Lipoprotein(a) level and MIF gene variant predict incident metabolic syndrome and mortality

Altan Onat,[1] Günay Can,[2] Neslihan Çoban,[3] İbrahim Dönmez,[4] Hakan Çakır,[5] Evin Ademoğlu,[6] Nihan Erginel-Ünaltuna,[3] Hüsniye Yüksel[1]

[1]Department of Cardiology, Istanbul University, Istanbul, Turkey
[2]Department of Public Health, Cerrahpaşa Medical Faculty, Istanbul University, Istanbul, Turkey
[3]Department of Genetics, Institute for Experimental Medical Research, Istanbul University, Istanbul, Turkey
[4]Department of Cardiology, Izzet Baysal University, Abant, Istanbul, Turkey
[5]Darıca Farabi State Hospital, Istanbul, Turkey
[6]Department of Biochemistry, Medical Faculty, Istanbul University, Istanbul, Turkey

Correspondence to
Dr Altan Onat, Department of Cardiology, Istanbul University, Nisbetiye cad. 59/24, Etiler, İstanbul 34335, Turkey;
alt_onat@yahoo.com.tr

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ABSTRACT

Owing to the scarcity of available information, we aimed to assess the association of migration inhibitory factor (MIF)-173 G/C genotypes and serum lipoprotein(Lp)(a) with incident metabolic syndrome (MetS) and all-cause mortality, respectively. In population based, middle-aged adults (n=1297), stratified by gender and presence of MetS, we used Lp(a) quintiles to identify non-linear associations with outcomes using Cox regression models, adjusted for MIF genotype, age, smoking status, high density lipoprotein cholesterol, and systolic blood pressure. After 5.2 years of follow-up, 151 cases of incident MetS and 123 deaths were recorded. For incident MetS, adjusted HRs increased in each gender across four declining quintiles, starting from the highest quintile in men and from quintile 4 in women. The MIF CC-GC genotype appeared to contribute to the risk estimates in men. Similarly adjusted models in the whole sample disclosed that all-cause mortality tended to be inversely associated with Lp(a) quintiles and yielded an HR (2.42 (95% CI 1.03 to 5.81)) in men in quintile 2, whereas the MIF genotype additively predicted mortality (HR 1.79 (95% CI 1.01 to 3.18)) only in men. Excess risk of death was additively conferred on Turkish men by the MIF CC-GC genotype and by apparently reduced circulating Lp(a) assays, supporting the notion that 'low' serum Lp(a), mediating autoimmune activation, is a major determinant of metabolic disease risk and death. Damaged MIF protein and more complex autoimmune activation in women may be responsible from lack of relationship to MetS/mortality.

Significance of this study

What is known about this subject?

- Elevated plasma lipoprotein[Lp](a) and the cytokine macrophage migration inhibitory factor (MIF) possess pro-inflammatory and immunoregulatory functions.
- The significance for cardiometabolic outcomes of reduced Lp(a) and MIF gene variant has been scarcely studied.

What are the new findings?

- We assessed the association of *MIF*-173 G/C genotype and serum Lp(a) with incident metabolic syndrome (MetS) and all-cause mortality.
- In 1297 middle-aged adults, we used Lp(a) quintiles (Q) to identify non-linear associations with outcomes in multiadjusted Cox regression models.
- At 5.2 years of follow-up, 151 incident MetS and 123 deaths were recorded. Adjusted HRs increased in each gender across declining quintiles. Risk of death increased in men to a significant HR 2.42 in Q2 vs Q5. MIF genotype additively predicted mortality (HR 1.79) only in men.
- Excess risk of death is conferred in Turkish men additively by MIF CC-GC genotype and by apparently reduced circulating Lp(a) assays. Damaged MIF protein and more complex autoimmune activation in women may be responsible from lack of relationship to outcomes.

How might these results change the focus of research or clinical practice?

- These results invite focusing research on potential damage of certain plasma proteins in susceptible population segments.

INTRODUCTION

Elevated plasma lipoprotein (Lp)(a) is a recognized cardiovascular risk factor,[1 2] mediating systemic inflammation and endothelial dysfunction and using atherogenic and prothrombotic pathways. Coronary heart disease (CHD) risk is attributed to an elevated Lp(a) level inversely related to the kringle IV type 2 repeats.[3–5] One LPA SNP, rs10455872, was strongly associated with Lp(a) concentrations and a number of kringle IV-2 repeats and explained in European Caucasians the extent of Lp(a) variation sixfold that of kringle IV-2 repeats.[5] The question of whether apparently 'reduced' plasma Lp(a) levels might also predict atherogenic dyslipidemia[6] and cardiometabolic risk[7–9] has not received adequate attention. The lowest quintile of Lp(a) was found to be associated with the development of type 2 diabetes in the prospective Women's Health Study[7] and the EPIC-Norfolk study.[8]

