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Ethnicity and lipoprotein(a) polymorphism in Native Mexican populations

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Abstract

Background: Lp(a) is a lipoparticle of unknown function mainly present in primates and humans. It consists of a low-density lipoprotein and apo(a), a polymorphic glycoprotein. Apo(a) shares sequence homology and fibrin-binding with plasminogen inhibiting its fibrinolytic properties. Lp(a) is considered a link between atherosclerosis and thrombosis. Marked inter-ethnic differences in Lp(a) concentration related to the genetic polymorphism of apo(a), have been reported in several populations.

Aim: To study the structural and functional features of Lp(a) in three Native Mexican populations (Mayos, Mazahuas and Mayas) and in Mestizo subjects.

Methods: We determined the plasma concentration of Lp(a) by immunonephelometry, apo(a) isoforms by Western blot, Lp(a) fibrin-binding by immuno-enzymatic assay and STR polymorphic markers genetic analysis by capillary electrophoresis.

Results: Mestizos presented the less skewed distribution and the highest median Lp(a) concentration (13.25 mg/dL) relative to Mazahuas (8.2 mg/dL), Mayas (8.25 mg/dL) and Mayos (6.5 mg/dL). Phenotype distribution was different in Mayas and Mazahuas as compared to the Mestizo group. The higher Lp(a) fibrin-binding capacity was found in the Maya population. There was an inverse relationship between the size of apo(a) polymorphs and both Lp(a) levels and Lp(a) fibrin binding.

Conclusion: There is evidence of significative differences in Lp(a) plasma concentration and phenotype distribution in Native Mexican and the Mestizo group.

Introduction

The lipoprotein(a), Lp(a), particle is mainly found in primates and humans and is currently considered as one of the most prevalent inherited risk factors for atherosclerosis (Djurovic and Berg, 1997). Lp(a) composition is indeed similar to that of the atherogenic low density lipoprotein (LDL) particle in terms of lipid core content (cholesterol, triglycerides, phospholipids) and the apolipoprotein Apo B-100. The distinctive feature of Lp(a) is the glycoprotein apo(a). The apo(a) gene may have arisen from a duplicate copy of the plasminogen gene about 40 millions years ago (McLean et al., 1987). This time frame correspond to the time of separation of Old and New World monkeys and may explain why apo(a) is absent in the latter (Lawn et al., 1995).

Plasminogen, the precursor of plasmin the fibrinolytic enzyme, and apo(a) consist of a serine-protease region and several (respectively, 5 and 10 to 40) disulfide-bridged triple loop structural domains termed 'kringle' that share a high degree of amino acid sequence homology (McLean et al., 1987) (Fig. 1). Plasminogen binding to fibrin via kringles 1 and 4 is a necessary step in plasmin-mediated fibrin lysis (Ho-Tin-Noé et al. 2005). Apo(a) contains a single copy of plasminogen-like kringle 5 (KV) and multiple copies of kringle 4 (KIV) that have been classified in 10 different types on the basis of sequence homology. KIV type 1 and KIV type 3 to KIV type 10 are present in single copies whereas KIV type 2 varies in number given rise to more than 30 apo(a) isoforms (Marcovina et al., 1993). Since KIV type 10 contains a fibrin-binding site functionally identical to kringle 4 of plasminogen, and the protease region of apo(a) is inactive, Lp(a) may compete with plasminogen for binding to fibrin and inhibit fibrinolysis (De la Peña-Diaz et al., 2000). In summary, kringle KIV is the major basic structural unit of apo(a), which defines both its molecular size polymorphism and pathophysiological properties shared with Lp(a).

The apo(a) gene is a major determinant of Lp(a) concentration in plasma, accounting for greater than 90% of its variations (Boerwinkle et al., 1992). Epidemiological evidence indicates, indeed, that apo(a) polymorphism is in inverse relationship with Lp(a) concentrations (Gaubatz et al., 1990). A similar inverse relationship has also been found between apo(a) size and the ability of Lp(a) to compete with plasminogen for binding to fibrin (Hervio et al. 1996). Ethnicity-related differences in Lp(a) concentration and apo(a) phenotype have been described in European, African, Asian and Asian-Indian populations among others (Geethanjali et al 2003, Sandholzer et al 1991, Wong et al 1999, Sorensen et al 1994, Xiong et al 2004, Barkley et al 2003) with values markedly higher in Africans than Eurasians and Mexican-USA (Obisesan et al., 2004). In a population-based study of diabetes and cardiovascular disease, the San Antonio Heart Study, Mexican-USA had decreased Lp(a) concentrations relative to non-Hispanic whites (Haffner et al., 1992). In a random sample population of Mexico City an association of Lp(a) with myocardial infarction was observed but did not reach statistical significance (Cardoso-Saldaña et al., 1997). Their relationship with apo(a) fibrin-binding has not been reported as yet. Since Lp(a) plasma concentration remains stable through life and the KIV-encoding domain of apo(a) is highly polymorphic, definition of the Lp(a) profile in a given population may be a useful marker for cardiovascular disease. The purpose of our study was therefore to define this genetic marker in terms of Lp(a) concentration, apo(a) kringle number and binding of Lp(a) to fibrin in selected Native Mexican populations. This data were compared to those of the prevalent Mestizo population. Our results indicate that the Mestizos can be distinguished from by higher Lp(a) concentration and a less skewed distribution.

