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Serum lipid abnormalities are not associated with apoB 3' VNTR polymorphism in nephrotic children

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ABSTRACT. Apolipoprotein B (apoB) gene 3' variable number of tandem repeat (VNTR) is highly variable, and therefore can be an informative marker for associative analysis of lipid metabolism. This is the first report focusing on a possible association of apoB VNTR polymorphism with nephrotic hyperlipidemia. Genomic DNA was extracted from 500 children with primary nephrotic syndrome (PNS) and 500 healthy controls. The apoB genotype was determined by PCR analysis. Allele size distribution followed a unimodal curve, with the main peak at the hypervariable element 35 (HVE35); the most prevalent genotype was HVE35/35 in both control and PNS children. The genotype and allele distributions of apoB variants in PNS children were not significantly different from controls. There was significant variation in serum lipid profiles among different genotypes in control children. Individuals with the long (L) allele exhibited significantly higher total cholesterol, low-density lipoprotein cholesterol (LDL-C)

and apoB levels than those with the medium (M) or short (S) allele; consequently, M/L carriers had significantly higher total cholesterol, LDL-C and apoB concentrations than did S/S, S/M, S/L, or M/M carriers. However, in PNS children, no significant differences in serum lipid levels were observed among individuals with different genotypes and alleles of apoB 3' VNTR. We conclude that hyperlipidemia in nephrotic children is not associated with apoB 3' VNTR polymorphism.

Key words: Apolipoprotein B; Genetic variation; Hyperlipidemia; Child; Nephrotic syndrome

INTRODUCTION

Primary nephrotic syndrome (PNS) is the most prevalent renal disease in childhood and is characterized by elevated permeability of the glomerular filtration barrier and subsequent inability to restrict the urinary loss of protein (Gordillo and Spitzer, 2009). Hyperlipidemia (HLP) not only acts as a typical clinical manifestation of PNS but also is involved in cardiovascular disease and progressive glomerular damage (Mitsnefes, 2008; Hu et al., 2012). In addition, both of the above hazards may prove to be long-term factors that persist from childhood into adulthood (Lechner et al., 2004). Generally, increased concentrations of cholesterol, triglycerides (TGs), lipoprotein (a) [Lp(a)], low-density lipoprotein (LDL), very-LDL, and apolipoprotein B (apoB) are the common features of serum lipid abnormalities in nephrotic children (Hu et al., 2009a). Several mechanisms allegedly contribute to nephrotic HLP. According to the classic view, hypercholesterolemia is due to increased hepatic synthesis of apoB-containing lipoproteins, whereas hypertriglyceridemia results from impaired catabolism of TG-rich lipoproteins (Dixit and Hettiaratchi, 1979; Cohen et al., 1980). Furthermore, some studies have documented that genetics may play an important role in the onset of nephrotic HLP (Gong et al., 2000; Ruf et al., 2003; Hu et al., 2009b).

ApoB is the integral structure protein of hepatic very-low-density lipoprotein and serves as a principal ligand for LDL-receptor-mediated lipoprotein clearance (Segrest et al., 2001). The variability of the apoB gene may account in part for the genetic susceptibility to HLP by modulating the average effects of apoB variants of the lipidemic phenotype (Rantala et al., 2000; Hu et al., 2009c). The apoB gene maps to human chromosome 2p24 and comprises 29 exons spanning approximately 42 kb. Numerous polymorphisms have been identified on this gene. Among them, the apoB gene 3' variable number of tandem repeat (VNTR) polymorphism is located 482 bp downstream of the stop codon and exhibits a variable number of 11- to 16-bp (average variation, 15-bp) adenine- (A) and thymine- (T) rich sequences (Huang and Breslow, 1987). This locus is highly variable, and approximately 26 alleles have been reported (Destro-Bisol et al., 1994). Therefore, the apoB 3' VNTR is considered an informative marker for many applications, including linkage analysis, forensic identification, paternity testing, anthropological research, and phylogenetic studies (Batanian et al., 1998; Choong et al., 1999; Soares-Vieira et al., 2000; Verbenko et al., 2003; Khrunin et al., 2007).

