ORIGINAL ARTICLE

Is dyslipidemia sustained during remission of nephrotic syndrome genetically determined?

Evaluation of genetic polymorphisms of proteins involved in lipoprotein metabolism in children and adolescents with nephrotic syndrome

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KEY WORDS

adenosine triphosphate binding cassette transporter A1 (ABCA1), apolipoprotein E (apoE), CETP, dyslipidemia, genetic polymorphism

ABSTRACT

INTRODUCTION In same patients lipid profile disturbances persist during nephrotic syndrome remission.

OBJECTIVES The aim of the study was to evaluate the impact of the genetic polymorphisms of proteins involved in lipoprotein metabolism on persistent abnormal lipid profile in patients with nephrotic syndrome during remission.

PATIENTS AND METHODS 50 patients aged between 5.8 and 16.6 years (mean age 10.45 ±3.04) with nephrotic syndrome in remission of at least 8 weeks' duration, including 12 steroid-resistant and 38 steroid-dependent cases, participated in the study. We evalauated associations between lipid profile and genetic polymorphisms, V771M, V825I, and R1587K of the gene encoding the cassette ABCA1 (adenosine triphosphate binding cassette transporter A1) protein synthesis, a ε3 polymorphism of the gene encoding the type ε of apolipoprotein E (apoE) synthesis and that of the gene encoding the cholesterol ester transfer protein (CETP) synthesis.

RESULTS Dyslipidemia was observed in 10/13 (76.9%) patients with V825I polymorphism vs. 27/37 (73%) of non-carriers, and in 16/21 (76.2%) patients with R1587K polymorphism vs. 21/29 (72.4%) in the remaining subjects. V771M polymorphism was found only in 2 (4%) patients and one subject had abnormal lipid profile. In the presence of CETP gene polymorphism, hiperlipoproteinemia was detected in 22/31 (71%) vs. 15/19 (78.9%) in the remaining cases. The ε3ε3 apoE genotype (observed most commonly in the healthy population) was found in the majority (n = 35; 70%) of patients. This genotype was also seen in most patients with abnormal serum lipid profile (in 26/37; 70.3%). Analysis of the whole population (ANOVA) did not show significant correlations between parameters of lipid profile and any of the polymorphisms studied.

CONCLUSIONS The study did not confirm associations between genetic polymorphisms of ABCA1 transporter, CETP and apoE and abnormal serum lipid profile during remission of nephrotic syndrome.

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Received: August 27, 2008.
Revision accepted: October 29, 2008.
Conflict of interest: none declared.
Pol Arch Med Wewn. 2009;
119 (1-2): 11-17

TABLE 1 Characteristics of patients

Patients		Total	Males	Females	Steroid-resistant
Number (n)		50	35	15	12
%		100	70	30	24
Mean age (years)		10.45	10.5	10.35	
SD		3.04	3.13	2.82	
Duration of treatment (years)		7.09	6.88	7.55	
SD		2.88	3.14	2.19	
Histological diagnosis	MCNS	21	14	7	1
	MPGN	26	19	7	9
	FSGS	3	1	2	2

Abbreviations: FSGS – focal segmental glomerulosclerosis, MCNS – minimal change nephrotic syndrome, MPGN – mesangial proliferative glomerulonephritis, SD – standard deviation