METHODS

Subjects and population analysis. A total of 240 non-related healthy subjects, women and men, were selected at random from four different ethnic groups established in distinct geographic areas of Mexico, as follows: Mayos (n= 68) from Capomito, Estado de Sinaloa at the north-west of the country; Mazahuas (n= 60) from Santa Rosa and Pueblo Nuevo, Estado de Mexico; and Mayas (n= 36) from Tahdzibichen near Merida, Estado de Yucatan. The Mestizo (admixture of Native Mexicans and Spaniards) samples (n=41) were obtained in Mexico City where according to the gene proportion of Native Americans, European and South-Saharan African genes, the population structure components are 56.22%, 40.85%, and 2.93%, respectively (Lisker R. et al.1986). Besides the geographic localization, the historical, cultural and linguistic characteristics were different for each group as determined by the Instituto Nacional de Antropología e Historia de Mexico. However, to ascertain the validity of these criteria in the selection of the different ethnic groups, short tandem repeat (STR) markers were determined in two representative groups. The ethical committee of our institutions approved the study and an informed consent was obtained from all individuals.

Samples. After an overnight fast, blood (10 mL) drawn from the antecubital vein was collected into sodium EDTA tubes (1mg/ml) and kept on ice until separation of plasma by centrifugation at 2000g for 20 minutes at 4°C. The plasma was supplemented with a protease inhibitor (Aprotinin 100 IU/mL, Trasylo[®] Bayer) and stored at -70°C until analysis (less than 6 months from sampling).

Genetic markers analysis. In order to assess the allelic association between genetic markers in different ethnic groups, nine short tandem repeats (STR), CSF1PO, D3S1358, D5S818, D7S820, D13S317, FGA, TH01, TPOX, vWA (Edwards et al 1991, 1992a, Hammond et al 1994) were

studied. 184 Mestizos, 42 Mazahuas and 74 Mayas alleles were analyzed by using a capillary electrophoresis AmpFiSTR Profiler™ in an ABI-prism 310, Applied Biosystems (Perking-Elmer Co, Foster City, CA). The genetic distance between ethnic groups was estimated with the PHYLIP software (Phylogeny Inference Package) Version 3.57.

Alleles STR frequencies were compared between the Native Mexican groups and Mestizos, and also with those of two reference ethnic samples (Spaniards n=4172 and Afro-Americans USA n=393) obtained from the Distribution of the Human DNA-PCR Polymorphisms data base from a Cooperation Project of Institute of Forensic Medicine Institute of Human Genetics and Anthropology Heinrich-Heine–University Düsseldorf, Germany (The Distribution of the Human DNA-PCR Polymorphisms Supplement Volume I/II/III/IV/V (1998/1999/2000/2001/2002/2003) by Wolfgang Huckenbeck and Hans-Georg Scheil) and from (Yeh et al, Budolwle et al.1999).

Plasma Lp(a) concentration. Lp(a) in plasma was measured by kinetic immunonephelometry (Gillery et al., 1993) using a commercial antibody in an automated Array protein system (Beckman Co, Palo Alto, CA, USA). The intra-assay and inter-assay coefficients of variation were less than 8%.

Apo(a) isoforms identification. Plasma was adjusted to 15mg/dL of Lp(a) or directly diluted 1:2 in electrophoresis sample buffer. A mix of five recombinant apo(a) isoforms containing 10, 14, 18, 26 and 34 kringles were used as standard reference (Anglés-Cano et al., 1999). Samples and the recombinant apo(a) standard were electrophoresed on a 3.75% and 6% discontinuous polyacrylamide gel and immunoblotted as previously described (Cardoso-Saldaña et al., 2001).

Blots were scanned in a GS-670 Bio-Rad densitometer using the Molecular Analyst™/PC software. The procedure sensitivity was 50 ng. The apo(a) isoform size number was determined by reference to a standard curve made by plotting the relative migration of the five recombinant

apo(a) isoforms against the log of the number of kringle units. The inter and intra-assay coefficients of variation were less than 2%.

