Title: Lipoprotein (a) and its Position Among Other Risk Factors of Atherosclerosis

Authors: Zlatohlávek Lukáš[1], Zídková Kateřina[1], Vrablík Michal[1], Haas Tomáš[2], Prusíková Martina[1], Svobodová Helena[1], Češka Richard[1]

Institutes:

[1] 3rd Medical Department, 1st Faculty of Medicine and General Teaching Hospital, Charles University in Prague, Prague, Czech Republic

[2]Novartis International AG, Basel, Switzerland

Short title:

Lipoprotein (a), risk factors and promoter of apo(a) gene

Address for correspondence:

Lukas Zlatohlavek, MD

3rd Medical Department, 1st Faculty of Medicine and General Teaching Hospital, Charles University in Prague**,** U Nemocnice 1, 128 00, Prague 2**,** Czech Republic

phone: +420 728 110482, email: l.zlato@tiscali.cz

SUMMARY

Lipoprotein(a) [Lp(a)] comprises of an LDL particle and apolipoprotein(a) [apo(a)] and its elevated levels are considered a risk factor for atherosclerosis.

The aim of our study was to find out whether elevated Lp(a) levels are associated with increased risk of atherosclerosis in patients with multiple other risk factors. We further tested the association of three polymorphisms of the apo(a) gene promoter with Lp(a) levels.

No significant correlation was detected between Lp(a) levels and lipid and clinical parameters tested. The study demonstrated a significantly ($p=0.0219$) elevated Lp(a) level (mean 28 ± 35, median 14 mg/dl) in patients with coronary heart disease (CHD). In a group with premature CHD the correlation was not significant anymore. There was a significant correlation between polymorphic loci of the promoter region of apo(a) gene and Lp(a) levels (+93C>T, $p=0.0166$; STR, $p<0.0001$).

Our study suggests that elevated Lp(a) level is an independent risk factor of CHD in carriers of other important CHD risk factors. Observed association of sequence variants of the promoter of apo(a) gene with Lp(a) levels is caused in part due to linkage to a restricted range of apo(a) gene length isoforms.

KEY WORDS

lipoprotein (a), apolipoprotein (a), atherosclerosis, risk factors, coronary heart disease, gene polymorphisms

INTRODUCTION

Lipoprotein (a) comprises of an LDL (low-density lipoprotein) particle covalently bound to a specific glycoprotein, apolipoprotein (apo) (a), by apoB-100 (Hořejší and Češka, 2000). Apolipoprotein (a) determines the structural and functional properties of the lipoprotein. A number of prospective and retrospective studies demonstrated that increased levels of Lp (a) are associated with atherosclerosis and Lp (a) is therefore considered an independent risk factor of atherosclerosis (Berglund and Ramakrishnan, 2004; Evans et al., 2001; Marcovina and Koschinsky, 2002). In other trials, patients with Lp (a) levels ≥30 mg/dl had markedly higher risk of coronary heart disease (Foody et al., 2000; Paultre et al., 2000) and a susceptibility to occlusive complications after interventional procedures (percutaneous transluminal coronary angioplasty; stent placement) (Rifai et al., 2004).

Several mechanisms linking Lp (a) and development of atherosclerosis have been proposed. In arterial intima, Lp (a) is located only in atherosclerotic plaques, but not in the intact tissue. Lp (a) captured in the atherosclerotic plaque stimulates smooth muscle cells proliferation and its binding to extracellular matrix enhances lipid accumulation. As a non-functional structural homologue of plasminogen it can also negatively affect the process of fibrinolysis (Koschinsky and Marcovina, 2004; Shai et al., 2005a).

Plasma Lp (a) concentration is predominantly determined by genetic factors and is not affected by diet (Boerwinkle et al., 1992). Certain drugs (nicotinic acid and its analogues) and sexual hormones (androgenes, estrogenes, progesterone) have a lowering effect on Lp (a) levels (Šulcová et al., 2001). No effects of statins and fibrates on the Lp (a) were demonstrated (Lippi and Guidi, 2003).

