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Association between plasma biomarkers, *CDK5* polymorphism and the risk of Alzheimer's disease

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In this study we evaluated biochemical blood serum parameters and the association of Cyclin-dependent kinase 5 (*CDK5*) gene polymorphisms with the risk of Alzheimer's disease (AD) in the Polish population. We observed an elevated total cholesterol, low-density lipoproteins (LDL) and homocysteine levels and lower concentrations of high-density lipoproteins (HDL) and vitamin B_{12} in AD patients. However, the analyzed *CDK5* polymorphisms were not associated with the biochemical parameters. Moreover, we found no association between the studied polymorphisms and the risk of AD in the Polish population. The meta-analysis of previously published and current study was performed. In conclusion, our study demonstrated that alteration of cholesterol, LDL, HDL, homocysteine and B_{12} concentration may be an important factor in pathogenesis of AD.

Key words: Alzheimer's disease, CDK5, single nucleotide polymorphism, lipid metabolism, cholesterol, homocysteine, vitamin B_{12}

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disorder leading to irreversible cognitive impairment in the elderly. So far, there is no efficient method of prevention nor effective treatment affecting disease progression. AD has a complex pathogenesis, and neurodegenerative processes start many years before the clinical symptoms are observed. Because the risk of AD increases with age, demographic prognosis indicates a significant rise of population affected by AD.

So far, in the Alzgene database there has been information gathered about 695 genes and 2973 polymorphisms that could potentially contribute to AD risk (http://www.alzforum.org/. Accessed 22 October 2012). However, the only generally acknowledged risk factor remains *APOE4* (Corder et al. 1993, Strittmatter et al. 1993, Schmechel et al. 1993, Blacker et al. 1997, Wehr et al. 2003, Seshadri et al. 2010). The presence of ε4 allele of *APOE* significantly increases the risk of AD. It is noteworthy that *APOE4* is neither necessary nor sufficient for AD and about 50% of AD patients carry this allele. The recently published large genome-wide association studies suggest that other genes are significantly involved in AD risk, such as *CLU* (also known as *APOJ*), *PICALM* and *CR1* (Harold et al. 2009, Lambert et al. 2009). However, there are remaining genetic risk factors to be discovered.

Deregulation of protein phosphorylation has been implicated in the pathogenesis of AD. It was observed that expression and activity of many kinases (i.a. CDK5, GSK-3β, JNK, p38, PKA, PKB, PKC) and phosphatases (i.a. PP1, PP2A, calcineurin) are altered in AD brains (Chung 2009). Among the kinases, Cyclin-Dependent Kinase 5 (CDK5) is one of the most interesting functional candidates, as it is responsible for the aberrant phosphorylation of both microtubule associated

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protein (MAP) tau and amyloid β precursor protein (APP) (Baumann et al. 1993, Iijima et al. 2000). Hyperphosphorylated tau is the major component of the intracellular neurofibrillary tangles (NFTs), which besides extracellular deposits of amyloid β (Aβ) are pathological hallmarks of AD. Recently, the dual pathway hypothesis was proposed, suggesting that both Aβ and tau abnormalities may be linked through the common upstream triggers (Small and Duff 2008). CDK5 may evoke hyperphosphorylation of MAP tau and APP, but it can also affect other proteins potentially involved in the pathomechanism of AD, i.a. GSK-3β, NMDAr, p53, PSEN1 (Li et al. 2001, Lau et al. 2002, Xu et al. 2002, Morfini et al. 2004). Experimental models of AD and post-mortem analysis of AD brains confirmed the important role of CDK5 in the pathomechanism of Alzheimer's disease (Alvarez et al. 1999, Lee et al. 1999, Tseng et al. 2002, Lopes et al. 2010, Chu et al. 2012, Shukla et al. 2012).

The human gene *CDK5 (PSSALRE)* coding Cyclin-Dependent Kinase 5 lies on the long arm of chromosome 7, at loci 7q36, and consists of 12 exons. According to the NCBI database it contains 4098 base pairs and codes for 292 amino acids. Recently, a novel *CDK5* splicing variant, named as CDK5-SV, was cloned from the cDNA library of human testis (Li et al. 2010). *CDK5-SV* lacks the exon 7 and encodes a CDK5 protein built of 260 aa. However, expression of *CDK5-SV* was observed only in testis, skeletal muscle, colon, bone marrow and ovary, while CDK5 is ubiquitously expressed and active predominantly in neurons.

