SUMMARY OF THE PH.D. THESIS

MOLECULAR BOLOGICAL ANALYSIS OF GENETIC FACTORS PREDISPOSING TO CARDIOVASCULAR DISEASES

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1. INTRODUCTION

Molecular biological background of cardiovascular diseases

Morbidity and mortality statistics clearly demonstrate the importance of cardiovascular diseases in public health all over the world. According to recent epidemiological and molecular genetic investigations carried out in developed countries, it is widely accepted that both environmental and acquired/hereditary genetic factors are involved in the development of chronic diseases manifesting at young age. Cardiovascular diseases with multifactorial origins resulting from the interplay of genetic and environmental factors present a particular challenge to different medical disciplines. Moreover, the disorders of lipid metabolism caused by a number of genetic and environmental factors are frequently associated with acquired hypercholesterolaemia as well as the development and progression of atherosclerosis. These genetic abnormalities influence the synthesis and structure of proteins involved in lipid metabolism, among them the low density lipoprotein receptor plays a key role in controlling cholesterol metabolism.

Effects of LDL receport gene alterations on cardiovascular diseases

The low density lipoprotein is the major carrier of cholesterol in blood. It has a diameter of 22 nm, contains esterified cholesterol molecules and phospholipid molecules. The B-100 protein component of the LDL binds to a specific receptor protein on the plasma membrane of hepatic and nonhepatic cells. The LDL receptor protein consists of 5 domains and has a molecula weight of 115kD. Defects at any part of the receptor leads to disturbancy of the LDL metabolism in the cells. The gene coding the receptor located at the short arm of chromosome 19 is composed by the promoter region followed by 18 exons. The transcription of the gene is under feed-back control regulated by the endproduct, the intracellular cholesterol. The monogenic disease resulting from the structural and functional alterations of low density receptor gene (LDL-RG) can be characterised by lifelong elevation of serum LDL cholesterol level and its frequency in the populations of Europe and North America averages about 0.2 per cent. However, in some regions of the world it is much higher due to

effects consequent on emigration or due to local customs, which encourage consanguineous marriages. Studies carried out in the last decades identified a large number of genetic defects which strongly alter the synthesis and consecutively the function of the LDL receptor and clinically result in the manifestation of hypercholesterolaemia in human. Recently identified aberrations in different FH (familiar hypercholesterolaemic) families have proved to vary extensively, ranging from large and small deletions and insertions to nonsense and missence types of single nucleotide alterations predisposing to the premature onset of atherosclerotic vascular disease. In most populations of the world, including the population of North America and Europe, more than 150 different genetic variants of the gene involving major gene rearrangements or single nucleotide alterations have been identified so far each of which can give rise to the disease. It can also be seen that genetic variability of the LDL receptor gene exist in case of a single country. For example in Italy and in Finland different type of deletions could be identified in different ethnic communities. In case of the so-called Helsinki type of familiar hypercholesterolaemia a deletion from intron 16 to exon 18 exists, but the LDL receptor gene in patients belonging to the North Karelian ethnic group has a loss of 7 bp sequence in the exon 6. The identification of these deletions characteristic for different groups of Finnish people resulted in the development of a special screening test in Finland, which can be used to test patients being at high risk for deletion abnormalities.

Effects of apoE polymorphisms on cardivascular diseases

Apolipoprotein A1, B, Lp(a) have already been routinely measured in laboratories, but only sporadic data are available on apoE alterations in different lipid disorders. ApoE is known more than 20 years, and it has been associated with the risk of developing cardiovascular diseases.

Human plasma apoE is a 299-amino acid protein which is found in chylomicrons, chylomicron remnants, very low density lipoproteins, intermediate density lipoproteins and high density lipoproteins. ApoE is taken up by apoE specific receptors and the LDL receptors of the cells. It is synthesized in most organs and cells of the body, especially in liver, brain, spleen, kidney and macrophages.

Human apolipoprotein E has several functions in humans such as regulation of cell growth and proliferation, lipid and hormone transports, proteolytic activation of coagulation factors. Among them, its role in the cholesterol transport and the metabolism of lipoprotein particles seems to be the most important: apoE is thought to play a crucial role in lipid metabolism through the regulation of cellular uptake of certain lipoproteins (chylomicron remnants, VLDL, IDL, HDL) by two types of receptors, an apoE specific remnant receptor and the LDL-receptor.

