Impact of Apo(a) Length Polymorphism and the Control of Plasma Lp(a) Concentrations
Evidence for a Threshold Effect

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Abstract—Plasma lipoprotein(a) [Lp(a)] levels are believed to be controlled predominantly by the apolipoprotein(a) [APO(a)] gene, which encodes the apo(a) glycoprotein, a key constituent of the Lp(a) particle. Previously, it has been accepted that the plasma Lp(a) level is inversely proportional to apo(a) length. To examine this relationship in greater detail, 1500 unrelated, homogeneous (sex, race, age, plasma lipids) subjects were studied, from which 769 were identified with a single-expressing APO(a) allele. A bimodal frequency distribution of apo(a) isoforms was observed. As expected, there was a general inverse relationship between apo(a) isoform size and Lp(a) level. However, when groups with equivalent single-expressing apo(a) isoforms were studied, it was clear that although smaller isoforms were associated on average with higher levels, they were also associated with the greatest variability in level. After logarithmic transformation of Lp(a) data, the overall contribution of the apo(a) length polymorphism was calculated to be 38%. However, in subjects with apo(a) isoforms of \#20 kringle-4 (K-4) repeats, only 9% of the variability in Lp(a) concentration is explicable on the basis of the apo(a) length polymorphism. In those with apo(a) isoforms of \textgreater{}20 K-4 repeats, the corresponding contribution is 10%. We conclude that the contribution of the apo(a) isoform size to the control of plasma Lp(a) level is considerably lower than previously calculated, because the variability in plasma Lp(a) concentration is not uniform across the apo(a) size spectrum. (Arterioscler Thromb Vasc Biol. 1998;18:1870-1876.)

Key Words: apolipoprotein(a) ■ lipoprotein(a) ■ polymorphism ■ phenotyping

Lipoprotein(a) [Lp(a)], a cholesteryl ester–rich lipoprotein, comprises a particle of LDL to which is attached a large, hydrophilic glycoprotein, apolipoprotein(a) [apo(a)]. Elevated plasma levels of Lp(a) confer an increased risk of coronary artery disease and have recently been confirmed to be an independent risk factor for this disease. Because of the association between high plasma Lp(a) concentrations and coronary artery disease, the factors controlling plasma Lp(a) concentrations have been the subject of active research in recent years as potential therapeutic targets.

The APO(a) gene was first implicated as a major determinant of plasma Lp(a) levels when it was noted that the apo(a) glycoprotein varied in size over a wide range and that its length tended to be inversely related to plasma concentrations of Lp(a). The corresponding size variation in the apo(a) glycoprotein is due to length polymorphism in the APO(a) gene, which contains multiple tandem repeats of a 5.5-kb sequence encoding a cysteine-rich protein motif resembling the kringle 4 (K-4) of plasminogen. From sib-pair analyses, it has been estimated that \textgreater{}90% of the interindividual variation in plasma Lp(a) can be attributed to sequence differences at, or closely linked to, the APO(a) locus, whereas 42% of the variability in plasma Lp(a) levels between unrelated individuals has been attributed to the size polymorphism in the apo(a) protein. We have previously used high-resolution apo(a) phenotyping in conjunction with apo(a) genotyping to help elucidate underlying mechanisms of plasma Lp(a) level control. One aspect of our previous studies on plasma Lp(a) concentration control highlighted the possibility of an interesting relationship between plasma Lp(a) concentration and apo(a) isoform length that was more complex than the simple inverse association proposed and confirmed many times in the literature.

Because of the large degree of variation in plasma Lp(a) concentrations and apo(a) size polymorphism in subjects, any examination of this relationship with greater resolution can only be attempted if a large number of relatively homogeneous subjects are studied. Furthermore, in a selected large group of subjects who express only a single APO(a) allele, it would be possible to examine the relationship between circulating Lp(a) level and apo(a) phenotype without the potentially confounding effects of dual expression of 2 different-size isoforms in the same subjects. From the West of

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Scotland Coronary Prevention Study (WOSCOPS) biobank, a sufficiently large number of such subjects were available. This is the largest study examining the relationship between apo(a) isoform size, as determined by high-resolution immunoblotting and plasma Lp(a) concentration, and the results clearly demonstrate, for the first time, that apo(a) isoform size and plasma Lp(a) concentration do not have a simple inverse relationship. This study also demonstrates that the apo(a) length polymorphism exerts much less control on the plasma Lp(a) concentration than previously accepted.