We decided to study SNPs that in previously published analyses gave contradictory results to further elucidate their role in AD (Rademakers et al. 2005, Reiman et al. 2007, Li et al. 2008, Arias-Vasquez et al. 2008, Vázquez-Higuera et al. 2009). We have focused on three single nucleotide polymorphism (SNP) sites in *CDK5* gene (7q36): rs2069454 (G>C), rs9278 (G>A) and rs2069442 (C>G). So far, many environmental and biochemical factors, including the serum level of cholesterol, homocysteine and vitamin B_{12}, have been suggested to increase risk and/or to affect progression of AD (Ikeda et al. 1990, Galbete et al. 2000, Leblhuber et al. 2000, Koudinov and Koudinova 2005, Flicker et al. 2008). There are several direct and indirect indications suggesting a possible interaction between CDK5 kinase and serum level of cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), homocysteine and B_{12} (Vafai and Stock 2002, Zhu et al. 2005, Sanghera et al. 2008, Ma et al. 2009, Dong et al. 2009, Jones et al. 2010). Cholic acid, a cholesterol derivative, activates CDK5 (Zhu et al. 2005). Cell culture experiments and animal studies indicated a strong dependence between cholesterol level and Alzheimer's disease (Eckert et al. 2005). The increased cholesterol level, which is a risk factor for Alzheimer's disease (Solomon et al. 2009), may enhance CDK5 activity, leading to deregulation of phosphorylation processes, including hyperphosphorylation of Microtubule-associated protein (MAP) tau. Cholesterol-dependent modulation of tau phosphorylation in cultured neurons was demonstrated previously (Fan et al. 2001). Cholesterol may increase expression of laminin, which in turn may affect activity and localization of CDK5 (Konings et al. 1994, Pigino et al. 1997). Moreover, it was demonstrated that cholesterol enhances proinflammatory signaling in macrophages, including expression of TNF-α, and TNF-α increases CDK5 activity (Memon et al. 1993, Utreras et al. 2009). However, reverse relationship is also possible, as CDK5 may phosphorylate eNOS, which activity may affect plasma cholesterol level (van Haperen et al. 2002, Lee et al. 2010). Furthermore, other association between CDK5 and lipid metabolism was recently demonstrated. According to Sanghera and coworkers (2008), *CDKAL1* (CDK5 regulatory subunit-associated protein 1-like 1) polymorphism (rs7754840) is significantly associated with decreased HDL-cholesterol levels.

A decrease of vitamin B_{12} concentration and related increase of homocysteine level were observed in LOAD group compared to the control group. Homocysteine may affect progression of AD by interaction with methylation processes. It was demonstrated that homocysteine inhibits Protein Phosphatase 2A (PP2A) methylation in the brain leading to reduction of ABαC heterotrimer formation, drop of activity and thus to tau hyperphosphorylation (performed by GSK-3β and CDK5) (Vafai and Stock 2002, Zhang et al. 2008). Moreover, homocysteine may increase expression of Cyclin D1, which is a binding partner of CDK5 (Lazaro et al. 1997, Outinen et al. 1999). Thus, investigation of the association of these factors with polymorphism of *CDK5* gene was also included in the study.

Table I

Serum levels of lipids, homocysteine, folic acid and vit. B_{12} in healthy controls, EOAD and LOAD patients

	Control	EOAD	LOAD	AD (EOAD+LOAD)
Total cholesterol (mg/dl)	190.60 ± 32.61 (*n*=80)	220.21 ± 38.22 (*n*=68)***	213.54 ± 46.29 (*n*=189)***	215.30 ± 44.32 (*n*=257)***
	189 (125–304)	220.5 (135–324)	210 (182–238)	213 (184–241)
Triglycerides (mg/dl)	111.825 ± 54.98 (*n*=80)	102.55 ± 54.65 (*n*=67)	113.72 ± 65.65 (*n*=189)	110.8 ± 63.04 (*n*=256)
	102 (74.5–131)	87 (62–138)	99 (74–135)	94 (70.5–136)
HDL (mg/dl)	65.91 ± 17.64 (*n*=80)	60.57 ± 17.85 (*n*=68)	59.39 ± 16.67 (*n*=187)**	59.70 ± 16.96 (*n*=255)**
	64 (53.5–74.5)	59 (46–74.5)	57 (49–68)	58 (47–69)
LDL (mg/dl)	102.29 ± 29.44 (*n*=80)	137.68 ± 37.37 (*n*=68)***	131.59 ± 39.62 (*n*=188)***	133.21 ± 39.05 (*n*=256)***
	97 (79–122.5)	132 (116.5–164)	129 (107–154.5)	130 (107.5–158)
Homocysteine (μmol/l)	12.62 ± 3.94 (*n*=90)	12.97 ± 4.20 (*n*=25)	16.53 ± 7.83 (*n*=157)***,##	16.04 ± 7.53 (*n*=182)***
	11.95 (10.55–13.93)	12.32 (10.35–14.7)	14.94 (12.32–18.34)	14.4 (11.98–18.13)
Folic acid (ng/ml)	8.58 ± 3.76 (*n*=99)	10.72 ± 16.69 (*n*=37)	8.32 ± 3.88 (*n*=167)	8.75 ± 7.90 (*n*=204)
	7.6 (5.8–10.59)	7.6 (6.3–9.22)	7.6 (5.4–10)	7.6 (5.6–9.9)
Vitamin B_{12} (pg/ml)	411.52 ± 182.05 (*n*=102)	354.50 ± 135.71 (*n*=37)	307.32 ± 152.82 (*n*=165)***,#	315.96 ± 150.63 (*n*=202)***
	405.5 (280–531.5)	333.8 (263.8–433.8)	287 (202–378.8)	291.45 (208.7–384.4)