INTRODUCTION Dyslipoproteinemia is seen in the majority of patients with idiopathic nephrotic syndrome. The compensatory mechanism of increased hepatic synthesis of lipoproteins triggered by proteinuria is regarded as the major underlying cause. Proteinuria is an independent risk factor of cardiovascular complications.[1] Among secondary dyslipidemias reduced conversion of low-density lipoprotein cholesterol (LDL-C) to high-density lipoprotein cholesterol (HDL-C) and abnormal distribution of subclass HDL have particularly unfavorable effect.[2] Adenosine triphosphate binding cassette transporter A1 (ABCA1) has a crucial role in regulating cellular amounts of cholesterol and oxysterols as well as modulating the serum HDL-C level. This protein is the major modulator of removal of HDL-C fraction and phospholipids from cell membranes. It facilitates binding of intracellular phospholipids and cholesterol to apolipoprotein A1 (apoA1) associated with HDL, which determines the cholesterol efflux from vascular walls.[3-5] Non-estrified fatty acids, present in LDL-C, facilitate activation of polymorphic serum cholesterol ester transfer protein (CETP), which determines the size of lipoprotein molecules and their susceptibility to the catabolic process. An increase in CETP activity promotes conversion of HDL2 to HDL3 with decreased affinity to apoA1. It also diminishes the incorporation of apoA1 to HDL molecules, increasing their renal clearance and leading to hyperlipoproteinemia.[6,7] Extent and persistence of abnormalities in lipid profile might be associated with apolipoprotein E (apoE) deficiency which is related to apoE genetic polymorphism. A specific genetic variant accounts for a certain lipoprotein level. The presence of ε2 allele correlates with decreased cholesterol and increased triglyceride levels. ApoE deficiency facilitates LDL-C and very low-density lipoprotein (VLDL) accumulation in renal glomeruli and promotes proliferation of cells and mesangial matrix.[8,9] There have been conflicting reports on associations between specific apoE variants and renal disease. Some investigators have suggested higher incidence of specific genotypes in patients with nephritic syndrome and end-stage renal disease. Other reports have failed to show any difference in frequencies of individual apoE haplotypes between the healthy and the nephrotic subjects.[10,11] Clinical trials have demonstrated that in about half of the nephrotic cases lipid profile disturbances persist during remission, which due to their clinical outcomes worsens further prognosis.[12,13]

The aim of the study was to evaluate the specific genetic polymorphisms of selected proteins participating in metabolism of lipoprotein, including ABCA1, CETP and apoE and to test the hypothesis that they are associated with sustained lipid abnormalities observed during remission of nephrotic syndrome.

PATIENTS AND METHODS Overall 50 patients aged between 5.8 and 16.6 (mean age 10.45 ±3.04) years, including 12 cases of steroid-resistant and 38 of steroid-dependent nephrotic syndrome, were evaluated. Duration of the disease ranged from 2.6 to 13.8 (mean 7.09 ±2.88) years. In 46 patients lipid profile was evaluated during complete remission of at least 8 weeks' duration and in 4 patients during partial remission. Seven patients were treated with prednisone, 42 subjects received prednisone combined with cyclosporine (which was in 7 subjects substituted to mycophenolate mofetil), and one individual took angiotensin-converting enzyme inhibitor combined with angiotensin type 1 receptor blocker. Clinical characteristics of the patients are shown in TABLE 1.

Blood samples were drawn after the 12-hour overnight fast.

Total cholesterol and triglyceride levels were assayed with enzymatic methods cholesterol oxidase phenol 4-aminoantipyrine (CHOD-PAP) using the Spin-Reack set. LDL-C and VLDL-C lipoproteins were separated by ultracentrifugation and chemical precipitation using polyvinyle sulphate, HDL-C was isolated by chemical precipitation in presence of ions magnesium with use of volframic acid, and then fractions of cholesterol were estimated. The oxidized LDL-C level was ameasured using an ELISA (Bio-Medica reagents). Lipoproteins apoA1 and apoB were assayed with

TABLE 2 Median values of lipid profile parameters in patients with dyslipidemia (n = 37) and normolipidemia (n = 13) during remission of nephrotic syndrome

