Monogenic hypercholesterolaemias – an evaluation of apolipoprotein B100 and LDL receptor gene polymorphisms

Robert Plewa[1], Michał Łuczak[2], Paweł Burchardt[4], Andrzej Bolewski[3], Jerzy Wierzchowiecki[3], Tomasz Siminiak[4]

[1]Department of Physiology and Animal Development Biology, Adam Mickiewicz University, Poznań, Poland
[2]Department of Biochemistry and Molecular Biology, University of Medical Sciences, Poznań, Poland
[3]Department of Cardiology, District Hospital, University of Medical Sciences, Poznań, Poland
[4]Cardiac and Rehabilitation Hospital Kowanówko, University of Medical Sciences, Poznań, Poland

Abstract

Introduction: Normally the N-terminal domain of the LDL receptor (LDLR) binds to low-density lipoprotein through apolipoprotein B100. Mutations within the LDL receptor and/or apolipoprotein B-100 genes compromising this process may lead to congenital monogenic hypercholesterolaemias known as familial hypercholesterolaemia or familial defective apolipoprotein B-100. These diseases are inherited as autosomal dominant traits.

Aim: To search for LDLR and apoB100 gene mutations in a Polish population of patients with hypercholesterolaemia and to determine their types and locations. An attempt was also made to evaluate the influence of identified gene mutations on the modification of protein product sequence and the severity of its functional impairment.

Methods: LDLR and apoB100 gene analyses using PCR, SSCP and automated sequencing techniques were performed in 190 hypercholesterolaemic patients. Flow cytometry was used to measure the influence of Cys152Trp mutation in the LDLR gene on ligand binding and internalisation. The OLA method was used for the preparation of adequate genetic markers allowing rapid detection of one of the most deleterious mutations of the apoB100 gene.

Results and conclusions: Three brand new mutations, not reported so far, have been detected – Pro2712Leu and Ile3532 in the apoB100 gene, and Cys347Ser in the LDLR gene – and numerous changes in the nucleotide sequence of the LDL receptor gene have been confirmed. The observations of functionality of the mutated receptor gene with flow cytometry suggested the dysfunction of LDLR due to Cys152Trp polymorphism reported in many studies.

Key words: familial hypercholesterolaemia, familial defective apolipoprotein B-100 hypercholesterolaemia, dyslipidaemias

Kardiol Pol 2006; 64: 127-133

Introduction

Primary hypercholesterolaemia is a congenital disorder of lipid metabolism of monogenic or polygenic origin. Clinical manifestations include a high level of total cholesterol (above 200 mg%) and low density lipoprotein (LDL) cholesterol (above 150 mg%) as well as low levels of high density lipoprotein (HDL) cholesterol. Polygenic hypercholesterolaemias may appear in subjects in whom endogenous factors are amplified by environmental factors (e.g. nutritional patterns). In this group the risk of cardiovascular disease is twice that of the healthy population, being however lower than in monogenic forms of the disorder.

Monogenic hypercholesterolaemia is inherited as an autosomal dominant trait. Two forms may be distinguished: heterozygotic and homozygotic, clinically different with respect to incidence in the general population (1:500 for heterozygotic and 1:1,000,000 for homozygotic), levels of total cholesterol (250-500 mg% and 300-1000 mg%, respectively) and LDL fraction, as

Address for correspondence:
Prof. Tomasz Siminiak, Pracownia Hemodynamiki, Szpital Rehabilitacyjno-Kardiologiczny, ul. Sanatoryjna 34, 64-600 Kowanówko k. Obornik Wlkp., Poland, tel.: +48 61 296 11 12, fax: +48 61 297 75 00, e-mail: tomasz.siminiak@usoms.poznan.pl
Received: 10 May 2005. **Accepted:** 8 July 2005.

Table I. Clinical characteristics of studied patients (n=190)

	Females	Males
Mean age [years] (n)	49	53
Cholesterol above 250 mg% (n)	65	107
LDL above 175 mg% (n)	62	111
Triglycerides above 200 mg% (n)	18	37
HDL below 39 mg% (n)	6	12
One myocardial infarction (n)	25	59
More than one myocardial infarction (n)	3	19
Diabetes mellitus (n)	31	21
Arterial hypertension (n)	56	40

well as the progression rate of atherosclerotic lesions within the cardiovascular system [1, 2]. The causes of monogenic lipid metabolic disturbances should be sought in the impairment of LDL cholesterol transport and binding (by protein domain of apoB100), and defective cholesterol internalisation by the receptor. On molecular grounds these changes result from partially missing allele, complete deletion or other LDL receptor (LDLR) gene mutations [3] – or may be associated with much worse known mutations within the apolipoprotein B100 (apoB100) gene [4-6]. This disorder is known as familial hypercholesterolaemia (FH) with defective apoB 100 (FDP) [7, 8].