Data were described by means ± SD, median (25^{th} percentile–75^{th} percentile). *,**,*** P<0.05, 0.01, 0.001 as compared to control group, respectively; #,##,### P<0.05, 0.01, 0.001 as compared to EOAD group, respectively.

METHODS

Sample description

The EOAD group consisted of 71 patients (F –46 (64.79%), M –25 (35.21%), mean age of onset = 57.25 ± 4.19 and 57.05 ± 4.32 years, respectively). LOAD group consisted of 204 patients (F – 146 (71.57%), M – 58 (28.43%), mean age of onset = 73.50 ± 5.04 and 74.04 ± 5.30 years, respectively). The groups did not differ in gender. Both groups were recruited by the Alzheimer Outpatient Clinic of the Department of Neurodegenerative Disorders (Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw). In all affected Alzheimer's disease was diagnosed as probable according to the NINCDS-ADRDA criteria. All patients obtained neurological, neuropsychological [MMSE, Global Deterioration Scale, Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) and Blessed Dementia Rating Scale] and psychiatrical evaluation. In addition, a CT scan with an assessment of hippocampal fissure was obtained for each patient. The control group consisted of 178 non-demented people [F-129 (72.47%), M-49 (27.53%), mean age = 70.86 ± 7.08 and 72.43 ± 5.79 years, respectively]. The control group was recruited from the general population. These individuals were neurologically tested and exhibited no apparent neurological disease or psychiatric syndrome.

They had no memory impairment and obtained MMSE score >27 (as further described in Styczyńska et al. 2008). The same control group was applied for the study of EOAD and LOAD patients, representing a healthy population sample. All human studies were approved by the appropriate ethics committee and were performed in accordance with the ethical standards of the Declaration of Helsinki (1964). All participants gave their informed consent prior to their inclusion in the study.

APOE genotypes in the subgroup of the control individuals and AD patients were determined according to previously method (Henderson et al. 2002). Distribution of *APOE* genotypes and alleles in tested groups is presented in Appendix Table I. Patients and the control individuals were stratified into two subgroups, according to *APOE* status: those carrying at least one *APOE4* allele (*APOE4*+) and *APOE4* non-carriers (*APOE4*-).