The amount of synthesized apo (a) is considerably different among individuals in a population. There is an apo (a) gene length polymorphism that accounts for about 40-60 % of the variance (Cohen et al., 1993; Kraft et al., 1992). Part of the variance could be attributed to polymorphic sites either in the coding sequence or in the transcription regulatory sequences of

apo (a) gene. Four polymorphisms with possible relation to Lp (a) levels have been identified in the promoter region of the apo (a) gene. These are three single nucleotide substitutions (+121 G>A, +93 C>T, and -772 G>A) and one pentanucleotide TTTTA repetition (7-11 repeats) designated as the STR (short tandem repeat) locus (Trommsdorf et al., 1995; Zysow et al., 1995). In contrast to the other polymorphic sites the -772 G>A substitution was not reported to be functional.

The aim of our study was to test if Lp (a) levels are associated with coronary heart disease and premature manifestation of coronary atherosclerosis in patients with other important risk factors of atherosclerosis. We analysed the relationship between Lp (a) levels and serum lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, apoB-100) and other risk factors of atherosclerosis (type 2 diabetes mellitus, arterial hypertension, smoking, and overweight). Finally, we investigated an association between selected polymorphic sites in the apo (a) gene promoter region and Lp (a) plasma levels.

METHODS

Subjects

3.915 out-patients followed at the Centre of Preventive Cardiology of the General Teaching Hospital, Charles University in Prague with dyslipidemia and other CHD risk factors (e.g. type 2 diabetes mellitus, hypertension, overweight, metabolic syndrome) were included in the study. Characteristics of the study group is shown in Table 1.

The study group was divided into quintiles according to Lp (a) level. We randomly selected a representative sub-group of 650 individuals (each sixth patient from each quintile). 100 patients with inflammatory diseases, malignancies, renal insufficiency or other diseases, which could affect the Lp (a) level were excluded. The presence of type 2 diabetes mellitus, hypertension, smoking, overweight [defined as BMI (body mass index) over 25] and of CHD (defined as a documented myocardial infarction, revascularization procedure, angina, positive

stress test or positive coronary angiography) were recorded in the final group of 550 individuals. The final group did not differ from the principal group in lipid parameters and it included 12.8% of patients with type 2 diabetes mellitus, 35.4% of patients with arterial hypertension, 26.5% of smokers, 12.7% patients with documented CHD and 7,8% patient with premature CHD (men <55 years of age, women <65 years of age). When subgroups of diabetics and non-diabetics, hypertonics and non-hypertonics, smokers and non-smokers, males and females and those with CHD and without CHD were mutually compared, no significant differences were observed in selected lipid parameters. The study was approved by the ethics committee of the General Teaching Hospital in Prague and all study participants gave their informed consent.

Biochemical analysis

Venous blood was collected after 12 hours fasting and Lp (a) concentrations were measured using frozen serum (-20°C), separated within two hours after blood collection, by the immuno LEIA® Lp (a) method (reproducibility is over 90% and the coefficient of variability is under 4%, the minimal detected concentrations: 1,5 - 2,0 mg/dl, the sensitivity is < or = 3 mg/dl) (Technoclone GmbH, Vienna, Austria). We used internal control samples as well as control samples and standards provided by the manufacturer during each assay.

Plasma concentrations of total cholesterol, HDL [high-density lipoprotein] cholesterol, triglycerides, and apo-B were determined on automatic analyzers (Modular SWA, Roche, Switzerland). The LDL cholesterol level was calculated using the Friedewald equation (LDL-c= TC-HDL-c-TG/2,2).

DNA analysis

DNA was isolated from fresh or frozen whole blood using a salting-out method according to Miller et al. (1988). Subjects were then directly genotyped for +121 G>A, +93 C>T, and -772 G>A apo (a) gene polymorphisms. The sequence of the proximal promoter and the pentanucleotide microsatellite (STR locus) from the distal promoter were amplified using

