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APOPROTEIN(A) ISOFORMS AND PLASMA LP(A) CONCENTRATION IN MEMBERS OF FOUR FAMILIES

IZOFORME APOPROTEIN(a) I KONCENTRACIJA LP(a) U PLAZMI KOD ČLANOVA ČETIRI PORODICE

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Summary: Apoprotein(a) is a multikringle protein which shows a genetically inherited size polymorphism. The APO(a) gene is located at the telomeric region of chromosome 6q2.6-q 2.7. Apo(a) size polymorphism is a major determinant of Lp(a) levels. The aim of this study is to describe the influence of apo(a) size polymorphism on the plasma Lp(a) levels in the members of four families. K_3EDTA plasma was obtained from every subject after over-night fast. Apo(a) isoforms were determined by 3–15% SDS-PAGE followed by Western immunoblot technique. Plasma Lp(a) level was determined with immunonephelometric method. Every child inherited one isoform from its mother and the other from its father. The children from the first family had Lp(a) levels similar to those measured in their parents. The daughters from the second and fourth family inherited the dominant S3 apo(a) isoform from their mothers and also mother's high Lp(a) levels (0.365 g/L – daughter from the second, and 0.465 g/L and 0.446 g/L – daughter from the fourth family respectively). The elder daughter from the third family, carrier of double banded S4S1 apo(a) isoform, had the highest Lp(a) level among the children from all four families. We found out a generation decrease of the Lp(a) level in two families. On the basis of our findings we concluded that the inheritance of the apo(a) isoforms in the members of all four families is in accordance with the simple Mendelian's model and that the apo(a) size polymorphism influences the Lp(a) level in the blood of the examined subjects.

Key words: apoprotein(a) isoforms, lipoprotein(a) concentration, heritage

Kratak sadržaj: Apoprotein(a) je multikringle protein koji se odlikuje genetski nasleđenim polimorfizmom. Humani gen APO(a) nalazi se u telomeričkom regionu hromozoma 6q2.6-q 2.7. Polimorfizam apo(a) glavna je determinanta nivoa Lp(a). Cilj studije je da opiše uticaj polimorfizma apo(a) na nivoe Lp(a) u plazmi članova četiri porodice. K_3EDTA plazma je dobijena od svakog člana porodice posle celonoćnog posta. Izoforme apo(a) određene su uz pomoću 3–15% SDS-PAGE-a, a zatim tehnikom »Western imunoblot«. Nivoi Lp(a) određivani su imunonefelometrijskom metodom. Svako dete nasledilo je jednu izoformu od majke, a drugu od oca. Deca iz prve porodice nasledila su apo(a) izoforme i to po jednu od oba roditelja i kod nih su nivoi Lp(a) bili slični onima koji su izmereni kod njihovih roditelja. Ćerke iz druge i četvrte porodice nasledile su dominantnu majčinu S3 apo(a) izoformu, kao i majčin visok Lp(a) nivo (0,360 g/L u drugoj odnosno 0,465 g/L i 0,446 g/L u krvi ćerke četvrte porodice). U krvi starije ćerke, treće porodice, nosioca S4S1 apo(a) izoforme izmeren je najviši nivo Lp(a) u krvi (0,540 g/L). Uočeno je generacijsko snižavanje nivoa Lp(a) kod dece u trećoj i četvrtoj porodici. Na osnovu dobijenog zaključeno je da se nasleđivanje kod članova svih porodica odvija po jednostavnom Medeljejevom sistemu i da apo(a) izoforme utiču na nivoa Lp(a) u krvi.

Ključne reči: apoprotein(a) izoforme, koncentracija lipoprotein(a), nasleđivanje

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Introduction

Lipoprotein(a) [Lp(a)] due to its composition and properties is still an exclusive, unique and an enigmatic particle in the class of lipoproteins. The peculiarity of this lipoprotein arises from: 1) the presence of high molecular weight protein, named apoprotein(a) [apo(a)]; 2) the plasma Lp(a) levels that vary over

1000-fold between individuals and 3) the remarkable intraindividual stability of Lp(a) during the lifetime of any individual (1). Lp(a) was discovered in 1962 by the Norwegian scientist Kare Berg (2).