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Macrophage migration inhibitory factor (MIF) is a cytokine identified as an inhibitor of the random migration of macrophages,[10] possessing pro-inflammatory and immunoregulatory functions mediating innate and adaptive immune responses.[11] The human MIF gene is expressed especially in T cells and macrophages[12] and is strongly expressed in atherosclerotic lesions.[13] Systemic concentrations of MIF are elevated in obesity,[14 15] impaired glucose tolerance, and diabetes mellitus,[16 17] promising to be a novel biomarker for the risk of cardiometabolic disorders.

Expression of MIF polymorphism, a recognized mediator of host immune responses, might contribute to individual susceptibility to chronic inflammatory disease. Turkish adults, being prone to metabolic syndrome (MetS),[18] and women, to diabetes,[19] constitute a good sample to examine the impact of Lp(a) levels and MIF variants on MetS and overall mortality. By adjusting the Lp(a) concentration for the effects of the LPA rs10455872 A>G polymorphism, age, gender, total cholesterol, and fasting insulin, we estimated the expected Lp(a) concentration in each of the 1669 participants to determine the quotient between observed and expected Lp(a) values. Cox regression models (also comprising smoking status, systolic pressure, and serum HDL-cholesterol) disclosed that, compared with the mid-tertile, both low and high Lp(a) quotient tertiles significantly predicted incident CHD, especially in women, after 5.1 years of follow-up.[20] This was consistent with the notion that 'low' serum Lp(a) reflects autoimmune activation.[9] An overwhelming majority of participants were shared in the previous and current study.

Postulating a non-linear relationship between Lp(a) and all-cause mortality and/or MetS risk, we investigated prospectively the risk of both outcomes using Lp(a) quintiles in multivariable Cox regression models. We included the MIF rs755622 polymorphism in the analyses to determine both direct associations with the stated outcomes and its impact on the associations with Lp(a). Findings revealed fundamental aspects of pathophysiology contributing to the pro-inflammatory state and associated autoimmune activation, and underlined several important sex differences.

METHODS

Population sample

The Turkish Adult Risk Factor Study (TARF) is a prospective survey on the prevalence of cardiac disease and risk factors in adults in Turkey carried out periodically, almost biennially, since 1990, in 59 scattered communities.[21] It involves a random sample of the Turkish adult population, representatively stratified for sex, age, geographical region, and for the rural–urban distribution.

Measurements of serum Lp(a) were made between the surveys 2003 and 2013 (median 2005) of the TARF[22] residing in all seven geographical regions of Turkey. Participants who had a first serum Lp(a) measurement, available genotyping of the MIF polymorphism rs755622 and a follow-up in the biennial surveys (n=1297) formed the sample. Baseline values for other variables analyzed herein represent those of the same survey.

The TARF study conformed to the principles embodied in the Declaration of Helsinki and was approved by the Istanbul University Ethics Committee. Written informed consent was obtained from all participants. Data were obtained from a history of the past years via a questionnaire, physical examination of the cardiovascular system, and recording of a resting ECG.

Measurement of risk factors

Waist circumference was measured with the subject standing, at the end of gentle expiration, at the level midway between the lower rib margin and the iliac crest. Cigarette smoking status was categorized as current, former and never-smokers. Blood pressure (BP) was measured in the seated position on the right arm using an aneroid sphygmomanometer (Erka, Bad Tölz, Germany), after 5 min of rest, and the mean of two recordings was computed. Physical activity was graded by the participant himself into four categories of increasing order with the aid of a scheme.[21]

Sera were obtained from venous blood after an overnight fast (>11 h) and measurements were made with commercially available kits in a central laboratory in Istanbul. Serum concentrations of total cholesterol, fasting triglycerides, glucose, creatinine, and high density lipoprotein (HDL) cholesterol (directly without precipitation) were determined using enzymatic kits from Roche Diagnostics (Mannheim, Germany). Concentrations of insulin, total testosterone and sex hormone binding globulin (SHBG) were measured by a chemiluminescence immunoassay utilizing an Elecysys 1010 immunautoanalyzer. Apolipoprotein (apo) A-I, apo B, Lp(a), and C reactive protein (CRP) were measured by means of particle enhanced immunonephelometry with the Behring nephelometer (Behring Diagnostics). Serum concentrations of uric acid were determined enzymatically (uricase) by an InfinityTM (Thermo Electron, Victoria, Australia) kit utilizing the modified Trinder method.