primers described in our previous study (Zídková et al. 2007). The promoter fragment was then restricted in two separated 10-μl reaction mixtures using 5 units of the *Hpy*188III (New England Biolabs, GmbH, Frankfurt am Main, Germany) enzyme for the +121G>A variant detection and 5 units of the*Hpy*CH4IV (NEB) enzyme for the +93C>T variant detection. Restricted fragments were then subjected to 2% agarose gel electrophoresis and visualized in UV light after ethidium bromide staining. The STR locus product varied in size according to the number of TTTTA repeat units from 164 bp (7 repeats) to 184 bp (11 repeats) and was measured by fragmentation analysis conducted on ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Correlation between Lp (a) levels and serum lipid concentrations, apo-B plasma levels, risk factors of atherosclerosis, and prevalence of CHD was evaluated using Pearson's correlation coefficient (r). The Lp (a) plasma levels in subgroups of diabetics and non-diabetics, hypertonics and non-hypertonics, smokers and non-smokers, and those with heart disease and without heart disease were compared by Mann-Whitney test. The logistic regression analysis was used to establish predicted probability. The ANOVA test was employed to reveal an association of polymorphic loci with Lp (a) levels. Each polymorphic locus was tested for significant deviation from Hardy-Weinberg equilibrium using the χ^2 test ($p<0.05$; 1d.f.).

RESULTS

No statistically significant correlation was observed between Lp (a) levels and serum lipids and age in the overall patient group of 3915 individuals. For relevant values of Pearson´s correlation coefficient see Table 1. The same correlation analyses were conducted in the final group of 550 individuals (Table 2), which had correlation coefficients not markedly different from those in the basic group.

When we compared the mean Lp (a) plasma levels between the subgroup of type 2 diabetics and non-diabetics (p=0.3935), hypertonics and non-hypertonics (p=0.2329), and smokers and non-smokers (p=0.2682), no statistically significant differences were detected.

We found a statistically significant increase of mean Lp (a) levels in the subgroup of patients with CHD (p=0.0219). In the logistic regression analysis we utilized the Lp (a) plasma level as a predictor of CHD. Lipoprotein (a) levels significantly affected the probability of CHD (p=0.0234). The predicted probability represents the likelihood of CHD development among patients with a given Lp (a) level (Graph 1). When we analyzed the individuals with premature manifestation of CHD (78 individuals) the association of Lp (a) levels with the development of CHD was not significant (p=0.1471).

Allelic frequencies of the two single nucleotide substitutions were as follows: +121G>A (0.794/0.206±0.017), +93C>T (0.865/0.135±0.010). Genotypes and relevant mean Lp (a) levels are summarized in Table 3. The most frequent allele of the STR locus had 8 repeats (0.711±0.019), allelic frequencies of other alleles were: STR with 7 repeats (0.008±0.003), STR with 9 repeats (0.151±0.015), STR with 10 repeats (0.111±0.013), and STR with 11 repeats (0.019±0.006). All genotypes detected in the final group and related Lp (a) levels are given in Table 4. Distribution of the genotype frequencies were in Hardy-Weinberg equilibrium.

No effect of the +121G>A polymorphism on Lp (a) level was observed (p=0.2515), but there was a significant association of the +93C>T polymorphism with Lp (a) levels (p=0.0166). There was a highly significant association of different length of the STR locus with Lp (a) levels ($p<0.0001$). Our results indicate that mean Lp (a) levels increase with a decreasing number of TTTTA repeats. Moreover, when we analyzed a combined effect of all three polymorphic sites on Lp (a) levels the association of the +93C>T polymorphism became attenuated and was no more significant (p=0.6001), whereas the effect of the STR locus was still persistent (p=0.0029).

DISCUSSION

The study was carried out in a group of patients of the Centre of Preventive Cardiology and thus it includes individuals with different risk factors of atherosclerosis. The purpose of the study was to find out whether Lp (a) levels are still associated with CHD in patients with other cardiovascular risk factors (type 2 diabetes, arterial hypertension, smoking, obesity) and if Lp (a) levels are independent on these risk factors. We also tested the association of three polymorphic loci from the apo (a) gene promoter region with Lp (a) levels. The polymorphic sites were chosen for the analysis based on the data from previous studies (Brazier et al., 1999; Kalina et al., 2001).