In a previous study, we identified the higher repeat alleles of the apoB 3' VNTR as a potential risk factor for HLP in Han children from central China (Hu et al., 2010). However, what remains to be resolved is whether the apoB 3' VNTR polymorphism effect on lipid metabolism also exists in nephrotic children.

MATERIAL AND METHODS

Subjects

To avoid unnecessary interruptions in the treatment of the disease and steroid-to-lipid metabolism and proteinuria, 500 pediatric patients with PNS aged between 2 and 14 years (mean age, 8.4 ± 3.6 years) were recruited to the study at their first visit, and none was undergoing steroid therapy or other medications. All patients had nephrotic onset with proteinuria >50 $mg \cdot kg^{-1} \cdot day^{-1}$, hypoalbuminemia <30 g/L, edema, and HLP of varying degrees (Gipson et al., 2009). The control group consisted of 500 healthy volunteers aged between 3 and 14 years (mean age, 7.8 ± 3.2 years) without allergies or renal disease. The ethnicities of both groups were Han Chinese, and recruited individuals were unrelated and not members of the same family or related. The study was approved by the Ethics Committee of the Medical Facility, and written informed consent was obtained from the parents of all subjects before study entry.

Laboratory analysis

Blood samples for the measurement of serum lipid parameters were collected after an overnight fast during the first visit to the hospital. Serum was separated within 4 h and stored. Subsequent analysis of serum Lp(a) was performed using an assay based on a sandwich enzyme-linked immunosorbent assay that was insensitive to the presence of plasminogen (RANDOX, UK). The sensitivity and specificity of the enzyme-linked immunosorbent assay were 98.2 and 95.7%, respectively, and this test was not validated against manufacturer claims. Serum total cholesterol (TC), TG, and high-density lipoprotein cholesterol (HDL-C) were measured using standard enzymatic methods (RANDOX). Serum LDL-C was calculated with the Friedewald formula (Bairaktari et al., 2000). Serum apolipoprotein A1 (apoA1) and apoB were measured using immunoturbidimetric methods (RANDOX). All analyses were performed in duplicate, and the examiners were blinded to the clinical and laboratory results.

Genotyping of the apoB gene 3' VNTR locus

DNA was extracted from blood with a salting out method using phenol-chloroform as described by Noguera et al. (2000). The amplification of the apoB gene 3' VNTR locus was undertaken with polymerase chain reaction. The sequences of the forward and backward primers used were 5'-ATG GAA ACG GAG AAA TTA TG-3' and 5'-CCT TCT CAC TTG GCA AAT AC-3' (Boerwinkle et al., 1989). The reaction mixture (50 µL) was prepared with 10 pmol of each primer, 100 mM deoxyribonucleotide triphosphates, 1 U Taq polymerase (TaKaRa, Dalian, China), buffer containing 1.5 mM $MgCl_2$, 1% dimethyl sulfoxide, and 500 ng DNA sample. The amplification process consisted of an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, coiling at 58°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. Negative controls (no DNA added) were included in every polymerase chain reaction run to check for contamination. To separate the 3' VNTR alleles, 10 µL amplified DNA was run on a 3% agarose gel with ethidium bromide. The gel was visualized under ultraviolet light and photographed (Figure 1). The length of each band was determined using an ABI 3100 Automated DNA 114 Sequencer (Applera,

San Francisco, CA, USA). The number of tandem repeats was calculated with the following equation: repeat number = [fragment length (bp) - 138 bp] / 15 bp (Ruixing et al., 2007).