Methods

All reagents were purchased from BDH Laboratory Supplies unless otherwise stated.

Subjects

All subjects studied were participants in the WOSCOPS, and their characteristics are summarized in detail elsewhere. In brief, these subjects were unrelated white men between 45 and 64 years old (mean, 55.2 years) with a mean baseline plasma total cholesterol of 272 mg/dL (SD, 23 mg/dL), LDL cholesterol of 192 mg/dL (SD, 17 mg/dL), and HDL cholesterol of 44 mg/dL (SD, 9 mg/dL). They also had normal renal and hepatic function with no history of myocardial infarction or organ transplantation. From a survey of 1500 WOSCOPS participants taken at random from the total cohort of 6595, a group of 769 subjects were selected who had a single-expressing APO(a) allele ranging in size from 11 to 38 K-4 repeats. Blood sampling of human subjects was approved by the Glasgow Royal Infirmary Ethics Committee.

Plasma Lp(a) Assay

Fasting venous blood samples were collected into tubes containing Na2EDTA (final concentration, 1.5 mg/mL). Plasma was isolated by low-speed centrifugation at 1000g for 15 minutes at 4°C, divided into aliquots, and stored at −70°C for up to 3.5 years until Lp(a) assays were performed. Lp(a) levels have been shown to be stable for up to 8.5 years in samples stored at −20°C. Plasma Lp(a) concentrations were measured with the commercially available Innogenetics Lp(a) assay (Innogenetics NV). This sandwich ELISA consists of a solid-phase mouse monoclonal anti-Lp(a) and a sheep anti-apo(a) polyclonal second antibody, which is labeled with the enzyme horseradish peroxidase (HRPO). This labeled antibody binds to any solid-phase antibody/Lp(a) complex because it recognizes the apoB moiety of the Lp(a) complex. This assay does not use the anti-apoB antibody used in the apo(a) phenotyping system. The detection limit of the plasma Lp(a) assay is 1 mg/dL. The units used to report Lp(a) level, milligrams per deciliter, refer to the total lipoprotein mass of Lp(a).

Apo(a) Isoform Determination by Immunoblotting

Plasma aliquots prepared as above were stored for up to 6 years at −70°C. Apo(a) isoform analysis was performed according to a modification of the immunoblotting method described by Kamboh et al. A 12.5-μL aliquot of plasma was mixed with 30 μL reducing buffer [1:2:10 ratio of β-mercaptoethanol, 0.5% (wt/vol) bromphenol blue in 5% (vol/vol) glycerol, and 5% (vol/vol) SDS] and boiled for 5 minutes. A 10-μL aliquot of this mixture was loaded onto a 2% agarose (Ultra Pure DNA Grade Agarose, Bio-Rad) gel (20×24 cm) in 90 mmol/L Tris base, 90 mmol/L boric acid, 2 mmol/L Na2EDTA, and 0.1% (wt/vol) SDS. A mixture of equal quantities of 6 apo(a) isoforms, with 15, 18, 21, 24, 28, and 32 K-4 repeats, from fully characterized subjects was run on each gel as size standards, and the size of each isoform was calculated by comparison with this ladder of standards. In addition, a plasma control from a subject with an apo(a) phenotype of 15/30 was run on every gel to control for interassay variation in sensitivity.

Electrophoresis was performed in a horizontal gel apparatus (model HRH, IBI Inc) with 45 mmol/L Tris base, 45 mmol/L boric acid, 2 mmol/L Na2EDTA, and 0.1% (wt/vol) SDS at 60 V for 30 minutes and then for 20.5 hours at a constant 140 V at 4°C. After electrophoresis, the proteins were transferred to a pure nitrocellulose membrane (Hybon-ECL, Amersham) by electroblotting using a Hoefer Transphor Cell TE42 (Hoefer Scientific Instruments) at 90 V for 3 hours in 10 mmol/L Tris base, 40 mmol/L glycine, and 5% (vol/vol) methanol at 4°C. Before electroblotting, the gel was soaked in prechilled transfer buffer for 5 minutes, and the membrane was soaked first in distilled water for 1 minute and then in prechilled transfer buffer for 10 minutes.