Determination of the MIF rs755622 and LPA rs10455872 genotypes

DNA was extracted from peripheral blood leukocytes using a QIAmp DNA Maxi KIT (Qiagen, Hilden, Germany). The DNA concentrations were standardized and stored in an 8×12 format at −20°C. Unselected subjects were examined for their MIF-173 G/C genotypes. Genotyping was performed using hybridization probes in a Real-Time PCR LightCycler 480 device. DNA amplification was set up in 96 well plates (ABGENE Ltd). A typical 10 µL PCR reaction consisted of a 2 µL LightCycler 480 genotyping master ready mix (Roche), 0.2 µL probes and 0.2 µL primers, and 6.6 µL distilled water. Genomic DNA (1 µL (15 ng)) was added to the PCR mixture. PCR was carried out on LightCycler 480 using the following conditions: 95°C for 10 min, 95°C for 5 s, 62°C for 5 s, and 72°C for 7 s (45 cycles). Melting curve analysis was assessed using the LightCycler 480 genotyping software.

LPA rs10455872 was similarly genotyped. Probes were allele specifically labeled with one of the fluorescent dyes FAM (6-carboxy-fluorescein; major allele) and VIC (proprietary dye of Applied Biosystems, Foster City, California, USA; minor allele) and contained a minor groove binder group and a dark quencher.[23] The sequences of primers and probes were designed in-house using Primer Express software (V.2.0; Applied Biosystems)—primers: ACTCTCAGCTGCCTTCCT CCTT and CATGTTTGTCTTGGGTAACAAGTGA, probes: FAM-CAGAACCCAATGTGTTTA-MGB and VIC-AACCCA

GTGTGTTTAT-MGB. Primers and probes were synthesized by Applied Biosystems.

Definitions

Individuals with diabetes were diagnosed using the criteria of the American Diabetes Association,[24] namely when plasma fasting glucose was ≥7 mmol/L (or 2 h postprandial glucose >11.1 mmol/L) and/or the current use of diabetes medication. MetS was identified when three of the five criteria of the National Cholesterol Education Program ATP-III were met, modified for pre-diabetes (fasting glucose 5.56–6.95 mmol/L)[25] and further for male abdominal obesity using as a cut-off point ≥95 cm,[26] as assessed in the Turkish Adult Risk Factor study. Death was identified via information from first degree relatives, records of local health personnel, and/or the nationwide Identity Participation System.

Data analysis

Follow-up was lacking in only 6% of subjects with the available Lp(a) measurement and MIF genotype. Two-sided t-tests and Pearson's χ^2 tests were used to analyze the differences in means and proportions between groups. The whole sample was analyzed by stratifying for the presence of baseline MetS and gender. Only the baseline values of Lp(a) and other independent variables were considered in the analyses. Quintiles of serum Lp(a) were formed by cut-offs of <3.2/<4.2; 6.63/8.82; 12.4/15.3; and >24.2/≥31.0 mg/dL in men and women, respectively, used as independent variables in predicting outcome risk. In analyzing the associations of the MIF-173 G/C variant, the GG genotype served as the referent. HR estimates and 95% CIs were obtained for Lp(a) quintiles, and the MIF variant with incident MetS and overall mortality by use of Cox proportional hazards analyses in models that controlled for potential confounders. In predicting MetS, cases with prevalent MetS were excluded in the prospective analysis. Events time was estimated as the period between baseline examination and the survey date of incident MetS or recorded month of death. A value of $p<0.05$ on the two-tailed test was considered statistically significant. Statistical analyses were performed using SPSS-10 for Windows (SPSS Inc, Chicago, Illinois, USA).

RESULTS

Total follow-up of the sample consisted of 6769 person-years (mean 5.22±2.1 years, $p=0.21$ across gender) during which 123 participants were deceased. The mean annual death rate was 17.5 per 1000 participants. Incident MetS was recorded in 151 of 646 individuals (23.4%) free of MetS at baseline. In the MIF 173-G/C polymorphism, GG homozygosity constituted 67.8% and GC+CC genotypes 32.2% of the sample.

Baseline characteristics of the sample (n=1297) are listed in table 1 stratified by survivorship and gender. Deceased individuals were 15 years older, generally male, and had higher SHBG and lower physical activity grades. They had similar genotype groups as the survivors. Deceased females had significantly higher systolic BP, low-density lipoprotein (LDL)-cholesterol, and uric acid levels and tended to be never-smokers, whereas male deaths were characterized by significantly higher fasting glucose, creatinine and CRP levels, and had a significantly higher prevalence of diabetes and of former smokers.

