소아 미세변화 신증후군 환자에서 Apolipoprotein E 유전자형에 관한 연구

경희대학교 의과대학 부속병원 소아과학 교실, *동서 의학 대학원
김성도, 배영민, 조병수, 조여원*, 김일수*

= Abstract=

Increased Frequency of Apolipoprotein E4 Genotype in Childhood Minimal Change Nephrotic Syndrome (MCNS)

Sung-Do Kim, M.D., Young-Min Bae, M.D., Byoung-Soo Cho, M.D.,
Yoe-Won Cho*, M.D., Il-Soo Kim*, M.D.

Department of Pediatrics, School of Medicine, Kyunghee University,
**Kyunghee Oriental and Western medical graduate school.*

Purpose: We studied to find out apo-E genotype polymorphism in minimal change nephrotic syndrome(MCNS) and IgA nephropathy(IgAN) and to determine the relationship between apo-E genotype and clinical course of MCNS.

Materials and Method: 43 MCNS patients and 15 IgAN patients were examined for apo-E polymorphism. 50 healthy blood donors were examined for apo-E genotype as control. Genomic DNA was prepared from peripheral blood leukocytes according to standard procedures.

Results: As compared with control group, e4 allele frequency was significantly increased in MCNS ($P<0.01$). However, in IgAN e2 allele frequency, however, was 2.6 times higher than normal control ($P<0.01$). The frequency of e4 allele of frequent relapser group was 4.6 times higher than normal control and was 2 times higher than infrequent relapser group.

Conclusion: We think that apo-E typing might be one of the parameters, which should be considered to predict the course of MCNS in children. MCNS with risky HLA profile and E4/4 genotype could indicate the need for a longer steroid administration. And apo-E genotype needs to be considered for the evaluation of therapeutic responses to other drugs.

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Key words: apolipoprotein E (apo-E), Polymorphism, Minimal Change Nephrotic Syndrome (MCNS), IgA nephropathy (IgAN)

Introduction

Apolipoprotein E(apo-E) is a plasma protein that serves various functions, including maintenance of the structure of the lipoprotein particles and regulation of the metabolism of several different lipoproteins[1,2]. Apo-E, a protein with relative molecular mass of 34000, is a constituent of liver-synthesized very low density lipoproteins(VLDL), which functions primarily in the transport of triglyceride from the liver to peripheral tissues, and subclass of high density lipoproteins(HDL), which participates in cholesterol redistribution among cells. In addition, apo-E becomes a major protein constituent of intestinally synthesized chylomicrons, which transport dietary triglyceride and cholesterol. A major physiologic role for apo-E in lipoprotein metabolism is its ability to mediate high affinity binding of apo-E containing lipoproteins to the low density lipoprotein(LDL) receptor, also referred to as the apo-B, E(LDL) receptor[2,5,6]. Lipoprotein binding to the receptors initiates the cellular uptake and degradation of the lipoproteins, which leads to the use of the lipoprotein cholesterol in the regulation of intracellular cholesterol metabolism. Apoli- poprotein E shares this function with apo-B, the protein constituent of plasma LDL.

The polymorphic nature of apo-E was established by Utermann and his associates[20], using iso-electric focusing(IEF), and further clarifed by Zannis and Breslow[24], using two-dimensional electrophoresis. The three major isoforms of apo-E, referred to as apo-E2, E3, and E4, are products of three alleles(e2,e3,e4) at single gene locus. Three homozygous phenotypes(apo E 2/2, E3/3, and E4/4) and three heterozygous phenotypes(apo-E2/3, E4/3, and E4/2) arise from the expression of any two of the three alleles [8,10,19,20].

The most common phenotype is apo-E3/3 and the most common allele is e3; therefore apo-E3 is considered as the parent form of the protein, and apo-E4 and E2 are varients. Apolipoprotein E2 is the most common form of apo-E associated with type III hyperlipoproteinemia and is defective in receptor binding[2,10]. Apolipoprotein E4 displays nomal binding but is associated with elevated plasma cholestrol and LDL[8].

The molecular basis for apo-E polymorphism was elucidated by analysis of amino acid sequences of the three isoforms[19]. Amino acid substitutions accounted for the differences among apo-E4, E3,and E2. Apolipoprotein E4 differs from apo-E3 in that in apo-E4 arginine is substituted for the nomally occurring cysteine at amino acid residue 112. The most common form of apo-E2 differs from apo-E3 at residue 158, where cysteine is substituted for the normally occuring arginine.