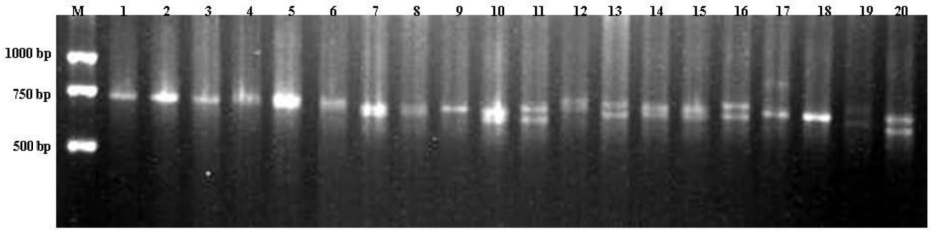

Figure 1. Representative gel photogragh of PCR product amplified for apoB 3' VNTR. *Lane M* = DNA ladder markers; *lane 1* = hypervariable element (HVE)40/40; *lane 2* = HVE38/38; *lane 3* = HVE37/37; *lane 4* = HVE36/36; *lane 5* = HVE35/35; *lane 6* = HVE34/34; *lane 7* = HVE30/32; *lane 8* = HVE30/32; *lane 9* = HVE32/32; *lane 10* = HVE28/30; *lane 11* = HVE26/32; *lane 12* = HVE32/36; *lane 13* = HVE28/32; *lane 14* = HVE28/30; *lane 15* = HVE26/30; *lane 16* = HVE26/32; *lane 17* = HVE30/44; *lane 18* = HVE28/28; *lane 19* = HVE24/30; *lane 20* = HVE22/24.

Statistical analyses

A P value of <0.05 was considered to be significant. The results are reported as means ± standard deviations or percentages. The allelic frequencies and genotype distribution were calculated with a gene-counting method (Yasuda and Kimura, 1968). The chi-square test or the Fisher exact test was used to evaluate the allelic and genotypic frequencies and estimate Hardy-Weinberg equilibrium. One-way analysis of variance and the Student *t*-test were performed to determine differences in lipid parameters. Statistical analyses were performed using SPSS version 11.5.

RESULTS

Demographic and laboratory characteristics

The clinical and biochemical characteristics of children in PNS and control groups are shown in Table 1. Gender ratio and age were very close in the two groups (P > 0.05). However, serum concentrations of Lp(a), TC, TG, HDL-C, LDL-C, and apoB in the PNS group were significantly higher than those in the control group (P < 0.01).

Table 1. Clinical and biochemical characteristics of controls and primary nephrotic syndrome (PNS) children.

	Controls (N = 500)	PNS children (N = 500)	P
Gender ratio (M/F)	276/224	282/218	0.702
Age (years)	7.5 ± 3.6	8.3 ± 3.7	0.732
Lp(a) (mg/L)	354.9 ± 28.6	839.6 ± 35.2	0.001
TC (mM)	3.7 ± 0.8	9.1 ± 1.3	0.001
TG (mM)	1.1 ± 0.4	2.6 ± 0.4	0.001
HDL-C (mM)	1.0 ± 0.4	1.9 ± 0.3	0.003
LDL-C (mM)	3.2 ± 0.7	7.3 ± 1.2	0.001
ApoA1 (mM)	1.7 ± 0.2	1.8 ± 0.3	0.947
ApoB (mM)	0.8 ± 0.3	1.6 ± 0.5	0.001

Data are reported as means ± SD. For abbreviations, see legend to Table 4.

Allelic frequencies and genotype distribution

Fourteen alleles of apoB 3' VNTR comprising 22 to 44 hypervariable elements (HVEs) were identified in our populations. They were HVE22, HVE24, HVE26, HVE28, HVE30, HVE32, HVE34, HVE35, HVE36, HVE37, HVE38, HVE40, HVE42, and HVE44. With the exception of HVE44, all of these alleles were present in controls, whereas HVE22 and HVE42 were absent in children with PNS (Figure 2). The genotype distribution in the populations did not follow the Hardy-Weinberg equilibrium but rather displayed a unimodal curve in both groups with the main peak at HVE35, a second peak at HVE34, and a third peak at HVE36 (58.0 *vs* 55.4, 16.8 *vs* 18.7, and 11.5 *vs* 10.8%, respectively). Thirty-seven genotypes were detected in the control and PNS groups, with the most frequent being HVE35/35; the second most frequent was HVE34/35, and the third was 35/36 (36.4 *vs* 33.4, 20.6 *vs* 21.2, and 18.4 *vs* 16.6%, respectively; Table 2).