Parameter mg/dl	n = 13	n = 37	p[a]
TC (mg/dl)	175.8	232	<0.0001
HDL-C (mg/dl)	55	49	NS
LDL-C (mg/dl)	100.66	163.29	<0.0001
VLDL-C (mg/dl)	14.2308	21.6	0.0015
TG (mg/dl)	77.2222	156.25	0.00007
ApoB (g/dl)	0.8067	1.22	0.000004
ApoA1 (g/dl)	1.52	1.51	NS
Oxy-LDL (mU/ml)	278.28	504.9	0.0022
GPX (u/gHb)	32.30	30.71	0.0016
Lp(a) (mg/dl)	10.20	15.62	0.0184
Albumin (g/l)	40.73	37.52	0.0014

a Mann-Whitney's test

Abbreviations: apoA1 – apolipoprotein A1, apoB – apolipoprotein B, GPX – glutathione peroxidase, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, Lp (a) – lipoprotein (a), NS – not significant, oxy-LDL-C – oxidized LDL-cholesterol, TC – total cholesterol, TG – triglycerides, VLDL-C – very low-density lipoprotein cholesterol

TABLE 3 Number of patients with ABCA1 genetic polymorphisms

Patients	Polymorphic variants						
	V771M	V825I		R1587K		V825 I + R1587K	
	GA	GA	AA	GA	AA	GA-GA	GA-AA
s – d. ns n = 38	2	9	1	11	2	5	1
s – r. ns n = 12	–	3	–	6	2	2	1
Overall n = 50	2 (4%)	13 (26%)		21 (42%)		9 (18%)	

Abbreviations: AA, GA, GG – variants of polymorphism of ABCA1 gene, s-d ns – steroid-dependent nephrotic syndrome, s-r ns – steroid-resistant nephrotic syndrome, V771M, V825I, R587K – non-synonymous single nucleotide polymorphisms of ABCA1 cassette gene

the immunoturbidic method using the Orion-Diagnostica kits. Lipoprotein (a) and apoE were assayed with radioimmunodiffusion on agarose gel by using specific antibodies. Lecithine acetyltransferase was determined by the enzymatic method using the Wak-Chemie kit. Glutathione and glutathione peroxidase in isolated erythrocytes were measured by the spectrophotometric method using the Oxis assay.

The results were assessed in comparison with normal reference values coming from the evaluation of the healthy Warszawa population aged between 6 and 20, published previously by investigators from the Children's Memorial Health Institute.[14]

In all subjects the following genetic polymorphisms were evaluated: – three non-synonymous single nucleotide polymorphisms variants of the ABCA1 gene (V771M, V825I and R1587K), a polymorphism of the CETP gene and polymorphisms of the apoE gene.

The apoE gene variants were genotyped by the modified polymerase chain reaction restrictive fragments length polymorphism (PCR-RFLP) method developed in 1990 by Hixson and Vernier.[15] It consists in DNA genome amplification with a pair of starters, sense and nonsense primers, and subsequent digestion of the reaction product restriction enzyme *Hha I*, and next separation of restriction fragments by electrophoresis in 4% agarose gel with ethidine bromide. Heterozygotic genotypes were identified on the basis of the presence of restriction fragments specific to specific alleles. Three single nucleotide variants of guanine (G) > adenine (A) transition of the ABCA1 gene such as V771M, V825I i R1587K that determine the HDL-C plasma level were genotyped by the self-developed method, using the restriction enzyme *Tail* for determining polymorphism V771M, the restriction enzyme *MboI* for polymorphism V825I and the restriction enzyme *Bgl* II for R1587K polymorphism. Transition G to A in position 2310 cDNA ABCA1 caused substitution of valine to methionine in position 771 of the polypeptide chain of ABCA1 protein (polymorphism V771M-G2310A). Transition G to A in position 2472 cDNA caused substitution of valine to isoleucine in position 825 of the polypeptide chain of ABCA1 protein (polymorphism V825I-G2472A). Transition G to A in position 4759 of cDNA caused substitution of arginine to lysine in position 1587 of the polypeptide chain of ABCA1 protein (polymorphism R1587K-G4759A). A CETP gene polymorphism was determined similarly to ABCA1, with the amplification DNA genome method, using DNA amplification with a pair of starters for nucleotide restriction in position –629 and –38.

