Association of Polymorphisms of the Apolipoprotein(a) Gene With Lipoprotein(a) Levels and Myocardial Infarction

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Background—Serum lipoprotein(a) [Lp(a)] concentration is largely determined by variability at the apolipoprotein(a) gene locus. Most prominent effects relate to polymorphisms in the promoter (a pentanucleotide [PN] repeat) and coding regions (a kringle IV [K4] repeat), the latter of which also affects Lp(a) particle size. The impact of these polymorphisms on cardiovascular risk is poorly understood.

Methods and Results—We studied both polymorphisms and Lp(a) levels in 834 registry-based myocardial infarction (MI) patients (38% women) and 1548 population-based controls. Lp(a) concentrations were inversely related with the numbers of K4 and PN repeats. However, the effect of the PN polymorphism was restricted to subjects producing small Lp(a) particles (≤8 PN 66.1 mg/dL versus >8 PN 8.7 mg/dL; P<0.0001). The odds to present with MI were elevated in individuals producing small Lp(a) particles (≤22 K4 repeats; OR 1.47 for men and 1.69 for women; P<0.002) and in women with ≤8 PN repeats (OR 1.46, P=0.009). Interestingly, in women, the frequent haplotype with ≤8 PN and ≤22 K4 repeats, which is related to high levels of small Lp(a) particles, resulted in an elevated OR for MI (1.79; P=0.01) independently of Lp(a) serum concentration.

Conclusions—The K4 and PN repeat polymorphisms largely explain the high variability of serum Lp(a) levels. A haplotype with ≤8 PN and ≤22 K4 repeats is characterized by high concentrations of small Lp(a) particles. Our observation that this haplotype was associated with MI independently of Lp(a) serum levels may suggest that Lp(a) particle size in addition to its concentration may modulate MI risk in women. (Circulation. 2003;107:696-701.)

Key Words: apolipoproteins □ polymorphism □ myocardial infarction □ women □ population

Lipoprotein(a) [Lp(a)] serum levels have been repeatedly associated with coronary heart disease or myocardial infarction (MI) in large observational studies.1–7 Interestingly, such associations were found to be particularly prominent in young and middle-aged rather than elderly men8 and women.9,10 Thus, Lp(a) serum levels may modulate the risk for premature arteriosclerosis and MI.

The interindividual variability of Lp(a) levels is very high and is largely determined by the polymorphic apolipoprotein(a) gene on chromosome 6q27.11–14 The apolipoprotein(a) kringle IV (KIV) repeat polymorphism (38 known alleles) that generates apolipoprotein(a) molecules ranging from 250 to 800 kDa appears to determine about half of the variability of Lp(a) serum levels, with an inverse relationship between the number of KIV repeats and Lp(a) serum concentration.15,16 However, the variability of Lp(a) concentration is high, particularly in carriers of a low number of KIV repeats, ranging from 0 to >200 mg/dL.15,16,17 Other polymorphisms in this gene, particularly those located in the 5′-flanking region, were related to serum Lp(a) levels as well, presumably by modulating the transcriptional activity of the apolipoprotein(a) gene.15–19 A pentanucleotide repeat polymorphism (7 known alleles) at position −1373 from the ATG site may be of particular interest, because a consistent association with Lp(a) levels was found in small populations.16,19,20 In addition to potential effects on transcriptional activity, this polymorphic site was found to be in linkage disequilibrium with other functional 5′-polymorphisms and with the KIV repeat polymorphism.16

Despite the evidence for genetic determination of Lp(a) serum levels, data demonstrating linkage or association with coronary artery disease are rare. Some studies have shown association of the KIV polymorphism with coronary artery disease phenotyped by angiography.21,22 No study has shown linkage or association of any of the Lp(a) polymorphisms with MI. Furthermore, it is not known whether certain haplotypes of the pentanucleotide and the KIV repeat polymorphism are associated with high Lp(a) concentrations or MI. This
may be of interest, because such haplotypes may affect both Lp(a) serum concentration and Lp(a) particle size, with potential implications for atherogenicity.

Methods

The Ethics Committee at the University of Regensburg approved the study protocol, and all participants gave informed consent.