Hyperlipoproteinemia in MCNS is characterized by high concentrations of cholesterol and, less frequently, of triglycerides[22]. In all likelihood, these disorders play a role in the development of atherosclerosis and, possibly, in the progression of the renal disease[12]; their mechanism, which might involve an increased

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책임저자 : 김일수
서울 영등포구 신길동 451-5
성애병원 소아과
전화: 02) 8407-217 FAX : 02) 680-7162
e-mail: kimsungdo@empal.com

synthesis and perhaps decreased clearance of some categories of lipoproteins, mainly those containing apolipoprotein-B and remains unclear[12]. To date, the possibility that an abnormal apo-E phenotype might contribute to hyperlipoproteinemia in the nephrotic syndrome has been alluded[13] but not substantiated.

Phenotypic studies of the e2 and e4 alleles in renal disease differ with regard to their frequency[14]. The apparent variation in gene frequency may be partly due to methodological problems. Thus, comparisons of phenotyping by IEF with genotyping have shown marked discrepancies. After the sequencing of the apo E gene[18] and the recognition of the base substitutions responsible for the polymorphism, these problems have been eliminated by methods that determine genotype directly by using the polymerase chain reaction(PCR) and hybridization with radiolabeled oligonucleotide probes. The Amplification Refractory Mutation System(ARMS) extends the PCR to allow the rapid analysis of known mutations in genomic DNA[15-17]. By ARMS method, we had genotyed a group of MCNS patients and IgA nephropathy to compare the frequency of apo-E genotype of MCNS with normal control. The present study was also carried out to determine the relationship between apo-E genotype and clinical course of MCNS.

Methods and Materials

Patients : All patients were diagnosed and received medical care in Kyunghee University Hospital in Seoul. Among 58 children ; 30 patients were diagnosed as frequent relapser (FR), 13 patients were nonrelapser or infrequent relapser(IR) MCNS according to diagnostic criteria of ISKDC, and 15 patients were diagnosed as IgAN(Table 1). Careful family history was taken and no patient were related. Fifty healthy unrelated Korean blood donors were examined for ApoE genotype as control.

Table 1. Clinical Classification and Sex Distribution of Subjects

Classification		Male	Female	Total
MCNS	IR	9	4	13
	FR	26	4	30
IgAN		8	7	15
Control		25	25	50
Total		68	50	108

IR: infrequent relapser, FR: frequent relapser

DNA preparation

Genomic DNA was prepared from peripheral blood leukocytes according to standard procedures.

Oligonucleotide primers

The oligonucleotide primers were prepared with the EXPEDITI Nucleic Acid Synthesis System (PerSeptive Biosystems, USA) and used without further purification. The orientation of the alleleic- specific primers with in Apo-E gene is illustrated in Fig. 1. The base sequence of the alleleic specific apo-e2 ARMS primer used to detect the variation at nucleotide 3884(amino acid 158) were Apo1 (112-cys) 5'-CTGGGCGCGGACATGGAGGAC GTT C-3', Apo3 (158-cys) 5'-CCCCGGCC TGGTACACTGCCAGGTG-3', and those of the apo-e3 primer were;Apo4 (158-arg), 5'-CC CCGGCCTGGTACA CTGCCAGGTA-3,' Apo1

(112-cys), 5'-CTGGGCGCG GACATGGAGG ACGTTC-3'. The base sequence of the allele-specific ARMS e4 primer used to detect both amino acid 112 and 158 arginine were Apo2(112-arg) 5'-CTGGGCGCGGACATGGAG GAC GTTT-3',andApo4(158-arg) 5'-CCCCG GCCTGGTA CACTGCCAGGTA-3'.