Statistical analysis The medians of lipid profile parameters were compared with reference values and between groups, divided in terms of presence or absence of lipid abnormalities during remission. The correlations between specific genetic polymorphisms and lipid profile abnormalities were analyzed. The normality of variable distribution was tested with Kolmogorov-Smirnov and Shapiro-Wilk tests. As the variables were not normally distributed, comparison of medians between groups was performed with Mann-Whitney's test. Analysis of correlations (in the whole population) was performed with non-parametric ANOVA Kruskal-Wallis's test. The study protocol, including genetic and biochemical assessment, was designed in adherence to the Declaration of Helsinki. Written informed consent was obtained from all parents and the adolescent patients older than 16 years of age. A protocol of biochemical and genetic investigations was approved by the Ethical Committee of the Children's Health Center.

TABLE 4 Distribution of significant (compared to reference data) lipid abnormalities in subgroups of patients divided with regard to ABCA1 genetic polymorphisms

Polymorphism / Disturbances	V771M n = 2 (4%)		V825I n = 13 (26%)		R1587K n = 21 (42%)		V8251 + R1587K n = 9 (18%)	
	GA n = 2	AA	GA n = 12	AA n = 1	GA n = 17	AA n = 4	GA-GA n = 7	GA-AA n = 2
↑ TC	–	–	1	–	2	–	1	–
↑ TC + ↑ TG	1	–	2	–	3	1	2	2
↑TC + ↑ TG + ↓ HDL-C	–	–	2	–	4	1	1	–
↑TC + ↓ HDL-C	–	–	1	–	–	1	–	–
↑ TG + ↓ HDL-C	–	–	1	–	1		1	–
+ ↓ HDL-C	–	–	2	1	2	1	1	–
Overall	1 50%	–	10 76.9%		16 76.2%		7 77.8%	
No disturbances	1 50%	–	3 23.1%		5 33.8%		2 22.2%	

Abbreviations: AA, GA, GG – variants of polymorphism of ABCA1 gene, HDL-C – fraction HDL of cholesterol, TC – total cholesterol, TG – triglycerides, V771M, V825I, R587K – non-synonymous single nucleotide polymorphisms of ABCA1 cassette gene

TABLE 5 Distribution of significant (compared to reference data) lipid abnormalities in subgroups of patients divided with regard to CETP gene polymorphisms

Polymorphism / Disturbances	GA variant n = 25 (50%)	AA variant n = 6 (12%)
↑ TC + TG	3	2
↑TC + TG + ↓ HDL-C	3	2
↑ TG + ↓ HDL-C	1	–
↑ TC + ↓ HDL-C	1	–
↑ TC	4	1 –
↓ HDL-C	4	1
Overall	22/31 71%	
No lipid disturbances	9/31 29%	

Abbreviations: AA, GA, GG – variants of polymorphism of the gene, HDL-C – fraction HDL of cholesterol, TC – total cholesterol, TG – triglycerides

RESULTS Persistent lipid profile abnormalities were present in 37 of 50 (74%) patients during remission of nephrotic syndrome. Comparison and analysis of median values of the lipid parameters in dys- and normolipidemic patients are presented in TABLE 2. Significantly higher values of the majority of parameters were observed in dyslipidemic patients. There was no significant difference between steroid-dependent and steroid-resistant cases in this term. The only significant difference was observed in serum albumin levels, which were lower in steroid-resistant patients (p = 0.028).

The number and distribution of specific gene polymorphisms of ABCA1 are presented in TABLE 3. The V771M of ABCA1 gene polymorphism of GA variant was confirmed in 2 (4%) patients. The V825I polymorphism of ABCA1 gene of GA variant was confirmed in 12 of 50 (24%) cases. The V825I polymorphism of AA variant was confirmed in 1/50 (2%) case with the low HDL-C serum level. Overall, the V825I polymorphism was demonstrated in 13 of 50 (26%) patients. The R1587K polymorphism of ABCA1 gene of GA variant was detected in 17 of 50 (34%) patients. The R1587K polymorphism of AA variant was shown in 4 of 50 (8%) patients. In all 4 the lipid profile was abnormal. Overall, the R1587K polymorphism was found in 21 of 50 (42%) patients. The presence of 2 gene polymorphisms (V825I and R1587K) was detected in 9 of 50 (18%) patients. In this group GA-GA variants were confirmed in 7 and GA-AA variants in 2 patients. Abnormal lipid profile was present in 7 of 9 (77.8%) of these patients. The distribution of significant (compared to reference values) lipid profile abnormalities in subgroups is shown in TABLE 4. Data on not significant parameters of lipid profile were not included in the table to make the presentation clearer. Polymorphisms of the ABCA1 gene were detected in 27 (54%) patients.