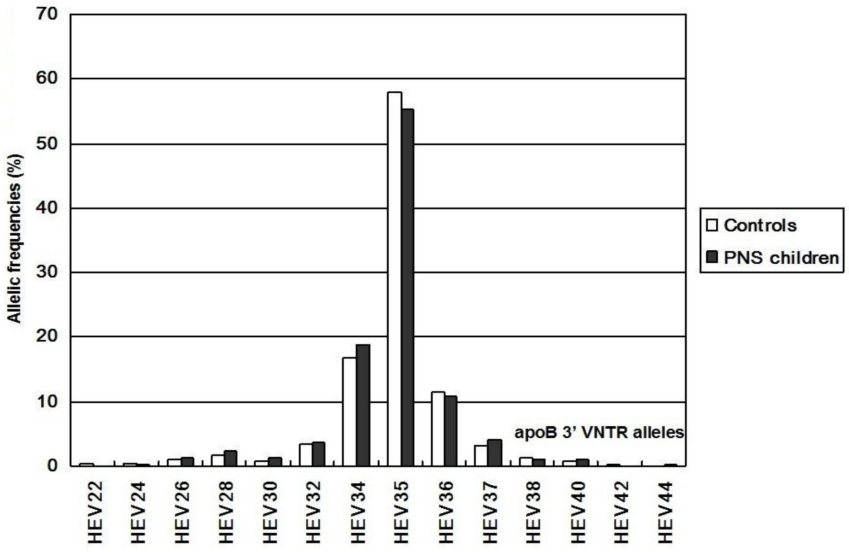

Figure 2. Allelic frequencies of apoB 3' VNTR in control and primary nephrotic syndrome (PNS) children. Allelic distribution followed a unimodal curve in controls and PNS with the main peak at hypervariable element (HVE)35, the second at HVE34 and the third at HVE36 (58.0 *vs* 55.4, 16.8 *vs* 18.7, and 11.5 *vs* 10.8%, respectively).

To make the analysis uncomplicated, we coded alleles in a three-allele model. Up to 32 repeats were kept in the short (S) allele group, the most common alleles (34-38 repeats) were in the medium (M) group, and the long (L) allele group included more than 40 repeats. Three homozygous phenotypes (S/S, M/M, and L/L) and three heterozygous phenotypes (S/M, S/L, and M/L) arise from the expression of any two of the three alleles (Dixit et al., 2008). The genotype and allelic frequencies of the apoB 3' VNTR locus in the control and PNS groups are shown in Table 3. The genotype distribution in children with PNS was not significantly different from that in controls (chi-square = 2.170, P = 0.830). Similarly, no significant differences in allelic frequencies were observed for the apoB 3' VNTR in children with PNS compared with those in controls (chi-square = 0.220, P = 0.900).

Table 2. Genotype distribution of apoB 3' VNTR in control and primary nephrotic syndrome (PNS) children.