After electroblotting, the membrane was incubated in blocking solution [Tris-buffered saline (TBS) (0.5 mol/L NaCl and 35 mmol/L Tris base, pH 7.4), 5% (wt/vol) powdered skimmed milk] on an orbiting platform for 1 hour before a 30-minute incubation in TBS plus 1% (vol/vol) NP-40 and 15 μg/100 mL of 1A′ Anti-apolipoprotein(a) human, catalog No. 1399 314, Boehringer Mannheim]. This antibody concentration was chosen empirically. After incubation with the first antibody, the membrane was washed 4 times for 10 minutes each in 100 mL washing buffer [TBS plus 1% (vol/vol) NP-40, 0.25% (wt/vol) deoxycholic acid, and 0.1% (wt/vol) SDS] on an orbiting platform.

The membrane was then incubated with a 1:15 000 dilution of a sheep anti-mouse HRPO-linked antibody (catalog No. NA931, Amersham). Again, this antibody concentration was chosen empirically. The 4-cycle wash procedure was repeated and the membrane developed with ECL Western blot detection reagents (Amersham) and subjected to autoradiography using Kodak XLS-5 film (IBI Inc) for 1 minute, 5 minutes, and overnight. The latter is used to detect any very faint bands that might otherwise be designated as “null.” The ECL Western blot detection system is a light-emitting, nonradioactive method for detection of immobilized specific antigens, conjugated directly or indirectly with HRPO-labeled antibodies.

Statistical Methods

Previous studies investigating the relationship between the apo(a) length polymorphism and plasma Lp(a) concentration have analyzed the raw, untransformed plasma Lp(a) measurements. The methods used make the assumption of homogeneity of variability in Lp(a) concentration across different apo(a) isoform sizes. The present study is large enough to establish that this assumption is untenable. However, after transformation of the Lp(a) concentrations to a logarithmic scale, the assumption of homogeneity appears to be quite reasonable. The percentage of variability in the population Lp(a) concentrations explained by knowledge of the apo(a) isoform size was calculated by the method of Boerwinkle and Sing with the addition of prior logit transformation of the data.

Results

The plasma Lp(a) concentrations in the 1500 subjects ranged from 1 to 284 mg/dL, with a mean value of 41 mg/dL and a median value of 19 mg/dL. The frequency distribution of plasma Lp(a) is shown in Figure 1. As has been repeatedly demonstrated in white populations throughout the world, the distribution is positively skewed toward lower plasma levels, with the majority of subjects having a plasma Lp(a) concentration <20 mg/dL.

All subjects were apo(a) phenotyped by the high-resolution immunoblotting method described above. This phenotyping was carried out within a 12-month period to minimize interexperiment variation. Furthermore, to ensure consistency throughout this time period, all gels were run with the same size standards and controls.

Of the 3000 APO(a) alleles studied indirectly by examination of their apo(a) isoform protein products, 2205 (73.5%) were associated with a detectable protein product on the
immunoblots. The remaining APO(a) alleles were designated “null.” As described elsewhere, this is an operational definition that may change as the sensitivity of the phenotyping system improves. Currently, we estimate that our system will detect all apo(a) isoforms associated with a plasma Lp(a) concentration of 0.05 mg/dL, ie, this phenotyping system is ~20 times more sensitive than the Lp(a) ELISA used. The size distribution of these apo(a) isoforms is shown in Figure 2. The 32 different apo(a) isoforms detected ranged in size from 7 K-4 repeats to 40 K-4 repeats. The nomenclature used for these isoforms will be apo(a)-7 to apo(a)-40. The distribution of the apo(a) isoforms in the whole group is bimodal, with 2 peaks at apo(a)-19 and apo(a)-27, respectively. This bimodal distribution of apo(a) isoform sizes has been noted before when fewer subjects were studied.

In 769 subjects (51.3%), only a single apo(a) isoform was detectable, and these subjects were assumed to be heterozygous for a “null” allele and an expressing APO(a) allele. Because these individuals had only a single-expressing APO(a) allele, they will be referred to as “single expressers.”