		112	158	
	5'	TGC	TGC	3'
E2	NH2	Cys	Cys	COOH
E3	5'	TGC	CGC	3'
	NH2	Cys	Arg	COOH
E4	5'	CGC	CGC	3'
	NH2	Arg	Arg	COOH

Fig. 1. Amino acid and DNA Sequences of each Apolipoprotein E

Table 2. Sequences of ARMS Primers

Primer name	Primer sequence
Apo1 (112-cys)	5'-CTGGGCGCGGACATGGAGGACGTTC-3'
Apo2 (112-arg)	5'-CTGGGCGCGGACATGGAGGACGTTT-3'
Apo3 (158-cys)	5'-CCCCGGCCTGGTACACTGCCAGGTG-3'
Apo4 (158-arg)	5'-CCCCGGCCTGGTACACTGCCAGGTA-3'

The underline base, next to the alleleic specific 3'-base, is the base that has been deliberately destabilized by substituting T for G to ensure absolute allele-specificity. After the conditions necessary to achieve successful allele-specific amplification of the ARMS primers alone were established, we tested several possible internal primers for amplification under the same conditions. Only those primers spanning the 636-bp fragment of β-globin gene co-amplified with the ARMS primers. These primers produce an internal control amplification product whether or not there is amplification with the ARMS primers.

ARMS analysis of genomic DNA.

Three reactions were set up for each samples. Two of the four ARMS primers were used with the internal control primers. All reactions were carried out with 1 μg of genomic DNA and 1 unit of Taq DNA polymerase in a volume of 50 μL. The final concentrations of other constituents per liter 10 mmol of Tris(Ph 8.3), 1.5 mmol of MgCl, 50 mmol of KCl, 0.1 g of gelatin, 200 μmol of all four deoxynucleoside triphosphates, and 10μ moL of dimethyl-sulfoxside. The reaction mixture was overlaid with 50 μL of paraffin oil. The first step of DNA synthesis consisted of denaturation at 94℃ for 5min and primer annealing at 70℃ for 1min followed by 30cycles of primer extension at 74℃ for 5min denaturation at 94℃ for 0.5min, and primer annealing at 70℃ for 1min. Finally, there was one cycle of primer extention for 10min at 70 ℃. Reactions were carried out with Turbo-Thermalcycler (Bioneer, Korea)

Detection of ampilfication products.

A 10μL of DNA product was mixed with loading buffer and was seperated on 2% agarose gel electroporesis at 130V for 20min in 40mM Trisacetate-1mM EDTA. A molecular mass marker was included in each gel.

Statstical analysis for individual apo-E genotype frequency and comparing plasma albumin, choles- terol, triglyceride and apo-E phenotype were done using the Chisquare test and ANOVA(analysis of variation). We consi-

dered it to be statistically sig- nificant if the P value was less than 0.05.

Results

1. Amplification of samples of known genotype

The observed and expected ARMS results obtained from 12 samples previously genotyped. All samples gave the correct amplification patterns of a paticular genotype as expected. The e3e3 sample were obtained with primers Arg158 and Cys 112, e2e2 sample were abtained with primers Cys158 and Cys 112 and e4e4 sample were obtained with primers Arg 158 and Arg 112 respectively(Fig. 2). One of the heterozygotes, e4e2 has an amplification with Arg158 and Arg112, Cys158 and Cys158, e3e4 has those in Arg158 Arg112 and Arg158 and Cys 112(Fig. 3).

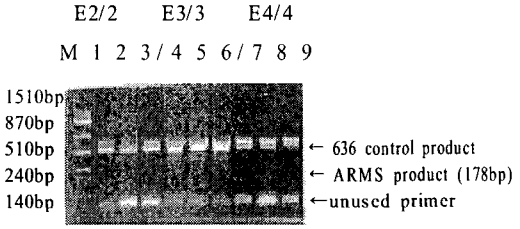

Fig. 2. ARMS analysis of subjects of genotype e2/2, e3/3, e4/4 homozygotes ARMS primers are follows, tract 1,4,7 : E2 primers(apo1 and apo3 : cysteine 112, cysteine158), tract 2,5,8: E3 primers (apo1, apo4 : cysteine 112, arginine158), tract 3,6,9; E4 primers(apo2 and apo4 : arginine 112, arginine 158) M, size marker

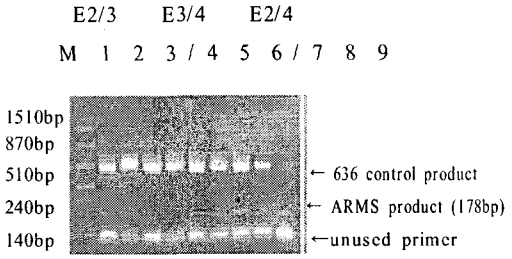

Fig. 3. ARMS analysis of ApoE genotype e2/3 e3/4, e2/4 heterozygotes ARMS primers are follows, tract 1,4,7 : E2 primers(apo1 and apo3 : cysteine 112, cysteine158), tract 2,5,8 : E3 primers (apo1, apo4 : cysteine 112, arginine 158), tract 3,6,9 : E4 primers (apo2 and apo4: arginine 112, arginine 158) M, size marker

ARMS therefore sucessfully distinguished between the six genotypes and corresponding six genotypes.