The LDL receptor is 115 kD glycoprotein presenting on the cell surface, consisting of 860 amino acid residues, clustered to form five domains [9, 10]. The first domain is responsible for ligand binding (292 amino acid residues). The second one closely resembles the precursor of the epidermal growth factor (EGF). The third domain, rich in serine and threonine residues with O-linked carbohydrates, undergoes glycosylation within the Golgi apparatus during maturation of the protein (receptor). The fourth domain is responsible for membrane receptor stability, whereas the fifth C-terminal chain constitutes the cytoplasmic portion of the protein and anchors the receptor in the clathrin-coated membrane pits [11-13].

Hypercholesterolaemia may also result from a functional defect of the protein component of apoB100, which is one of the forms of apolipoprotein B. The other one is apoB48. Both molecules originate from the same mRNA template, but the deamination of cytidine changes the normal Gln codon into a stop codon. ApoB100, constituting a part of the LDL particle, is responsible for LDLR ligand binding.

The aim of the study was to search for LDLR and apoB100 gene mutations in a population of Polish patients with hypercholesterolaemia, and to determine their types and locations. An attempt was also made to evaluate the influence of identified gene mutations on the amino acid sequence and the severity of functional impairment of the resultant protein.

Methods

Patients

The study involved 190 patients with cholesterol levels over 200 mg%, and with suspected or known atherosclerosis or ischaemic heart disease. Patients with a history of myocardial infarction (MI) were treated according to the standards of the Polish Cardiac Society (PCS) at that time or the treatment was modified during subsequent hospitalisations to match the PCS guidelines and included angiotensin-converting enzyme inhibitors, β-blockers, aspirin, ticlopidine and statins. Clinical characteristics of the study group are shown in Table I. The mean body mass index in patients with or without LDLR and apoB100 gene mutations was 29.21 and 28.08, respectively.

Genetic tests

Blood samples collected from the patients were used for isolation of genomic DNA with salt-extraction approach. Subsequently, the purity of obtained DNA was assessed with electrophoresis on 1% agarose gel. Using polymerase chain reaction (PCR), analyses of coding sequences of the LDLR gene and five fragments of the apoB100 gene were performed for each patient. PCR primers were selected from LDLR and apoB100 gene sequences taken from the Entrez Gene database. The products of DNA amplification were analysed using single strand conformation polymorphism (SSCP) analysis (Figure 1).

DNA fragments were separated by electrophoresis and underwent further silver staining. Beckman-Coulter Genetic Analysis System CEQ 2000XL with capillary electrophoresis technology and fluorescence detection option was used for automated sequencing, allowing more accurate determination of nucleotide sequence changes essential for the identification of gene structure and function.

An effort also was made to determine the dysfunction of the LDL receptor for one of the known LDLR polymorphisms (Cys152→Trp). For this purpose lymphocytes were isolated from blood samples collected from a patient with known FH and from a healthy individual. The lymphocyte suspension was aliquoted into five parts to determine the receptor density on the cell before and after ligand addition (test 1 – negative control; test 2 – receptor density measurement before ligand addition; tests 3, 4, and 5 – after 5, 10 and 15 minutes of incubation with apoB100, respectively). The cells were coated with CD3PerCP and

CD14PE antibodies, and then monoclonal antibodies I and II against LDL receptor were added. Using Becton-Dickinson FACSort flow cytometer and CellQuest software, mean fluorescence intensity was measured after 5, 10 and 15 minutes following apoB100 addition, and the results were compared with that of the healthy individual (Table II).

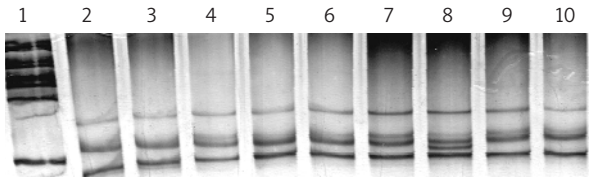
Oligonucleotide ligation assay was applied for known apoB100 gene mutation (codon 3500aa) in order to prepare the genetic markers for the purpose of rapid testing. Two synthetic primers were used for flanking polymorphism regions. One of them had a mutation inserted within the 3-terminal end.