The apoE is a structural gene, containing 4 exons and 3 introns, and resides in chromosome 19. In the last decade apoE has come to the fore because of the association of certain isoforms with the risk of developing cardiovascular (mainly coronary heart) diseases. The human apoE gene is polymorphic: two variable nucleotides, located 138 bases from each other in the 4th exon of the gene, result in cysteine-arginine interchanges at residues 112 and 158 in the polypeptide sequence. These interchanges determine three common alleles (ε2, ε3, ε4) coding for three isoforms (E2, E3, E4), resulting in six different isotypes (E2/2, E2/3, E3/3, E3/4, E2/4, and E4/4) in the human population. The apoE isoforms have different affinities for the LDL receptors and the catabolism of apoE-containing lipoprotein remnant particles is strongly related to apoE phenotypes, which may lead to up- or down-regulation of hepatic LDL-receptors . In accordance with these experimental findings, numerous population studies demonstrated close relationships between apoE alleles and serum lipid concentrations . Numerous studies regarding the relationship between apo E polymorphism and disturbances of lipid metabolism have shown that patients with at least one ε2 allele tend to have lower level, whereas patients with at least one allele of ε4 have higher level of plasma cholesterol in comparison to individuals who are homozygous for the ε3 allele. Thus at present, the consensus appears to be that the overall effect of ε2 allele is to decrease, while that of ε4 allele is to increase significantly the serum cholesterol concentration. On the basis of these findings it is generally accepted that ε4 allele, and consequently the E3/4, E4/4 isotypes confer a relative predisposition to atherosclerosis, while ε2 allele has a protective effect.

A north to south decreasing gradient can be observed in the frequency distribution of the ε4 allele in Europe: its frequency is higher in the Northern countries while lower in the Souhern populations, thus it might explain – at least partially – the prevalence of cardiovascular diseases in these countries.

2. AIMS

2.1. LDL RECEPTOR GENE ANALYSES

No study have been carried out on the background of cardiovascular diseases in Hungary similarly to other Central and Eastern European countries, moreover so far no hot spots characteristic for the LDL receptor gene were identified among the different ethnic communities. To study the possible DNA damages none of the developed tests can be adapted because of the very variable genetic composition of the Hungarian population. A new molecular biological assay should be developed which allows to screen the whole gene and identify deletions at any sites of the gene. The PCR assay is the only one among the molecular biological assays, which can fulfill this requirement. Thus we decided

- to develop a molecular biological method which can give the possibility to screen the whole coded region of the LDL receptor gene composed of 17 introns and 18 exons, most of which correlate with functional domains previously defined at the protein level and can be used in most routine diagnostic and risk assessment laboratories. For this purpose the multiplex polymerase chain reaction (PCR) assay seems to be the most appropriate among the routinely available molecular biological methods, which can fulfil these requirements.

2.2. APOE ANALYSES

Although studies supporting the hypothesis that certain apo E allele predispose to atherosclerosis while others have a protective factor against it, were carried out on many different ethnic groups, subjects have been exclusively healthy, but results specifically for hypercholesterolaemic groups are not available. Thus, it seems to be important to test the validity of the generally accepted hypothesis about the predictive value of apoE isotype on hypercholesterolaemic groups. According to the apo E allele frequency in the Hungarian population only one data is available: it is based on genetic analysis of 202 blod donors and has contradicoty results. The elevated cholesterol level measured in the E3/2 subgroup cannot be explained by the protective role of the ε2 allele, that was found in many ethnic groups so far. This discrepancy

indicated to carry out a genetic analysis in a hypercholesterolaemic population to determine the apo E polymorphism in Hungary. The general goal of our apo E studies were

- to determine apo E allele and genotype frequencies in the Eastern Hunagrian hypercholesterolaemic population;
- to determine whether the apo E allele frequency measured in the Eastern Hungarian hypercholesterolaemic population significantly differ from that in the Hunagarian general population using PCR RFLP methodology;
- to develop a one-step, fast diagnostic assay for analysing apo E polmorphisms that can be used even in routine diagnostic laboratories replacing the time- and labour consuming traditional PCR RFLP method.

3. MATERIALS AND METHODS

3.1. SAMLPE PREPARATIONS FOR THE GENETIC ANALYSES

Isolation of genomic DNA

Genomic DNA isolated with salt extraction from peripheral white blood cells – according to Lahiri's method – served samples for the genetic studies (Nucleic Acid Research, 19:5444, 1991).