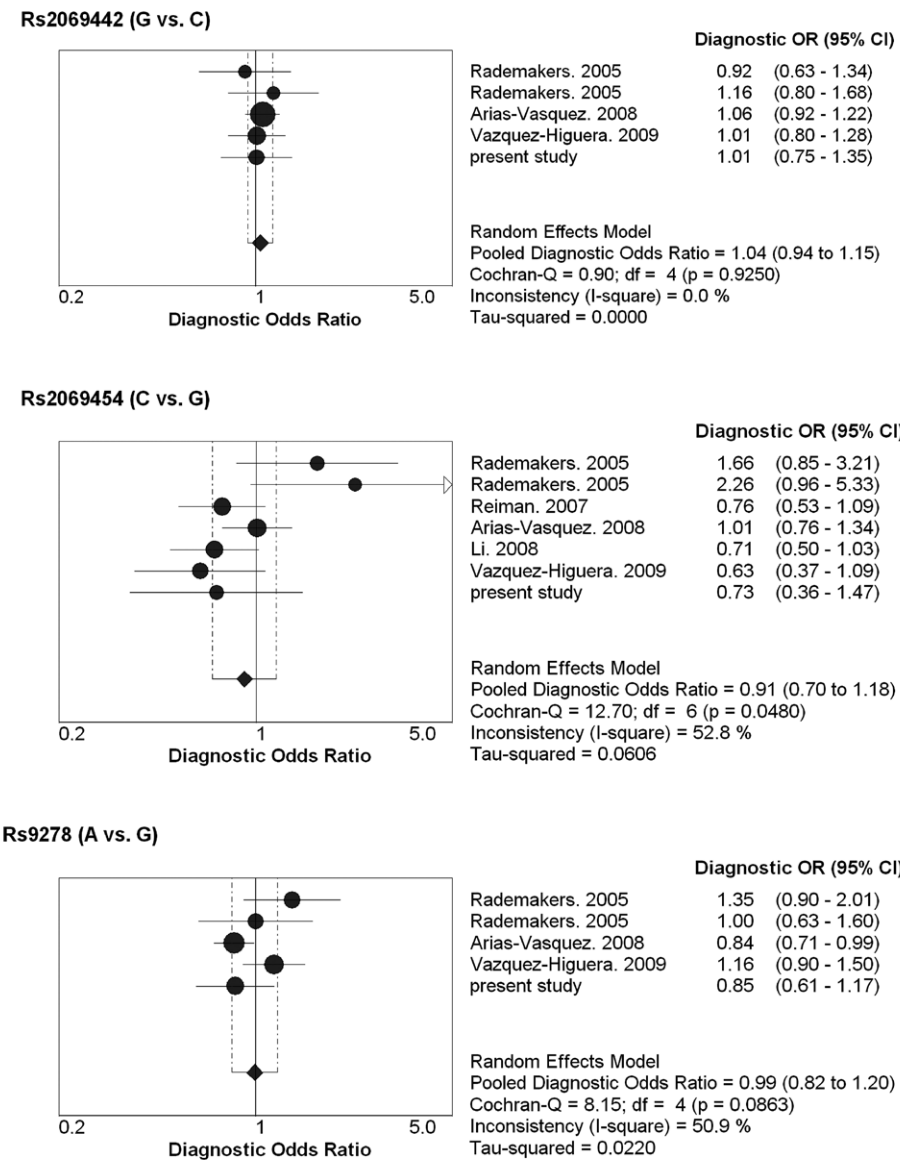

Fig. 1. The meta-analysis of all available studies on the association of *CDK5* polymorphism and AD, including present study (Rademakers et al. 2005, Reiman et al. 2007, Li et al. 2008, Arias-Vasquez et al. 2008, Vázquez-Higuera et al. 2009). All the studies included a total of 6 675/1 373, 7 928/2 931 and 6 616/1 408 control individuals / AD cases for rs2069442, rs2069454 and rs9278, respectively. Meta-analysis was performed with Meta-Disc ver. 1.4.

A set of biochemical parameters in the blood plasma (homocysteine, vitamin B_{12}, cholesterol and other lipids) was previously published in the small subgroup of subjects (subgroup of 100 patients with AD and 100 control individuals) (Styczyńska et al. 2008). The currently published results were determined in enlarged subgroups of the control individuals, EOAD and LOAD patients, extend those previously published data (Table I).

Genotyping

Genomic DNA was extracted from leukocytes using the Miller's protocol (Miller et al. 1988). Genotyping of rs9278, rs2069442 and rs2069454 in *CDK5* was performed using commercially available TaqMan Probe Genotyping Assays (ABI Biosystems, Foster City, CA) (Heid et al. 1996). Reliability of the applied method was verified in randomly selected samples by DNA sequencing and PCR-RFLP, as described previously (Rademakers et al. 2005). No inconsistencies were detected.

Statistical analysis

Statistical analysis was performed using Statistica 7.1 software (Statsoft). Continuous variables for the groups were tested for normality of distribution using the Shapiro–Wilk test. Since all distributions were significantly different from normal, data were described not only by mean ± SD but also by median (25^{th}–75^{th} percentile). Kruskal-Wallis test followed by Mann-Whitney test as well as χ^2 test and Fisher exact test were used in further statistical analyses. Haplotype frequencies were analyzed with application of Haploview 4.0 software (Barrett et al. 2005). Meta-analysis was performed with Meta-Disc ver. 1.4 (Zamora et al. 2006). *P* values less than 0.05 without correction for multiple comparisons were considered significant. The statistical power for comparison of allele frequencies between our AD (n=275) and control (n=178) groups was sufficient to detect with 80% probability the true effect size measured as odds ratio (OR) equal to 1.52 or 0.64 for rs2069442, 1.55 or 0.61 for rs9278 and 2.37 or 0.22 for rs2069454.

RESULTS

Genotypes of the three analyzed SNPs in *CDK5* gene were determined in 178 control individuals, and 275 AD patients (71 EOAD and 204 LOAD cases). Three SNPs in *CDK5* gene (rs9278, g.11346603:G>A; rs2069454, g.11348605:G>C; rs2069442, g.11350828:C>G) were analyzed. All genotype frequencies are according to the Hardy-Weinberg equilibrium in each group. There was no difference in the distribution of genotypes and alleles of analyzed SNPs, when EOAD and LOAD patients were analyzed separately and as a combined group "AD" (Table II). Moreover, similar distribution of genotype or allele frequencies was observed when the groups were stratified by gender or age (data not shown).