In 13 subjects (0.9%), no apo(a) band was detectable on the immunoblot. This is identical to the percentage of null/null subjects found by another group studying a different white population.

In the 769 single expressers, the general inverse relationship between apo(a) isoform size and plasma Lp(a) level is supported, as shown in Figure 3, with all apo(a) isoforms associated with plasma Lp(a) concentrations <50 mg/dL. The distribution of apo(a) isoforms in these single expressers is shown superimposed on the whole group in Figure 2. The apo(a) size distribution in this subset shadows almost exactly the distribution in the whole group, with a bimodal distribution and a range from apo(a)-11 to apo(a)-38. The most commonly occurring apo(a) isoform in the single expressers was apo(a)-26.

When the plasma Lp(a) concentrations associated with subsets of single expressers with equal-size apo(a) isoforms are examined, a distinct pattern appears, as shown in Figure 4 and the Table. Interestingly, although the smaller apo(a) isoforms are associated with the highest plasma Lp(a) levels, they are also associated with the widest variation in levels. There appears to be a marked decline in the variability of plasma Lp(a) concentration with isoforms >20 K-4 repeats in length. However, from apo(a)-21 through apo(a)-33, there is a gradual decline in the variability of associated Lp(a) levels ranging from 2 to 108 mg/dL with apo(a)-21 to 1 to 24 mg/dL with apo(a)-33. The plasma Lp(a) levels associated with apo(a) isoforms of <16 K-4 repeats and >33 K-4 repeats are not included in Figure 4 or the Table because <10 subjects each had these single-expressing isoforms, and no valid conclusions can be made with such small numbers.

This phenomenon is exemplified in greater detail in Figure 5. Here, 54 subjects with a single-expressing apo(a)-20 isoform had plasma Lp(a) levels ranging from 3 to 284 mg/dL (median, 64 mg/dL), whereas in 50 subjects with a single-expressing apo(a)-30 isoform, the plasma Lp(a) level ranged from 1 to 28 mg/dL (median, 11 mg/dL).

After logarithmic transformation of the Lp(a) data, the apo(a) length polymorphism accounts for 38% of the vari-
ability in plasma Lp(a). This figure is close to the 41.9% previously calculated in a group of 473 subjects but not based on logarithmically transformed data. Because of the marked step in the variability of plasma Lp(a) observed between apo(a) isoforms with ≥20 and <20 K-4 repeats (Figure 4), these subgroups were analyzed separately. The variability in plasma Lp(a) concentration explained by the apo(a) length polymorphism in subjects with an apo(a) isoform of ≥20 K-4 repeats is only 9%. In those subjects with circulating Lp(a) containing only apo(a) isoforms <20 K-4 repeats in length, the resulting explained variability is 10%.

**Discussion**

This study, using high-resolution apo(a) phenotyping in a large, well-characterized data set, is the first report of a hitherto unexplained level of complexity underlying the relationship between plasma Lp(a) concentration and apo(a) isoform size.

The main finding of this study is that the contribution of the apo(a) isoform size to the plasma Lp(a) concentration is not the result of a smoothly varying relationship over the size distribution but rather of a quantum change at ∼20 K-4 repeats. Thus, the proportion of variability in Lp(a) concentration explained by the apo(a) length polymorphism is significantly less than previously estimated, leaving greater potential for control by other, perhaps as yet unidentified, polymorphisms in the APO(a) gene.

The molecular mechanism responsible for the size variation in apo(a) was first suggested by the structure of the apo(a) cDNA. McLean et al proposed that the variations in the size of the apo(a) protein were due to differences in the numbers of K-4 repeats in the APO(a) gene. Direct proof that the apo(a) size variation was due to differences in the number of K-4 repeats awaited the development of a method to analyze the APO(a) gene directly. Lackner et al demonstrated that the length of the K-4 encoding region of the APO(a) allele was correlated directly with the size of its apo(a) protein product. Thus, the major size variation in apo(a) isoforms was shown to be due to a length polymorphism in the APO(a) gene and not to post-translational modifications.