Lp(a) is an LDL-like macromolecular complex. It is built of a low density lipid core rich with cholesterol--ester (30–45% of weight) and a high density protein wrapping which contains two major proteins, apoprotein B_{100} [$ApoB_{100}$] attached by a disulfide bridge to highly glycosylated apoprotein(a) (3). Apo(a) is a hydrophilic, multikringle protein with little affinity for lipids and it circulates in the plasma as part of the Lp(a) particle and as a free apo(a) fragment (4, 5). The cDNA (6) and amino acid sequence analysis (7) of apo(a) revealed a highly significant structure homology to plasminogen. The variable number of Kringle IV in apo(a) share a variable degree of structure homology (78–88%) with K–IV in plasminogen, and serine-protease domain and Kringle V (over 88% and up to 94%, respectively) with their counterparts in plasminogen (8).

Apo(a) is a member of a Kringle-containing protein family that includes prothrombin, factor XII, tissue plasminogen activator (t-PA), urokinase plasminogen activator (u-PA), plasminogen, etc. Based on the plasminogen nomenclature, the kringles have been named as kringle-IV (K–IV) and kringle-V (K–V). K–IV in PLG is present as a single copy, but K–IV of apo(a) is built of ten different units named from type 1 to type 10. Nine of the K–IV apo(a) units (K–IV type 1 and type 3–10) occur as a single copy and have a different amino acid sequence. K–IV type 2 vary in copy number (3–42 among individuals) and they are completely identical at the amino acid sequence level. The variable number of K-IV type 2 repeats affects the apo(a) size polymorphism that varies from >300 to < 800 KDa (9).

Initially, six human apo(a) isoforms were detected by SDS-polyacrilamide gel electrophoresis (SDS-PAGE) with the pioneer's work of Utermann (1). Later, Marcovina identified 34 apo(a) isoforms by SDS-agarose gel electrophoresis (SDS-AGE) among American blacks and whites (10). These six isoforms were designated as F (faster), B (similar to $ApoB_{100}$), S_1, S_2, S_3, and S_4, (slower), according to their relative mobility compared with $ApoB_{100}$. F, B, S_1, and S_2, are known as low-molecular weight (LMW) apo(a) isoforms and S_3 and S_4 as high molecular weight (HMW) isoforms. Individuals with the LMW apo(a) phenotype, on average, have a higher Lp(a) concentration and those with HMW and »null« phenotype express lower Lp(a) values. Thus, there is an inverse association between the molecular weight of apo(a) isoforms and Lp(a) plasma concentration (1).

More than 90% of the variability in Lp(a) concentration is almost completely determined by the variation at the high polymorphic APO(a) gene that encodes the apoprotein(a) synthesis (11, 12). Human APO(a) gene is localized on the telomeric region of chromosome 6(6q26–27). Several polymorphisms have been identified in the APO(a) gene which affect Lp(a) plasma levels. First, a strong and direct influence have a variable number of 5,6 kb repeat units that code the protein motif, called kringle IV type 2 (13). The number of K-IV type 2 tandemly repeats, is negatively associated with Lp(a) levels in plasma; it has been explained that the number Kringle IV type 2 repeats was responsible for only 30–70% of the total variability in Lp(a) concentration because it has been investigated that same sized apo(a) produced by different alleles may express up to 200-fold difference in the Lp(a) concentration (14).

The second locus that affects Lp(a) concentration is a pentanucleotide repeat polymorphism (PNRP), located in the 5' flanking region of the APO(a) gene. This polymorphism contributes to variations of Lp(a) levels in Caucasians with 10–14% (15).

The third intragenetic polymorphism is +93C/T transition, located in 5'untranslated region of the APO(a) gene; the study done by Kraft has shown that C/T transition has been associated with significant reduction in Lp(a) levels only in Africans, and not in Caucasians (16).