Genomic DNA agarose gel electropgoresis

The genomic DNA samples were checked in agarose gel electrophoresis using 1% agarose and EcoR I HindIII λ DNS ladder.

DNA concentration measurement

DNA samples were quantified by GeneQuant II. RNA/DNA calculator (Pharmacia Biotech) using spectrophotometric method using 100 x dilution. Only those samples were used in the genetic analyses which ratios (A_{260nm}/A_{280nm}) was higher than 1.6.

3.2. LDL RG ANALYSES

Study population

To develop the new LDL RG assay DNA samples derived from healthy individuals were used, while for validating the test we used DNA samples with confirmed FH Helsinki gene aberration. For the genetic analyses among the Eastern Hunagian hypercholestetrolaemic population we used DNA samples from 20 Eastern-Hungarian heterozygous FH patients involved in the Regional Lipid Clinic of the Medical and Health Science Centre at Debrecen University.

FH status of the patients was characterized previously by standard criteria. The diagnostic criteria of FH were 1) serum total cholesterol level greater than 7.8 mmol/l,

2) serum LDL cholesterol level greater than 4.9 mmol/l, 3) normal triglyceride level, 4) the presence of tendon xathomas, 5) the presence of hypercholesterolaemia and/or tendon xanthomas in at least one first-degree relative, 6) positive family history for early myocardial infarction. The involved individuals agreed to provide peripheral blood samples for LDL receptor analysis simultaneously with samples for a "lipid-panel" (cholesterol, HDL-C, LDL-C, apoA-1 {apolipoprotein A-1}, apoB {apolipoprotein B}, Lp(a) {lipoprotein (a)}, triglyceride and lipid electrophoresis) before starting with any dietary and/or therapeutic intervention. Patients with chronic diseases, such as diabetes, hypertension or hyperlipidaemia, or receiving anticoagulant drugs were excluded from the study. None of the study group had heart failure, renal, liver or thyroid disease. The study had been approved by the Ethical Committee of the University Medical School, Debrecen, Hungary.

Multiplex PCR

The whole coded region of the LDL receptor gene (promoter and 18 exons) was amplified by PCR. For the amplification of exons 2, 3, 4, 5, 6, 10, 11, 12, 13, 14, 15, 16 we have used self-designed primer pairs and for synthesis of promoter region and exons 1, 7, 8, 9, 17 and 18 oligonucleotides designed by Hobbs were applied [3]. Primer pairs were synthesized by Pharmacia Biotech and sequences of them are given in Table 1. The primer sets of different reaction mixtures are listed in Table 2. Each 50 µl reaction mixture consist of 0.3 µg of genomic DNA, 10 mmol/L Tris-HCl (pH 9.0), 50 mmol/L KCl, 1.5 mmol/L each of dATP, dTTP, dCTP, and dGTP (Pharmacia Biotech), 1-1 µmol/L of both primers (Pharmacia Biotech), 2.5 U Taq polymerase (Pharmacia Biotech) and 10% dimethyl-sulfoxide (Sigma Aldrich). Reaction mixes number 1, 2, 3, 4, 5, 6, and 7 contain 6.0; 4.7; 4.5; 5.6; 4.4; 4.5, and 4.3 mmol/L $MgCl_2$ (Promega), respectively. After initial denaturation 35 PCR cycles were performed in a programmable DNA thermal cycler (Gene Machine Junior, UNITEK) using the temperature profile of 1 minute at 94 °C, 1 minute at annealing temperature and 2 minutes at 72 °C. The primer extension of the 35th cycle was extended to 12 minutes. In case of reaction mixes 1, 2, and 3 the 51°C annealing temperature was applied, while in case of the other mixes this temperature was 47°C. After amplification the post-PCR samples were concentrated under vacuum by Automatic Environment SpeedVac (AES 1000 Savant) for 30 minutes. The products were

electrophoresed on a 3.5% agarose gel (A9539, Sigma Aldrich) in a buffer containing 90 mmol/L Tris-borate (pH 8.4) and 4 mmol/L EDTA for 2 hours at a voltage of 70V. The fragments were dyed with ethidium bromide (10 mg/ml; Sigma Aldrich) and were visualised by ultraviolet illumination. Large quantities of the pre-made reaction mixes were prepared in advance and stored at -86°C. For analysis a tube was thawed, DNA and Taq polymerase were added and the reaction was performed in an automatic thermocycler.