No correlation was detected between Lp (a) levels and risk factors of atherosclerosis examined. Our results are in agreement with conclusions of other studies (Shai et al., 2005b, Hernández C et al., 2005, Gazzaruso C et al., 1996), but e.g. in the work of Heller et al. 1993 there was a positive correlation of Lp (a) and diabetes mellitus. Major limitation of this study is that it included patients with renal complications (albuminuria and renal insufficiency), which increase Lp (a) levels. So we support the independent role of Lp (a) on studied risk factors of atherosclerosis.

There is a lack of dependence of Lp (a) levels and apo-B, which forms a part of Lp (a), in our study, but such correlation was also missing in other trials (e.g. Lippi and Guidi, 2003). In most individuals, the Lp (a) concentration is very low in comparison with the abundance of other lipoproteins containing apo-B. It seems that even markedly decreased apo-B plasma levels are still not limiting for the rate of Lp (a) synthesis.

A significantly increased mean Lp (a) level (mean 49 ±54 mg/dl) was found in a subgroup of patients suffering from CHD. However, such significant correlation was absent when only patients with premature CHD were included. An insufficient sample size of the subgroup with premature CHD could be the possible reason for our results, because in other trials a possitive correlation was demonstrated (e.g. Stein and Rosenson, 1997, Berlung and

Ramakrishnan, 2004). Nevertheless, our results support the notion that increased Lp (a) levels are associated with CHD in patients other CHD risk factors.

Gene variants affecting apo (a) gene transcription may contribute to the high variability of Lp (a) plasma concentrations. The study by Suzuki et al. (1997) indicated a potentially positive role of the +121 G>A substitution on apo (a) gene transcription. However, this was not reproduced by others (Wu and Lee, 2003). We detected no association of the +121 G>A polymorphism with Lp (a) levels. A statistically significant association of the +93 C>T polymorphism with Lp (a) levels is in accordance with its direct negative effect on apo (a) production (Zysow et al., 1995). Nevertheless, in Caucasians the effect is usually masked by the strong linkage disequilibrium with intermediate apo (a) gene length isoforms (Brazier et al., 1999).

Several studies have reported a correlation between the STR locus of apo (a) gene promoter and plasma Lp (a) concentrations (Brazier et al., 1999; Kalina et al., 2001). The correlation was independent of apo (a) size isoforms and it accounted for up to 14 % of Lp (a) level variation (Brazier et al., 1999; Trommsdorff et al., 1995). Our findings are consistent with these data. It is supposed to be due to the linkage disequilibrium of different STR alleles to the distinct range of apo (a) isoforms and to other functional changes influencing apo (a) production rate. The STR allele with 9 repeats was reported to be in a linkage disequilibrium with the +93 T promoter variant (Holmer et al., 2003) and with apo (a) middle- sized isoforms (Puckey et al., 1997). It seems that the association of +93 C>T polymorphism with Lp (a) levels was actually caused by the STR allele with 9 repeats at least in part due to the linkage with restricted apo (a) gene length isoforms. Such a conclusion is in agreement with results of the combined analysis where the effect of +93 C>T polymorphism lost its significance and the effect of the STR locus was preserved. Thus, our results are in compliance with previously published data (Kraft et al., 1998; Puckey et al., 1997).

Our study suggests that elevated Lp (a) level is independently associated with CHD in patients with other important risk factors of atherosclerosis. Sequence variants of the regulatory regions of apo (a) gene are associated with Lp (a) plasma levels, in particular due to the linkage to a restricted range of apo (a) gene length isoforms.

ACKNOWLEDGMENTS

This work was supported by grant IGA NR 8328-3, IGA NR/9411-3 and research project MSM 0021620807.