In the case when apo(a) isoforms cannot be visualized with immunoblott analyses on the nitrocellulose membrane, the explanation is the existence of »null« alleles in the APO(a) gene (1).

The aim of the study was to analyzed the influence of apo(a) size polymorphism on plasma Lp(a) concentration in the members of four families.

Material and Methods

Members of four families were included in this study. Venous blood was collected from the subjects after a 12 hour over-night fast in two different tubes (one with K_3EDTA and the second without anticoagulant). Plasma was separated by low-speed centrifugation, subdivided into several small samples and immediately frozen and stored at –80 °C until analyzing. The lipid parameters were analyzed in fresh sera after low-speed centrifugation of the blood.

Apo(a) phenotyping was performed by 3–15% gradient SDS–PAGE in Mini-Protean II Bio-Rad Vertical System. The procedure lasted for three days. The first day, 12 mini gradient gels were cast. The second day, the treated plasma samples with SDS, glycerol, bromphenol blue and β-mercaptoethanol and standard samples (IMMUNO-AG, Vienna, Austria) were applied in the wells of the gels. The electrophoresis ran in the Tris-glycine buffer, until the blue front departed the gel. Then the proteins were transferred to nitrocellulose membrane (S&S NC; BA 83; 0.2 mm) by electroblotting in Tris-glycine-methanol buffer, using Hoefer TE 22 Mini Tank Transfer Unit which was connected to a cooling bath, set to +10 °C. The third day, the apo(a) isoforms were visualized immunochemically using an Lp(a) phenotyping kit (Immuno-AG, Vienna).

According to their relative electrophoretic mobility on SDS-PAGE, compared with $ApoB_{100}$, apo(a) isoforms were designated, using Utermann's terminology, into single banded F, B and S1, S2, S3, and S4 and into respective double-band phenotypes. The phenotype was defined as »null« when the blots showed no bands at all (1).

The molecular mass of each apo(a) band in samples was estimated by comparison with the molecular mass of apo(a) bands presented in the standard, loaded on the gel in an immediately adjacent lane, using Pharmacia-Biotech laser scanner, with Image Master Software.

Biochemical measurements

The concentration of Lp(a), ApoA-1, and ApoB was determined with the immunonephelometric method kits (Dade-Behring, Germany) on a Behring Nephelometer Analyzer. Triglycerides and total cholesterol were determined in fresh sera, using standard enzymatic CHOD-PAP test kits (Randox Laboratories). HDL-cholesterol was measured by assaying the cholesterol concentration in the supernatant obtained after precipitation of the other lipoproteins by a mixture of phosphovolframate and $MgCl_2$. LDL-cholesterol was calculated by the Friedewald's formula (17).

Glucose was analyzed by glucose oxidize technique on a Beckman Glucose Analyzer.

Statistical analyses

Data are expressed as means ± standard deviation (SD) and the concentration of lipoprotein(a) additionally as median (18).

Results

The plasma samples taken from members of one family were loaded on one PA gel, with standard samples.

Family 1. The immunoblott with separated apo(a) isoforms of the members of family 1 is shown in *Figure 1*. This family consisted of 6 members: father, mother, daughter and the daughter's own family: her husband and her two sons.

Figure 1 shows that the father is a double-banded carrier of two HMW >S4S4 apo(a) isoforms and measured plasma Lp(a) concentration of 0.292 g/L. The mother manifests a single banded HMW <S4 apo(a) phenotype. The daughter is also a single banded carrier and she inherited the S4 isoform from her father and »null« phenotype from her mother. In both (mother and daughter) we measured the same plasma Lp(a) concentration (0.123 g/L).