Agarose gel elelectrophoresis

The synthethised amplicons were electrophoretised in 3.5% agarose gel using 1x TBE and pBR322 HaeIII DNS marker.

3.3. APOE POLYMORPHISM ANALYSES

Study population

Patients, who were admitted to the health care program of the Eastern-Hungarian Regional Lipid Center at the University School of Medicine, Debrecen, Hungary between January 2, 1995 - December 30, 1999, were invited to participate in this study (n=247). None of them refused to participate. The diagnostic criteria of FH were the same as in case of LDL RG studies. We applied a population of blood donor volunteers for reference in our studies that is considered as a representative sample from Hungarian general population (n=202; Hallman et al, Am J Hum Gen, 49:338, 1991).

PCR RFLP

Apo E genotyping was carried out according to Hixon's PCR RFLP method (J Lipid Res, 31:545, 1990). Briefly, the region of apoE DNA containing the two common polymorphic sites in the exon 4 was amplified by polymerase chain reaction (PCR). Each 25 µl reaction mixture contained 0.15 µg of genomic DNA, 5.1 mM $MgCl_2$ (Promega), 10 mM Tris-HCl (pH 9.0), 6 mM dNTP (Pharmacia), 1-1 µM of both

primers (Pharmacia), 0.25 U Taq polymerase (Pharmacia). After 5 min of initial denaturation, 35 cycles, each for 1 min at 94 °C, 1 min at 63 °C and 2 min at 72 °C followed by a final extension step for 10 min at 72 °C, were performed in a DNA thermal cycler (Gene Machine Junior, UNITEK, UK). The PCR product was subjected to HhaI (Sigma) digestion for 3 hours at 37 °C.

Polyacrilamide gel electrophoresis

The visualisation of the digested fragments was carried out by standard polyacrylamide gel elctrophoresis using 9% neutral acrylamide gel, 0.2% ammonium persulphate and 0.15% TEMED. For the electrophoresis of the DNA fragments 1xTBE buffer and pBR322 HaeIII DNS marker were applied.

Mutation analysis using melting point analysis

The apo E single nucleotide polymorphism genotyping by melting point analysis was carried out according to Aslanidis' method using LightCycler real time PCR instrument (Roche) (Clin Chem, 45:1094, 1999).
Statistical methods

STATA 5.0 statistical package was used for the analysis. Odds ratios were used to estimate relative risk. Relative allele and genotype frequencies and odds ratios were computed. ApoE 3/3 genotype was chosen as reference category. 95% confidence interval of odds ratios were calculated by Cornfield approximation [20]. Considering the facts that the ε4 allele distribution is 12,90% in the general Hungarian population and having a study power of 80% with 5% significance level, applying the sample size of 247 hypercholesterolaemic and 202 reference subjects 22,85% increase in apoE allele frequency could be detected.

4. RESULTS

4.1. Results of the LDL RG analyses

- We have developed a rapid, effective screening test enabling the simultaneous analysis of DNA alterations in the entire LDL receptor gene. In a relatively simple multiplex PCR system all the 18 exons and the promoter region of the gene can be amplified and analyzed using primer pairs flanking completely the coding region of the gene. Sizes of amplified fragments and reaction conditions were taken into consideration to evolve the PCR assay.
- Primer compositions were positioned to have efficient amplification yield of all relevant DNA regions under approximately similar conditions in the same test tube, and to synthesize DNA fragments that could be size-fractionated in a fully informative way by a single electrophoretic run. Using the system 83-386 bp long DNA sequences were synthesised at not more than two annealing temperatures in seven different reaction mixtures.
- Using this simple assay deletions affecting more than 10 bp in any part of the LDL receptor gene can be easily detected by a single agarose gel electrophoresis. Further analysis of small deletions, insertion mutation and other genetic aberrations in the gene can be studied by using the same PCR products by single-strand conformation polymorphism (SSCP) analysis.
- In case of 20 Hungarian hypercholesterolaemic patients all these regions of the gene were amplified presenting no FH-Helsinki DNA mutation can be found in the LDL receptor gene. Further analysis of the entire coding region of the gene indicated that no large deletions or other large rearrangements in the LDL receptor gene were present in any of the Eastern-Hungarian hypercholesterolaemic patients.

4.2. Results of the apoE analyses

- Apo E genotyping was carried out in case of 247 hypercholesetrolaemic patients aged 25-65 years. The isotype frequency distribution didn't show a significant difference between the reference and hypercholesterolaemic group using χ^2 test.

