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# Influence of apolipoprotein E, low-density lipoprotein (LDL) receptor related protein (LRP) and interleukin 1β polymorphisms and of plasma lipids level on dementia treatment

*Wpływ polimorfizmu apolipoproteiny E, białka zbliżonego do receptora LDL (LRP) i interleukiny 1β oraz poziomu lipidów osocza na leczenie otępienia*

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**SUMMARY**

***Aim***. *The aim of the work was to study a possible influence of several genes polymorphism and of lipids on the effectiveness of one year dementia treatment with cholinesterase inhibitors.*

***Methods***. *The group consisted of 68 patients – 48 with Alzheimer's disease and 20 with mixed dementia. Apolipoprotein E alleles, two polymorphisms of LRP and two of interleukin 1β genes were identified by DNA analysis. Lipid levels were determined in plasma using enzymatic methods.*

***Results***. *No one apolipoprotein E carrier was in the group of bad responders to treatment. A higher frequency of ε4 allele carriers showed bad response to treatment as compared with ε2 allele carriers. A higher frequency of carriers of the longer 92 allele of [TTTC]n LRP polymorphism and of carriers of T allele of the C766T LRP polymorphism and of persons with plasma LDL cholesterol level >135 mg/dl was observed in the group of bad responders comparing with their frequency in the good responders group. The more of those disadvantageous factors had an individual the significantly worse were his treatment results. It was concluded that APOE and LRP polymorphism as well as LDL cholesterol levels could have an influence on the effectiveness of treatment with acetylcholinesterase inhibitors in patients with dementia. The effect was stronger in Alzheimer's disease patients than in patients with mixed dementia.*

***Conclusions***. *Our results showed that APOE and LRP polymorphism as well as LDL cholesterol levels could modify the effectiveness of treating patients with dementia with acetylcholinesterase inhibitors.*

**STRESZCZENIE**

***Cel***. *Celem pracy było zbadanie wpływu polimorfizmu niektórych genów oraz wpływu lipidów osocza na skuteczność leczenia otępienia przy użyciu inhibitorów cholinesterazy.*

***Metody***. *Grupa składała się 68 pacjentów – 48 z chorobą Alzheimera i 20 z otępieniem typu mieszanego. Allele apolipoproteiny E, dwa rodzaje polimorfizmu białka zbliżonego do receptora lipoprotein niskiej gęstości (LRP) i dwa rodzaje polimorfizmu interleukiny 1β identyfikowano przy pomocy analizy DNA. Poziom lipidów w osoczu oznaczano stosując metody enzymatyczne.*

***Wyniki***. *W grupie osób źle odpowiadających na leczenie nie było ani jednego nosiciela allelu ε2 apolipoproteiny E. W grupie tej częstość allelu ε4 była większa w porównaniu z częstością w grupie z dobrą odpowiedzią na leczenie. Częściej również w grupie ze złą odpowiedzią obserwowano występowanie nosicieli dłuższego allelu (92) polimorfizmu [TTTC]n receptora LRP oraz nosicieli allelu T polimorfizmu C766T tego receptora, jak również częściej występowały w tej grupie osoby z poziomem cholesterolu LDL osocza przewyższającym 135 mg/dl. Wyraźniejsze różnice obserwowano u pacjentów z chorobą Alzheimera niż u pacjentów z otępieniem typu mieszanego. Im większa ilość powyższych niekorzystnych czynników charakteryzowała poszczególnych pacjentów tym w istotny sposób częściej znajdowali się oni w grupie ze złymi wynikami leczenia.*

***Wnioski***. *W konkluzji można uznać, że polimorfizm apolipoproteiny E i receptora LRP oraz poziom cholesterolu LDL w osoczu mogą mieć wpływ na efektywność leczenia pacjentów z otępieniem inhibitorami cholinesterazy.*

**Key words:** dementia treatment / apolipoprotein E / LDL receptor related protein / interleukin 1β / cholesterol
**Słowa kluczowe:** leczenie otępienia / apolipoproteina E / białko zbliżone do receptora LDL / interleukina 1β / cholesterol

Apolipoprotein E (APOE) polymorphism plays a significant role both in Alzheimer's disease (AD) and in atherosclerosis ε4 allele showing a disadvantageous effect in both these conditions [1, 2, 3, 4]. ApoE 4 isoform favours the essential symptom in AD i.e. β-amyloid storage in the brain. It favours also the development of atherosclerotic plaques.

According to recent opinions most patients with dementia have a mixed pathology combining both degenerative and vascular components which may interact [5].

In Alzheimer disease (AD) neurodegenerative symptoms are dominating, in a subgroup called mixed dementia (MD) the contribution of degenerative and vascular factors is similar.