Lp(a) fibrin-binding assay. The experiments were performed with fibrin surfaces prepared and characterized as previously described (Fleury and Anglés-Cano, 1991). Buffer A (0.05 mmol/L sodium phosphate, pH7.4, 0.08 mmol/L NaCl, 0.01% (w/v) Thymerosal, 0.01 % (w/v) Tween 20) supplemented with bovine serum albumin (BSA) where indicated, was used in all experiments. To allow plasminogen/Lp(a) equilibrium competition for fibrin binding, a volume of 50 μ L of plasma (diluted 1:16 in buffer A supplemented with 40 mg/mL BSA) was incubated in duplicate with fibrin surfaces for 12h at 4°C. Unbound proteins were removed by washing three times with buffer A supplemented with 2mg/mL of BSA and a volume of 50 μ L of a peroxidase-conjugated monoclonal mouse antibody, directed against apo(a), was added. This antibody does not cross-react with plasminogen. After 2h at 37°C the plate was washed and a solution of 1 mg/mL 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulphonic acid (ABTS, Boehringer Mannheim) was used for colour development. The change in absorbance ($\Delta A_{405/min}$) was measured with a microtitration plate counter (MR 5000, Dynatech). The amount of Lp(a) bound is expressed in nmol/L of apo(a) by referring to a calibration curve. The standard curve was obtained from a reference standard composed of equimolar amounts of five recombinant apo(a) isoforms of different lengths (10 to 34 kringles) (Anglés-Cano et al., 1999).

Statistical methods. The overall shape of the distribution of plasma Lp(a) concentration and apo(a) kringle IV phenotypes was compared between populations using the Kolmogorov-Smirnov test. Because of the highly skewed distribution of Lp(a) levels in the four ethnic groups, non parametric rank-sum test, Kruskal-Wallis test and U-Mann-Whitney post-hoc test were used to compare median Lp(a) levels between ethnic groups. The Spearman's rank correlation

between Apo(a) phenotype and Lp(a) level and fibrin binding was calculated for each ethnic group. The prevalence of higher Lp(a) levels ($> 30\text{mg/dL}$) were compared with the χ^2 tests. Statistical significance was considered when $p < 0.05$. The Statistical Package for Social Science (SPSS) V. 10 was used.

Results

Two hundred and forty subjects from 4 different ethnic groups were studied: 41 Mestizos, 68 Mayos, 36 Mayas and 60 Mazahuas. The criteria (historical, cultural and linguistic as defined by the Instituto Nacional de Antropología e Historia, Mexico City) used to select the Native American populations were supported by the results of the short tandem repeats analysis.

Population structure. As shown in the genetic tree, figure 2, the genetic distances between Mestizos, Mazahuas, Mayas and Europeans (Spain) were 0.127363, 0.220427 and 0.264046 respectively. In the same figure is shown the genetic distances between Mestizos and Mazahuas (0.06518) and between Mestizos and Mayas (0.109329).

According with Spearman correlation, no associations of Lp(a) were found with age, gender, weigh, height, and body mass index.

The distribution of plasma Lp(a) levels in each of the four ethnic groups is shown in figure 3. In all cases the distribution is skewed toward low concentrations. However, the plasma levels of Lp(a) were less skewed in the Mestizo group than in the other groups ($p < 0.001$). The median Lp(a) concentration (Table 1) was significantly higher in Mestizos than Mayos ($p < 0.01$), Mayas ($p < 0.05$) and Mazahuas ($p < 0.01$). Median values for Lp(a) fibrin binding were significantly higher ($p < 0.001$) in Mayas when compared to each of the other ethnic groups. (Table 1).

All groups considered, a total of 26 different apo(a) phenotypes were identified, and each individual's plasma presented either one or two isoforms. The distribution of apo(a) phenotypes in the four ethnic groups is shown in figure 4. Phenotype distribution was significantly different in Mayas and in Mazahuas as compared to the Mestizo group ($p=0.004$). Differences were also observed between Mazahuas and Mayos ($p<0.05$). There was an inverse relationship ($p<0.05$) between apo(a) kringle number and fibrin binding for Mestizos, Mazahuas and Mayos, Table 2; the Maya population studied showed a direct relationship between apo(a) kringle IV number and fibrin binding.

Discussion

Several reports have suggested that the plasma Lp(a) concentration is determined by the apo(a) gene locus (Kraft et al., 1992, Boerwinkle et al., 1992). However, variations among populations have been observed. As a matter of fact, in western Eurasian populations the apo(a) gene size explains 40 to 60% of plasma Lp(a) concentration, whereas in other ethnic groups this proportion varies from as low as 28% in Sudanese to as high as 70% in Chinese (Scholz et al., 1999). Indeed, the number of kringle IV type 2 that define the apo(a) polymorphism is inversely related not only to the plasma concentration of Lp(a) (Utermann et al., 1987) but also to its ability to bind to fibrin (Hervio et al., 1996). Therefore, the profile of Lp(a) in a given population may be better assessed by estimation of the relationship between these parameters. The availability of a reliable fibrin-binding assay and a well-defined standard composed of recombinant apo(a) isoforms of known kringle number allowed us to perform the first population study integrating Lp(a) concentration, apo(a) kringle IV number analysis and the functional fibrin-binding assay in well genetically characterized Native Mexican groups, as has been shown by the genetic markers analysis. The markers analysis also suggest deviations from Hardy-Weinberg equilibrium for Mazahuas and Mayas, probably associated to within-group mating as these are closed

populations (Mayas>Mazahuas). The high difference in genetic distances may be explained by an increased frequency of STR markers homozygosis.