- No statistically significant association was observed between any types of apoE alleles, isoforms and hypercholesterolaemia between hypercholestetrolaemic and reference group.
- For the faster genotyping we introduced the ultrafast single mutation analysis of the apo E gene in our laboratory. The time of the whole genotyping dropped: 32 samples can be analysed in 50 minutes that is a significant reduction in time against the traditional method that requires 12 hours for 96 samles.

5. DISCUSSION

As genetic alterations of the LDL receptor gene are very variable among ethnic communities the identification of these deletions characteristic for different ethnic groups is needed for developing special screening tests, which can be used to test patients being at high risk for genetic abnormalities. The availability of new methods that speed up the process of finding genetic aberrations in genes has allowed the identification of an ever-increasing number of LDL receptor gene variants that cause hypercholesterolaemia. The aim of these studies is to identify disease-associated genes and to examine the structure and function of the encoded protein [11]. Through these studies the functional consequences of this alteration and the molecular mechanism also can be defined. Human molecular genetics offers the possibility of improved disease diagnosis prevention and treatment through genetic testing and mechanism based therapy [12]. The fact that relatively efficient therapeutic measures can today be offered for both heterozygous and homozygous FH patients emphasises the importance of early diagnosis and intervention of FH. Our new multiplex PCR assay is designed so, that hypercholesterolaemic patients subsequently can be screened for larger than 10 bp deletions in the LDL receptor gene. The simplicity, specificity, and versatility of the assay make it an ideal system for routine screening of LDL receptor gene mutations in large population samples. This PCR assay can be recommended for screening the gene for genetic aberrations even in groups at high risk and individuals of cardiovascular diseases.

Analysis of the entire coding region of the LDL RG of Eastern Hungarian hypercholesterolaemic patients using the newly developed assay indicated that no large deletions or other large rearrangements in the LDL receptor gene were present in any of those patients. Further analysis of small deletions, insertion mutation and other genetic aberrations in the gene can be studied by using the same PCR products by single-strand conformation polymorphism (SSCP) analysis.

Although apoE polymorphism studies in different populations revealed significant heterogeneity in apoE type frequencies, in all population studied so far apoE3 was found to be the predominant isoform. Studies regarding the relationship between apo E polymorphism and serum lipid levels have shown that individuals with at least one

ε2 allele tend to have lower level, whereas those with at least one allele of ε4 have higher level of plasma cholesterol in comparison to persons who are homozygous for the ε3 allele. On the basis of some clinical and epidemiological studies it is generally accepted that the presence of ε2 allele has protective, while that of ε4 allele has permissive effect on the development of atherosclerosis. This assumption is in a good harmony with the results of several studies reporting reduced binding affinity of apoE2 of LDL-receptors which may lead to the accumulation, and consequently increased plasma concentration of apoE containing lipoproteins as well as with that demonstrating decreased LDL clearance and LDL receptor down-regulation in the presence of the ε4 allele.

Although it is generally accepted that apoE does affect plasma cholesterol levels, questions as whether the effect of different apoE types on plasma cholesterol concentration is the same across different ethnic groups are not unequivocally answered yet.

In the Cardiovascular Risk in Young Finns Study the influence of selected genetic markers on the intra-individual long-term variability in serum lipid levels was studied and association between apoE phenotypes and LDL-cholesterol levels was not found in females. In nine different populations studied by Hallman et al. the Sudanese group had the highest frequency of ε4 allele, and unexpectedly, not only the average total cholesterol level was found to be the lowest in this population, but the cholesterol value for the E4/3 group was lower than that of the E3/3 group. Similarly, in Malays the ε4 allele seemed to have an anomalous cholesterol-lowering effect. In Hungarians no difference was observed between the average cholesterol values for the E3/2 and E4/3 groups.

Thus it seems to be rather obvious that instead of creating rules on the basis of data obtained on healthy populations, studies on different hypercholesterolaemic populations should be carried out.

In our present study, the data obtained in case of hypercholesterolaemic subjects do not show a significant shift of ε4 allele frequency in comparison with the general

population. Thus, we can conclude, that the frequency distribution of the apoE alleles, as well as, that of the apoE isotypes in the hypercholesterolaemic population do not differ significantly from the values observed in the group of healthy volunteers. These results do not support the hypothesis that individuals homozygous or heterozygous for the ε4 allele of apoE gene have increased risk of hypercholesterolaemia, and on the basis of these findings it cannot be excluded that the high prevalence of coronary heart disease in the Eastern-Hungarian population is strongly independent on the metabolic events in which apoE isoforms participate. It should be noted that the supposed link between apo E4 isotype and the higher systolic blood pressure was also not sustained with the data from the Dutch, Zutphen and Greenland Inuit population studies.