Study Populations

Group 1: MI Cases and Controls From the Augsburg MONICA Center

In 1996/1997, MI patients from the population-based MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease) MI registry Augsburg who had experienced their first MI before 60 years of age were examined in a study center (n=609). MI had been verified according to standard criteria.21 The control population for this group of MI patients was drawn as a subgroup from the third MONICA survey of the general population of the city of Augsburg in 1994/1995 that originated from a sex/age-stratified cluster sample of all German residents of the Augsburg study area (age 25 to 74 years; participating n=1674).23–25 All individuals were studied by a standardized interview, clinical examination, and biochemical and molecular analyses.

Group 2: Female MI Cases and Controls From the Regensburg MI Sib-Pair Registry

A second independent case-control population was recruited from cardiovascular rehabilitation centers throughout Germany from 1997 to 2000. A total of 281 female MI patients were examined by a physician at a home visit according to a similar protocol.25,26 All MI patients had experienced their first MI before 60 years of age and were characterized by a positive family history for coronary heart disease. To serve as a control population for this group of MI patients, 310 nonaffected sisters in-law of MI patients were examined by the identical protocol.

Serum Lipoprotein Levels

Serum for determination of lipoprotein levels was stored at −20°C for up to 3 months before the assay. Serum total cholesterol, LDL cholesterol, and HDL cholesterol were measured by standard enzymatic methods.21 Lp(a) serum concentrations were determined by an Immuno latex-enhanced immunoassay (Immuno AG).

Apolipoprotein(a) KIV Repeat Polymorphism

The number of KIV repeats in the apolipoprotein(a) gene was determined from serum by immunoblotting of SDS-PAGE.21,22

Apolipoprotein(a) Pentanucleotide Polymorphism

Genotyping of the apolipoprotein(a) pentanucleotide polymorphism was performed after DNA purification by PCR amplification as described by others.19 Determination of Lp(a) concentration, genotyping of the pentanucleotide polymorphism, and apolipoprotein(a) phenotyping were available in 589 MI patients and 1272 controls from Augsburg, as well as 245 MI patients and 273 controls from Regensburg.

Statistical Analysis

The statistical software package SPSS/PC 10.0 was used. Because of skewness of Lp(a) levels, the nonparametric Mann-Whitney U test was used for comparison of Lp(a) concentrations. Linkage disequilibrium between the pentanucleotide and the KIV polymorphism was determined with the Arlequin (version 2.000) program. Frequencies of genotypes were studied in male and female MI patients and respective sex-matched controls by a χ² test. The influence of the KIV and the pentanucleotide polymorphism on the odds of being in the MI group was also calculated in all MI cases and controls in a sex-stratified multiple logistic regression analysis that included risk factors such as age, systolic blood pressure, presence of diabetes mellitus, any history of cigarette smoking, and, in women, menopausal status and estrogen replacement therapy as covariates. To dissect a genetic influence that is present after correction for Lp(a) concentration, all models were performed with or without logarithmically transformed Lp(a) concentration as an independent variable.

Results

Baseline Characteristics

Cardiovascular risk factors were more prevalent in MI patients than in controls (Table 1). The 2 female MI populations showed no relevant differences.

Association of Lp(a) Concentration With MI

Lp(a) concentrations were significantly higher in MI patients than in the general population (median in MI patients 16.0 versus 12.1 mg/dL in controls; P<0.0001). The OR for MI in individuals with Lp(a) above a cutoff of 28 mg/dL, which represents the upper quartile of the Lp(a) distribution of the general population, was estimated to be 1.76 (CI 1.36 to 2.26) in men and 2.12 (CI 1.62 to 2.77) in women (P<0.0001).
The allele with 9 pentanucleotide repeats was found to be in strong linkage disequilibrium (Figure 1; \( P < 0.0001 \)). The KIV repeat and the pentanucleotide repeat polymorphism were found to be in strong linkage disequilibrium for all individuals, as well as each group of pentanucleotide genotypes. Figure indicates strong linkage disequilibrium of KIV repeat and PNR polymorphism (left), as well as largely differing Lp(a) concentrations, depending on particular haplotypes (right).