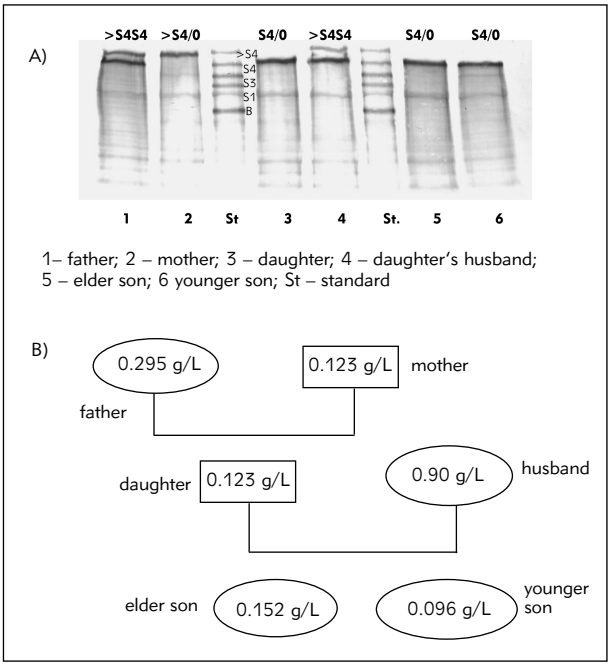

Figure 1 NC membrane with apo(a) isoforms (A) and a simple pedigree diagram with Lp(a) concentration (B) in members in family 1.

Table I The concentration of lipid parameters in the members of family 1.

Variables	Father	Mother	Daughter	Daughter's husband	Daughter's elder son	Daughter's younger son
Total cholesterol (mmol/L)	4.4	5.4	5.3	6.4	4.8	3.5
HDL-cholesterol (mmol/L)	1.6	1.4	1.4	1.6	1.5	1.0
LDL-cholesterol (mmol/L)	2.4	3.5	3.3	4.3	2.89	2.1
Triglycerides (mmo/L)	0.8	1.1	1.2	1.1	0.9	0.8
Apo A1 (g/L)	1.617	1.561	1.482	1.595	1.100	1.146
Apo B (g/L)	0.803	1.288	0.980	1.079	0.843	0.815
Glucose (mmol/L)	5.4	6.6	4.8	4.9	4.5	4.3

The daughter's husband is a carrier of double banded, HMW, >S4S4 apo(a) isoforms; we measured 0.098 g/L Lp(a) concentration in his blood. Both children are descendents to a single phenotype, as well as their mother; we visualized only one HMW S4 apo(a) isoform for them which they have inherited from their father, and the »null« phenotype from their mother. We determined 0.152 g/L Lp(a) level for the elder and < 0.096 g/L Lp(a) level for the younger son.

The concentration of lipid parameters, ApoA and Apo B in plasma and sera of members in family 1 were within the reference limits (*Table I*).

The heritage of apo(a) isoforms in *the Family 2* is shown in *Figure 2*.

It can be seen that the father is the HMW single banded S4 apo(a) carrier, (MM-703.60 kDa) and measured Lp(a) level of <0.096 g/L in his blood. The mother is a HMW double banded >S4S3 apo(a) carrier, with determined MM-759.30 and 592.00 kDa of each band respectively. The immunoblott shows that she has a dominant S3 apo(a) isoform. Although she has two HMW apo(a) isoforms, we measured a high plasma Lp(a) concentration of 0.325 g/L for her. The daughter has inherited the father's S4 apo(a) isoform with determined MM-708.69 kDa, and the mother's dominant S3 apo(a) isoform (MM-597.01 kDa). We also determined a high Lp(a) level of 0.364 g/L in her blood, as well as in her mother (*Figure 2*).

Only the concentration of the total cholesterol was increased in all members of *family 2* above the recommended 5.17 mmol/L, whereas LDL cholesterol was increased only in the mother. The other parameters were within reference limits (*Table II*).

