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Molecular genetic of apolipoprotein (a) in Taiwan

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í ëñ gã (a)(Lp(a)) ä ǵ ǵ]é ë ìî (>25-30 mg/dl) ê ìî Ĉ ǧ ê ő|â Ą ě ę ä æ ő í ¥ ø ë ë ä þ fä zâ ªâ å]ß Lp(a) ä ǵ ǵ]é ě ò Ö å é ă ,¨ä ę ç 0.1 æ Ī ę 200 mg/dl å ã ã § í ß ä đ ø ė đ ñ Ö å ã ã ç ý đ ą ã ß ` ç ù ]Ą tï Ę ±â ,apo(a) ñ gã ê Ħ |é ę ê ă ǵ ĝ Lp(a) ý ]é ê ä ő ę ê ş đ ą Ā â ã ç ß ä è ǧ ±è þ ä ǧ ǵ ê ę ±â ,Lp(a) è ä ǵ ǵ ]é ě å ô ¹ï ä û ã ą (ã å ß (14.3 mg/dl, â â Ī ú 9.0 mg/dl, n=3453) ß `ä ù]â Ä ǧ ©ã ä î º í ¤â eê îî Ā æ ê ę ±ó -ð ¹,coronary heart disease(CAD) ï µè ê å Lp(a) ë (33.0 ± 21.9 mg/dl,n=211) ä ǵ ę ę æ |ä CAD è (23.6 ± 17.6 mg/dl, n=154)(p<0.0001) ß `ã ª]â Ħ ,ï å Ę ê ñã SDS-polyacrylamide û ä ¯è -â ă Ħ â Ħ]ê Ù Ā fê © µû ç ¹ã apo(a) â 16 ø ä û Ħ ð å ã ,ã zê ^5 ë F-form,1 ë B-form,9 ë S-form, â Ĉ ë null-form ß æ ó ú ã ã ,74 % ä ǧ ă ã dï Ķ,23.1 % ä ã]ï Ķ,â 2.6 % Ă ě ç null-type ß æ F-form è ê S-form è ê ñ ö Ĺ è Lp(a) ë (45.6 ± 19.5 V.S. 21.5 ± 13.1 mg/dl,p<0.0001) ß ï ä null form è ,æ ę ǵ ĝ Lp(a) ý]é ê û ,ä ä ő ę ä Ħ Ħ ø]Ė æ ǧ õ Ĺ â `ä ą ô ¯apo(a) ä û Ħ å ã (S5-S9) â æ ó ë è ,æ ę -â ǧ ¤â ê ę ë đ fâ `ç æ ä õ á ® apo(a) (S1-S4) â å µè (R.R.=0.51, n=164 V.S. R.R.=0.68, n=93; p<0.01) ß Apo(a) ä û Ħ ð å ã ã ą ô ¯ã `ä ®ç ]é -â ǧ ¤â ê ę ä þ fä æ â ]ä ã ą ê \ß `å {â å û §ã]æ _â þ ä capo(a) î ä z5′ ø ê æ Ī ĉ ă ê ą `ä |é ę ê ä ǵ ĝ Lp(a) ý]é ä å ß ê ę ç ,ã ä ě Ģ ç é -è ê î Ĉ ǧ ê ő|â Ą å ã ß ì Ę Ę ã tó ð Lp(a) ä ǧ ş]é ë ê -â ǧ ¤â å þ fä zâ ªâ å]ä đ £ è ð]æ apo(a) ä û Ħ å ã ã å ã ß ñ æ ę Ī ì Ą kä å ç å {â ÿ ^ö đ ù]Ą tï Ę ±â Lp(a) â ş đ ą ã ,ã ě ě -â ǧ ¤â §å ő ªâ æ â ,Gï ¯

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High plasma level of Lp(a) (>25~30 mg/dl) is one of the major risk factors for coronart atherosclerosis and its major complication, but is different from LDL by the large glycoprotein named apolipoprotein(a) (apo(a)). The kringle IV domain of apo(a) is variously repeated form about 15 to 40 times in individual, resulting in a large number of molecular weight isoforms of the protein. The significance of Lp(a) on coronary heart disease (CHD) and the genetic bases for the variation in plasma Lp(a) concentration for population in Taiwan are, however, not understood. In a prospective Chin-Shan Community Cardiovascular (CCC) study, the Lp(a) plasma level showed a skew distribution (mean 14.3 mg/dl, medium 9.0 mg/dl, n=3453). A hospital-based study in National Taiwan University Hospital (NTUH) disclosed higher Lp(a) levels in patients with than those without significant coronary lesions (33.0 ± 21.9 versus 23.6 ± 17.6 mg/dl, n = 381, P<0.0001). Sixteen isoforms of apo(a) have been identified based on electromobility, including 5 F-forms, 1 B-form, 9 S-forms and a null form. Seventy four percent of the subjects have two bands, 23.1 % have single band and 2.6 % are null type, and six cases of them contain both S-and F-forms. Subjects containing F- or null form have high Lp(a) level than subjects containing S-form (45.6 ± 19.5 versus 21.5 ± 13.1 mg/dl, p<0.0001). Subjects containing larger apo(a) isoforms (S5-S9) have lower risk of CAD than those having smaller isoforms (S1-S4) (0.51, n = 164 versus 0.68, n = 93; p<0.01). For subjects (n = 47) with plasma Lp(a) level disproportion to its apo(a) size, the TTTTA-repeats in the 5'-control region of the apo(a) gene was analyzed by polymerase chain reaction and nucleotide sequencing. The