The frequency of *APOE4* in LOAD (0.63) and AD (0.60) is significantly (P<0.00001) increased in comparison with the control individuals (0.24). There were no significant differences in the *APOE4* frequency between the EOAD group (0.30) and the control individuals (P=0.61). In the studied groups no differences in *APOE2* distribution was detected. Moreover, no association between *APOE4* genotype and tested *CDK5* genotypes (Appendix Table II) or biochemical parameters (data not shown) was found.

There were four haplotypes encompassing rs9278-rs2069454-rs2069442 loci: G-G-C, G-G-G, A-G-C and G-C-C, with frequencies in all combined groups equal to 0.45, 0.296, 0.219 and 0.035, respectively. Their combinations formed nine of ten possible diplotypes (G-C-C / G-C-C was not found in any subject). No significant differences were detected in the distribution of these *CDK5* haplotypes and diplotypes between the healthy control group and EOAD, LOAD or AD groups (data not shown).

The analysis of serum biochemical parameters was performed in the subgroup of AD patients and in the control group. Significant differences were found in the total, HDL- and LDL-cholesterol levels, but not in the triglycerides' level between the control group and AD patients (Table I). Moreover, lower concentration of vitamin B_{12} and higher level of homocysteine were observed in the AD group compared to the control group. No differences in the folic acid level were observed. No significant associations were found between the analyzed polymorphism in *CDK5* genotypes and the subjects' age, gender and AD risk.

Because a single study may be not sensitive enough to detect interactions of gene variations with AD risk, the meta-analysis of all available studies on *CDK5* polymorphisms was performed, including present study. Five eligible studies on six populations were identified in the Pubmed and Alzgene databases (Rademakers et al. 2005, Reiman et al. 2007, Arias-

Vasquez et al. 2008, Li et al. 2008, Vazquez-Higuera et al. 2009). All the studies included a total of 6 675/1 373, 7 928/2 931 and 6 616/1 408 control individuals / AD cases for rs2069442, rs2069454 and rs9278, respectively (Appendix Table III). Because the studies tested both EOAD and LOAD, we pooled all AD patients into one group "AD" for the meta-analysis purposes. The meta-analysis of all previously published data and our results revealed that the rs2069442, rs2069454 and rs9278 polymorphisms have no effect on AD risk (Fig. 1). Comparing to the previously published meta-analysis (Alzgene database, http://www.alzgene.org), our results gave similar results (Bertram et al. 2007). However, the high level of heterogeneity and inconsistency for rs2069454 and rs9278 (P<0.05 heterogeneity using Cochran's Q test) suggested that variations across the studies are greater than expected. The data were re-analyzed after excluding the results published by Rademakers and colleagues (2005) and, as shown on Figure 2, this operation considerably changed the results of meta-analysis. The heterogeneity of analyzed studies was excluded by Cochran-Q test, P=0.4294 and P=0.0954 for rs2069454 and rs9278, respectively. Surprisingly, despite all previous studies gave negative results, the meta-analysis indicated an association (P<0.05) between rs2069454 polymorphism and the