Best linear covariates of Lp(a) concentrations

A highly significant multiple linear regression analysis for covariates of circulating Lp(a) was constructed comprising sex, age, two SNPs, serum total cholesterol, and fasting insulin levels (table 2). LPA genotype, sex, and serum total cholesterol emerged as significant covariates, the latter only in men, while fasting insulin tended to be inversely associated.

Joint evaluation of Lp(a) quintiles and MIF genotypes in predicting mortality and MetS

Table 3 shows the results of Cox regression analysis of the whole study sample at baseline for all-cause mortality, whereby HRs, estimated for Lp(a) quintiles and MIF genotypes, were adjusted for sex, age and smoking status, HDL-cholesterol and systolic BP. In men, carriage of 173-C allele (HR 1.78) and Lp(a) quintile 2 (HR 2.43 (95% CI 1.02 to 5.82)) significantly and additively predicted mortality, while HRs increased, albeit non-significantly, with declining Lp(a) quintiles 5 to 2. The MIF genotype in males was positively associated with the risk of death; no association was found in women.

Table 4 demonstrates findings of the Cox regression analysis of participants free of baseline MetS for incident MetS, adjusted for sex, age, smoking status, MIF genotype, and two MetS components (HDL-cholesterol and systolic BP). Covariates smoking status, HDL-cholesterol level, and systolic BP were selected because these are parameters (outside the apoB containing lipoproteins) highly relevant to the risk of MetS or overall mortality. Waist circumference and fasting glucose as MetS traits are, in our opinion, intimately related to Lp(a), insofar as abdominal obesity induced oxidative stress leads to lower serum Lp(a) assay (associated with a higher HOMA index), in turn yielding an increased risk of diabetes. Adequate power was lacking so that significant associations emerged with the independent variables for the female sex and the MetS components. A non-significant trend towards increasing MetS risk with declining Lp(a) quintiles was additively observed in each sex; in men in quintiles 5 through 2; and in women in quintiles 4 through 1, as illustrated in (figure 1).

DISCUSSION

With the purpose of assessing the impact of the MIF 173-G/C variant and detecting apparent non-linear associations of Lp(a) values with fatal outcome and with incident MetS in a middle-aged population sample, we used Lp(a) quintiles and Cox regression analyses over a mean follow-up of 5.2 years. Although not attaining significance, declining quintiles down to quintile 2 were paralleled by increasing multi-adjusted risk of death and of incident MetS, added to the MIF 173-G/C polymorphism, HDL-cholesterol, and systolic BP. The MIF genotype, tending in men to be linearly associated with baseline circulating Lp(a), seemed to predict incident MetS and significantly predicted all-cause mortality. Lp(a) quintiles tended in men to be inversely associated with both outcomes, and a significant HR for overall mortality was found in quintile 2. No association with mortality emerged in women. We concluded that the MIF173-G/C

Table 1 Baseline characteristics of the sample, by death and gender (n=1297)

	Survivors n=1174					p Value*		Deaths n=123			
	Men n=535			Women n=639				Men n=71		Women n=52	
	Total n=	mean	SD	mean	SD	Men	Women	mean	SD	mean	SD
Age, years	1297	51.8	10.7	51.6	10.8	0.001	0.001	**67**	10.5	**66.8**	10.6
Lipoprotein(a)†, mg/dL	1297	8.83	2.97	11.4	2.88	0.14	0.91	10.78	2.81	11.3	3.15
Systolic BP, mm Hg	1290	122.5	20	128	23	0.001	0.002	131.4	19	**138**	24.6
Diastolic BP, mm Hg	1290	79.6	11	80.7	12	0.27	0.12	79	11	83	13
Waist circumference, cm	1291	96.5	11	94	12	0.11	0.74	94	10.6	94.5	16
Total cholesterol, mg/dL	1297	190	42	201.6	44	0.54	0.56	187	36	214	51
LDL cholesterol, mg/dL	891	115	36	120.3	35.6	0.51	0.003	111.5	30	**138**	42
HDL cholesterol, mg/dL	1296	40.6	10.6	48.6	12.6	0.37	0.55	42	11.2	50	14
F. triglycerides†, mg/dL	910	148.4	1.65	126.7	1.59	0.62	0.19	143.2	1.82	140	1.64
Apolipoprotein A-I, g/L	1017	1.344	0.33	1.49	0.26	0.70	0.78	1.325	0.26	1.50	0.29
Apolipoprotein B, g/L	1025	1.005	0.25	1.04	0.26	0.26	0.17	0.963	0.265	1.10	0.31
Fasting glucose, mg/dL	1283	98	31	98	37	0.001	0.85	**123**	57	99	26
Creatinine, mg/dL	1229	0.967	0.23	0.82	0.53	0.001	0.60	**1.18**	0.67	0.86	0.18
C reactive protein†, IU/L	820	2.76	3.98	3.50	3.79	0.001	0.52	**6.30**	4.24	2.93	3.79
Uric acid, mg/dL	1031	5.88	1.38	4.63	1.3	0.80	0.03	5.83	1.66	**5.15**	1.81
Testosterone†, μ	740	9.52	2.2	0.45	2.79	0.45	0.31	10.56	2.18	0.37	3.55
SHBG†, nmol/L	551	40.4	1.6	49.4	1.73	0.04	0.011	**48.2**	1.87	**70**	1.57
Physical activity grades, I–IV	1282	2.45	1.22	2.08	0.63	0.005	0.001	**2.03**	0.84	**1.60**	0.70
MIF genotype. GC+CC and GG, n, %	1297	184; 34.4	351	203; 31.8	436	0.15	0.88	18; 28.4	53	16; 30.8	36
Current smokers, n, %	1278	202	38.5	79	12.5			19	27.5	2	3.8
Former, never-smokers, n, %	1278	168	154	34	520	0.009	0.082	**35**	15	1	49
Diabetes prevalence, n, %	1297	53	9.9	57	8.9	<0.001	0.31	24	**33.8**	7	13.5

