes mellitus and hypertension which are risk factors for CHD. Additionally, they did not suffer from stroke, cancer, dementia or other age-related diseases. They were referred to our lipid clinic for their annual routine physical examination. The majority of individuals (88%) from the LS group were referred to us for increased LDL cholesterol levels according to NCEP ATP III guidelines [18, 19]. The remained individuals were self-referred (hospital personnel) to the study for their exceptional high HDL levels. Heavy drinking, hepatic disease, renal disease, and hypothyroidism were among the exclusion criteria. Hypertension was defined as systolic arterial blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or as current treatment with antihypertensive drugs. All subjects in both groups had a body mass index (BMI) <30 kg/m^2. Diabetes was defined according to the diagnostic criteria of the World Health Organization or as current treatment of diabetes [21]. Only 15% of subjects were smokers in each group. None of the subjects was taking any medication. Triglycerides levels were < 150 mg/dl (1.68 mmol/l) in both groups and mean LDL-cholesterol levels were < 130 mg/dl (3.3 mmol/l) in non-LS group. These levels are normal according to the NCEP ATP III guidelines [18, 19]. The mean value of LDL cholesterol in LS group was above 160 mg/dl (4 mmol/l), which is raised according to the NCEP ATP III guidelines [18, 19].

The Onassis Cardiac Surgery Center ethics committee approved the protocol of this study. All patients signed an informed consent form.

Gene Polymorphisms

CETP TaqIB and I405V genotyping was performed by polymerase chain reaction and restriction fragment length polymorphism as previously described [22-25].

Biochemical Analyses

Plasma total cholesterol, triglycerides and HDL cholesterol were measured using enzymatic colorimetric methods on a Roche Integra Biochemical analyzer with commercially available kits (Roche). The serum low density lipoprotein (LDL) levels were calculated using the Friedewald formula [26].

Statistical Analysis

The normality of continuous variables was tested using the Shapiro-Wilk statistic. None of the continuous variables had a non-normal distribution.

Continuous variables are presented as mean ± standard deviation, while qualitative variables are presented as absolute and relative frequencies. Contingency tables were constructed to evaluate the association between genotypes, carriers and LS. The statistical evaluation for the categorical variables was based on the calculation of the chi-square and Fisher's exact criteria. Comparison of mean values between study groups was performed with the Student's t-test. Estimations of the multivariate association of the B2 allele with LS were performed by the calculation of the odds ratio (OR) and the corresponding confidence intervals through logistic regression analysis, after adjusting for age and BMI.

All tests were two-sided and p-values of 0.05 were considered significant. Data were analyzed using STATA™ (version 9.0, Stata Corporation, College Station, TX, USA).

RESULTS

Clinical and Laboratory Parameters

Clinical characteristics and laboratory parameters of the non-LS and LS groups are shown in Table **1**. BMI was lower in the LS compared with non-LS group. The mean age difference between the 2 groups was 6 years. Serum total LDL and HDL cholesterol were higher while plasma triglyceride concentration was lower in LS group compared with the non-LS group. Moreover the LDL/HDL cholesterol ratio was higher in the non-LS group and statistically significant in comparison with LS group.

Distribution of CETP Genotypes

Table **2** shows the CETP genotype and allele frequencies in the 2 groups. No significant differences were found concerning the genotype frequency of TaqIB and I405V polymorphisms, between the 2 groups. However, the frequency of B2B2 genotype in LS group was twofold higher in comparison with the same genotype frequency in the non-LS group. The B2 allele frequency was significantly higher in the LS compared with the non-LS group, while B1 allele frequency was significantly higher in the non-LS compared with the LS group. Concerning the I and V alleles, there was a trend (p=0.053) with the latter being more frequent in the LS group. There was no significant correlation between the alleles of the TaqIB or I405V polymorphism and HDL levels with groups.

Those with LS had 69% more odds of having the B2 allele, compared with the non-LS, adjusting for age (Table **3**). Although not significant, it should also be noted that the same participants had 64% more odds of having the B2 allele than the non-LS, adjusting for BMI (Table **3**). When fully adjusted for BMI and age, there was not any statistically significant difference concerning the frequency of the B2 allele between LS and non-LS. When adjusted for triglycerides and age our result remained significant. When adjusted for the other lipids the significance was lost.

DISCUSSION

Our subjects with LS were more frequently carriers of the B2 allele of the TaqIB polymorphism than healthy subjects. The aim of the study was to evaluate whether subjects with LS in the family who potentially will live longer than the average life expectancy, have favourable CETP gene variants compared with healthy subjects. This genetic distinction may be important in the management of patients.