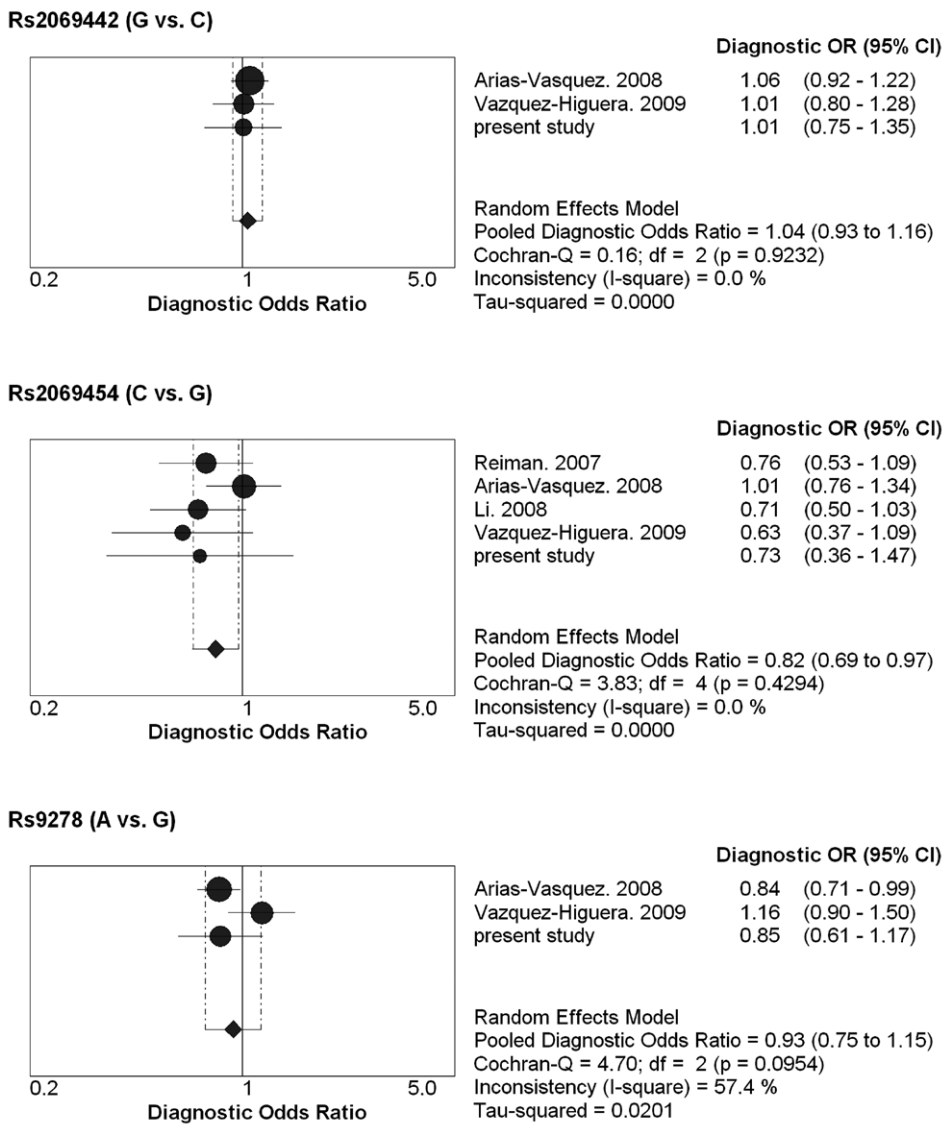

Fig. 2. The meta-analysis of studies on the association of *CDK5* polymorphism and AD after excluding the results of Rademakers and colleagues (2005). Meta-analysis was performed with Meta-Disc ver. 1.4.

risk of AD (Fig. 2). Polymorphisms rs2069442 and rs9278 were not associated with the risk of AD.

DISCUSSION

In the present study we focused on three SNPs in *CDK5* gene, rs2069454, rs9278 and rs2069442. CDK5 has been proposed as an important factor involved in the deregulation of phosphorylation processes in Alzheimer's disease. Previous analysis of above-mentioned SNPs gave contradictory results (Rademakers et al. 2005, Reiman et al. 2007, Li et al. 2008, Arias-Vasquez et al. 2008, Vázquez-Higuera et al. 2009). Our results indicate that in Polish population there is no significant association between *CDK5* polymorphism (rs9278, rs2069442 and rs2069454) and the risk of Alzheimer's disease.

Polymorphism rs2069442 is located upstream of exon 1, rs2069454 in intron 5 and rs9278 in 3' untranslated region (3'UTR) of *CDK5* gene. Although they do

Table II

Frequencies of *CDK5* genotypes and alleles analysis in healthy controls, EOAD and LOAD patients

rs9278								
	total	GG	GA	AA	allele G	allele A	OR (95% CI) [a]	*P* [a]
	n	*n* (freq.)	*n* (freq.)	*n* (freq.)	*n* (freq.)	*n* (freq.)		
controls	178	104 (0.58)	64 (0.36)	10 (0.06)	272 (0.76)	84 (0.24)		
EOAD	71	43 (0.61)	23 (0.32)	5 (0.07)	109 (0.77)	33 (0.23)	0.980 (0.619–1.553)	1
LOAD	204	130 (0.64)	67 (0.33)	7 (0.03)	327 (0.80)	81 (0.20)	0.802 (0.568–1.133)	0.218
AD (EO+LO)	275	173 (0.63)	90 (0.33)	12 (0.04)	436 (0.79)	114 (0.21)	0.847 (0.615–1.166)	0.324
rs2069454								
	total	GG	GC	CC	allele G	allele C		
	n	*n* (freq.)	*n* (freq.)	*n* (freq.)	*n* (freq.)	*n* (freq.)		
controls	178	163 (0.92)	15 (0.08)	0 (0)	341 (0.96)	15 (0.04)		
EOAD	71	67 (0.94)	4 (0.06)	0 (0)	138 (0.97)	4 (0.03)	0.659 (0.215–2.021)	0.608
LOAD	204	191 (0.94)	13 (0.06)	0 (0)	395 (0.97)	13 (0.03)	0.748 (0.351–1.595)	0.563
AD (EO+LO)	275	258 (0.94)	17 (0.06)	0 (0)	533 (0.97)	17 (0.03)	0.725 (0.357–1.471)	0.461
rs2069442								
	total	CC	CG	GG	allele C	allele G		
	n	*n* (freq.)	*n* (freq.)	*n* (freq.)	*n* (freq.)	*n* (freq.)		
controls	178	87 (0.49)	77 (0.43)	14 (0.08)	251 (0.71)	105 (0.29)		
EOAD	71	40 (0.56)	27 (0.38)	4 (0.06)	107 (0.75)	35 (0.25)	0.782 (0.501–1.220)	0.321
LOAD	204	99 (0.49)	82 (0.40)	23 (0.11)	280 (0.69)	128 (0.31)	1.093 (0.802–1.489)	0.582
AD (EO+LO)	275	139 (0.50)	109 (0.40)	27 (0.10)	387 (0.70)	163 (0.30)	1.007 (0.752–1.349)	1