It has also been noted previously that the apparent molecular mass of the apo(a) isoform tends to be inversely related to the plasma concentration of Lp(a), and it has been estimated that ∼42% of the variability in plasma Lp(a) levels between unrelated individuals is attributable to the length polymorphism in the apo(a) protein. Previous metabolic studies have indicated that it is the variability in the apo(a) production rate rather than its clearance rate that primarily controls the apo(a) and, in turn, the Lp(a) plasma concentration. The apo(a) production rate itself also has a number of component steps: APO(a) gene transcription, apo(a) mRNA translation, apo(a) isoform transport through the cell, and secretion onto the hepatocyte surface, where it is believed to join with the apoB-100 moiety of LDL to form mature Lp(a).

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**Summary Statistics for the Plasma Lp(a) Distributions for Each Group of Subjects With the Same-Size Single-Expressing apo(a) Allele**

<table>
<thead>
<tr>
<th>Apo(a) Isoform (No. of K-4 Repeats)</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
<th>27</th>
<th>28</th>
<th>29</th>
<th>30</th>
<th>31</th>
<th>32</th>
<th>33</th>
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<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>21</td>
<td>53</td>
<td>53</td>
<td>36</td>
<td>26</td>
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<td>71</td>
<td>71</td>
<td>66</td>
<td>50</td>
<td>58</td>
<td>37</td>
<td>30</td>
</tr>
<tr>
<td>Minimum</td>
<td>6</td>
<td>47</td>
<td>1</td>
<td>3</td>
<td>2</td>
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<td>2</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Maximum</td>
<td>156</td>
<td>210</td>
<td>197</td>
<td>284</td>
<td>108</td>
<td>94</td>
<td>92</td>
<td>56</td>
<td>55</td>
<td>65</td>
<td>65</td>
<td>46</td>
<td>46</td>
<td>35</td>
<td>28</td>
<td>40</td>
<td>24</td>
</tr>
<tr>
<td>Median</td>
<td>72.0</td>
<td>104.0</td>
<td>68.0</td>
<td>64.0</td>
<td>9.0</td>
<td>12.0</td>
<td>11.5</td>
<td>26.0</td>
<td>25.0</td>
<td>17.0</td>
<td>15.0</td>
<td>14.0</td>
<td>14.0</td>
<td>11.0</td>
<td>9.0</td>
<td>9.0</td>
<td>7.5</td>
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<tr>
<td>Mean</td>
<td>80.9</td>
<td>110.1</td>
<td>84.6</td>
<td>79.0</td>
<td>18.5</td>
<td>19.5</td>
<td>27.0</td>
<td>26.4</td>
<td>23.1</td>
<td>18.4</td>
<td>15.6</td>
<td>15.3</td>
<td>15.0</td>
<td>10.9</td>
<td>10.7</td>
<td>10.1</td>
<td>8.5</td>
</tr>
<tr>
<td>SD</td>
<td>44.4</td>
<td>46.2</td>
<td>63.0</td>
<td>71.1</td>
<td>24.9</td>
<td>22.9</td>
<td>27.8</td>
<td>17.3</td>
<td>16.3</td>
<td>15.6</td>
<td>13.0</td>
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</table>
The simple inverse relationship between apo(a) length and Lp(a) level has been explained by the increasing difficulty in synthesizing and secreting a very large glycoprotein. The present study immediately demonstrates that this explanation is inadequate and supports other indirect evidence in the literature for a more complex relationship between apo(a) length and Lp(a) level. In primates, the amount of hepatic apo(a) mRNA tends to be correlated with the plasma concentration of Lp(a), but a number of exceptions have been described. However, no correlation was found in these animals between apo(a) mRNA length and hepatic apo(a) mRNA levels. In the present study, we have demonstrated that a short apo(a) isoform [eg, apo(a)-20] may be associated with a very wide range of plasma Lp(a) levels (Figure 5). As such, there appears to be no inherent property of a small apo(a) isoform that makes it consistently more easily synthesized and secreted than a large isoform.

Two potentially confounding factors in the present study require discussion before further analysis of the findings. In the present study, high-resolution apo(a) phenotyping is used as a surrogate for apo(a) genotyping. This methodology provides equally valid information on APO(a) allele size in all but one situation. This exception is important and merits further discussion. When a subject has an apparent (null, X) phenotype, it is not possible to differentiate the genotype as a (null, X) or an (X, X) by immunoblotting, because these 2 potential genotypes appear identical. However, because only ~2.9% of the white population are homozygotes with 2 APO(a) alleles of the same size, virtually all of the subjects assigned as (null, X) will indeed be (null, X) and not (X, X). Because similar proportions of homozygotes are likely to be incorrectly assigned across the apo(a) isoform size spectrum, this will not influence the main conclusions of the study.