2. The frequency of apo-E genotype polymorphism of the control subjects

Among 50 control subjects, Fourty-two were apoE3/3 genotype, one was apo- E2/2 , two were apo-E3/4 and three were apo-E2/3 (Table 3). e2,e3,e4 allele frequencies were 5(frequency 0.05), 91(0.91), 4(0.04).

Table 3. Amplification patterns of ApoE geno type in control group

Genotype	No. of subjects (n=50)	ARMS primer amplification			
		Cys 112	Arg 112	Cys 158	Arg 158
E2E2	1	+	-	+	-
E3E3	42	+	-	-	+
E4E4	0	-	+	-	+
E3E2	3	+	-	+	+
E4E3	4	+	+	-	+
E2E4	0	+	+	+	+

Table 4. Amplification patterns of ApoE geno- type in disease group

Genotype	No. of subjects (n=58)	ARMS primer amplification			
		Cys112	Arg112	Cys158	Arg158
E2E2	3	+	-	+	-
E3E3	38	+	-	-	+
E4E4	3	-	+	-	+
E3E2	7	+	-	+	+
E4E3	7	+	+	-	+
E2E4	0	+	+	+	+

3. The frequency of apoE genotype polymorphism of MCNS

In group of MCNS, there were three e4/4 homozygotes, seven e4/3 heterozygotes, two e2/2 homozygotes(Table 5).

Table 5. Amplification of apo E genotype in MCNS

Genptype	MCNS		Total
	IR	FR	
E2/2	0	2	2
E3/3	9	18	27
E4/4	0	3	3
E2/3	2	2	4
E3/4	2	5	7
E2/4	0	0	0

The e2,e3,e4 allele frequency was 8(0.09), 65(0.76), 13(0.15). As compared with control group, e4 allele frequency was significantly increased in MCNS ($P<0.05$).(Table 6)

Table 6. The ·allele frequencies of apo-E o MCNS

Genotype	Control %(n=100)	MCNS %(n=86)	Significance
E2	5(5)	93(8)	$P>0.05$
E3	91(91)	76.5(65)	
E4	4(4)	15.1(13)	$P<0.01$

The frequency of e4 allele of frequent relapser(FR) group was 4.6 times higher than normal control and was 2 times higher than infrequent relapser(IR) group(Table 7)

Table 7. The allele frequencies of apo-E in FR and IR group

Genotype	MCNS		Significance (IR vs FR)
	IR %(n=26)	FR %(n=60)	
E2	7.7(2)	10(6)	NS
E3	84.6(22)	71.7(43)	
E4	7.7(2)	18(11)	$P=0.056$

4. The frequency of apoE genotype polymorphism of IgA nephropathy

As shown in Table 8, e2 allele was 4(frequency 0.13), e3 allele were 26(0.87), e4 were 0(0.00). There were significant differences between e2 allele frequency of IgAN and normal control ($P<0.01$). The e2 frequency was 2.6 times higher than control group.

Table 8. The frequencies of apoE genotype in IgAN

Genotype	Control %(n=100)	IgAN %(n=30)	Significance
E2	5(5)	13.3(5)	$P<0.01$
E3	91(91)	87.7(25)	
E4	4(4)	0(0)	

5. The frequency of apoE phenotype polymorphism by classifying three groups: normal E3, E4 variants(including e3/4, e4/4), E2 variant (including e2/2, e2/3).

In control group, E4 variants was 4(0.08), E2 variant was 2(0.04), E2/4 variant was zero (0). In MCNS group, E4 variants was 10(0.23), E2 variant was 6(0.14).