The Lp(a) concentration frequency distribution in the Native Mexican group was skewed to levels (<10 mg/dL) lower than those observed in Mestizo, Western Eurasian, Chinese, South-Saharan African, African-American and Mexican-American populations (Haffner et al., 1992; Gaw et al., 1994; Kraft et al., 1996). Another major finding in this study was the significant difference in Lp(a) plasma concentration between Mestizo and Native Mexican populations. The Mestizo population currently living in the Mexican Republic arose after the Spanish colonization started in 1502. Note that the mean concentration of Lp(a) found in the Mexican Mestizo group (16.6 ± 18 mg/dL) is close to that found in Spanish populations (18.2 ± 14.8 mg/dL) (GomezGerique et al. 1996, Muros et al. 1996). However, the prevalence of Lp(a) greater than the conventional cut-off excess level (>30 mg/dL) is higher in apparently healthy western Eurasian populations (15-20 %) than in the Native Mexican groups (5.6 – 14.5 %) (Table 1). This difference is probably related to a less skewed distribution of Lp(a) concentrations in the Spanish population relative to the distribution observed in our study. It may reflect, in a certain way, cross genetic influences resulting from the admixture of Native Mexicans with Spaniards as suggested by the results of STR markers.

An interesting finding was the direct correlation between apo(a) phenotype and Lp(a) concentration in the Maya population we have studied (Table 1) . This finding suggests that the number of repetitive sequences in the apo(a) gene coding for kringle IV type 2, and therefore the size of apo(a), is not a major determinant of Lp(a) concentration in this population. Mayas also showed the greatest fibrin-binding but no correlation was found with apo(a) size. These observations may be of great value to our understanding of the influence of apo(a) size on both Lp(a) concentration and binding to fibrin in this population.

Conclusions

There is evidence of significant differences in Lp(a) plasma concentration and phenotype distribution in the Native Mexican and the Mestizo group. Lp(a) distribution in the autochthonous populations is highly skewed toward lower levels relative to the Mestizo population. Apo(a) size distribution in Native Mexicans also differs significantly from the apo(a) size distribution in Mestizos. Our data provide further support to a genetic hypothesis for the difference in Lp(a) parameters among Native Mexicans and Mestizos.

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Table 1. Lp(a) plasma concentrations and fibrin-binding by ethnic group.

| | Lp(a) Concentration | | | | Fibrin-binding | |
|----------|---------------------|--------|----------------|----------------|----------------|----------------|
| | n | Median | p ^a | >30 mg/dl* (%) | Median | p ^a |
| Mayos | 68 | 6.55 | 0.001 | 7.4 | 0.10 | ns |
| Mayas | 36 | 8.25 | 0.012 | 5.6 | 0.28 | ns |
| Mazahuas | 60 | 8.20 | 0.001 | 11.7 | 0.09 | 0.011 |
| Mestizos | 76 | 13.25 | - | 7.5 | 0.14 | - |

Lp(a) concentration is expressed in mg/dL. Lp(a) binding is expressed in nanomol bound.

a= Mann-Whitney U test vs. Mestizos.

* Conventional cut-off excess value

Table 2. Correlation between apo (a) size and Lp(a) fibrin-binding and concentration.

| Ethnic Group | Apo (a) phenotype/Lp (a) level | Apo(a) phenotype/fibrin-binding |
|--------------|--------------------------------|---------------------------------|
| Mayos | -0.361* | -0.07 |
| Mayas | 0.541* | -0.261 |
| Mazahuas | -0.007 | -0.248 |
| Mestizo | -0.422** | -0.003 |

* p < 0.05, ** p < 0.005

Figure Legends

Figure 1. Population tree, based on the analysis of nine short tandem repeats markers.

Data were analysed with the software Phylip V. 3.5. and the genetic distances determined.

Figure 2. Distribution of lipoprotein Lp(a) concentrations in the populations studied. Difference between Mestizos and Native Mexicans is significant ($p < 0.001$).

Figure 3. Distribution of apolipoprotein(a) isoforms by kringle number. The X axis indicates the total number of plasminogen-like kringles including the single copy of kringle V. Kolmogorow-Smirnov Z test: Mayas $p = 0.05$, Mazahuas $p < 0.001$, vs Mestizos.