All subjects recruited into the West of Scotland Study, from which the present study group was taken, were relatively healthy middle-aged men with moderate hypercholesterolemia. A further potential confounding factor in the present study is therefore the use of all male subjects, 45 to 64 years old, with moderate hypercholesterolemia (LDL cholesterol, 155 to 232 mg/dL). There is thought to be no significant sex or age effect on plasma Lp(a) concentration; therefore, a study in middle-aged men should provide conclusions that are equally valid in the whole population and is more likely to add homogeneity rather than heterogeneity to the study. The association between plasma Lp(a) concentration and other plasma lipid levels is more controversial. The median Lp(a) level in this cohort is higher than in some other reported white groups. This may have a number of reasons. Most importantly, this group is exclusively male, middle-aged, and with moderate hypercholesterolemia. As such, it is perhaps not surprising that the levels do not exactly correspond with other healthy cohorts. Another important point is that very marked ethnic differences have been observed in plasma Lp(a) levels throughout the world. Even within white groups, there is variability, and never before has a Scottish cohort been studied in this way. Perhaps Scottish Lp(a) levels are higher than those of other white groups, a conclusion not inconsistent with the observation that the Scottish population has a very high incidence of coronary artery disease.

We have previously demonstrated a bimodal distribution of APO(a) allele sizes in a group of 174 white subjects, and Marcovina and her colleagues have recently confirmed this in a larger group of whites. The present study shows the phenomenon much more clearly. This is not explained simply by the fact that individuals have 2 different-size alleles, because the superimposed graph of apo(a) isoform sizes in the single expressers is also clearly bimodal (Figure 2). This pattern would suggest that there are 2 overlapping and normally distributed groups of apo(a) isoforms: those with an average length of ~20 K-4 repeats and those with an average length of ~28 K-4 repeats.

It is difficult to explain this phenomenon. It could be an artifact resulting from some observer bias in reading the apo(a) immunoblots. This is unlikely, however, because 1 of the size standards used is an apo(a)-24, which is exactly the apo(a) size that appears to be underrepresented in Figure 2. If there were no size standard at this point, it might be argued that the operator could misassign apo(a)-22 through apo(a)-25 to smaller and larger sizes.

Alternatively, we must seek a physiological explanation. One possibility is that we are observing in this population an admixture of 2 genetic groups. However, because the bimodality observed here in a West of Scotland population was also previously observed in a US white group whose genetic roots were considerably more diverse, this explanation seems unlikely.

Finally, we may consider an evolutionary explanation. The APO(a) gene polymorphism, which directly controls the apo(a) isoform length polymorphism, is thought to have arisen as a result of multiple duplication and deletion events in prehistory. The result of this process has been the generation of a large number of APO(a) alleles ranging across the size spectrum: in this study, from 7 to 40 K-4 repeats. Some APO(a) alleles may be less easily generated by this process, perhaps because they are thermodynamically less stable, and this would account for the lack of normality in the frequency distribution of the apo(a) isoforms, as shown in Figure 2.

The striking comparison between the variability of plasma Lp(a) concentrations associated with apo(a)-20 and apo(a)-30 shown in Figure 5 clearly demonstrates the relative difference in control on the plasma Lp(a) levels exerted by the size of the apo(a) isoform.

Increased variation with increased mean value is a natural consequence for a variable with a positive value. This phenomenon is seen in many biological contexts, and although it may be one explanation of the observed low variability in Lp(a) concentration at the upper end of the apo(a) size spectrum, it does not fully explain the patterns observed.
How can Lp(a) concentration vary so widely in 54 subjects, all of whom have a single population of circulating Lp(a) particles all carrying the same-size isoform with 20 K-4 repeats, if the apo(a) length is a major controlling factor? Although the APO(a) gene, or sequences closely linked to it, is believed to account for >90% of the variability in the plasma Lp(a) level, we know that apo(a) isoforms of the same length do not necessarily possess the same internal sequence. The apo(a) length polymorphism alone has previously been accountable for 42% of the variability. However, this figure must be considered more closely.