This study may serve as a starting point for further examinations to determine the distribution of apoE allele frequencies among patients with different type of hypercholesterolaemia and to estimate the predictive value of apoE genotype in risk assessment for coronary heart disease and other vascular diseases. Today, when more and more laboratories are planning to introduce apoE polymorphism determination as a diagnostic test for increased risk of atherosclerosis, it is important to emphasize that more data should be collected on the relationship between apoE polymorphism and cholesterol level in different populations. It is conceivable that the effects of genetic variation may be less important in a population with a very high dietary fat content. Further studies on the interrelationships between genetic polymorphisms of different genes involved in the lipid metabolism and different forms of hypercholesterolaemia are therefore indicated.

Public health importantce of the results

Morbidity and mortality statistical data clearly demonstrate the importance of cardiovascular and malignant diseases in public health concerning the Hungarian population. In the School of Public Health the Hungarian population's premature mortality pattern according to specific causes and settlement size was analyzed. Our data provided convincing evidence of regional inequalities as well as the high excess of avoidable mortality and the importance of rational/differential preventive

programs. Therefore it is reasonable to perform molecular epidemiological screening tests targeting the early diagnosis of susceptibility to common diseases, early treatment and in order to extend the disease free years at individual level.

So far no hot spots characteristic for any of the gene alterations involved in the lipid metabolism were identified among the different ethnic communities. Because of the very variable genetic alterations in different populations none of the assays, developed for the detection of different mutations/deletions in the regions of the gene, can be adapted to study the possible DNA damages. Intensive investigations are being performed in order to increase the general knowledge about the genetic background of ischaemic heart disease. The main goals of this studies are to detect the increased risk determined by genetic alterations thus to provide tailored therapy for individuals at increased risk. According to the literature, the differences in genetic predisposition for certain diseases show geographical, regional as well as ethnic variability. It is impossible to design a national genetic epidemiological screening program based on international data the prerequisite is the exploration of differences between regions and different ethnic groups on which a screening program might be launched. These data are completely missing in Hungary, therefore suggestions for screening programs targeting the primary, and secondary (possibly tertiary) prevention of cardiovascular diseases as well as for the formulation of treatment algorithms is very important. The planned screening program for cardiovascular and malignant diseases having been introduced, the prevention and the treatment of these diseases can be based on conceptionally new principles. The detection of genetic susceptibility offers new possibilities for primary (presymptomatic) prevention of these diseases resulting in quality improvement in life expectancy at individual level as well as cost reduction in treatment of these diseases in health economics. Pointed at frequently occurred alterations used in routine diagnostics will improve the quality of medical diagnosis.

APPENDIX

In extenso publications involved in the thesis

Pocsai Zs, Paragh Gy, Ádány R: Molecular biological background of cardiovascular diseases: genetic alterations affecting lipid metabolism as biomarkers of increased susceptibility (A kardiovaszkuláris megbetegedések molekuláris biológiai háttere: a lipidmetabolizmus genetikai defektusai, mint a veszélyeztetettség biomarkerei) Klinikai Kísérletes Laboratóriumi Medicina, 23:169-179 (1996)

IF: -

Pocsai Zs, Paragh Gy, Ádány R: Multiplex PCR assay for screening deletions in the low density lipoprotein receptor gene. Clinica Chimica Acta 307:7-12, (2001)

IF: 1,035

Pocsai Zs, Paragh Gy, Ádány R: Apolipoprotein E polymorphism among patients with hypercholesterolaemia in Eastern-Hungary. Cardiology, 6:S7-8 (1997)

IF: 0,692

Pocsai Zs, Paragh Gy, Ádány R: Is the apoE ε4 allele associated with hypercholesterolaemia? *(közlésre elküldve)*

Other in extenso publications

Varga Cs, Pocsai Zs, Kertai P: Genotoxicity studies on urine and bone marrow samples of rats bearing transplanted nephroma. Mutagenesis, 10:253-255 (1995)

IF: 2,176

Varga Cs, Pocsai Zs, Kertai P: Urinary and serum mutagenicity studies with rats bearing experimental tumours. Mutagenesis, 10:43-45 (1995)