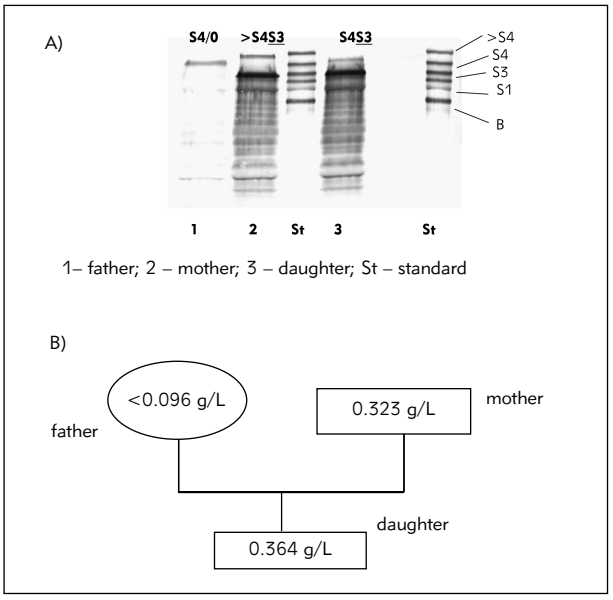

Figure 2 NC membrane with apo(a) isoforms (A) and simple pedigree diagram with Lp(a) concentration (B) of members in family 2.

Table II The concentration of lipid parameters in the members of family 2.

Variables	Father	Mother	Daughter
Total cholesterol (mmol/L)	5.7	6.2	5.4
HDL-cholesterol (mmol/L)	1.5	1.6	1.5
LDL-cholesterol (mmol/L)	3.8	4.2	3.4
Triglycerides (mmo/L)	0.9	0.8	1.0
Apo A1 (g/L)	1.730	1.996	1.725
Apo B (g/L)	1.063	1.067	0.881
Glucose (mmol/L)	5.3	5.0	4.9

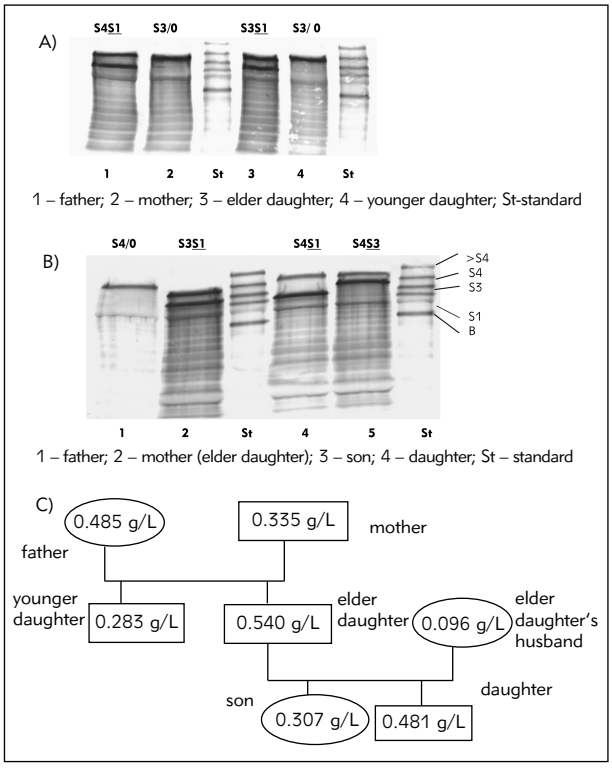

Figure 3 The NC membrane with apo(a) isoforms of family-3-a (A) the elder's daughter family 3-b (B) and simple pedigree diagram with plasma Lp(a) concentration of the whole family 3 (C).

Family 3, as well as family 1, actually consisted of 2 narrowed families with 7 members: mother, father their two daughters and the family of the elder daughter (husband, son and daughter) (*Figure 3*).

The immunoblot 3-a shows that the father is a carrier of two apo(a) isoforms, one HMW-S4 with determined MM-681.00 kDa and a LMW apo(a) isoform MM-531.56 kDa; we measured a high Lp(a) level of 0.485 g/L in his blood. The mother was a single banded HMW-S3 apo(a) phenotype carrier (MM-634.94

Table III The concentration of lipid parameters in the members of family 3-a.