*p Value and indicated significant values (highlighted in bold) refer to differences between mortality and survivorship.
†Log-transformed values. 1 SD in these variables denotes the factor to be multiplied or divided by the geometric mean.
BP, blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; MIF, migration inhibitory factor; SHBG, sex hormone binding globulin.

polymorphism weakly mediates in Turkish men the development of MetS and significantly impacts the risk of death, in addition to rendering autoimmune activation of circulating Lp(a) and thereby yielding an apparent inverse association of the latter with both outcomes.

Determinants of Lp(a) concentrations

In agreement with data in the multi-ethnic SHARE study,[5] our multiple linear regression analysis showed LPA SNP rs10455872 to be the major determinant of Lp(a) concentrations, followed by female sex. The MIF GC-CC genotype and, inversely, fasting insulin tended to be weakly associated with circulating Lp(a) only in men.

Risk conferring for MetS and mortality

Regression analyses for incident MetS–comprising seven confounders–failed among men to disclose significant predictors other than the expected HDL-cholesterol, but the MIF GC-CC genotype tended to be so and Lp(a) Q2 and 3 presented higher HRs than Q4 and 5, suggesting an inverse association with the risk of MetS. In women, sex and the two MetS components were significant predictors, whereas

Table 2 Linear regression analysis for Lp(a) concentrations in the whole sample, by gender

	Total, n=1256			Men, n=584			Women, n=672		
	β-coefficients	SE	p Value	β-coefficients	SE	p Value	β-coefficients	SE	p Value
Female sex	**1.20**-fold	1.06	0.002						
LPA GA vs AA	**3.81**-fold	2.0	<0.001	**4.09**-fold	1.33	<0.001	**3.62**-fold	1.27	<0.001
T. cholesterol, 35 mg/dL	**1.056**-fold	1	0.023	**1.086**-fold	1	0.025	1.04-fold	1	0.23
Insulin* 2-fold	0.69-fold	1.10	0.054	0.64-fold	1.14	0.093	0.76-fold	1.15	0.28
MIF GC/CC vs GG	1.06-fold	1.067	0.34	1.16-fold	1.10	0.106	0.98-fold	1.09	0.82
Age, 11-year	1.02-fold	1.03	0.48	1.005-fold	1.00	0.18	0.98-fold	1.05	0.86
constant	**5.71**	1.24	<0.001	**5.0**	1.35	<0.001	**10.3**	1.31	<0.001

The combined model explained 6% of Lp(a) variation (p<0.001). Significant values are highlighted in bold.
*Log-transformed values. 1 SD in independent variables denotes the factor for change in Lp(a).
MIF, migration inhibitory factor.