REFERENCES

BERGLUND L, RAMAKRISHNAN R: Lipoprotein (a): an elusive cardiovascular risk. *Arterioscler. Thromb. Vasc. Biol.* **24**(12): 2219-2226, 2004

BOERWINKLE E, LEFFERT CC, LIN J, LACKNER C, CHIESA G, HOBBS HH: Apolipoprotein (a) gene accounts for greater than 90% of the variation in plasma lipoprotein (a) concentrations. *J. Clin. Invest.* **90**: 52-60, 1992

BRAZIER L, TIRET L, LUC G, ARVEILER D, RUIDAVETS JB, EVANS A, CHAPMAN J, CAMBIEN F, THILLET J: Sequence polymorphisms in the apolipoprotein(a) gene and their association with lipoprotein(a) levels and myocardial infarction. The ECTIM study. *Atherosclerosis.* **144**: 323-333, 1999

COHEN JC, CHIESA G, HOBBS HH: Sequence polymorphisms in the apolipoprotein (a) gene-evidence for dissociation between apolipoprotein (a) size and plasma lipoprotein (a) levels. *J. Clin. Invest.* **91**, 1630-1636, 1993

EVANS RW, SHPILBERG O, SHATEN BJ, ALI S, KAMBOH MI, KULLER LH: Prospective association of lipoprotein (a) concentrations and apo(a) size with coronary heart disease among men in the Multiple Risk Factor Intervention Trial. *J. Clin. Epidemiol.* **54**: 51-57, 2001

FOODY JM., MILBERG JA, PEARCE GL, SPRECHER DL: Lipoprotein(a) associated with coronary artery disease in older women: age and gender analysis. *Atherosclerosis.* **153**(2): 445-51, 2000

HOLMER SR, HENGSTENBERG C, KRAFT HG, MAYER B, POLL M, KURZINGER S, FISCHER M, LOWEL H, KLEIN G, RIEGGER GA, SCHUNKERT H: Association of polymorphisms of the apolipoprotein(a) gene with lipoprotein(a) levels and myocardial infarction. *Circulation.* **107**(5): 696-701, 2003

GAZZARUSO C, BUSCAGLIA P, GARZANITI A, BONETTI G, SAVINO S, MARIOTTI S, JUCCI A, FINARDI G, GEROLDI D: Lipoprotein(a) plasma concentrations, apoliporpotein (a) polymorphism and family history of coronary heart disease in patiens with esential hypertension. *J Cardiovasc Risk* **3**(2): 191-7, 1996

HERNANDEZ C, FRANCISCO G, CHARON P, SIMO R: Lipoprotein(a) as a Risk Factor for Cardiovascular Mortality in Type 2 Diabetic Patiens, A 10-year follow-up study *Diabetes Care* **28**: 931-933, 2005

HOREJSI B, CESKA R: Apolipoproteins and atherosclerosis. Apolipoprotein E and apolipoprotein (a) as candidate genes of premature development of atherosclerosis. *Physiol. Res.* **49**(Suppl. 1): 63-S69, 2000

KALINA Á, CSASZAR A, FUST G, NAGY B, SZALAI C, KARADI I, DUBA J, PROHASZKA Z, HORVATH L, DIEPLINGER H: The association of serum lipoprotein (a) levels, apolipoprotein (a) size and $(TTTTA)_n$ polymorphism with coronary heart disease. *Clinica. Chimica. Acta.* **309**:45-51, 2001

KRAFT HG, KOCHL S, MENZEL HJ, SANDHOLZNER C, UTERMANN G: The apolipoprotein (a) gene: a transcribed hypervariable locus controlling plasma lipoprotein (a) concentration. *Hum. Genet*. **90**: 220-230, 1992

KRAFT HG, WINDEGGER M., MENZEL HJ, UTERMANN G: Significant impact of the +93 C/T polymorphism in the apolipoprotein (a) gene on Lp(a) concentrations in Africans but not in Caucasians: confounding effect of linkage disequilibrium. *Hum. Mol. Genet.* **7**: 257-264, 1998

KOSCHINKY ML, MARCOVINA SM: Structure-function relationships in apolipoprtein (a): insights into lipoprotein (a) assembly and pathogenicity. *Curr. Opin. in Lipidol.* **15**(2): 167-174, 2004

LIPPI G, GUIDI G: Lipoprotein(a): An emerging Cardiovascular Risk Factor. *Crit. Rev. Clin. Lab. Sci.* **40**(1): 1-42, 2003

MARCOVINA SM, KOSCHINSKY M: A critical evaluation of the role of Lp(a) in cardiovascular disease: Can Lp(a) be useful in risk assessment? *Semin.Vasc. Med.* **2**(3): 335-344, 2002

