

Pentanucleotide repeat (TTTTA)_n polymorphism in the 5 control region of the apolipoprotein (A) gene and atherothrombotic serum lipoprotein (A) concentration, in a pediatric population

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Lipoprotein (a) [Lp(a)] is a complex of apolipoprotein (a) [apo(a)] and low density lipoprotein, which is an independent major risk factor associated with atherothrombotic disease¹. Unlike other lipoproteins, interindividual differences in Lp(a) plasma concentration is almost entirely due to inheritance, most of them being attributed to sequence differences linked to the apo(a) locus.^{2,3,4} The primary structure of apo(a) encompasses a protease domain, a kringle V domain and a variable number of kringle IV repeats, which is homologous to the same domain in the plasminogen molecule and is thought to be responsible for the antifibrinolytic activity of Lp(a). The gene contains a pentanucleotide repeat (TTTTA)_n polymorphism, 1.4 Kb upstream from the apo(a) gene reading frame. Eight different alleles have been detected to date, with the number of TTTTA repeats ranging from 5 to 12.⁵ This polymorphism, first described by Wade *et al* in 1993,⁶ has been suggested to be important in the control of apo(a) gene expression. Alleles containing more repeats were generally associated with lower plasma Lp(a) concentration.^{1,5,7}

The aim of this study was to investigate a possible link between the number of TTTTA repeats and atherothrombotic serum Lp(a) concentrations [Lp(a) > 30 mg/dL]⁷.

We studied 96 unrelated pediatric patients, without chronic disease or history of thromboembolic events. These patients - 51 with serum Lp(a) concentrations above 30 mg/dL (Group 1) and 45 with concentrations below 30 mg/dL (Group 2) - were age- and gender-matched; the median age of group 1 was 11.13 years (range: 2.9 to 18 years, SD: 4.6 years) and of group 2 was 13.1 year (range: 4 to 18.1 years, SD: 3.57 years), respectively; male: female distribution, 1:0.71. The mean (± SD) value for Lp(a) plasma concentration, performed by an immunoturbidimetric method, was 84.89 ± 46.4 mg/dL (range: 34 to 165 mg/dL) and 14.83 ± 6.39 mg/dL (range: 1 to 29 mg/dL), for Groups 1 and 2, respectively.

Genomic DNA was extracted from peripheral blood leukocytes using standard methods. Genotyping of the polymorphism in the 5' control region of the apo(a) gene was performed by fluorescence labeled polymerase chain reaction with primers described by Mooser *et al.*⁵ The amplified DNA fragments were separated by automated capillary electrophoresis and subsequently analyzed with the ABI GeneScan program (Applied Biosystems) (Figure 1). The number of TTTTA repeats was confirmed in samples of homozygous patients for (TTTTA)₈ and (TTTTA)₉, by automated sequencing.

The c2 test was used to compare the apo(a) allele frequency in each group. A p-value less than 0.05 was considered to be statistically significant.

In this population, the apo(a) allele with (TTTTA)₈ was the most common with a frequency of 76.04%. Alleles with 9, 10, 11, and 7 TTTTA repeats accounted for 13.02, 8.33, 1.56 and 1.04% respectively (Table I). No difference was found in the genotype distribution between the two groups, with Lp(a) < and > 30 mg/dL (c2=2.527; p>0.05). Homozygosity for the (TTTTA)₈ allele was found in 51.1 % of children with Lp(a) levels below and in 64.7% with Lp(a) levels above the cut-off point, although this was not statistically significant (χ2=

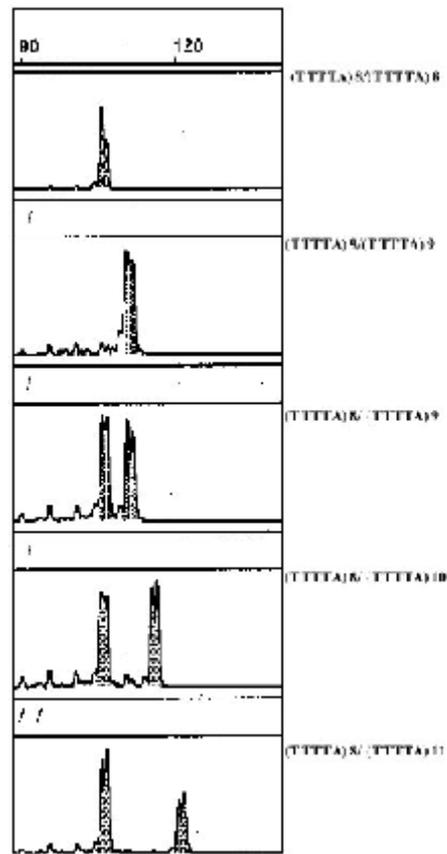


Figure 1.

1.302 ; p>0.05).

To date, no similar study has been carried out in a pediatric population. Nevertheless, since Lp(a) levels are almost entirely genetically determined, our data may be compared to other adult Caucasian data. As such the allele frequencies found here for the pentanucleotide repeat polymorphism were similar to those previously described for other adult Caucasian populations¹. We did not find statistically significant differences between the two groups of patients, suggesting that the polymorphism might not be directly linked with atherothrombotic serum lipoprotein (a) levels. Our sample size, however, might have some influence on the statistical significance of these findings.

The method that we used here is easy, reliable and inexpensive and allows for successful genotyping of the

Table 1.

Table 1 - Distribution of the apo(a) alleles (n and %) according to the number of TTTTA repeats.

		(TTTTA) ₇	(TTTTA) ₈	(TTTTA) ₉	(TTTTA) ₁₀	(TTTTA) ₁₁
Group 1	n	1	82	19	7	2
	%	0,98	80,1	9,8	6,88	1,96
Group 2	n	1	64	15	9	1
	%	1,11	71,11	16,59	10	1,11
Total	n	2	146	25	16	3
	%	1,04	76,04	13,02	8,33	1,36

pentanucleotide repeat polymorphism in the apo(a) gene.

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