A frequent way of Alzheimer's disease treatment consists in compensating the cholinergic deficit by giving cholinesterase inhibitors. This constitutes a merely symptomatic kind of therapy and could be influenced by genetic factors. It was observed that APOE polymorphism played a role in the effectiveness of such treatment [6, 7].

It is possible that besides ApoE, other genetic factors which play a role in the development of AD exert an influence on the effectiveness of its treatment. One of the candidate is a receptor occurring in the central nervous system named the low density lipoprotein (LDL) receptor related protein (LRP). This multiligand receptor binds ApoE, is involved in the catabolism of many proteins and plays a role in β-amyloid degradation. The association of AD with two kinds of polymorphism in the LRP gene has been studied by several authors – a tetranucleotide repeat number ([TTTC]n) polymorphism at the 3' end of Alu repeat [8] and a silent C766T polymorphism in exon 3 [9]. The longer allele of tetranucleotide repeats number polymorphism and T allele of the C766T polymorphism were considered to be unfavourable in AD development. The effect of those polymorphisms on the treatment effectiveness is also possible. Interleukin 1β (IL 1β) is a proinflammatory cytokine. In two kinds of its gene polymorphisms – the + 3993 one in exon 5 and the – 511 polymorphism in the promoter – one of the alleles determines enhanced expression of the gene and higher levels of the cytokine in the plasma i.e. the C allele of the first polymorphism defined as allele 2 and the T allele of the second polymorphism also defined as allele 2 [10, 11]. Both polymorphisms were considered to be risk factors in Alzheimer's disease [12]. Particular interleukin 1β alleles determine the stronger inflammatory reactions in dementia and their role in the response to medication is possible. The influence of LRP and interleukin 1β polymorphisms on the response to treatment has not been studied so far.

Recently it was observed that serum cholesterol level was another factor modulating the efficacy of cholinesterase inhibitors in AD treatment [13].

In this study we investigated the influence of being carriers or noncarriers of various APOE, LRP and IL1β alleles on the response to treatment in the patients with dementia. The influence of serum lipids was also investigated.

## SUBJECTS

The investigated group consisted of 68 individuals with dementia 27 men and 41 women, mean age 69 ± 8,9 years. All patients were subjected to a comprehensive medical evaluation, CT or MR examinations and neuropsychological tests. Dementia was diagnosed using ICD-10 criteria. The patients fulfilled the criteria for probable AD according to NINCDS ADRDA [14]. In 20 patients MD was diagnosed according to Hachinski Ischemia Scale [15].

*Treatment.* Patients included in the study were at the beginning of their treatment and were treated one year with the cholinesterase inhibitors mostly rivastigmine (Exelon) 6 mg daily. Only 8 persons were treated with donepezil (Aricept) 5 or 10 mg daily. The patients did not receive any other drugs improving cognitive function or cerebral blood flow. A bad response to treatment was recognized when the progress of the disease exceeded a two points difference on MMSE [16] scale. A good response was recognized when the progress of the disease was stopped or when it was very slow – up to a two point difference on the MMSE scale. In order to ascertain full objectivity the physician evaluating the course of the disease was blind for patients genotype.

Patients gave their informed consent for the investigations and the study was approved by the Ethics Committee of the Institute of Psychiatry and Neurology.

## METHODS

DNA was isolated from blood leukocytes. APOE genotype identification was performed by the Hixson and Vernier method [17], the tetranucleotide repeat polymorphism of the LRP gene was analysed basing on the Hatanaka et al. method [18] method and the exon 3 polymorphism on Kang's et al. method [9]. Both interleukin 1β gene polymorphisms were investigated according to the Takamatsu et al. method [19].

Total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were determined by enzymatic methods.

*Statistical evaluation.* Statistical analyses were performed using Statistica version 6. Quantitative data were expressed as mean values ± standard deviations. Statistical significance of the differences in the frequencies of qualitative variables was evaluated using $chi^2$ or Fisher exact tests. Differences in means between groups were tested using the Mann-Whitney nonparametric test. P-values lower than 0,05 were considered as statistically significant.