The present study reveals that a negative correlation between apo(a) length and plasma Lp(a) level is present. Using a method of apo(a) isoform sizing that can separate apo(a) isoforms differing in length by a single K-4 unit, we have the ability to view the apo(a) size spectrum with the greatest resolution possible. This, coupled with the large number of subjects studied and the decision to examine only those subjects with a single-expressing apo(a) isoform, means that for the first time, the true nature of the complex relationship between apo(a) length and plasma Lp(a) concentration can be unraveled.

Previous studies of this relationship have involved smaller and less-well-characterized data sets, but our overall estimate (38%) of the contribution of the apo(a) length polymorphism to plasma Lp(a) variability is in close agreement with that published previously, 41.9%. However, the present study has revealed important and marked differences in the variability of Lp(a) concentrations associated with different apo(a) isoform lengths across the apo(a) size spectrum. The intersisform variability in Lp(a) is not uniform but rather falls sharply beyond apo(a) lengths of 20 K-4 repeats (Figure 4). When the relationship between apo(a) length and Lp(a) level is examined above and below this threshold, the relationship is much weaker and the contribution made to the Lp(a) variability in each group is only ~10%. The stronger overall negative correlation between apo(a) length and Lp(a) level observed here, and we believe in other studies, is driven largely by the nonuniformity of Lp(a) variability as the apo(a) isoform increases in length.

Because the APO(a) gene is thought to control >90% of the variation in plasma Lp(a) levels in human populations, other levels of polymorphism at this locus, beyond the apo(a) length polymorphism, must now be sought. The remainder of the variation may be due to cis-acting sequences such as promoter elements regulating APO(a) transcription. Recently, 2 groups have found a pentanucleotide repeat (TTTTA) in the 5' flanking region of the APO(a) gene that may control the plasma Lp(a) level. Others have demonstrated polymorphisms and candidate control elements in the 5' untranslated region of the APO(a) gene that form the putative APO(a) promoter. It is possible that mutations in this 5' region of the APO(a) gene account for the marked differences in expression observed in the smaller apo(a) isoforms. However, if this is true, it is also interesting to note that no such mutations appear to be able to enhance APO(a) promoter activity, only to suppress it. This is clear from the observation that no large apo(a) isoform is associated with a high plasma Lp(a) concentration, but small isoforms may be associated with low as well as high levels.

Apart from transcription and translation, the apo(a) protein must be guided through and out of the hepatocyte to join LDL and form Lp(a). The mechanisms controlling this intracellular trafficking of such a large glycoprotein are not well understood but are likely to involve a number of other genes and their products. Conceivably, mutations in any of these may misdirect the protein and lead to its intracellular degradation, thus overriding any control of the plasma Lp(a) level exerted by the apo(a) length polymorphism.

Against this argument for the involvement of other genes is the finding that >90% of the interindividual variation in plasma Lp(a) concentrations is controlled by sequences at or closely linked to the APO(a) locus. This hypothesis leaves only <10% of the variability unexplained, so other genes may play a role but do not explain the variability in expression seen especially with smaller apo(a) alleles in this study, which may vary >100-fold.

**Appendix**

**West of Scotland Coronary Prevention Study Group**

Executive Committee: James Shepherd (chairman), Stuart M. Cobbe, A. Ross Lorimer, James H. McKillop, Ian Ford, Christopher J. Packard, Peter W. Macfarlane, Christopher Isles.


Cardiovascular End-Points Committee: Stuart M. Cobbe (chairman), Barry D. Vallance, Peter W. Macfarlane.


Data center staff: Liz Anderson, David Duncan, Sharon Kean, Audrey Lawrence, June McGrath, Vivette Montgomery, John Norrie.

Population screening: Melvyn Percy.

Clinical coordination, monitoring, and administration: Elspeth Pophrey, Andrew Whitehouse, Patricia Cameron, Pamela Parker, Fiona Porteous, Leslie Fletcher, Christine Kilday.

Computerized ECG analysis: David Shoat (deceased), Shahid Latif, Julie Kennedy.

Laboratory operations: M. Anne Bell, Robert Birrell.

Company liaison and general support: Margot Mellies, Joseph Meyer, Wendy Campbell.

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