Controls (N = 500)			PNS children (N = 500)		
Genotype	Cases	Percentage (%)	Genotype	Cases	Percentage (%)
HVE35/35	182	36.4	HVE35/35	167	33.4
HVE34/35	103	20.6	HVE34/35	107	21.2
HVE35/36	92	18.4	HVE35/36	83	16.6
HVE35/37	21	4.2	HVE35/37	25	5.0
HVE34/34	15	3.0	HVE34/34	20	4.0
HVE32/34	8	1.6	HVE34/36	9	1.8
HVE34/36	8	1.6	HVE32/34	7	1.4
HVE34/37	6	1.2	HVE34/37	7	1.4
HVE28/34	5	1.0	HVE28/34	6	1.2
HVE30/32	5	1.0	HVE30/32	6	1.2
HVE34/38	5	1.0	HVE34/38	6	1.2
HVE32/36	4	0.8	HVE32/35	5	1.0
HVE36/36	4	0.8	HVE36/36	5	1.0
HVE28/32	3	0.6	HVE28/32	4	0.8
HVE32/32	3	0.6	HVE32/32	4	0.8
HVE32/34	3	0.6	HVE32/34	4	0.8
HVE36/40	3	0.6	HVE36/40	4	0.8
HVE37/37	3	0.6	HVE37/37	4	0.8
HVE22/24	2	0.4	HVE26/26	3	0.6
HVE22/26	2	0.4	HVE26/28	3	0.6
HVE26/26	2	0.4	HVE26/30	3	0.6
HVE26/28	2	0.4	HVE28/28	3	0.6
HVE26/32	2	0.4	HVE28/30	3	0.6
HVE28/28	2	0.4	HVE24/36	2	0.4
HVE28/30	2	0.4	HVE32/38	2	0.4
HVE32/38	2	0.4	HVE28/40	2	0.4
HVE22/38	1	0.2	HVE37/44	2	0.4
HVE24/30	1	0.2	HVE34/40	1	0.2
HVE24/38	1	0.2	HVE38/38	1	0.2
HVE28/40	1	0.2	HVE38/40	1	0.2
HVE30/44	1	0.2	HVE40/40	1	0.2
HVE32/40	1	0.2			
HVE32/42	1	0.2			
HVE38/38	1	0.2			
HVE38/40	1	0.2			
HVE38/42	1	0.2			
HVE40/40	1	0.2			

Table 3. Genotype and allelic frequencies of apoB 3' VNTR in control and primary nephrotic syndrome (PNS) children.

Genotype or allele	Controls [N = 500 (%)]	PNS children [N = 500 (%)]	χ^2	P
S/S	26 (5.2)	29 (5.8)		
S/M	21 (4.2)	26 (5.2)		
S/L	4 (0.8)	2 (0.4)		
M/M	443 (88.6)	434 (86.8)		
M/L	5 (1.0)	8 (1.6)		
L/L	1 (0.2)	1 (0.2)	2.170	0.830
S	38.5 (7.7)	43 (8.6)		
M	456 (91.2)	451 (90.2)		
L	5.5 (1.1)	6 (1.2)	0.220	0.900

S = short; M = medium; L = long alleles.

Influence of apoB 3' VNTR polymorphism on lipid profiles

Serum lipid concentrations according to the genetic polymorphisms of apoB 3' VNTR

are presented in Table 4. Because only one nephrotic and one healthy child displayed L/L and two nephrotic children displayed S/L, the contributions of these genotypes to serum lipid components could not be evaluated. To probe the association of serum lipids with the alleles of the apoB 3' VNTR, we divided subjects into three carrier subgroups: S (S/S and S/M), M (M/M), and L (M/L and L/L). Individuals with S/L were excluded from the analysis because of the uncertainty about pooling with other genotypes. In controls, significant variation among the five genotypes was seen for serum levels of TC, LDL-C, and apoB. M/L carriers had TC, LDL-C, and apoB concentrations significantly higher than those of S/S, S/M, S/L, or M/M carriers ($P < 0.05$). In addition, the same trend was also observed among the three alleles. Individuals with the L allele exhibited significantly higher TC, LDL-C, and apoB levels than those with the M or S alleles ($P < 0.05$). However, in the PNS group, no significant differences in serum lipid levels were observed for variant genotypes and alleles of the apoB 3' VNTR ($P > 0.05$).

Table 4. Influence of apoB 3' variable number of tandem repeat polymorphism on lipid profiles in control and primary nephrotic syndrome (PNS) children.