### Association of Apolipoprotein(a) Polymorphisms With Lp(a) Concentration

The KIV repeat and the pentanucleotide repeat polymorphism were found to be in strong linkage disequilibrium (Figure 1; \( P < 0.0001 \)). The allele with 9 pentanucleotide repeats was linked to a high number of KIV repeats, and alleles with 10, 11, or 12 pentanucleotide repeats were found to be linked to small apolipoprotein(a) particles (number of KIV repeats \( \leq 22 \)). In contrast, homozygous individuals with 8 pentanucleotide repeats, which represents the most frequent genotype (48.2%) of the pentanucleotide repeat polymorphism, showed no apparent linkage with a particular range of KIV repeats. These analyses confirmed the significant association of the pentanucleotide repeat polymorphism with MI in both female study groups (Figure 2B). For further analysis, genotypes that had been associated with the highest Lp(a) concentration (6/8) were combined into 1 group versus all other genotypes. Individual analyses of case-control populations from Augsburg and Regensburg showed similar genotype distributions and revealed significant associations of these genotypes with MI in both female study groups (Figure 3).

#### Table 2. Median Lp(a) Concentration and Interquartile Range Stratified by KIV Repeat and Pentanucleotide Repeat Polymorphisms

<table>
<thead>
<tr>
<th>Pentanucleotide Repeat Genotypes</th>
<th>KIV Repeats</th>
<th>Frequency, n</th>
<th>( \leq 22 ) KIV Repeats</th>
<th>( &gt; 22 ) KIV Repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( 6/8, 7/7, 7/8, 8/8 )</td>
<td>310</td>
<td>66.1 (55.1)</td>
<td>8.7 (16.0)</td>
<td></td>
</tr>
<tr>
<td>( 6/8, 7/9, 8/9, 9/9 )</td>
<td>157</td>
<td>46.1 (42.6)</td>
<td>7.6 (16.0)</td>
<td></td>
</tr>
<tr>
<td>( 7/10, 8/10, 8/11, 8/12, 9/10, 9/11, 10/10, 10/11, 10/12, 11/11 )</td>
<td>182</td>
<td>12.4 (22.2)†</td>
<td>12.0 (11.8)†</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( 6/8, 7/7, 7/8, 8/8 )</td>
<td>422</td>
<td>65.4 (52.8)</td>
<td>9.0 (16.0)</td>
<td></td>
</tr>
<tr>
<td>( 6/8, 7/9, 8/9, 9/9 )</td>
<td>207</td>
<td>44.6 (51.7)†</td>
<td>10.0 (15.8)</td>
<td></td>
</tr>
<tr>
<td>( 7/10, 8/10, 8/11, 8/12, 9/10, 9/11, 10/10, 10/11, 10/12, 11/11 )</td>
<td>267</td>
<td>16.7 (37.3)†</td>
<td>10.4 (10.1)</td>
<td></td>
</tr>
</tbody>
</table>

Control individuals were stratified by major allele of KIV polymorphism (\( \leq 22 \) or \( > 22 \) KIV repeats). Genotypes of pentanucleotide polymorphism were grouped according to results of distribution analysis shown in Figure 1: carriers of \( \leq 8 \) repeats, carriers of at least 1 allele with 9 repeats, and carriers of \( \geq 10 \) repeats. Medians and interquartile ranges are given; Lp(a) values are in mg/dL.

### Association of Apolipoprotein(a) Polymorphisms With MI

The detailed distribution of all alleles in controls and MI patients is shown in Figure 2A. A low number of KIV repeats (\( \leq 22 \)) in the dominant allele that was found by Western blot analysis to have the most influence on Lp(a) concentration was more frequently found in male and female patients with MI than in controls (OR 1.470 and 1.687 versus \( > 22 \) repeats; \( P = 0.002 \) and \( < 0.0001 \), respectively).

No difference in genotype distribution of the pentanucleotide polymorphism was detected between male MI patients and controls (Figure 2B). In contrast, in women, homozygous carriers of 8 pentanucleotide repeats were more frequently found in the group of MI patients than in the controls (55.3% versus 46.3%; Figure 2B). For further analysis, genotypes that had been associated with the highest Lp(a) concentration (6/8) were combined into 1 group versus all other genotypes. Individual analyses of case-control populations from Augsburg and Regensburg showed similar genotype distributions and revealed significant associations of these genotypes with MI.
association of the KIV repeat polymorphism with MI both in men and in women (Figure 4). However, adjustment for Lp(a) concentration abolished this relationship. In contrast, \( \leq 8 \) pentanucleotide repeats were independently associated with MI in women even after controlling for Lp(a) serum concentration. Haplotypes with \( \leq 22 \) KIV repeats and \( \leq 8 \) pentanucleotide repeats that were associated with the highest Lp(a) concentrations showed the highest odds ratios for MI (Figure 4). Although a significant association was abolished by further adjustment for Lp(a) concentration in men, it persisted in women.