## RESULTS

Out of all the patients 19 subjects showed unsatisfactory response to treatment and 49 demonstrated positive outcomes. The initial and after treatment MMSE scores for the patients are shown on Table 1. The groups did not differ significantly in respect of age and lipid levels with the exception that LDL-C was borderline

Table 1. Clinical and biochemical characteristics of patients with bad and good response to treatment

| Variable analysed | AD+MD | | | | AD | | | | MD | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | bad response (n = 19) | | good response (n = 49) | | bad response (n = 14) | | good response (n = 34) | | bad response (n = 5) | | good response (n = 15) | |
| | mean | ± SD | mean | ± SD | mean | ± SD | mean | ± SD | mean | ± SD | mean | ± SD |
| Age | 69,4 | ± 7,1 | 69,8 | ± 9,6 | 68,2 | ± 7,4 | 69,0 | ± 10,4 | 72,8 | ± 5,4 | 71,6 | ± 7,3 |
| MMSE (initial) | 20,4 | ± 4,8 | 21,6 | ± 3,7 | 20,2 | ± 5,2 | 21,3 | ± 3,9 | 21,0 | ± 4,3 | 22,3 | ± 3,2 |
| DMMSE | –5,6 | ± 2,6 | –0,5 | ± 1,2* | –5,7 | ± 2,4 | –0,6 | ± 1,2* | –5,4 | ± 3,6 | –0,4 | ± 1,3** |
| TC | 226,5 | ± 50,4 | 214,7 | ± 40,6 | 238,9 | ± 52,5 | 215,5 | ± 40,7 | 196,7 | ± 31,6 | 212,7 | ± 41,9 |
| TG | 121,2 | ± 37,5 | 117,1 | ± 47,4 | 120,7 | ± 36,5 | 113,7 | ± 43,8 | 122,3 | ± 44,1 | 125,3 | ± 55,9 |
| LDL-C | 149,0 | ± 48,9 | 140,5 | ± 35,4 | 161,9 | ± 49,8 | 139,4 | ± 35,3# | 118,2 | ± 32,9 | 143,0 | ± 36,8 |
| HDL-C | 53,1 | ± 13,0 | 50,7 | ± 12,8 | 52,8 | ± 11,6 | 53,4 | ± 13,5 | 53,9 | ± 17,5 | 44,5 | ± 8,6 |
| TC/HDL | 4,48 | ± 1,35 | 4,43 | ± 1,10 | 4,69 | ± 1,34 | 4,24 | ± 1,11 | 3,97 | ± 1,37 | 4,87 | ± 0,98 |

* – $p < 0,00001$ good vs bad response
** – $p = 0,0009$ good vs bad response
\# – $p = 0,090$ good vs bad response
AD – Alzheimer Disease, MD – Mixed Dementia, MMSE – Mini Mental State Examination,
DMMSE – the difference between MMSE after treatment and initial MMSE

Table 2. The frequency of various APOE, LRP genotype and allele carriers and high LDL cholesterol level (LDL-C >135 mg/dl) in patients with bad and good response to treatment

| Variable analysed | AD+MD | | | | | AD | | | | | MD | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | bad response (n = 19) | | good response (n = 49) | | p | bad response (n = 14) | | good response (n = 34) | | p | bad response (n = 5) | | good response (n = 15) | | p |
| | n | % | n | % | | n | % | n | % | | n | % | n | % | |
| APOE | | | | | | | | | | | | | | | |
| ε2 allele carriers | 0 | 0 | 8 | 16,3 | 0,146 | 0 | 0 | 6 | 17,7 | 0,161 | 0 | 0 | 2 | 13,3 | 1,000 |
| ε3/3 | 9 | 47,4 | 26 | 53,1 | 0,880 | 7 | 50,0 | 18 | 52,9 | 0,895 | 2 | 40,0 | 8 | 53,3 | 1,000 |
| ε4 allele carriers | 10 | 52,6 | 15 | 30,6 | 0,159 | 7 | 50,0 | 10 | 29,4 | 0,200 | 3 | 60,0 | 5 | 33,3 | 0,347 |
| LRP [TTTC]n polymorphism | | | | | | | | | | | | | | | |
| 92 allele carriers | 18 | 94,7 | 39 | 79,6 | 0,248 | 14 | 100,0 | 27 | 79,4 | 0,090 | 4 | 80,0 | 12 | 80,0 | 1,000 |
| LRP C766T polymorphism | | | | | | | | | | | | | | | |
| T allele carriers | 6 | 31,6 | 9 | 18,4 | 0,394 | 5 | 35,7 | 4 | 11,8 | 0,098 | 1 | 20,0 | 5 | 33,3 | 1,000 |
| LDL-C > 135 mg/dl | 11 | 64,7 | 23 | 48,9 | 0,405 | 10 | 83,3 | 15 | 45,5 | 0,040 | 4 | 80,0 | 6 | 42,9 | 0,303 |
| More than 1 disadv, fact # | 16 | 94,1 | 28 | 59,6 | 0,020 | 12 | 100 | 18 | 54,5 | 0,012 | 4 | 80,0 | 10 | 71,4 | 1,000 |

AD – Alzheimer Disease, MD – Mixed Dementia
\# – disadvantageous factors: APOE ε4-allele, LRP 92 allele, LRP T allele and LDL-C > 135 mg/dl

significantly higher in the individuals with AD showing bad response to treatment.