Variables	Father	Mother	Younger daughter	Elder daughter
Total cholesterol (mmol/L)	5.4	6.1	4.9	6.5
HDL-cholesterol (mmol/L)	0.9	1.4	1.3	1.4
LDL-cholesterol (mmol/L)	3.7	3.3	3.1	4.6
Triglycerides (mmo/L)	1.6	3.1	1.0	1.0
Apo A1 (g/L)	1.007	1.529	1.636	1.672
Apo B (g/L)	1.406	1.165	1.024	0.955
Glucose (mmol/L)	5.2	6.2	5.7	5.0

kDa) which also manifested a high Lp(a) concentration of 0.335 g/L. The younger daughter was a single banded apo(a) phenotype carrier, as well as her mother, but she inherited the father's S4 apo(a) isoform (MM-689.54 kDa) and the »null« phenotype from her mother; we measured an Lp(a) level of 0.283 g/L in her blood. The elder daughter is a carrier of two apo(a) isoforms like her father, with an inherited S3 apo(a) isoform from her mother (MM-639.93 kDa) and a dominant LMW S1 apo(a) isoform from her father (MM-548.73 kDa). We measured the highest Lp(a) concentration of 0.540 g/L blood compared to all other members of family – 3.

The elder daughter's husband (*Figure 3-b*) is the carrier of a single HMW-S4 apo(a) isoform and he has a low Lp(a) level of < 0.096 g/L in his blood. Both children, shown in *Figure 3-b*, are double banded apo(a) carriers: S4S3 (daughter), and S4S1 (son). Both children have inherited the father's S4 apo(a) isoform. The daughter inherited the HMW-S3 apo(a) isoform from her mother and for her it was dominant compared to the father's S4; the Lp(a) concentration for the daughter was 0.480 g/L. The son inherited the LMW-S1 apo(a) isoform from his mother and for him it was dominant compared to the father's S4; in his blood we determined an Lp(a) concentration of 0.307 g/L. In spite of the fact that the daughter inherited mother's HMW S3 apo(a) isoform she had a higher Lp(a) concentration of 0.480 g/L compared to her brother (0.307 g/L).

The lipid status and the concentration of ApoA1 and Apo B for the whole family 3 are shown in *Table III* and *Table IV*.

The tables display that the total cholesterol is increased (above recommended >5.17 mmol/L) for the parents, the elder daughter, and particularly for her husband (7.0 mmol/L). For the two children, in addition to the increased Lp(a) values, the lipid status parameters were within the reference limits (*Table IV*).

The analysis of NC membrane in *Family 4* shows that the father is the »null« allele carrier and the mother an HMW S3apo(a) isoform carrier, with a rather high Lp(a) concentration of 0.741 g/L blood determined for

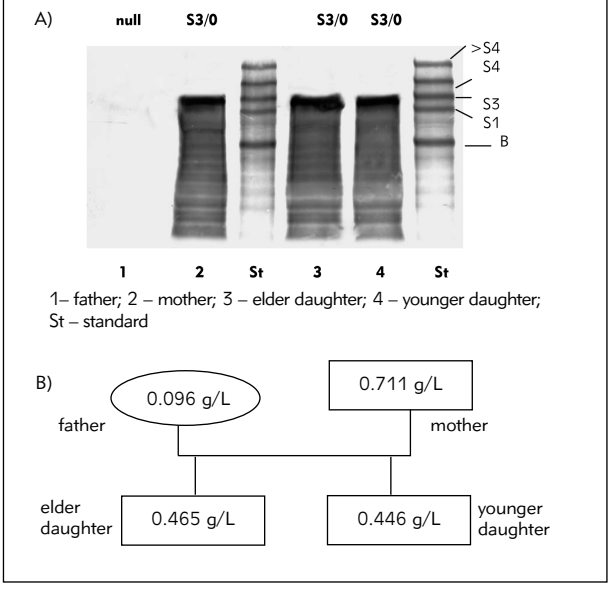

Figure 4 NC membrane with apo(a) isoforms (A) and simple pedigree diagram with Lp(a) concentration (B) of members in family 4.

Table IV The concentration of lipid parameters in the members of family 3-b.