Table 3 Cox regression analyses of adjusted Lp(a) quintiles and MIF-173 genotypes for prediction of overall mortality

	Total 121/1274*		Men 69/592*		Women 52/682*	
Whole sample n=	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**
Gender, female	0.91	0.56 to 1.47				
Age, 11 years	**3.09**	2.58 to 3.73	**3.09**	2.38 to 4.07	**3.15**	2.38 to 4.19
Current smoking	1.65	0.92 to 2.94	**2.22**	1.07 to 4.59	0.93	0.22 to 3.95
Former smoking	1.49	0.90 to 2.47	**1.98**	1.08 to 3.66	0.49	0.07 to 3.55
HDL-cholesterol, 12 mg/dL	1.00	0.83 to 1.20	1.01	0.78 to 1.31	0.99	0.77 to 1.27
Systolic blood pressure, 25 mm Hg	1.08	0.86 to 1.31	1.03	0.76 to 1.42	1.10	0.84 to 1.49
MIF genotype GC+CC vs GG	1.48	0.91 to 2.22	**1.79**	1.01 to 3.18	1.03	0.57 to 1.88
Lp(a) quintile 2 4.9/6.5 mg/dL	1.65	0.90 to 3.02	**2.42**	1.03 to 5.81	1.19	0.50 to 2.86
Lp(a) quintile 3 9.5/12 mg/dL	1.27	0.70 to 2.28	1.59	0.69 to 3.70	0.97	0.39 to 2.43
Lp(a) quintile 4 18.3/23 mg/dL	1.31	0.73 to 2.38	1.52	0.64 to 3.64	1.31	0.57 to 3.02
Lp(a) quintile 5 >24.2/≥31 mg/dL	1.43	0.77 to 2.60	1.44	0.58 to 3.53	1.59	0.69 to 3.67
Death rate. per 1000 person-year	**17.5**	**21.7**	**13,9**			

The MIF GG genotype was carried in 2/3 of the sample. The referent was the lowest quintile with <3.2/<4.2 mg/dL (n=276).
Significant values are highlighted in bold.
*Number of deaths/number at risk.
HDL, high density lipoprotein; MIF, migration inhibitory factor.

the MIF GC-CC genotype was not associated, and decreasing Lp(a) quintiles from 4 downwards tended to exhibit an inverse association with the risk of incident MetS.

The described features paralleled the ones related to overall mortality. The MIF genotype among women was again unrelated to all-cause mortality, while in men both the MIF 173-G/C polymorphism and the Lp(a) Q2 were additive significant predictors of MetS risk, and the four highest Lp(a) quintiles displayed an inverse risk shape.

Studies examining the relationship between the MIF 173-G/C polymorphism and MetS are sparse. A cross-sectional analysis of serum MIF concentrations in a limited number of Koreans found a sex divergence[27] converse to the polymorphism divergence in this study. Serum levels of MIF were similar in men with or without MetS, whereas in female patients with MetS, MIF concentrations were significantly higher than in healthy controls, as well as in males with MetS.

Such gender disparity of circulating MIF (or of MIF 173-G/C polymorphism) has been reported in other ethnic groups and in females developing gestational pre-eclampsia, and is indicative of a specific significance. Similar to Korean men with MetS, German men in the KORA/Augsburg case cohort study exhibited no association between either the MIF gene variant or MIF concentration and the subsequent development of diabetes, while in women this association was significant.[28] Furthermore, the same group of Italian workers examining the association of maternal MIF concentrations with gestational pre-eclampsia, who had found elevated circulating MIF

Table 4 Cox regression analyses of adjusted Lp(a) quintiles and MIF-173 genotypes for prediction of incident MetS

	Total 146/626*		Men 55/307*		Women 91/319*	
Free of MetS	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**
Gender, female	**2.48**	1.61 to 3.83				
Age, 11 years	1.12	0.94 to 1.33	1.09	0.81 to 1.46	1.14	0.91 to 1.41
Current smoking	1.31	0.85 to 2.03	1.30	0.62 to 2.74	1.30	0.73 to 2.34
Former smoking	1.54	0.82 to 2.59	1.66	0.79 to 3.52	1.36	0.54 to 3.42
HDL-cholesterol, 12 mg/dL	**0.76**	0.63 to 0.91	**0.71**	0.51 to 1.00	**0.78**	0.62 to 0.98
Systolic blood pressure, 25 mm Hg	**1.31**	1.05 to 1.60	1.13	0.74 to 1.72	**1.35**	1.03 to 1.72
MIF genotype GC+CC vs GG	1.14	0.80 to 1.63	1.35	0.72 to 2.51	1.01	0.65 to 1.58
Lp(a) quintile 2 4.9/6.5 mg/dL	1.01	0.60 to 1.70	1.52	0.64 to 3.63	0.83	0.43 to 1.63
Lp(a) quintile 3 9.5/12 mg/dL	0.97	0.58 to 1.61	1.55	0.66 to 3.63	0.75	0.39 to 1.45
Lp(a) quintile 4 18.3/23 mg/dL	0.83	0.50 to 1.37	1.12	0.47 to 2.65	0.72	0.39 to 1.35
Lp(a) quintile 5 >24.2/≥31 mg/dL	1.01	0.61 to 1.67	1.16	0.48 to 2.80	0.95	0.51 to 1.78
MetS incidence per 1000 person-year	**41**	**31**	**49.3**			

The MIF GG genotype was carried in 2/3 of the sample. The referent was the lowest quintile with <3.2/<4.2 mg/dL (n=128).
Significant values are highlighted in bold.
*Number of incident MetS/number at risk.
HDL, high density lipoprotein; MIF, migration inhibitory factor.