MILLER SA, DYKES DD, POLESKY HF: A simple salting out procedure for DNA extraction from human nucleated cells. *Nucl. Acid. Res.* **16**: 1215, 1988

PAULTRE F, PEARSON TA, WEIL HF, TUCK CH, MYERSON M, RUBIN J, FRANCIS CK, MARX HF, PHILBIN EF, REED RG, BERLUNG L: High levels of Lp(a) with a small apo(a) isoform are associated with coronary artery disease in African American and white men. *Arterioscler. Thromb. Vasc. Biol.* **20**(12): 2619-2624, 2000

PUCKEYLH, LAWN RM, KNIGHT BL: Polymorphisms in the apolipoprotein (a) gene and their relationship to allele size and plasma lipoprotein (a) concentration. *Hum. Mol. Genet*. **6**:1099-1107, 1997

RIFAI N, MA J, SACKS FM, RIDKER PM, HERNANDEZ WJ, STAMPFER MJ, MARCOVINASM Apolipoprotein (a) size and lipoprotein (a) concentration and future risk of angina pectoris with evidence of severe coronary atherosclerosis in men: Physicians Health Study. *Clin. Chem.* **50**(8): 1364-1371, 2004

SHAI I, RIMM EB, HANKINSON SE, CANNUSCIO C, CURAHN G, MANSON JE, RIFAI N, STAMPFER MJ, MA J: Lipoprotein (a) and coronary heart disease among women: beyond a cholesterol carrier? *Eur. Heart. J.* **26**(16): 1633-1639, 2005 a

SHAI I, SCHULZE MB, MANSON JE, STAMPFER MJ, RIFAI N, HU FB: A prospective study of lipoprotein (a) and risk of coronary heart disease among women with type 2 diabetes. *Diabetologia.* **48**(12): 2691-2692, 2005 b

SUZUKI K, KURIYAMA M, SAITO T, ICHINOSE A: Plasma lipoprotein(a) levels and expression of the apolipoprotein(a) gene are dependent on the nucleotide polymorphisms in its 5'-flanking region. *J. Clin. Invest*. **99**(6): 1361-1366, 1997

STEIN JH, ROSENSON RS: Lipoprotein Lp (a) excess and coronary heart disease. Archives od Internal Medicine, **157** (11),1997

SEDA O: Comparative Gene Map of Hypertriglyceridaemia. *Folia Biologica* **50**: 43-57, 2004

SULCOVA J, HILL M, MASEK Z, CESKA R, NOVACEK A, HAMPL R, STARKA L: Effects of transdermal application of 7-oxo-DHEA on the levels of steroid hormones, gonadotropins and lipids in healthy men. *Physiol. Res.* **50**: 9-18, 2001

TROMMDORF M, KOCHL S, LINGENHEL A, KRONENBERG F, DELPORT R, VERMAAK H, LEMMING L, KlAUSEN IC, FAERGEMAN O, UTERMANN G: A pentanucleotide repeat polymorphism in the 5´ control region of the apolipoprotein(a) gene is associated with lipoprotein(a) plasma concentration in Caucasians. *J. Clin. Invest.* **96**: 150-157, 1995

WU JH, LEE IN: Studies of apolipoprotein (a) promoter from subjects with different plasma lipoprotein (a) concentrations. *Clin. Biochem.* **36**(4): 241-246, 2003

ZIDKOVA K, KEBRDLOVA V, ZLATOHLAVEK L, CESKA R: Detection of variability in apo(a) gene transcription regulatory sequences using the DGGE method. *Clin. Chim. Acta.* **376**(1-2): 77-81, 2007

ZYSOW BR, LINDAHL GE, WADE DP, KNIGHT BL, LAWN RM: C/T polymorphism in the 5´untranslated region of the apolipoprotein (a) gene introduces an upstream ATG and reduces in vitro translation. *Arterioscler. Thromb. Vasc. Biol.* **15**: 58-64, 1995

Table l: Clinical and lipid characteristics of the principal out-patient group, serum lipid parameters and Pearson correlation coefficients to Lp (a)