Table 1. Clinical Characteristics of the Non-LS and LS Group

	non-LS		LS		
	Mean	**SD**	**Mean**	**SD**	**p**
Age (years)	56	13	49	20	0.008
BMI (kg/m^2)	26	4	23	3	<0.001
TC (mg/dl)	210	43	270	49	<0.001
TG (mg/dl)	93	34	81	36	0.043
HDL (mg/dl)	53	13	84	12	<0.001*
LDL (mg/dl)	128	27	168	45	<0.001
LDL/HDL ratio	2.6	0.8	2	0.6	<0.001

LS: longevity syndrome, BMI: body mass index, TC: total cholesterol, TG: triglycerides, HDL: high density lipoprotein cholesterol, LDL: low density lipoprotein cholesterol, SD: standard deviation.
* HDL was a criterion for the selection of patients.
To convert cholesterol from mg/dl to mmol/l divide by 38.67 and to convert triglycerides from mg/dl to mmol/l divide by 88.57.

Table 2. Distribution of CETP Genotype and Allele Frequencies in the 2 Groups

	Genotype or Allele	non-LS n (%)	LS n (%)
TaqIB	B2B2	13 (19)	26 (38)
	B1B2	36 (54)	31 (45)
	B1B1	17 (25)	12 (17)
	B1[a]	70 (53)	55 (40)
	B2[a]	62 (47)	83 (60)
I405V	VV	1 (2)	5 (7)
	IV	18 (42)	39 (56)
	II	24 (56)	25 (36)
	V	20 (23)	49 (35)
	I	66 (77)	89 (64)

CETP: cholesteryl ester transfer protein, LS: longevity syndrome.
[a] p = 0.03

Table 3. Multiple Logistic Regression of LS on the B2 Allele, after Adjusting for BMI and Age

Dependent Variable	Explanatory Variable	Odds Ratio	95% Confidence Interval	*p*
B2 allele[a]	LS vs non-LS	1.643	(0.968-2.789)	0.07
B2 allele[b]	LS vs non-LS	1.687	(1.017-2.795)	0.04

LS: longevity syndrome, BMI: body mass index.
[a]Adjusted for BMI, [b] adjusted for age

CETP may have pro- or anti-atherogenic properties depending upon the lipid-metabolic setting [27]. The HDL particle is influenced by CETP activity. CETP promotes the exchange of cholesteryl esters for triglycerides between HDL and triglyceride-rich lipoproteins [28] Furthermore, the HDL particle is involved in reverse cholesterol transport [29]. The cholesterol from extrahepatic cells is incorporated into the HDL particle and is transported to the liver [29]. Then, the excess cholesterol is removed *via* cholic acids to the intestine [29]. Low HDL levels (<40 mg/dl) are considered as an independent risk factor for CHD [2-7]. Ordovas *et al*. suggested that increased HDL cholesterol levels resulting from lower CETP activity appear to be associated with a lower risk of CHD in male subjects [30].

There are controversial studies reporting that CETP polymorphisms can be associated with either increased CHD risk or longevity [31-35]. Also, significant associations of the B1B1 genotype with higher plasma CETP concentration and/or CETP activity and lower HDL cholesterol were found in several studies [30, 36, 37], but this is not consistently observed [38, 39]. In our study, we did not measure CETP activity, and we may only speculate that LS subjects had lower CETP concentration and activity since they had normal BMI [40] and normal triglyceride levels [41].

The West of Scotland Coronary Prevention Study tested the association of CETP polymorphisms with the risk of cardiovascular events [42]. In this study, those homozygous for the B2 allele had a 30% reduced risk of a cardiovascular event (OR 0.70, CI (95) 0.51-0.96, p=0.03) compared with B1 homozygotes. Furthermore, in a large population-based cohort, the B2 allele was associated with a less atherogenic LDL particle size distribution, consisting of decreased levels of the more atherogenic small LDL subfraction and increased levels of the less atherogenic large LDL [43-45]. In the Framingham Offspring Study, the B2 allele was associated with a reduced risk of CHD in men [30], and this was confirmed in the Veterans Affair HDL cholesterol Intervention Trial (VA HIT) [46]. In contrast, no association was found between any of the TaqIB genotypes and CHD in subjects with a history of myocardial infarction in the Coronary and Recurrent Events Study [47] and in a cohort of healthy middle-aged U.S. physicians [48]. Brousseau *et al*. [46] reported that in men with CHD and HDL deficiency (VA HIT study), the frequency of the B2B2 genotype was reduced. However, there are studies suggesting that B2 allele carriers have higher HDL cholesterol levels than B1 allele carriers; paradoxically they have an increased risk for CHD [49, 50, 22]. However, a meta-analysis of 7 studies (Physicians' Health Study, Northwick Park Heart Study, Reykjavik, EC-TIM, OPERA, EARS and the study of Arca *et al*), reported a lower cardiovascular risk in B2 compared with B1 homozygotes [37].