	S/S	S/M	S/L	M/M	M/L	S	M	L
Controls								
N = 500	26	21	4	443	5	47	443	6
Lp(a) (mg/L)	348.3 ± 32.7	359.6 ± 38.5	351.5 ± 62.3	362.5 ± 21.3	365.1 ± 55.3	353.1 ± 34.2	362.5 ± 21.3	366.3 ± 50.6
TC (mM)	3.6 ± 1.2	3.8 ± 0.9	3.7 ± 1.4	3.8 ± 0.7	4.3 ± 1.4*	3.6 ± 0.8	3.8 ± 0.7	4.4 ± 1.2*
TG (mM)	0.8 ± 0.3	0.9 ± 0.3	1.0 ± 0.5	1.0 ± 0.5	1.1 ± 0.6	0.8 ± 0.3	1.0 ± 0.5	1.1 ± 0.4
HDL-C (mM)	1.5 ± 0.4	1.6 ± 0.6	1.5 ± 0.6	1.3 ± 0.1	1.5 ± 0.8	1.5 ± 0.5	1.3 ± 0.1	1.5 ± 0.6
LDL-C (mM)	2.5 ± 0.7	3.2 ± 0.8	2.7 ± 0.9	3.1 ± 0.5	3.6 ± 1.1*	2.7 ± 0.7	3.1 ± 0.5	3.5 ± 0.9*
apoA1 (g/L)	1.5 ± 0.5	1.5 ± 0.4	1.4 ± 0.6	1.6 ± 0.2	1.4 ± 0.4	1.5 ± 0.4	1.6 ± 0.2	1.3 ± 0.5
apoB (g/L)	0.7 ± 0.2	0.9 ± 0.4	0.8 ± 0.4	0.9 ± 0.2	1.3 ± 0.3*	0.8 ± 0.4	0.9 ± 0.2	1.3 ± 0.3*
PNS children								
N = 500	29	26	2	434	8	55	434	9
Lp(a) (mg/L)	835.3 ± 44.2	841.0 ± 42.5	-	831.1 ± 40.3	846.4 ± 67.9	837.6 ± 36.5	831.1 ± 40.3	843.9 ± 65.4
TC (mM)	8.9 ± 1.5	9.1 ± 1.6	-	9.3 ± 1.5	9.5 ± 1.8	8.9 ± 1.3	9.3 ± 1.5	9.4 ± 1.6
TG (mM)	2.5 ± 1.0	2.7 ± 1.3	-	2.8 ± 0.6	2.8 ± 1.3	2.5 ± 0.8	2.8 ± 0.6	2.8 ± 1.4
HDL-C (mM)	1.9 ± 0.5	2.1 ± 0.5	-	2.0 ± 0.3	1.8 ± 0.6	2.0 ± 0.6	2.0 ± 0.3	1.9 ± 0.6
LDL-C (mM)	6.9 ± 1.1	7.3 ± 1.4	-	6.9 ± 1.5	7.5 ± 2.2	7.0 ± 1.2	6.9 ± 1.5	7.4 ± 1.9
apoA1 (g/L)	1.8 ± 0.6	1.8 ± 0.4	-	2.0 ± 0.4	2.1 ± 0.6	1.8 ± 0.6	2.0 ± 0.4	2.0 ± 0.4
apoB (g/L)	1.6 ± 0.3	1.8 ± 0.6	-	1.7 ± 0.4	1.9 ± 0.7	1.6 ± 0.5	1.7 ± 0.4	1.9 ± 0.7

Data are reported as means ± SD. *$P < 0.05$. In control children, M/L carriers had significantly higher total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and apolipoprotein B (apoB) concentrations than did S/S, S/M, S/L, or M/M carriers ($P < 0.05$); accordingly, individuals with the L allele exhibited significantly higher TC, LDL-C, and apoB than those with M or S allele ($P < 0.05$). Lp(a) = lipoprotein (a); TG = triglycerides; HDL-C = high-density lipoprotein cholesterol; apoA1 = apolipoprotein A1. S = short; M = medium; L = long alleles.