Among 13 subjects with confirmed V825I polymorphisms, abnormal lipid profile was shown in the majority of cases (10; 76.9%). Among the remaining 37 children (without ABCA1 gene polymorphisms) abnormal lipid profile was present in 27 (73%).

Among 21 patients with R1587K polymorphisms, abnormal lipid profile was present in 16 (76.2%). The presence of CETP gene polymorphisms was detected in 31 of 50 (62%) patients, including 25 cases of the GA and 6 of the AA variant. Within this group lipid profile abnormalities were shown in 22 (71%). Data are presented in TABLE 5.

TABLE 6 Distribution of significant (compared to reference data) lipid abnormalities in subgroups of patients divided with regard to apoE gene polymorphisms

Lipid disturbances	ApoE subtype					
	ε3ε3 n = 35 (70%)	ε3ε4 n = 6	ε2ε3 n = 5	ε2ε4 n = 1	ε4ε4 n = 2	ε2ε2 n = 1
↑TC + ↑TG + ↓ HDL-C	4	2	2	1	–	–
↑TG + ↓HDL-C	1	–	–	–	1	–
↓ HDL-C	5	1	1	–	–	1
↑TC	7	1	–	–	–	–
↑TC + ↑TG	7	1	–	–	–	–
↑TC + ↓ HDL-C	2	–	–	–	–	–
Overall	26 (74.3%)	5	3	1	1	1
No disturbances	9 (25.7%)	1	2	–	1	–

Abbreviations: apoE – apolipoprotein E, HDL-C – fraction HDL of cholesterol, TC – total cholesterol, TG – triglycerides

TABLE 7 Distribution of lipid disturbances prevalence in children with and without specific genetic polymorphisms(summary)

Polymorphism	Number of patients (n)	
	with polymorphisms and lipid abnormalities	with no polymorphisms and lipid abnormalities
V771M n = 2/50	1/2 (50%)	36/48 (76.6%)
V825I n = 13/50	10/13 (76.9%)	27/37 (73%)
R1587K n = 21/50	16/21 (76.2%)	21/29 (72.4%)
CETP n = 31/50	22/31 (71%)	15/19 (78.9%)
apo ε3ε3 n = 35/50	26/35 (74.35)	–
Other types apoE n = 15/50	12/15 (73.3%)	–

Abbreviations: apoE- polymorphism of apoE gene, CETP – polymorphism of CETP gene, V771M, V825I, R587K – non-synonymous single nucleotide polymorphisms of ABCA1 cassette gene

Among the remaining 19 subjects (not showing this polymorphism) abnormal lipid profile was observed in 15 patients (78.9%).

The apo ε3ε3 genotype (most commonly present in the healthy population) was found in 35 (70%) patients. Abnormal lipid profile was observed in 26 (74.3%) of these patients. Data are presented in TABLE 6.

The summary of the data on incidence of specific polymorphisms in normo- and dyslipidemic patients are presented in TABLE 7.

The results of analysis (ANOVA) of correlations between all consecutive genotypes in the whole population (n = 50) and all the studied parameters of lipid profile are shown in TABLE 8. No significant correlation was observed in any case. There was a non-significant trend (p = 0.067) in terms of association between the triglyceride level and R1587K genotype.