[a] for rare allele frequency in patients vs. controls

not affect the protein sequence, the effect on mRNA stability or translation can not be excluded. It was previously suggested that these SNPs are associated with an increased risk of Alzheimer's disease.

For SNP rs9278, no difference in genotype distribution between AD patients and the control group was observed in the Polish population. Similar results were previously obtained in the Swedish, Dutch and Spanish populations (Rademakers et al. 2005, Vazquez-Higuera et al. 2009). However, multi-sample analysis of the Dutch population performed by Arias-Vasquez and others (2008) indicated a significant trend (odds ratio 0.65 for the AA carriers and 0.84 for the GA carriers, compared to GG carriers). In our analysis a borderline significance (P=0.0596) trend was observed for the reduced frequency of AA genotype in the *APOE4*-non-carriers in the LOAD group (0.01) comparing to the control group (0.08) (Appendix Table I).

We did not find any difference in the distribution of rs2069454 genotypes. Rademakers and coauthors (2005) observed a 2-fold increased AD risk for the C allele carriers in both Dutch and Swedish populations. However, the following studies did not confirm this finding (Reiman et al. 2007, Li et al. 2008, Arias-Vasquez et al. 2008, Vazquez-Higuera et al. 2009). Our meta-analysis of all published studies combined with our data indicated significant (P=0.048) heterogeneity of results using Cochran's Q test (Fig. 1). As the study by Rademakers and others (2005) was the cause of high heterogeneity, it was excluded from further analysis (Fig. 2) what significantly reduced the heterogeneity of the data. The meta-analysis of the remaining studies indicated a slightly decreased AD risk (OR=0.82) for C allele carriers. The further studies would be necessary for the explanation of the reasons of high heterogeneity and for confirmation of the significance of rs2069454 polymorphism in AD.

Our analysis of rs2069442 did not indicate any association with AD risk. Arias-Vasquez and colleagues (2008) found that in the Dutch population the carriers of GG genotype without *APOE4* allele have significantly increased risk of AD. When limiting the analysis to incident AD cases without *APOE4*, a risk of AD related to GG genotype was increased by 1.9-fold. Studies of Rademakers and coauthors (2005) in Dutch and Swedish populations did not indicate any association, however, they did not control for the influence of *APOE4* genotype. Vazquez-Higuera and others (2009) did not find the difference in the genotype and haplotype distributions between AD cases and the control group, also after stratification by the *APOE4* status.

The presence of the *APOE4* allele is the most important genetic risk factor for late-onset AD (Schmechel et al. 1993, Strittmatter et al. 1993, Blacker et al. 1997). The frequency differs from 0.32–0.42 in AD patients to 0.13–0.17 in the control individuals. Our analysis confirmed the significant difference in distribution of *APOE4* allele between LOAD or AD groups compared to the control group. No association between *APOE* genotype and tested *CDK5* genotypes or biochemical parameters was found.

The analysis of serum biochemical parameters confirmed and extended our previously published results showing significant changes in AD patients (Religa et al. 2003, Styczyńska et al. 2008). The analysis indicated an elevation of total cholesterol, LDL and homocysteine level and lower concentrations of HDL and vitamin B_{12} in the serum of AD patients, comparing to healthy controls.

There is growing body of evidence indicating that dyslipidemia may be an important factor in pathogenesis of AD. Cholesterol, HDL, LDL and triglycerides have been involved in the pathomechanism of AD. High level of cholesterol may negatively influence the brain, however, cholesterol is necessary for normal neuronal function. As an essential component of lipid rafts, cholesterol is involved in neuronal cell adhesion, synaptic transmission, signal transduction and vesicular trafficking (Mathew et al. 2011). Cholesterol is also a precursor for neurosteroids synthesis, such as dehydroepiandrosterone and allopregnanolone, promoting neurogenesis and modulating neurotransmission. The role of cholesterol in AD is still the matter of debate. Several epidemiological studies indicated that elevated mid-life cholesterol level is associated with an increased risk of AD, but opposite results were also published (Solomon et al. 2009, Mielke et al. 2010). There are also controversies regarding treating the AD patients with cholesterol-lowering statins (Rockwood et al. 2002, Sano et al. 2011). Moreover, the analyses of alterations of cholesterol plasma level in AD patients gave also conflicting results (Raygani et al. 2006, Kölsch et al. 2010). Cholesterol may affect APP processing by regulation of activity of β-secretase, an enzyme existing predominantly in the cholesterol-rich micro domains (Puglielli et al. 2003). There was a positive correlation of the level of serum total cholesterol to the amount of Aβ N-42 in AD brains (Kuo et al. 1998). In experimen-