Results

In 32 of 190 studied patients the tests showed 17 different changes within the analysed DNA fragments. The following changes were found for the LDL receptor gene: four transitions (T2786C, G2728A, T1102C, T1103C), three transversions (C2623A, T1012A, C518G), and two deletions (ΔA2731, Δ2734-2746). In eight subsequent cases the deletions involved entire exons (Δe8, Δe9, Δe8-9). Sequence analysis of exon 18 of the LDL receptor gene, for which differences in agarose DNA fragment migration were found, revealed the following mutations: transition T→C in codon 2786, G→A in codon 2728, transversion C→A in codon 2623 and deletion of A in codon 2731 that changed the open reading frame GAC→GCT, and also another deletion (of 13 nucleotides) in codons 2734-2746.

Alteration of the amino acid sequence was caused by C518G – exon 4 (Cys152Trp), T1012A – exon 7 (Cys 317Ser), C2623A – exon 7 (Leu855Ile), T1103C – exon 8 (Cys347Ser) (Figure 2), T1102C – exon 8 (Cys347Arg).

This study showed three mutations in the apoB100 gene within exon 26. Two of them were transitions (G10580A leads to Arg3500Gln substitution, whereas C8216T leads to Pro2712Leu substitution) and one was transversion (A10677C – amino acid sequence remains intact – Ile 3532). Mutations A10677C and G10580A are localised in the region responsible for receptor binding. Variants of mutations Pro2712Leu and Ile 3532 have not been reported so far.



Lane 1 – molecular mass marker (3.3.10c); Lane 2 – patient no. 83; Lane 3 – patient no. 82; Lane 4 – patient no. 81; Lane 5 – patient no. 80; Lane 6 – patient no. 79; Lane 7 – patient no. 78; Lane 8 – patient no. 77; Lane 9 – patient no. 76; Lane 10 – patient no. 75

Figure 1. Diagram of fragments of DNA strand: exon 18 of LDLR gene in 8% PAA gel analysed with SSCP method

Flow cytometry was used for the evaluation of the influence of transversion Cys152Trp in the receptor gene on ligand binding capability. After adding apoB100 the number of receptors on the cell surface (expressed as mean fluorescence intensity) was 29.8% (for controls) and 58.07% (for patients with heterozygotic FH) in comparison to the baseline receptor density in each group (Table III).

In patients with identified substitution Arg3500Gln, which is the most frequently reported (5), confirmation was obtained with the OLA approach (Figure 3) using the specific flanking primers.

Discussion

In normal subjects the LDL fraction binds to the N-terminal domain of the receptor through apoB100 [9, 10, 12]. The entire complex is then placed in a clathrin-coated pit, which leads to vesicle formation and endocytosis. Subsequently, LDL dissociates from the receptor, which is redistributed to the surface. The LDL is cleaved by lysosomal enzymes. Cholesterol esters undergo reesterification, supplementing the free cholesterol pool, or are again changed into reserve esters [14-16].

Supplementation of exogenous LDL cholesterol inhibits endogenous production through interaction with HMG-CoA reductase and activation of formation of cholesterol esters (acyl-CoA: cholesterol acyltransferase). Excessive LDL intake results in inhibition of both gene expression of LDL receptor (down-regulation) and cholesterol ester hydrolase [16].

Table II. Composition of each test for coating the cells with monoclonal antibodies

Test no.	Lymphocytes	ApoB100	Incubation with ApoB100	CD3	CD14	Antibody 1	Antibody 2
1	+	–	–	+	+	–	+
2	+	–	–	+	+	+	+
3	+	+	5 min.	+	+	+	+
4	+	+	10 min.	+	+	+	+
5	+	+	15 min.	+	+	+	+