DISCUSSION

HLP is one of the most common pathophysiological features in nephrotic children (Gordillo and Spitzer, 2009). In the present study, patients with childhood PNS experienced profound dyslipidemia characterized by significant increases in Lp(a), TC, TG, HDL-C, LDL-C, and apoB levels. Although many factors appear to be implicated in the pathogenesis of PNS-related lipid abnormalities, the underlying mechanisms remain a matter of debate. Increasing evidence suggests that these disturbances and mass proteinuria may result from both hepatic overproduction and impaired catabolism (Dixit and Hettiaratchi, 1979; Cohen et al., 1980). In a previous study, we observed that serum lipid abnormalities in children with PNS

parallel the degree of urinary protein excretion (Hu et al., 2009a). Furthermore, some studies have documented that genetics may exert an important influence on the onset of nephrotic HLP (Gong et al., 2000; Ruf et al., 2003; Hu et al., 2009b).

ApoB has been identified as an important candidate gene for serum lipid abnormalities. Our previous data have demonstrated that genetic variations at restriction enzyme recognition sites of apoB (*Xba*I and *Eco*RI) might be the risk factors for HLP in both healthy and PNS children (Hu et al., 2009b,c). The apoB 3' VNTR is highly variable, and to date, approximately 26 alleles have been reported (Destro-Bisol et al., 1994). Therefore, this locus is considered an informative marker for association analysis with lipid metabolism. The results of our latest study, published in Clinica Chimica Acta, has suggested that the L allele of the apoB 3' VNTR might be a potential risk factor for HLP in Han children from central China (Hu et al., 2010). However, it remains to be determined whether the effects of apoB 3' VNTR polymorphism on lipid metabolism exist in nephrotic children.

This report is the first to focus on the association of apoB 3' VNTR polymorphism and nephrotic HLP. In the present study, 14 alleles comprising 22-44 HVEs were identified. The presence of high allelic variability at the apoB 3' VNTR is due to a complex mutational pattern. Earlier studies have reported that a stepwise mutational mechanism that reflects gain or loss of one or a few repeat units probably owing to replication slippage is responsible for the high polymorphism at the apoB 3' VNTR (Deka et al., 1992; Sajantila et al., 1999). The abovementioned alleles (except HVE44) were present in controls, whereas HVE22 and HVE42 were absent in children with PNS. Allele size distribution followed a unimodal curve, with a main peak at HVE35, and the most prevalent genotype was HVE35/35 in both the control and the PNS groups. The genotype and allele distributions in PNS children were not significantly different from those in controls.

Subsequent analysis demonstrated significant variation among the five genotypes for the serum lipid profiles of control children. M/L carriers had TC, LDL-C, and apoB concentrations significantly higher than those of S/S, S/M, S/L, or M/M carriers, and individuals with the L allele accordingly exhibited TC, LDL-C, and apoB levels significantly higher than those with M or S alleles. These results were consistent with those of other investigators (Hansen et al., 1993; Pan et al., 1998; Rebhi et al., 2008). However, in children with PNS, no significant differences in serum lipid levels were observed for variant genotypes and alleles of the apoB 3' VNTR. The reasons for the variable effects of apoB 3' VNTR polymorphism on serum lipid profiles between control children and children with PNS are unclear. The following explanations are possible.

First, nephrotic HLP is a secondary lipid disorder. In fact, all patients recruited into our study were under preliminary diagnosis, and renal pathological changes had not been identified. Thus, the effect of the apoB 3' VNTR polymorphism on lipid metabolism in a normal population may be hidden by the increased hepatic lipid synthesis triggered by primary glomerular damage (Wiecek et al., 1993). Second, these repeats are present in the 3' untranslated region and do not play any role in the apoB protein sequence. VNTR may be in linkage disequilibrium with other polymorphisms in the same gene or other nearby genes to develop lipid alterations (Pontrelli et al., 2004). Friedl et al. (1990) reported that the apoB 3' VNTR shows strong linkage disequilibrium with a polymorphic *Eco*RI site in exon 29, which might contribute, in part, to the elevated levels of serum cholesterol and apoB in L carriers. Finally, some environmental factors, such as diet modification and hormone secretion, may be also

involved in lipid regulation (Hokken-Koelega et al., 1990; Tovar et al., 2002). Further studies are warranted to elucidate the above hypotheses.

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