When compared MCNS with control group, there was significantly increased frequency of E4 variants in MCNS ($P<0.05$) (Table 9). Among 30 FR group, E4 variant was 8(0.27), E2 variant was 4(0.13). Among 13 IR group, E4 variants was 2(0.154), E2 variants was 2(0.154). The frequency of E4 variants of FR group was 1.8 times higher than IR group(Table 10). Among 15 IgAN, E2 variants were 4(0.24) E2 variants frequency was 3 times higher than control group.(Table 11)

Table 9. The frequencies of apoE phenotyp Variants in MCNS

Phenotype	Control %(n=50)	MCNS %(n=43)	Significance
Normal E3	88(44)	63(27)	
E4 variants	8(4)	23(10)	$P<0.05$
E2 variants	4(2)	14(6)	NS
E2/4	0(0)	0(0)	

Table 10. Comparison of apoE phenotype i MCNS according to clinical course

Phenotype	IR %(n=13)	FR %(n=30)	Significance
Normal E3	69.2(9)	60(18)	
E4 variants	15.4(2)	27(8)	$P>0.05$
E2 variants	15.4(2)	13(4)	
E2/4	0(0)	0(0)	

IR: infrequent relapser, FR: frequent relapser

Table 11. The frequencies of apoE phenotype variants in IgA nephropathy

Phenotype	Control %(n=50)	IgAN %(n=15)	Significance
Normal E3	76(42)	73(11)	
E4 variants	8(4)	0(0)	
E2 variants	8(4)	23(4)	$P>0.05$
E2/4	0(0)	0(0)	

6. The plasma albumin, cholesterol, and triglyceride level in E4 variants

We compared plasma albumin, cholesterol, triglyceride level of E4 variants group with those normal E3 group. There was no significant difference among them($P>0.05$) (Table12).

Table 12. Comparison of Plasma Albumin an Cholesterol and Triglyceride concentration with ApoE Genotype

Phenotype	albumin (g/dL)	cholesterol (mg/dL)	triglyeride (mg/dL)	significance
E3 (n=16)	1.42(±0.541)	563±135	432(±337)	NS
E4 variants (n=7)	1.67(±0.411)	497(±111)	550(±302)	NS

Discussion

The most difficult problem in the care of children with MCNS continues to be the occurrence of frequent relapses in patients who respond initially to treatment with steroids. Repeated and continuous administration of steroids, although usually effective, is frequently associated with toxicity.

Apolipoprotein E is a plasma protein that serves as a ligand for low density lipoprotein

recepters and, through its interaction with these receptors, participates in the transport of cholesterol and other lipids among various cells of the body. A mutant form of apolipoprotein E that is defective in binding to low density lipoprotein receptors is associated with familial type III hyperlipoproteinemia[20], a genetic disorder characterized by elevated plasma cholesterol levels and accelerated coronary artery disease. Apolipoprotein E also appears to be involved in the repair response to tissue injury: for example, markedly increased amounts of apolipoprotin E are found at sites of peripheral nerve injury and regeneration. Other functions of apolipoprotein E are immunoregulation and modulation of cell growth and differentiation. The apo-E gene show polymorphism. The three common alleles-e2, e3, and e4-are inherited co-dominantly and code for three apo E proteins(isoforms): E2, E3, and E4. The isoforms differ at amino acid residues 112 and 158. Isoform E2 has cysteine residues at both sites, E4 has arginine residues at both sites, and E3 has a cysteine at position 112 and an arginine at position 158[19]. Compared with the most frequent and fully functional isoform E3, an increased frequency of E4 is believed to be associated with hypercholesterolemia and, to a lesser extent, with hypertriglyceridemia. To date, the possibility that an abnormal apoE phenotype might contribute to hyperlipoproteinemia in the nephrotic syndrome has been alluded[13] but not substantiated.

The possibility that the apo-E polymorphism might contribute to hyperlipidemia in the nephrotic syndrome has been suggested by one study showing the e4 allele frequency being 4.8 times higher in patients than in the control. They studied only Apo-E phenotype by IEF in small group of 13 patients with adult nephrotic syndrome. The frequencies of control group apoE phenotype were E3/3 (11subjects,frequency 0.65) E3/4(4, 0.25), E3/2 (2, 0.11). However, in the group of nephrotic syndrome, only one patients had the common phenotype E3/3; all others displayed the isoform E4, either E4/4(n=3) or E4/3(n=9). We evaluated apo-E genotype polymorphism by ARMS in 43 MCNS patients. In our study there was a significant difference in the apo-E4 genotype frequencies between MCNS and controls($P<0.01$). Although e4 allele frequency of NS was only 15.1%, it was 3.8 times higher than in the controls(4%). We compared E4 freqeuncies of frequent relapser (FR) with non or infrequent relaper(IR). The e4 allele frequency of FR group was 2.0 times higher than IR group. This is the first report about Apo-E polymorphism associated with clinical course of MCNS.