No one carrier of the ε2 allele showed a bad response to treatment. A higher frequency of AD + MD ε4 allele carriers showed bad response to treatment as compared with ε2 allele carriers, the difference was borderline significant (40,0% vs 0,0%, respectively, $p = 0,071$) (data not shown). Among bad responders the frequency of ε4 allele carriers was higher comparing to their frequency in the good responders group (Table 2).

A higher frequency of carriers of the longer 92 allele of [TTTC]n LRP polymorphism and of carriers of T allele of the C766T LRP polymorphism was also observed in the group of bad response to treatment comparing with their frequency in the good responders group. In case of both these polymorphisms the difference was borderline significant in the AD subgroup. Comparison of frequencies of patients with high LDL cholesterol level (LDL-C > 135 mg/dl) between bad and good responders to treatment showed that the frequency in AD subgroup was significantly higher ($p = 0,04$) (Table 2).

The more of those disadvantageous factors had an individual i.e.: ε4 allele of apolipoprotein E, the longer allele of the tetranucleotide repeats number polymorphism and T allele of the C766T polymorphism in the LRP gene and an elevated LDL cholesterol level the significantly worse was the influence on his treatment results. The effect was stronger in AD group. As shown on Table 3 in AD group in simultaneous carriers of

Table 3. The frequency of bad response to treatment in carriers of different number of investigated disadvantageous factors i.e., APOE ε4 allele, LRP 92 allele, LRP T allele and LDL-C >135 mg/dl

| Number of factors # | AD + MD (n = 64) | | AD (n = 45) | | MD (n = 19) | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| 0 | 1/4 | 25,0 | 0/2 | 0,0 | 1/2 | 50,0 |
| 1 | 0/16 | 0,0 | 0/13 | 0,0 | 0/3 | 0,0 |
| 2 | 8/27 | 29,6 | 5/18 | 27,8 | 3/9 | 33,3 |
| 3 | 5/12 | 41,7 | 4/8 | 50,0 | 1/4 | 25,0 |
| 4 | 3/5 | 60,0 | 3/4 | 75,0 | 0/1 | 0,0 |
| P | 0,037 | | 0,014 | | 0,692 | |
| 0 or 1 | 1/20 | 5,0 | 0/15 | 0,0 | 1/5 | 20,0 |
| 2, 3 or 4 | 16/44 | 36,4 | 12/30 | 40,0 | 4/14 | 28,6 |
| P | 0,020 | | 0,012 | | 1,000 | |

# – disadvantageous factors: APOE ε4-allele, LRP 92 allele, LRP T allele and LDL-C > 135 mg/dl

3 unfavourable alleles and having also high LDL-C level the frequency of bad response to treatment was 75%, while all noncarriers of these factors or carriers of only one factor responded well to treatment.

IL 1β polymorphism did not show differences in the response to treatment between carriers of various alleles (data not shown).

## DISCUSSION

Poirier [6] and Richard et al. [7] reported that APOE allele ε4 carriers were more resistant to pro-cholinergic treatment than noncarriers of this allele. It is probable that the cholinergic deficit is stronger in APOE ε4 allele carriers and therefore it is more difficult to compensate. This observation was however not confirmed by some authors [20]. We did observe a difference in the frequency of carriers and noncarriers of the ε2 or ε4 of apolipoprotein E alleles in the groups showing a good or bad response to treatment. The difference was not significant most probably because a small quantity of carriers of particular alleles in the investigated groups.

Our results showed that LRP polymorphism could also as one of the disadvantageous factors contribute to a greater resistance to treatment in AD individuals possibly resulting from a greater cholinergic deficit.

The observation that plasma cholesterol level is a bad prognostic factor in the effectiveness of pro-cholinergic treatment in AD patients [13] was confirmed in our observations.

It has to be mentioned that our observations showed a more accentuated effect of investigated factors in AD i.e. in this form of dementia where neurodegenerative symptoms are the prevailing ones.

The influence of genetic traits on the response to not only pro-cholinergic treatment of dementia was also shown [20] and recent data justify the opinion that the knowledge concerning genetic factors in other kinds of dementia treatment will develop fast [21].

## CONCLUSION

Our preliminary results showed that APOE and LRP polymorphism as well as LDL cholesterol levels could modify the effectiveness of treating patients with dementia with acetylcholinesterase inhibitors. The effect of disadvantageous individual traits was stronger in AD patients as compared with mixed dementia.

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