Variables	Mother (elder daughter)	Husband	Son	Daughter
Total cholesterol (mmol/L)	6.5	7.0	4.4	4.6
HDL cholesterol (mmol/L)	1.4	0.9	1.0	1.6
LDL cholesterol (mmol/L)	4.6	4.9	2.9	2.8
Triglycerides (mmo/L)	1.0	2.7	1.2	0.5
Apo A1 (g/L)	1.672	1.363	1.439	1.529
Apo B (g/L)	0.955	1.432	0.939	0.616
Glucose (mmol/L)	5.0	4.9	4.6	4.2

Table V The concentration of lipid parameters in the members of family 4.

Variables	Father	Mother	Elder daughter	Younger daughter
Total cholesterol (mmol/L)	4.8	9.5	4.5	5.6
HDL cholesterol (mmol/L)	1.2	1.1	1.0	1.5
LDL cholesterol (mmol/L)	3.3	7.3	3.4	3.6
Triglycerides (mmo/L)	0.7	2.3	1.3	1.2
Apo A1 (g/L)	1.429	1.506	1.516	1.639
Apo B (g/L)	0.965	2.344	0.832	1.621
Glucose (mmol/L)	4.7	5.9	5.0	4.6

her. Both daughters inherited the »null« phenotype from their father, and the dominant HMW S3 apo(a) isoform from their mother. We noticed a generation decrease of Lp(a) concentration in both girls, with the measured values of 0.465 g/L for the elder daughter and 0.446 g/L for the younger one compared to their mother (*Figure 4*).

We found a hyperlipidemia in the mother of family 4. The parameters of the lipid status in the rest of the family 4 were within reference limits (*Table V*).

Discussion

Twin, sib pair, family and ethnic groups studies, have demonstrated that the Lp(a) plasma levels are genetically determined, and that the inheritance follows the Mendelian's model (19–22).

During the 1990s, by analyzing the Lp(a) concentration in monozygotic and dizygotic twins it was proved that there is a strong correlation regarding the Lp(a) concentration between twins ($r = 0.94$). Sixty five paires out of 125 bi-ovarian twin girls had exactly the same apo(a) phenotype, which confirms the strong determination of apoprotein (a) and Lp(a) (21).

The apo(a) isoforms inheritance examined among the members of four families was in accordance with the Mendelian model, i.e. children inherited one isoform from each parent.

In Family 1, both for the mother and the daughter, an identical concentration of Lp(a) (0.123 g/L) was measured although they were carriers of different apo(a) isoforms. For the younger of the daughter's sons, we found the lowest concentration (<0.096 g/L) compared to all Family 1 members, that was due to his sport activities, which has been proved by Durstin's studies, as well as by our research project (23, 24).

In Family 2, the daughter has inherited one isoform from her father and the dominant HMW S3 isoform from the mother. The molecular mass of two measured apo(a) bands in the daughter was higher compared to those inherited from the parents, which is probably due to the additional protein glycosylation. In spite of the fact that the daughter has inherited the isoform from her mother, her lipid status was more like the lipid status of her father.

Regarding the Family 3 members, we have found that the elder daughter, carrier of the double banded S3S1 apo(a) isoform, manifested the highest concentration of Lp(a) of 0.540 g/L blood from all Family 3 members. The daughter's husband was a carrier of the S4 isoform which was inherited by both children. The son inherited the S1 apo(a) isoform from his mother, whereas the daughter inherited the larger S3 apo(a) isoform, which manifested a higher Lp(a) value in her (0.481 g/L) compared to her brother (0.307 g/L). Fortunately, in both children we found generation decrease of Lp(a) concentration compared to the mother, which has already been noticed by other authors (12, 14).

In Family 4, the absence of a detectable apo(a) isoform in the father's line is interpreted as resulting from homozygosity for an operational »null« allele (11) and his allele is insignificantly contributing to the daughter's Lp(a) concentration. In the mother's blood we measured a much higher Lp(a) level (0.741 g/L), value which was the highest measured Lp(a) level among all participants, S3 apo(a) carriers (healthy subjects and patients) in our projects and my doctoral thesis (25, 26). Although the daughters have inherited the dominant S3 apo(a) isoform from their mother, it manifested a lower Lp (a) value of 0.464 and 0.446 g/L for them. So, in this family we found a generation decrease of Lp(a) levels as well as in the children of Family 3-b.