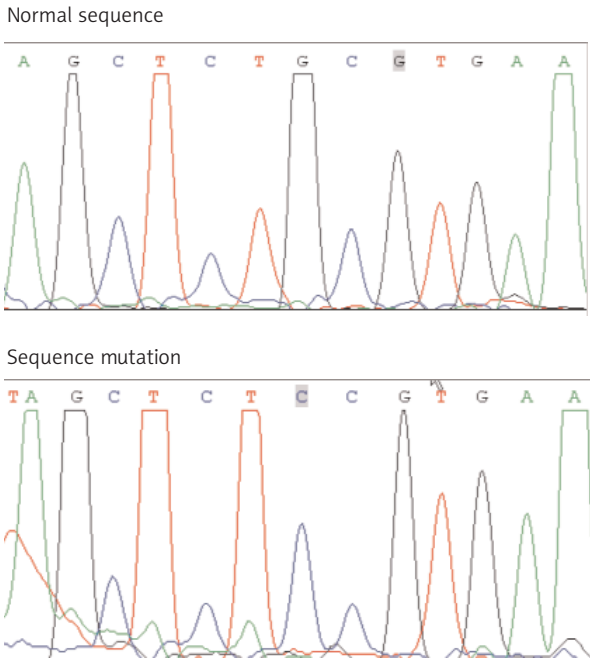


Figure 2. Results of DNA sequencing: exon 8, Cys347Ser, G1102C

The whole process is determined by ligand-receptor interaction. The impairment of this process caused by mutations within the LDLR and apoB-100 genes leads to congenital monogenic hypercholesterolaemias known as FH or FDP, respectively [5, 6].

The LDLR gene is located on chromosome 19. It consists of 18 exons and 17 introns. It has been shown that exon 1 codes a signal sequence consisting of 21 amino acid residues, directing the protein receptor towards the membrane. Exons 2-6 encode the ligand-binding domain of the receptor. The second domain of LDLR is determined by exons 7-14. The glycosylated fragment of the receptor containing serine and threonine residues is coded by exon 15. The information in exon 16 and 5' end of exon 17 codes the 22 amino acid transmembrane segment of LDLR. The cytoplasmic domain is determined by part of exons 17 and 18, and the 3'UTR region is coded by the terminal fragment of exon 18 [11, 13].

The apoB100 gene is located on chromosome 2. It consists of 29 exons and 28 introns. Exon 26 is the longest (7,572 kb) and is responsible for interaction between the receptor and the LDL particle.

World literature reports a range of various mutations within the LDLR gene that are associated with FH [17]. Deletions of the entire protein coding sequence have seldom been observed; these more frequently affect one or a few exons. The most common mutations include transversions and transitions of single nucleotides within the ligand-binding region that is responsible for the detection of and binding to apoB100. Approximately 30% of them involve loss or exchange of one of the conservative cysteine residues [18]. This is of key importance because it results in blocking of disulphide bond formation leading to entire receptor destabilisation.

Important studies on many novel gene polymorphisms in FH have also been carried out in Poland as well [19, 20]. Several mutations caused by decreased affinity of the (affected) apoB100 to the receptor leading to FDP have been reported. Our earlier studies revealed new, previously unknown, polymorphisms within the apoB100 gene (Pro2712Leu and Ile 3532), and one within the LDLR gene (Cys347Ser) (Figure 2). Additionally, a variety of changes in the nucleotide sequence within the LDLR gene were confirmed, including mutations Cys347Arg and Cys317Ser in the study patients with FH. The first one was found by Coutre et al. to cause FH due to its location in the B domain, very close to the ligand-binding domain. The other one was described by Maruyama et al. and referred to Cys in position 317 located in domain A and acting as the ligand-binding region stabiliser [21].

The presented observations of functionality of the mutated receptor gene with flow cytometry suggest the dysfunction of LDLR caused by Cys152Trp polymorphism reported in many studies. After adding apoB100, the number of receptor molecules that remained on the cell

Table III. Evaluation of binding and internalisation capabilities of LDL receptor

		Negative control	Test without apoB100	5 min.	10 min.	15 min.
				After adding apoB100		
MFI	C	0.31	21.14	6.30	6.74	15.26
	146	0.48	14.12	8.20	8.15	10.80
%	C	1.47	100	29.80	31.88	72.18
	146	3.40	100	58.07	57.72	76.49

MFI – mean fluorescence intensity expressing the count of receptor particles on the cell surface:
C – control (normal receptor): 146 – patient (receptor mutation): % – percentage of receptor particles on the surface after internalization

surface (expressed as mean fluorescence intensity) was 29.8% (for controls) and 58.07% (for patients with heterozygotic FH) in comparison to the baseline values, indicating about 40% decrease in LDL binding capacity in patients with the gene mutation (Figure 3).