We examined the frequency of apo-E polymorphism in 15 patients with IgAN. There was significant increased frequency of apo-E2 genotype than normal control. In two patients with FSGS, one was apo-E2/2 and the other was E2/3, there might be increased e2 frequency in FSGS. If this is the case, it would be very useful to determine the patients with FSGS. However the sample size was too small to make such decision. Further study with many patients should be necessary.

Recent studies have shown that the LDL-receptor, expressed in several tissue, including the liver plays a major role in the uptake of VLDL and remnants from plasma in vivo[7].

Interestingly, the three common isoforms have different affinities for the LDL-receptor. Apo-E3 and E4 have the same affinity for this receptor, whereas E2 shows defective binding affininty.

VLDL and remnants containing apo-E2 are slowly removed from the plasma and upregulation of liver LDL-receptor and thus a low concentration of plasma cholestrol. VLDL-apoE4 particles are removed faster from plasma than VLDL-ApoE3 particles, including down regulation of the LDL-receptor. VLDL-ApoE4 phenotype is thus associated with higher concentration of circulating cholesterol.

One important field of investigation in lipoprotein metabolism will be the determination of the respective contribution of each apo-E receptor to the clearance of the apo-E containing lipoproteins and the effect of apo-E polymorphism on these phenomena in vivo. The structure of the apoE isoforms might explain the different affinity of this apolipoprotein for its different receptors in both physiological and pathological conditions. We also studied the relationships between apo-E genotypes and plasma cholesterol level in MCNS. There was no significant difference of cholesterol and trigly- ceride between common E3 and E4 variants pheno- type.

As a consequence of its biological importance apo-E polymorphism has been investigated extensively in many clinical and research laboratories, with research focusing on the development of rapid and accurate method for typing apo-E isoforms. Apo-E polymorphism is usually determined through phenotyping by means of IEF (isoelectric focusing) techniques, which allow the detection of charge variations consequent upon the minor sequence differences between the principal isoforms[9,25].

Originally, IEF was done on delipidated VLDL followed by protein staining. However, this method requires a large volume of serum and is timeconsuming and expensive, because ultrafiltration is required to isolate VLDL it thus is not suitable for large studies. At the same time as the technological development of IEF, two-dimensional electrophoresis has proved increasingly important in the study of a large fraction of gene expression and regulatory activity of cell.

This tecnique has the power required to overcome some of the problems concerning posttranslational modifications associated with IEF[3].

However, two-demensional electrophoresis is also a time-consuming and expensive technique. To avoid these problems of phenotyping, apoE genotyping has been developed. Fig. 4 summaries several methods that have been used to assign the common apo-E polymorphism.

After polymerase chain reaction(PCR) of the apo-E genomic sequence containing the common polymorphic sites, several different approaches have been proposed: HhaI endonuclease digestion[11], use of allelespecific oligonucleotide(ASO)[23], singlestrand conformation polymorphism(SSCP) technique[3], and sequencing. Apo-E genotyping by HhaI endonuclase digestion uses PCR amplification with oligonucleoties containing to the gene sequence related to the fragment between amino acid 112 and 158. The amplified products are then digested with HhaI and the electrophoresis