**Discussion**

The present investigation evaluated 2 features of the apolipoprotein(a) gene locus that appear to affect Lp(a) plasma concentrations and, potentially, the risk of MI. First, individuals with \( \leq 22 \) KIV repeats were found to present with elevated Lp(a) levels and an elevated risk for MI. Our observation of a significant association between the KIV repeat polymorphism with MI in both men and women is...
consistent with many previous studies that reported an association between elevated serum Lp(a) levels and coronary heart disease.3,8–10,28–30

The second feature of the apolipoprotein(a) gene that might affect the risk of MI is the pentanucleotide repeat polymorphism in the promoter region. Although previous analyses demonstrated a significant, albeit moderate effect of this polymorphism on Lp(a) concentration in general,16,19,20 the present study demonstrated a strong influence of the pentanucleotide polymorphism, specifically in individuals who have low numbers of KIV repeats. Consequently, the pentanucleotide repeat polymorphism appears to determine the number of small Lp(a) particles. The distribution of the KIV and the pentanucleotide polymorphism does not occur at random but is confined to a highly significant linkage disequilibrium.

Interestingly, one of the frequently found haplotypes with ≤22 KIV repeats and ≤8 pentanucleotide repeats was identified to result in extremely high serum concentrations of small Lp(a) particles. With respect to MI, it may therefore be of no surprise that this haplotype with ≤22 KIV repeats and ≤8 pentanucleotides was related to an elevated risk of MI. However, it may be rather surprising that this association persisted in women after inclusion of Lp(a) serum concentrations in the logistic regression model. Caution is warranted in the interpretation of such statistical evaluations. However, it may be tempting to speculate that large numbers of small Lp(a) particles may confer a specific risk, as has been shown for the quantity and particle size of LDL and HDL cholesterol.31,32 Indeed, a recent report specifically associated small Lp(a) molecules with poor endogenous fibrinolytic activity.33 If our finding is reproducible in additional studies, it appears that in women, knowledge of a specific haplotype that produces large numbers of small Lp(a) particles provides predictive information beyond serum Lp(a) concentration.

Given that the association between the ≤22 KIV/≤8 pentanucleotide repeats haplotype with MI was significant only in women, one might speculate that Lp(a) may have a more pronounced effect in women than in men. The association of high Lp(a) levels with coronary atherosclerosis and acute coronary events appears to be more pronounced in women than in men in the present study and in a number of previous reports.9,10,34

A limitation of the present study may be the rather arbitrary grouping of genotypes based on the number of KIV and pentanucleotide repeats, although similar distinctions have been used previously.1,28 In addition, dichotomized stratification by the number of KIV repeats may result in an under-adjustment of the possible confounding effect of the KIV polymorphism. A further limitation of the study is its retrospective character. In particular, we cannot exclude that mortality after the acute MI was different in the respective genotype groups. However, age at time of MI and the time of follow-up after MI were similar in the respective genotype groups, and genotype distribution was not dependent on age (data not shown). Thus, we have no indication of selection bias due to mortality.

In summary, the present study suggests that at least 3 factors affect the association between lipoprotein(a) and the risk of MI. First, the KIV polymorphism is related to MI in both men and women, probably owing to modulation of both Lp(a) concentration and particle size. Second, a pentanucleotide polymorphism significantly affects the risk of MI in women only, which suggests a sex-specific modulation of the risk increase due to elevated Lp(a) particles. Third, a specific and frequent haplotype (≤22 KIV repeats and ≤8 pentanucleotide repeats) is associated with MI in women after correction for Lp(a) concentrations. This finding indicates that this particular haplotype, which results in high concentrations of small Lp(a) particles, may confer a specifically high risk that is not entirely reflected by conventional measurement of serum Lp(a) concentration.

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