majority have 7 (8.5%), 8 (42.5%), or 9 (42.5%) repeats. Only 1 case (2.1%) for 4, 5, and 6 repeats, individually. There is no significant correlation between the number of TTTTA repeats and plasma Lp(a) concentration. We conclude that the apo(a) size polymorphism, but not TTTTA repeats in the 5' control region, significantly correlated with the plasma Lp(a) level, which in turn correlated with the prevalence of CAD in our study group. These results would be helpful in the prevention and treatment of CAD in Taiwan.

Key words: lipoprotein(a), apo(a), polymorphism, coronary atherosclerosis,

Background

Lp (a) resembles low-density lipoprotein (LDL) in its content of cholesterol, phospholipids and apolipoprotein B-100 (apo B-100), but is different from LDL by the large glycoprotein named apolipoprotein(a) [apo(a)] which is attached to the particle by disulfide linkage to apo B-100 (Gaubatz, Heideman *et al.* 1983). Plasma Lp(a) level varies widely in the human population, ranging from less than 0.1 to more than 200 mg/dl. LP(a) production, rather than catabolism, has been shown to be the key determinant for its plasma concentration (Rader *et al*., 1993).Based on population studies, high plasma level of Lp(a) (>25-30 mg/dl) is one of the major risk factors for atherosclerosis and its major complications (Utermann 1989; Scanu, Lawn *et al.* 1991; Lawn 1992). An unusual feature of the apo(a) cDNA is the large number of times that one domain homologous to Kringle IV of plasminogen is repeated (McLean, Tomlinson *et al.* 1987). This variation in the number of kringle IV repeat results in a large number on molecular weight isoforoms of the protein (Gavish, Azrolan *et al.* 1989; Koschinsky, Beisiegel *et al.* 1990; Lackner, Boerwinkle *et al.* 1991). There is a inverse relationship between the molecular weight of apo(a) and plasma level of LP(a) (Boerwinkle, Menzel *et al.* 1989; Gavish, Azrolan *et al.* 1989; Gaubatz, Ghanem *et al.* 1990; Lackner, Boerwinkle *et al.* 1991). Moreover, individuals with different plasma Lp(a) levels were noted to have 5' control regions of the apo(a) gene different both in nucleotide sequence and *in vitro* transcription activity (Wade, Clarke *et al.* 1993; Zysow, Lindahl *et al.* 1995) In the case of the smaller size apo(a) alleles (Mr of apo(a) isoform ß Ë 660 kDa, presumed kringle IV repeats ß Ë 13)with 8[TTTTA] repeats in the 5' control region, the association with Lp(a) excess was stronger (Hamaguchi, Yamakawa-Kobayashi *et al.* 1996).

In a prospective Chin-Shan Community Cardiovascular (CCC) study, the Lp(a) plasma level showed a skew distribution (mean 14.3 mg/dl, medium 9.0 mg/dl, n=3453). In this project, the relationship between Lp(a) level, apo(a) size polymorphism, and the severity of coronary artery atherosclerosis were investigated. The possible control mechanism of Lp(a) level for Chinese in Taiwan was elucidated in analyzing the apo(a) phenotype and the 5' control region of apo(a) gene. The apo(a) size polymorphism, but not TTTTA repeats in the 5' control region, significantly correlated with the plasma Lp(a) level, which in turn correlated with the prevalence of coronary atherosclerosis in our study group.

Methods

Blood samples obtained from 381 individuals who underwent coronary angiography at National Taiwan University Hospital were analyzed for plasma Lp(a) level by using TintElize Lp(a) enzyme-linked immunoassay (ELISA) kit according to manufacture's instruction (Pasel & Lorei Co., Frannkfurt, FRG). Apo(a) size polymorphism was analyzed by SDS-PAGE followed by immuno-blotting against anti-

apo(a) immunoglobulin (monoclone antibody M1A2, Boehringer mannheim, FRG) (Kraft, 1988). The correlation between plasma Lp(a) level and apo(a) size polymorphism was analyzed. Nucleated cells were isolated from 10 ml of heparinzed blood and subjective to proteinase K followed by restriction enzyme *Kpn*I digestion. Fragment length polymorphs were resolved by pulse field agarose gel electrophoresis and detected by hybridization with radioisotope labeled cDNA probe specific to kringle IV of gene encoding apo(a). Individuals who have plasma Lp(a) level disproportion to the apo(a) size (beyond one standard deviation of the mean value) were subjective to analyze [TTTTA]repeats in the 5'-control of the apo(a) coding gene (n=53). Briefly, buffy coat was prepared from 10 ml of heparinized venous blood and genomic DNA was purified form the nucleated cells embedded in the low melting point agarose. A 404 bp fragment, encompassing the region -1447 to -1044 relation to the translation start site of apo(a) gene, was amplified by PCR using dig-nucleotides LPA3 (forward): 5'-gaattcatttgcggaaagattg-3' and LPR3 (antisense): 5'-ccttcctattctagtagttgtg-3'. A 104 bp fragment encompassing the TTTTA repeats was near amplified by using oligonucleotides LPA100 (forward): 5'-gcggaaagattgatactatgc-3', and LPR100 (antisense): 5'-cacgtcagtgcacttcaac-3'. The amplicon was cloned into TA-cloning vector transformed into Solopack Gold Supercompetent Cell (Stratagene, La Jolla, California). The number of TTTTA repeats was identified by nucleotide sequencing.