Characteristics	N[a]	Mean ± SD[c]	Median	Minimum	Maximum	Pearson[b]
age (years)	3915	50.95 ±16.34	53.00	19	94	0.14131
total cholesterol (mmol/l)	3869	6.66 ± 2.30	6.50	2.77	37.63	0.06036
LDL cholesterol (mmol/l)	3241	4.19 ± 1.64	4.01	0.56	18.80	0.13002
HDL cholesterol (mmol/l)	3644	1.39 ± 0.44	1.35	0.10	4.04	0.07100
triglycerides (mmol/l)	3863	2.85 ± 4.71	1.80	0.15	88.35	-0.08923
apo-B (mmol/l)	3838	1.28 ± 0.44	1.24	0.18	5.52	0.12770
Lp (a) (mg/dl)	3915	28 ± 35	14	0.00	410	—

[a] number of individuals with available data

[b] value of Pearson correlation coefficient (r) to Lp (a), the r value of 0,3 was considered as significant

[c] value of standard standard deviation

Table 2: Clinical and lipid characteristics of the final group, serum lipid parameters and Pearson correlation coefficients to Lp (a)

Characteristics	N[a]	Mean ± SD[c]	Median	Minimum	Maximum	Pearson[b]
age (years)	550	49.56 ±12.54	51.50	30	81	0.15007
Diabetes mellitus	70	—	—	—	—	—
Arterial hypertension	195	—	—	—	—	—
Smoking	146	—	—	—	—	—
Overweight (BMI over 25)	215	—	—	—	—	—
CHD	70	—	—	—	—	—
premature CHD	78	—	—	—	—	—
total cholesterol (mmol/l)	550	6.44 ± 1.94	5.90	3.04	20.78	0.05542
LDL cholesterol (mmol/l)	550	4.35 ± 1.82	4.12	0.62	9.60	0.12788
HDL cholesterol (mmol/l)	550	1.30 ± 0.37	1.33	0.40	3.12	0.1050
triglycerides (mmol/l)	550	2.52 ± 5.01	1.95	0.45	45.35	-0.09024
apo-B (mmol/l)	550	1.29 ± 0.34	1.25	0.19	5.48	0.13040
Lp (a) (mg/dl)	550	27 ± 56	13,5	0.00	340	—

[a] number of individuals with available data

[b] value of Pearson correlation coefficient (r) to Lp (a), the r value of 0,3 was considered as significant

[c] value of standard standard deviation

Table 3: Number of observed genotypes, mean Lp (a) plasma levels and standard deviation (SD) for the +121G>A and +93C>T polymorphic sites from the apo (a) gene promoter region in quintile based group.

Genotypes			Lipoprotein(a) (mg/dl)		
		N	Mean ± SD	Minimum[a]	Maximum
+121	G/G	348	39 ± 37	0.0	205
	G/A	177	45 ± 46	0.0	190
	A/A	25	54 ± 66	0.0	268
+93	C/C	412	44 ± 43	0.0	268
	C/T	128	34 ± 36	0.0	131
	T/T	10	19 ± 18	0.0	50

[a] the lowest non-zero detected Lp (a) level was 1 mg/dl

Table 4: Number of observed genotypes, mean Lp (a) plasma levels and standard deviation (SD) for the STR polymorphic site from the apo (a) gene promoter region in quintile based group.

Genotype		Lipoprotein(a) (mg/dl)		
$TTTTA_n$ repetition	N	Mean ± SD	Minimum[a]	Maximum
7/10; 7/8	9	76 ± 47	5.0	143
8/8	282	44 ± 43	0.0	268
8/9	115	38 ± 38	0.0	131
8/10	82	43 ± 40	0.0	160
8/11	13	27 ± 28	0.0	81
9/9	16	23 ± 35	0.0	132
9/10; 9/11	19	23 ± 19	0.0	67
10/10; 10/11; 11/11	14	10 ± 7	0.0	22

[a] the lowest non-zero detected Lp (a) level was 1 mg/dl

Graph 1: The predicted probability represents likelihood of coronary heart disease development among patients with a given Lp (a) level.