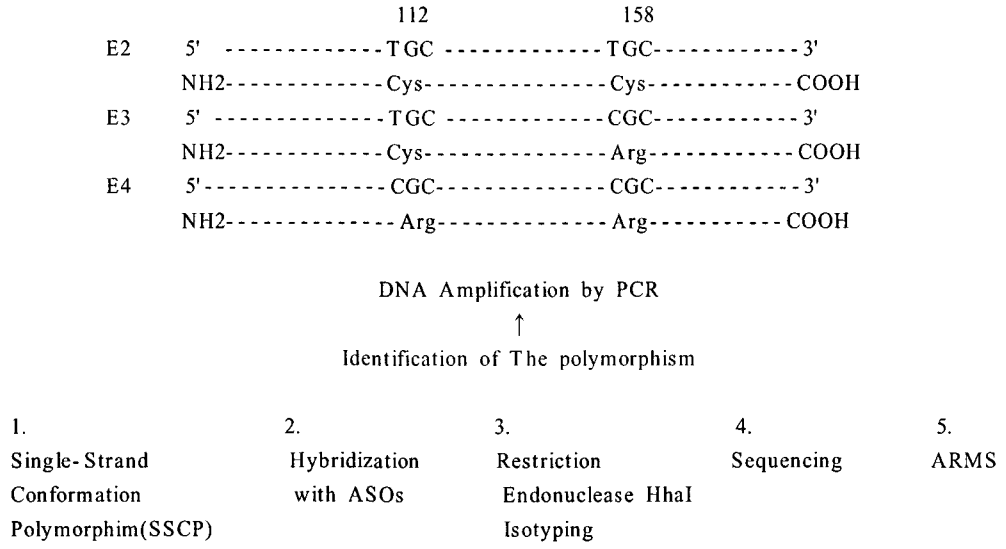

Fig. 4. Different Methods for Investigating Apo E Polymorphism at the Genomic Level

on polyacrylamide gel. This technique avoids the use of costly and time consuming hybridization and sequencing techniques. ASO methods detect apo-E after hybridization with oligoprobes, one containing the mutated fragment and the other not. This method requires isotopic labeling of the oligonucleotide probes.

Analysis of apo-E genotype by the amplification refractory mutation system is based on the use of an oligonucleotide specific for the mutation site as one of the PCR primers. If the primer is mismatched at the 3' oligonucleotide, amplification will not occur. The allele specific primer is therefore synthesized in two forms one with the mutant and the other with nonmutant 3' oligonu- cleotide. Amplification will occur with the mutant primer if the mutation is present in the DNA or with the non mutant primer if the mutation is absent, so that hybridization with labled ASOs unnecessary.

We firstly used RFLP with HhaI. since there were other restriction site on amplified product more smaller digested product made it difficult to discriminate apo-E genotype. We decided to use more simple and easy method, ARMS. We made primers with genotype specific sequence at 3'end. By using four allele-specific oligonucleotide primers, we could easily identify three common alleles of the apo-E polymorphism, e2, e3, e4.

This method was simple, reliable, and nonisotophic and obviates the need for digestion with restriction endonucleases or for hybridization with allele-specific oligonucleotide probes. These days, Many clinical reserch laboratories want to evaluate apo-E genotype for diagnosis of apoE- related diseases, such as Alzheimer disease, atherosclerosis, coronary

heart disease. Previous studies of apo-E genotype using ARMS used common primer and ARMS primers, however, we modified the method to use ARMS primers only. We firstly applied this method to type apoE in MCNS. Genotyping DNA by this method might be very useful for clinical diagnosis.

한 글 요 약

목 적 : 본 연구는 소아 미세변화 신증후군 환자와 IgAN에서 apoE 유전자형의 다형성을 알아보고, 스테로이드 반응과 빈발 재발 신증후군에서 apo-E 유전자형의 연관성을 관찰하므로 신증후군 예후인자로의 가능성을 밝히고, 신증후군에서 나타나는 고지질혈증과의 연관성을 알아보고자 하였다.

대상 및 방법 : 43명의 소아 신증후군 환자와 15명의 IgA신병증 환자를 대상으로 apo-E 유전자형을 조사하였다. 대조군은 50명의 혈연관계가 없는 건강한 혈액 공여자를 대상으로 하였다. Genomic DNA는 standard procedure에 따라 말초혈액의 백혈구로부터 분리하였다.

결 과 : 신증후군 환자에서 대조군보다 e4의 빈도가 유의하게 높았다.($P<0.01$). 그러나 IgAN에서는 e2가 대조군보다 2.6배나 높았다($P<0.01$). 빈발재발군 신증후군에서 e4의 빈도가 대조군 보다 4.6배, 비재발군 신증후군 보다 2배 높았다. 특히 e4/4는 빈발 재발군에서만 3명이 발견되었다. apo-E 유전자형에 따른 혈중 알부민, 콜레스테롤, 지질을 비교하였으나 정상 E3군과 E4 변이형에서 유의한 차이는 없었다($P>0.05$).

결 론 : 소아 미세변화 신증후군에서 apo-E 유전자형의 연구에서 e4가 대조군 보다 높은 빈도를 보였으며, IgAN에서는 e2와의 연관성을 보였다. e4 유전자형이 특히 빈발 재발군에서 비재발군에서 보다 2배나 높았으며, e4 homozygote는 빈발 재발군에서만 나타나 신증후군의 빈발재발과 스테로이드 의존성의 예후인자로 이용할 수 있으리라 생각된다.

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