Using OLA for confirmation of the known mutation in position 3500aa of the apoB100 gene, a new approach has been introduced that enables rapid identification of one of the most deleterious mutations. This may become a very valuable diagnostic tool for FDP screening. However, further molecular and clinical studies are required before these techniques become clinically relevant.

The development of molecular techniques enables more precise identification and understanding of the mechanisms of lipid disturbances; however, the treatment of dyslipidaemia still lacks high efficacy.

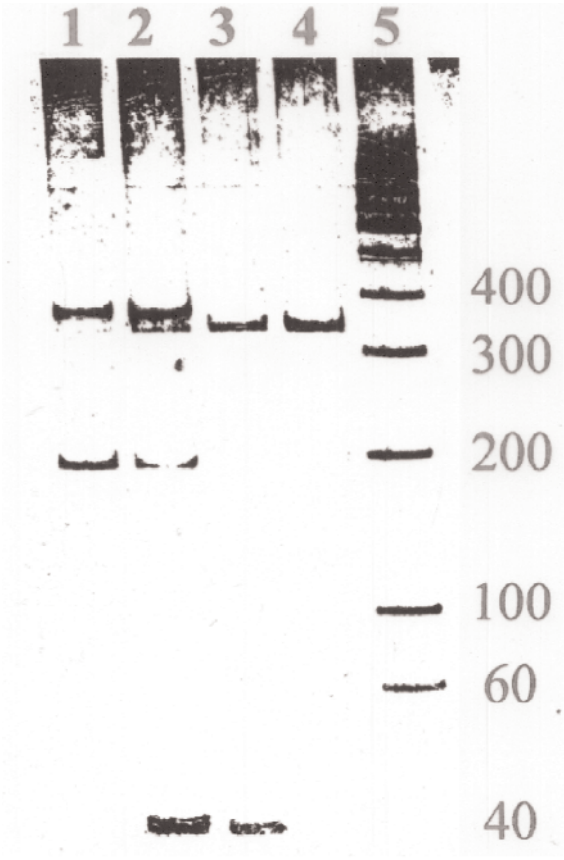
The results of many studies confirm the need of statin use in patients with heterozygotic forms of FH, which increase LDLR expression by inhibiting HMG-CoA reductase [22]. However, various different effects of the same 20 mg dose of atorvastatin in patients with heterozygotic FH have been shown, depending on the type of underlying mutations.

Recently, a therapy with ezetimibe [22, 23], which inhibits cholesterol absorption at low doses, was introduced. Co-administration of statins and ezetimibe is insufficient in homozygotic FH [24]. In these subjects the only effective method of treatment is LDL apheresis, although great expectations are pinned on microsomal triglyceride transfer protein inhibitor, a protein that participates in synthesis of the lipoproteins containing apoB100 [25]. Homozygotic and heterozygotic FDPs can be successfully treated with statins, which act through VLDL remnant elimination [22].

The mechanism of response to statins remains unknown in the studied patients, particularly in those with the new mutations. Determining the efficacy of such treatment requires further studies in larger groups of patients not on lipid-lowering drugs, which was impossible in our group with high LDL levels and past MI as such management was routinely implemented in these patients.

The insufficient number of patients with a history of MI and concomitant mutations within LDLR or apoB100 genes prevented determination of the mean age of the first incidence of acute coronary syndrome and comparison of these data to the control group. Published epidemiological data show that myocardial infarction occurred in FH or FDP patients in males aged 30-50 years and females aged 50-80 years [26].

Large prospective studies are required to determine the clinical relevance of lipid disorders and novel mutations in particular. Further improvement of the understanding of genetics of dyslipidaemia brings hope for using it for the purpose of diagnosis, but hopefully also for alternative methods of treatment based on genetic engineering.



Separation in PAA gel of amplification products obtained with OLA for identification of mutation Arg3500Gln:
Lane 1 – control primer without mutation (patient no. 37); Lane 2 – primer with mutation (patient no. 37); Lane 3 – primer without mutation (control); Lane 4 – primer with mutation (control); Lane 5 – molecular mass marker (3.3.10.)