It is well-known that a high molecular weight apo(a) isoform is associated with a lower Lp(a) value in the blood, but we found relatively high Lp(a) levels among the S3 apo(a) isoform carriers in Family 2, 3 and especially in the mother of Family 4, which are maybe due to the influence of an additional allele in their APO(a) gene.

While performing the APO(a) gene genotype analysis, Cohen et al. (27) discovered that four daughters in the same family who have inherited one isoform from their mother and one from their father, have rather different Lp(a) concentrations, i.e. three of them had very similar low concentrations whereas the fourth one had a much higher concentration compared to her sisters, yet lower compared to her mother. Having in mind that the inheritance isoform of the father had little contribution to the Lp(a) concentration, the authors have concluded that the inheritance allele from the mother is the one dominantly determining the Lp(a) concentration in daughters. Since the mother was a homozygote, they subjected her DNA to additional

analysis, i.e. SSCPs (single-strand DNA conformation polymorphism), which included PCR amplification, digestion and application of 6% polyacrylamide gel electrophoresis. At the same time, the researchers discovered another allele in the mother which was inherited by the daughter having a high Lp(a) level, whereas the other three daughters inherited only the apo(a) allele which was previously visualized by pulsed-field gel electrophoresis.

Gaubatz et al. analysed the apo(a) polymorphism pattern in a nine-member family composed of two parents and seven children. The parents were double-banded phenotype carriers, and had similar Lp(a) values. Four out of the seven children, had the inheritance based on Mendelian's model, i.e. genotype and phenotype two bands inherited one from each parent. However, the other three children showed an exception in the inheritance: two of them inherited one of the isoforms from their father, which was of rather low intensity in the father himself and manifested as dominant with strong intensity in the children. In addition, in the blood of the same children, after increasing the quantity of the applied serum, another isoform of low intensity was visualized which was not identified in their parents, i.e. it had a larger molecular mass compared to the common isoform in the parents. According to the author, both children have inherited the isoform from their mother, but the secondary modification of the polypeptide chain, through an intense glycosylation, has resulted in increased molecular mass of the isoform and its detection in another position from the one expected for the mother. The last child was the carrier of a single phenotype with a band inherited from the mother, whereas the inheritance isoform from the father was suppressed and thus could not be visualized (28).

By applying a parallel analysis of the APO(a) gene with pulsed-field agarose gel analysis and the manifested apoprotein(a) with SDS-PAGE and subsequent immunoblotting in the blood of family members, belonging to the white race, Boewrinkle et al. have determined that relatives who shared one or more of the same alleles (IBD-identical by descent) had surprisingly similar Lp(a) values (12). In addition, a 10 times more different Lp(a) concentration was measured in carriers of the same apo(a) genotype, in members of two unrelated families. According to investigators the reason for the differences lies, on one hand, in the possibility that the number of K-IV type 2 repeats in the APO(a) gene is not in balance with the adequate sequences responsible for the mediation of the APO(a) gene effect on the plasmatic Lp(a), and on the other hand, in the possibility that the alleles of the same size are different from each other in the K-IV sequence composition. The authors have come to the conclusion that the APO(a) gene is responsible for determining almost 90% of the Lp(a) level in the plasma, out of which 69% belong to the number of K 4 repetitions, vs. the previous 41% detected by the same group of authors, and 22% to other *cis*-active sequences in the APO(a) gene, which are yet to be defined (12, 14).

From the obtained results we can conclude that the polymorphic patterns for children in all 4 families were strictly in accordance with the simple Mendelian model of inheritance. Plasma Lp(a) levels are genetically determined and were very similar in those relatives who are carriers of the same apo(a) isoforms, who had alleles identical by descent (mother and daughter in family 2). Unrelated individuals from different families who had apo(a) isofroms of the same size had very different plasma concentrations of Lp(a). The carriers of HMW S3 apo(a) isoform manifested high Lp(a) levels, which was unexpected. The children from two families showed a generation decrease in Lp(a) concentration compared to their mothers.

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