DISCUSSION Reports on lipid profile in nephrotic patients consistently indicated that these abnormalities can also persist during remission.[13,16] Therefore, genetic background of disturbances in the activity of specific receptors or proteins involved in lipid metabolism are considered.[17] Among pathomechanims of such changes, hypoalbuminemia and the disturbed activity of hepatic LDL receptor have been listed.[18]

Some reports have suggested an influence of genetic variants of apoE on lipid abnormalities. There are few clinical studies on the frequency of specific alleles. Some reports have suggested the association between specific genotypes and focal segmental glomerulosclerosis (FSGS). Atilla et al. showed that prevalence of ε2 allele and ε2/ε3 genotype is higher in children with steroid-resistant nephrotic syndrome and FSGS.[10] Oda et al. demonstrated higher prevalence of apoε2 alleles in patients with chronic renal failure.[8] In patients participating in the current study, as in the report by Brushi et al., apoε2 and apo ε4 alleles in subjects with FSGS were more frequent.[11] The apoε3 was the predominant allele in these patients. Available data indicate the influence of sequence variants in ABCA1 and CETP on the plasma lipoprotein level in the general population.[17] However, there are no reports concerning genetic polymorphisms of these proteins and its possible clinical relevance in nephrotic patients. The results of the present study do not confirm the existence of such correlations. Among the causes which might explain persistent dyslipidemia in these patients is chronic pharmacotherapy, including cyclosporine A and steroids, with their unfavorable lipid-related side-effects.[19-21] These drugs were administered to almost all studied patients during remission, which was sustained by such treatment.

Of note, the significant limitation of the current study was a small number of patients (n = 50), therefore this observation should be verified in a multi-center trial involving a substantially larger population. Due to high costs of genotyping, we were unable to enrol a larger control group which could serve for analysis of distribution of specific genotypes in the healthy population. Recruitment of such a group would increase the statistical power of the analysis, and enable the use of parametric tests.

The results indicate the need for lipid profile monitoring not only during relapse, but also during remission of nephrotic syndrome, and for considering initiation of hypolipemic management in patients with persistent disturbances.

CONCLUSIONS Up to 74% of nephrotic patients demonstrated persistent abnormalities of lipid profile during remission of the disease.

TABLE 8 Summary results of statistical analysis (ANOVA) (numbers reflect the level of significance) of associations between values of lipid parameters and presence of specific gene polymorphisms in the whole population (n = 50)

Variable	V771M	V825I	R1587K	ApoE	CETP	Sum of mutations in one case
C	0.363	0.146	0.841	0.385	0.524	0.827
HDL-C	0.164	0.664	0.580	0.498	0.189	0.713
LDL-C	0.419	0.190	0.867	0.239	0.173	0.704
VLD-CL	0.834	0.660	0.130	0.251	0.323	0.717
Triglicerydes	0.959	0.404	0.067	0.647	0.462	0.578
ApoB	0.474	0.169	0.976	0.248	0.294	0.762
ApoA1	0.582	0.622	0.719	0.616	0.333	0.720
Oxy LDL-C	0.484	0.423	0.103	0.229	0.468	0.625
LCAT	0.099	0.285	0.763	0.470	0.592	0.927
GPX	0.247	0.591	0.259	0.659	0.157	0.663
GSH	0.582	0.463	0.812	0.513	0.263	0.520

Abbreviations: ApoA1 – apolipoprotein A1, ApoB – apolipoprotein B, GPX – glutathione peroxidase, GSH – glutathione, HDL-C – HDL-cholesterol, LCAT – lecithine acyltransferase, LDL-C – LDL-cholesterol, oxy-LDL-C – oxidized LDL-cholesterol, TC – total cholesterol, TG – triglycerides, VLDL-C – VLDL-cholesterol

Analysis of 50 patients did not confirm the significant impact of specific polymorphisms of genes coding selected proteins involved in lipoprotein metabolism on persistent lipid profile abnormalities during remission of nephrotic syndrome.

Acknowledgements The study was supported by the grant of Polish Ministry of Science (No 2 PO 5E 03628).

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