Figure 3. Result of OLA used for identification of mutation Arg3500Gln

References

1. Herold G. Zaburzenia metaboliczne. In: Myers AE (ed.). Choroby wewnętrzne. *Urban & Partner*, Wrocław 2002; 651-3.
2. Naruszewicz M. Cholesterol a miażdżyca. Zmienność opinii, niezmienność faktów. *Czynniki Ryzyka* 1997; 1-2: 11-16.
3. Goldstein JL, Hobbs HH, Brown MS. Familial hypercholesterolemia. In: Scriver CR, Beaudet AL, Sly WS, Valle B (eds.). The metabolic bases of inherited diseases. *McGraw-Hill*, New York 1995; Vol. 2: 1980-2037.
4. Dunning AM, Houlston R, Frostegard J, et al. Genetic evidence that the putative receptor binding domain of apolipoprotein B

(residues 3130 to 3630) is not the only region of the protein involved in interaction with the low density lipoprotein receptor. *Biochim Biophys Acta* 1991; 1096: 231-7.
5. Durovic S, Martz W, Sasa F, et al. Decreased Binding of Apolipoprotein (a) to Familial Defective Apolipoprotein B-100 (Arg 3500-Gln). *J Biol Chem* 1994; 48: 30320-5.
6. Srivastava RAK, Jiao S, Tang J, et al. In vivo regulation of low-density lipoprotein receptor and apolipoprotein B gene expressions by dietary fatand cholesterol inbred strains of mice. *Biochim Biophys Acta* 1991; 1086: 29-43.
7. Soria LF, Ludwig EH, Clarke HRG, et al. Association between a specific apolipoprotein B mutation and familial defective apolipoprotein B-100. *Proc Nat Acad Sci* 1989; 86: 587-91.
8. Pullinger CR, Hennesy LK, Chatterton JE, et al. Familial liagnd-defective apolipoprotein B: identification of a new mutation hat decreases LDL receptor binding affinity. *J Clin Invest* 1995; 95: 1125-34.
9. Chlebus H, Januszewicz W. Zaburzenia gospodarki lipidowej. In: Zarys kardiologii. *Państwowy Zakład Wydawnictw Lekarskich*, Warszawa 1992.
10. Lehninger AN, Nelson DL, Cox MM. Principles of Biochemistry. *Worth Publishers*, New York 1997.
11. Soutar AK, Knight BL. Structure and regulation of the LDL-receptor and its gene. *Br Med Bull* 1990; 46: 891-916.
12. Knott TJ, Rall SC, Innerarity TL, et al. Human Apolipoprotein B: Structure of carboxyl – terminal domains, sites of gene expresion and chromosomal localization. *Science* 1985; 230: 37-43.
13 Sudhof TC, Goldstein JL, Brown MS, et al. The LDL receptor gene: a mosaic of exons shared with different proteins. *Science* 1985; 228: 815-22.
14. Murray RK, Granner DK, Mayes PA, et al. Transport i magazynowanie lipidów. In: Harper HA (ed.). Biochemia. *PZWL*, Warszawa 1995: 294-313.
15. Badzio T, Dominiczak MH, Kabata J. Lipidy i lipoproteiny. In: Angielski S (ed.). Biochemia. *Wydawnictwo Perseusz*, Sopot 1995; 93-107.
16. Stryer L. Biosynteza lipidów i steroidów błon komórkowych. In: Harper HA (ed.). Biochemia. *PZWL*, Warszawa 1995: 744-8.
17. Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff. http://www.hgmd.cf.ac.uk/hgmd0.html
18. Laukkanen-Jalken J. Gene therapy and atheriosclerosis. Experimental studies with secreted macrophage scavanger receptor and platelet activating factor acetylhydrolase. *Kuopio University Publications* G.-A.I. Virtanen Institute for Molecular Sciences, Kuopio 2004.
19. Bednarska-Makaruk M, Bisko M, Pulawska MF, et al. Familial defective apolipoprotein B-100 in a group of hypercholesterolaemic patients in Poland: identification of a new mutation Thr3492Ile in the apolipoprotein B gene. *Eur J Hum Genet* 2001; 9: 836-42.
20. Górski B, Kubalska J, Naruszewicz M, et al. LDL-R and APO-B 100 gene mutations in polish familial hypercholesterolemia. *Hum Genet* 1998; 102: 562-5.
21. Maruyama T, Miyake Y, Tajima S, et al. Common Mutations in the Low-Density-Lipoprotein–Receptor Gene Causing Familial Hypercholesterolemia in the Japanese Population. *Arterioscler Thromb Vasc Biol* 1995; 15: 1713-18.
22. Gagne C, Gaudet D, Bruckert E. Efficacy and safety of ezetimibe coadministered with atorvastatin or simvastatin in patients with homozygous familial hypercholesterolemia. *Circulation* 2002; 105: 2469-75.
23. Rader DJ, Cohen J, Hobbs HH. Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. *J Clin Invest* 2003; 111: 1795-1803.
24. Thompson GR. LDL apheresis. *Atherosclerosis* 2003; 167: 1-13.
25. Wetterau JR, Gregg RE, Harrity TW, ET AL. An MTP inhibitor that normalizes atherogenic lipoprotein levels in WHHL rabbits. *Science* 1998; 282: 751-4.
26. Austin MA, Hutter CM, Zimmern RL, et al. Familial Hypercholesterolemia and Coronary Heart Disease: A HuGE Association Review. *Am J Epidemiol* 2004; 160: 421-9.