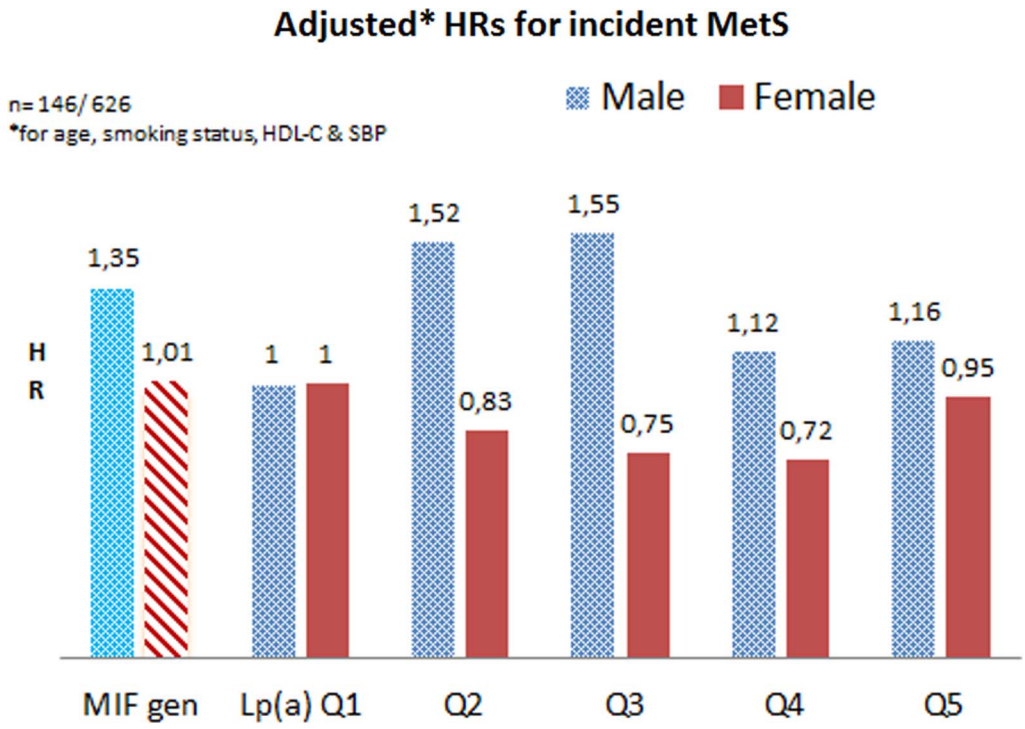

Figure 1 Graphic representation of multiadjusted HRs of Lp(a) quintiles and MIF-173 G/C genotypes for incident MetS in 626 male and female participants free of MetS at baseline. Noteworthy is the indirect evidence of Lp(a) aggregation and ensuing autoimmune activation in men manifesting with low HRs in the top two quintiles and reaching the highest HRs in Q2 and Q3 (presumably very few men with Lp (a) aggregation yielding Lp(a) as low as <3.2 mg/dL). In contrast, in women, HRs increase from quintile 4 onwards with declining quintiles down to Q1. The gender difference is partly linked to the LPA rs10455872 A>G polymorphism related properties of the top Lp (a) quintile in men harboring high susceptibility to MetS (rather than diabetes) and to diabetes in women (rather than MetS, documented in an as yet unpublished study). MetS, metabolic syndrome; MIF, migration inhibitory factor.

compared with pregnant women without pre-eclampsia,[29] newly reported in a prospective analysis of 127 pregnant women the existence of lower MIF concentrations in maternal serum before the onset of pre-eclampsia.[30]

MIF genotype and the presumable autoimmune process

After a bivariate genome-wide approach to MetS, the STAMPEED Consortium[31] concluded that qualitative and quantitative pleiotropic tests on pairs of traits indicated that common genetic variants could explain only a small portion of the covariation in these traits. Authors expressed the viewpoint that MetS is rather a consequence of interactions among different pathways. Collectively, our observations strongly suggest that the MIF gene variant contributes to the development of MetS or type 2 diabetes,[27 28] and also that the MIF protein may sustain damage to its epitope/DNA under conditions of prolonged oxidative stress, rendering a portion of it to escape from immunoassay and to a failure of its genetic expression, which is protective against a pro-inflammatory state in two-thirds of carriers.[32] The present and previous studies demonstrate that such oxidative damage to the MIF protein may be much more pronounced in one sex (confirmed in as yet unpublished observations) and that the involved gender may differ depending on ethnicity.