IF: 2,176

Varga Cs, Pocsai Zs, Horváth G, Timbrell V.: Studies on genotoxicity of orally administered crocidolite asbestos in rats: Implications for ingested asbestos carcinogenesis. Anticancer Research., 16: 811-814 (1996)

IF: 1,049

Szücs S, Kávai M, Varga Cs, Kertai P, Pocsai Zs, Karányi Zs, Ádány R.: Changes in superoxide anion production and phagocytosis by circulating neutrophils during tumor progression in a rat model. Cellular Immunology, 170:202-211 (1996)

IF: 2,142

Other publications (posters, lectures) published at national or international conferences

I. Ph.D. conference of the Medical University of Debrecen, Debrecen, 21-23 March 1996, Pocsai Zs: Multiplex PCR assay for analysis of LDL receptor gen alterations *(lecture)*

European Thrombosis Research Organization (ETRO) 2nd Working party on Population genetics of haemostatic risk factors for arterial vascular disease, Consorzio Mario Negri Sud, Santa Maria Imbaro (Italy), May 30-June 1, 1996. Zs Pocsai, Gy Paragh, R Ádány: Multiplex PCR assay for detecting deletions in the low density lipoprotein receptor gene *(lecture)*

46th Meeting of the Hungarian Association of Laboratory Diagnostics, Miskolc, 5-7 Sept, 1996. Pocsai Zs, Paragh Gy, Ádány R: Multiplex PCR assay for detecting deletions in the low density lipoprotein receptor gene *(lecture)*

5th International Symposium of Slovak Atherosclerosis Association, Bratislava (Slovakia), Jan 29, 1997. Zs Pocsai, Gy Paragh, R Ádány: Apolipoprotein E polymorphism among patients with hypercholesterolaemia in Eastern-Hungary *(lecture)*

II. Ph.D. conference of the Medical University of Debrecen, Debrecen, 6-8 March 1997, Pocsai Zs: Apolipoprotein E phenotype distribution among Eastern-Hungarian hypercholesterolaemic population *(lecture)*

XVI. International Society of Thrombosis Haemostasis Congress, Firenze, Italy 1997. június 6-13. Zs Pocsai, Gy, Paragh, R Ádány: Apolipoprotein E polymorphism among patients with hypercholesterolaemia in Eastern-Hungary *(poster)*

MEDLAB, 12th IFCC European Congress of Clinical Chemistry, Basel, 1997. augusztus 15-22, Zs Pocsai, Gy Paragh, R Ádány: Apolipoprotein E polymorphism among patients with hypercholesterolaemia in Eastern-Hungary *(poster)*

29th Conference of the Hungarain Association of Hygienics, Balatonföldvár, 24-26 Sept 1997. Pocsai Zs, Paragh Gy, Ádány R: Apolipoprotein E polymorphism among patients with hypercholesterolaemia in Eastern-Hungary *(lecture)*

7th Conference of the Hungarian association of Public Health, Pécs, 23-25 April, 1998. Pocsai Zs, Paragh Gy, Ádány R: Apolipoprotein B-100 Xba I allele frequency distribution among patients with hypercholesterolaemia in Eastern-Hungary *(lecture)*

47th Meeting of the Hungarian Association of Laboratory Diagnostics, Kecskemét, 2-5 Sept, 1998. Pocsai Zs, Paragh Gy, Ádány R: Apolipoprotein B-100 Xba I allele frequency distribution among patients with hypercholesterolaemia in Eastern-Hungary *(lecture)*

Other lectures, poters

20th Conference of the Hungarian Association of Oncology, Budapest, 3-5 Nov, 1993. Pocsai Zs., Varga Cs., Kertai P.: Tumormarker pseudouridin mutagenity in Ames test *(poster)*

3rd Conference of the Hungarian Association of Public Health , Gyula, 27-29 April, 1994. Varga Cs., Pocsai Zs., Kertai P.: Carcinogenity of asbestos fibres – a new approach. *(lecture)*

UICC International Cancer Congress New Delhi, Nov 1994. Varga Cs., Pocsai Zs., Kertai P.: Genotoxic substances produced during development of transplanted renal tumour *(poster)*

9th Conference of the Hungarian Association of Public Health, Hévíz, 13-15 April, 2000. Pocsai Zs, Fletcher T: Effects of passive smoking on childrens' respiratory health *(lecture)*