Hipercholesterolemie monogenowe – ocena polimorfizmów w genach apoproteiny B100 i receptora LDL

Robert Plewa[1], Michał Łuczak[2], Paweł Burchardt[4], Andrzej Bolewski[3], Jerzy Wierzchowiecki[3], Tomasz Siminiak[4]

[1]Zakład Fizjologii i Biologii Rozwoju Zwierząt, Uniwersytet im. Adama Mickiewicza, Poznań
[2]Katedra i Zakład Biochemii i Biologii Molekularnej, Akademia Medyczna, Poznań
[3]Oddział Kardiologii, Szpital Wojewódzki, Akademia Medyczna, Poznań
[4]Szpital Rehabilitacyjno-Kardiologiczny w Kowanówku, Akademia Medyczna, Poznań

Streszczenie

Wstęp: W prawidłowych warunkach domena N-końcowa receptora LDL łączy się z pomocą apoproteiny B100 z lipoproteiną o małej gęstości. Mutacje w obrębie genów receptora LDL i/lub apoproteiny B-100, powodujące zaburzenia tego procesu, mogą prowadzić do wrodzonych hipercholesterolemii monogenowych określanych odpowiednio jako hipercholesterolemia rodzinna lub rodzinny defekt apoproteiny B-100. Choroby te dziedziczone są w sposób autosomalny dominujący.

Cel: Poszukiwanie mutacji w genach LDLR oraz apoB100 w grupie chorych z hipercholesterolemią w populacji polskiej, a także określenie ich charakteru i lokalizacji. Podjęto próbę oceny wpływu stwierdzonych mutacji w genach na zmianę sekwencji aminokwasowej produktu białkowego oraz na stopień upośledzenia jego funkcji.

Metody: Analizę genów LDLR oraz apoB100 z wykorzystaniem technik PCR, SSCP i automatycznego sekwencjonowania wykonano w grupie 190 pacjentów z hipercholesterolemią. W celu określenia wpływu mutacji Cys152Trp w LDLR na procesy wiązania i internalizacji liganda, przeprowadzono badania z użyciem cytometru przepływowego. Wykorzystując technikę OLA, przygotowano odpowiednie markery genetyczne pozwalające na szybką identyfikację jednej z najbardziej letalnych mutacji w genie apoproteiny B100.

Wyniki i wnioski: Wykryto trzy nowe, dotychczas nieopisywane, mutacje: Pro2712Leu oraz Ile3532 dla genu apoB100 i Cys347Ser dla LDLR, a także potwierdzono występowanie szeregu zmian sekwencji nukleotydowych w obrębie genu receptora LDL. Przedstawiono również obserwacje dotyczące funkcjonalnej oceny zmutowanego receptora, uzyskane dzięki zastosowaniu cytometru przepływowego, które sugerują istnienie dysfunkcji LDLR, spowodowanej znanym z licznych doniesień polimorfizmem Cys152Trp.

Słowa kluczowe: hipercholesterolemia rodzinna, hipercholesterolemia związana z rodzinnym defektem apoproteiny B-100, dyslipidemie

Kardiol Pol 2006; 64: 127-133

Adres do korespondencji:
prof. Tomasz Siminiak, Pracownia Hemodynamiki, Szpital Rehabilitacyjno-Kardiologiczny, ul. Sanatoryjna 34, 64-600 Kowanówko k. Obornik Wlkp., tel.: +48 61 296 11 12, faks: +48 61 297 75 00, e-mail: tomasz.siminiak@usoms.poznan.pl
Praca wpłynęła: 10.05.2005. **Zaakceptowano do druku:** 8.07.2005.