A possible mechanism that explains the association of CETP genotype with CHD risk is the influence on plasma HDL. Kuivenhoven *et al*. [51] reported a strong association between B1 allele and low HDL cholesterol levels, which is in agreement with the results of others [36, 52, 53]. Moreover, our group reported a positive association between B1 genotype and postprandial lipemia [20]. Bruce *et al*. reported that the HDL cholesterol levels were higher in VV than IV and II men [54]. However, the increase in HDL cholesterol was only significant in VV men with plasma triglyceride > 165 mg/dl (1.85 mmol/l). In another study, the individuals homozygous for the V allele also had the highest HDL cholesterol levels [55]. Opposite to the beneficial influence of homozygosity of VV, Kakko *et al*., found that the VV genotype seems to be most harmful for men with the highest alcohol consumption [56]. In our study, the lack of an association between the TaqIB or I405V polymorphisms and HDL cholesterol observed within groups does not weaken the association between these polymorphisms and the presence of the LS. This was expected since each group was selected to have normal or very high HDL levels whose range was narrow. Therefore, analysis with a larger number of subjects is needed in order to assess such an association.

Populations with low CHD mortality rates, for example Eskimos, have high levels of HDL cholesterol (even with conjointly elevated LDL) which is similar to our study population [57]. Additionally, subjects with familial hypobeta and familial hyperalphalipoproteinaemia which are also associated with lower CHD rates, share a common characteristic, a ratio of LDL:HDL of approximately 1:1. This ratio of LDL:HDL is considerably lower than the usual 2.5:1 observed in the general population [58]. In our LS group, the ratio was 2:1 and in non-LS group 2.7:1 which is comparable with other studies [20].

The limitations in our study are the mean age difference (6 years) and the different BMI between the 2 groups. Although significant, these factors can not influence the gene distribution of our study population. The small number of our sample and the differences in lipid levels are disadvantages. When adjusted for triglycerides and age the statistical significance of our results remained. When adjusted for the other lipids there was not statistical significance concerning the B2 allele between LS and non-LS groups. However hyper-HDL-cholesterolemia and longevity represent various pathophysiological conditions and can not be represented solely from the genetic constitution of an organism, nor solely from the environmental factors. Additionally, our sample although small, was not genetically heterogeneous,

more specifically there was no population admixture, something that in past created inconsistencies to similar genetic association studies. Considering the existence of a strong familial component to extreme longevity, and the fact that previous studies investigated longevity in families [59] or in a preselected population, it was not possible to find a completely age-matched control group. For example, siblings of centenarians have an 8- to 17-fold higher probability of living past the age of 100 years, accounting for only approximately 1 of 10000 individuals in the general population [60].

In our study, LS subjects had higher B2 allele frequency and lower triglyceride levels compared with the non-LS group. In the LS group, total and LDL cholesterol levels were higher compared with non-LS individuals. Thus, the dilemma arises whether or not LS subjects should/should not receive hypolipidaemic treatment according to available guidelines. Until more knowledge is available the management of these individuals remains controversial. Some of the prerequisites could include that subjects with LS should not only have very high levels of HDL cholesterol but also favorable gene polymorphisms. However, further investigations with a larger sample and including other gene polymorphisms, are needed. Obviously, prospective studies in patients with LS have their limitations.

CONCLUSION

Individuals with LS had a higher B2 allele frequency of the TaqIB polymorphism than non-LS subjects. Gene polymorphisms could help decide whether individuals who have increased levels of both LDL cholesterol and HDL cholesterol require treatment.

DECLARATION OF INTEREST

This study was conducted independently; no company or institution supported it financially. Some of the authors have given talks, attended conferences and participated in trials and advisory boards sponsored by various pharmaceutical companies.

ABBREVIATIONS

BMI	=	Body mass index
CETP	=	Cholesteryl ester transfer protein
CHD	=	Coronary heart disease
HDL	=	High density lipoprotein
LDL	=	Low density lipoprotein
LS	=	Longevity syndrome
OR	=	Odds ratio

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