Results

1. Lp(a) plasma concentration of Lp(a) is a predictor of coronary atherosclerosis.---- The mean (+/- SD) serum Lp(a) concentration was significantly higher in patients with CAD than in those without CAD (33.0 +/- 21.9 mg/dl, n = 211, and 23.6 +/- 17.6 mg/dl, n = 154, respectively, p < 0.0001). The relative risk (R.R.) of CAD in subjects with plasma Lp(a) equal to or above 25 mg/dl was greater than that in subjects with Lp(a) below the indicated level (R.R.= 0.67, n = 211, and R.R. = 0.45, n = 154, respectively; odds ratio = 2.42, p <.001)

2. Apo(a) size inversely correlated with Lp(a) concentration.----Among 381 patients in whom apo(a) size polymorphism were checked, 16 different isoforms of apo(a) were observed, including 5 (F1-F5) faster than apo B-100, 9 slower (S1-S9) than apo B-100, one (B) migrated at the speed equivalent to apo B-100, and a null (N) type. There were 88 samples (23.1%) classified as single band and 283 samples (74.3%) as double bands. The mean Lp(a) level of patients containing F forms was significantly higher than the level of those containing S forms (45.6 +/- 19.5 mg/dl and 21.5 +/- 13.5 mg/dl, respectively, p< 0.0001). Subjects carrying null form apo(a) have higher mean (+/- S.D.) plasma Lp(a) concentration than those without (46.3 +/- 10.2 mg/dl, n = 10, and 28.7 +/- 20.7 mg/dl, n = 371, p = 0.0003).

3. Apo(a) size polymorphism tends to correlate with the prevalence of coronary atherosclerosis.-----Subjects containing apo(a) of higher molecular weight (isoforms S5-S9) have lower relative risk for CAD than those who have apo(a) of lower molecular weight (isoforms S1-S4) (0.51, n = 164, versus 0.68, n = 93, respectively; odds ratio = 1.30, p < 0.01).

4. The *KpnI* restriction fragment length polymorphs of apo(a) geneare normally distributed.----229 cases were subjective to the *KpnI* restriction fragment length

polymorphism analysis. The polymorphs are distributed in a nearly normal curve. There is no statistical significance between fragment length and the prevalence of coronary atherosclerosis.

5. There is no significant correlation between the number of TTTTA repeats and plasma Lp(a) concentration.---- For subjects (n = 47) with plasma Lp(a) level disproportion to its apo(a) size, the [TTTTA]n in the 5'-control region of the apo(a) gene was analyzed by polymerase chain reaction and nucleotide sequencing. The majority have 7 (8.5%), 8 (42.5%), or 9 (42.5%) repeats. Only 1 case (2.1%) for 4, 5, and 6 repeats, individually. Statistically, there is no significant difference in plasma Lp(a) concentration among subjects containing different repeats of TTTA.

Discussion

In Taiwan population, the positive correlation between Lp(a) plasma concentration and relative risk of coronary atherosclerosis is similar to other ethnic groups (Utermann, 1989; Scanu *et al*., 1991; Lawn, 1992; Rhoads *et al*., 1986; Rosengren *et al*., 1990). The plasma Lp(a) concentration varies from 5 to 100 mg/dl in our study group and correlated inversely with the molecular weight of apo(a). However, the variation in plasma Lp(a) concentration cannot be fully explained by the apo(a) size polymorphism. Firstly, the distribution of plasma Lp(a) level is highly skewed toward the lower end in Chin-Shan population and is toward Gaussian distribution in the NTUH-based study. Neither pattern can be explained by the nearly normal distribution of the *Kpn*I restriction fragment length polymorphism of apo(a) gene resolved by pulse field gel electrophoresis. Secondly, the plasma Lp(a) concentrations vary widely even in subjects with the same size of apo(a). Thus, other control mechanisms, including polymorphism in the 5' control region of apo(a) gene, are highly suspected. In our study group, 47 cases with plasma Lp(a) concentration beyond the range of mean ± S.D. were included for 5' control region TTTTA repeat analysis. Because the majority of subjects containing 8 or 9 repeats, no significant correlation between (TTTTA)n polymorphism and Lp(a) plasma level could be established. Other polymorphism, such as G/A at position –772, C/T at +93, G/A at +121, and *Nco*I at kringle 37, may be involved in the regulation of Lp(a) expression in Taiwan population.

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