By showing that the MIF 173-G/C polymorphism and lower (rather than higher) Lp(a) quintiles were significant additive predictors in men of overall mortality and tended to be so for the development of MetS, our study further suggests that the Lp(a) mediated pro-inflammatory state is substantially triggered by the MIF gene variant/damaged protein.

The reason why the inverse risk of Lp(a) does not extend to men to the lowest quintile may be as follows. Epitope damage to the Lp(a) protein, and the Lp(a) mass escaping from the immunoassay, are presumably decremental due to the declining inflammatory state in declining Lp(a) quintiles. For example, an involvement of 40–60% Lp(a) mass of the highest quintile and 10% of Q3 would concentrate the autoimmune process to Q3 and Q2, leaving male participants having the genuinely lowest Lp(a) quintile with the lowest MetS risk. The same process, valid also in females, seems to involve much more strongly the highest three quintiles, thus extending the higher MetS risk even to the bottom quintile.

The probable reasons for a clear sex difference among Turkish adults are as follows. Peri and postmenopausal women are burdened with a more enhanced low grade inflammation than men, as manifested by the higher degree of adiposity and higher levels of CRP, Lp(a), and fibrinogen. Autoimmune activation resulting from a pro-inflammatory state, a fundamental mechanism for numerous chronic diseases in people prone to MetS, is substantially more common in women, and these parameters constitute major determinants of risk for cardiometabolic diseases and death. Examples of sex disparity are systolic BP, which is clearly a risk factor in men, but less so in women, and current smoking, which acts as a risk factor in men but as a

protective determinant in women predisposed to autoimmune activation.

Lp(a) is not the only protein/polypeptide in autoimmune complexes. We have recently published evidence of serum creatinine being likewise involved and predicting future CHD risk in the general population,[33] or in people without metabolic disorders.[34] Similarly, low glycated hemoglobin has also been considered to represent an elevated risk state in non-diabetic adults.[35]

Implications: Recognition of a notion of autoimmune activation with elevated mortality or MetS risk has vast implications regarding public health and prevention in view of the high prevalence of autoimmune processes in the middle aged and elderly population. This notion entails renewed epidemiological research and novel immunoassay methods for polypeptides or proteins with damaged epitopes in an enhanced proinflammatory state.

Limitations and strength: We relied on single measurements of Lp(a) at baseline, albeit not a major limitation, given that this protein level is considered to change little over a lifetime. The sample, free of baseline MetS, offered relatively limited statistical power. Current findings may have lower or limited applicability to populations or population segments less susceptible to impaired glucose tolerance. Confounding by ethnicity may possibly result from allele frequencies of the MIF and LPA polymorphisms as well as from a variance in mortality rates and MetS incidence. This study is, to the best of our knowledge, the first to prospectively investigate the potential non-linear associations of assayed or estimated Lp(a) in conjunction with the MIF-173G/C variant, with the risk of overall mortality as well as MetS, using multivariable adjustments.

CONCLUSION

In a middle-aged, population based sample prone to MetS, we analyzed the association of the MIF rs755622 polymorphism, and the shape of the association curve of assayed Lp(a) concentrations with incident MetS and the risk of death, at a mean follow-up of over 5 years. Declining Lp(a) quintiles down to quintile 2 were paralleled by non-significant although increasing multi-adjusted risk of death in males, yielding in quintile 2 the significantly highest risk of death, added to MIF polymorphism, HDL-cholesterol, and systolic BP. The MIF genotype yielded a significant HR in men for overall mortality, but was not associated in women. We conclude that in Turkish men, but not in women, the MIF-173G/C polymorphism by inducing low-grade inflammation independently mediates weakly the development of MetS and significantly predicts the risk of death, in addition to triggering autoimmune activation comprising circulating Lp(a), rendering an apparent inverse association of the latter with all-cause death. The lack of a relationship in women may be attributed to MIF protein damage and more complex autoimmune activation.

Contributors AO conceived and designed the study, as well as analyzed and interpreted the data; GC performed the statistical analyses and revised the manuscript critically; NÇ and NE-Ü performed the genetic analyses and revised the manuscript critically; EA supervised the biochemical analyses and revised the manuscript critically; ID and HÇ collected data and revised the manuscript critically; HY drafted a major portion of the manuscript and obtained funding.

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