tal conditions high concentration of cholesterol stimulates Aβ production, but high Aβ load inhibits cholesterol synthesis. It seems that this negative feedback control mechanism is not effective in AD.

Low density lipoproteins also have been implicated in AD. Postmortem analyses indicated that serum LDL level clearly relates to density of neuritic plaques (Lesser et al. 2011). Warren et al. also confirmed the significant increase of LDL level in serum of patients with probable AD, comparing to healthy controls (Warren et al. 2012). Authors demonstrated also a decrease of HDL level in AD patients, however, the level of TG was not changed. Higher levels of HDL (>55 mg/dL) were associated with a decreased risk AD compared with lower HDL levels (Reitz et al. 2010).

The effect of vitamin B_{12} and folic acid deficiency is hyperhomocysteinemia, which is associated with an increased risk of developing AD. An increase of homocysteine level is considered as a potential risk factor or merely a risk marker. Recent data suggested that alterations of homocysteine concentration in plasma are not the primary risk factor, but just secondary reflection of changes in the determinants of homocysteine, like folate deficiency (Nilsson et al. 2012). In accordance to previous observations, we confirmed that AD patients had higher plasma levels of Hcy than age-matched controls. However, our analysis showed no association between tested *CDK5* genotypes and determined biochemical parameters.

CONCLUSIONS

In summary, we evaluated biochemical serum parameters and the association of *CDK5* polymorphisms with AD risk in the Polish population. Our results did not confirm the association of studied *CDK5* SNPs with the AD risk. However, the meta-analysis suggested that additional studies may be necessary for the exact determination of the role of genetic variations of *CDK5* gene in AD. Our analysis indicated an elevation of total cholesterol, low-density lipoproteins (LDL) and homocysteine level and lower concentrations of high-density lipoproteins (HDL) and vitamin B_{12} in AD patients. However, we did not confirm the association of studied *CDK5* SNPs with the analyzed biochemical parameters. In conclusion, our study demonstrates that alteration of cholesterol, LDL, HDL, homocysteine and B_{12} levels might constitute an important factor in AD development and pathogenesis.

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APPENDIX

Table I

Frequencies of *CDK5* genotypes in APOE4 carriers and non-carriers. *P*-value, OR and 95% CI refer to the genotype frequencies in AD patients vs. controls.

APOE4-				
	CONTROL	EOAD	LOAD	AD (EO+LO)
rs9278	freq.	freq. (*P*; OR; 95% CI)	freq. (*P*; OR; 95% CI)	freq. (*P*; OR; 95% CI)
GG	0.54	0.59 (0.79; 1.21; 0.41–3.53)	0.57 (0.74; 1.14; 0.59–2.18)	0.58 (0.75; 1.15; 0.62–2.14)
GA	0.38	0.35 (1.00; 0.91; 0.30–2.74)	0.41 (0.74; 1.17; 0.61–2.28)	0.40 (0.75; 1.12; 0.60–2.11)
AA	0.08	0.06 (1.00; 0.69; 0.08–6.12)	0.01 (0.0596; 0.15; 0.02–1.27)	0.02 (0.14; 0.24; 0.05–1.25)
rs2069454	freq.	freq. (*P*; OR; 95% CI)	freq. (*P*; OR; 95% CI)	freq. (*P*; OR; 95% CI)
GG	0.90	0.94 (1.00; 1.72; 0.20–15.03)	0.92 (0.78; 1.24; 0.40–3.88)	0.92 (0.78; 1.31; 0.44–3.92)
GC	0.10	0.06 (1.00; 0.58; 0.07–5.06)	0.08 (0.78; 0.81; 0.26–2.53)	0.08 (0.78; 0.76; 0.26–2.29)
CC	0	0 (n.a.)	0 (n.a.)	0 (n.a.)
rs2069442	freq.	freq. (*P*; OR; 95% CI)	freq. (*P*; OR; 95% CI)	freq. (*P*; OR; 95% CI)
CC	0.53	0.47 (0.79; 0.80; 0.28–2.29)	0.45 (0.41; 0.74; 0.39–1.42)	0.46 (0.43; 0.75; 0.40–1.40)
GC	0.40	0.41 (1.00; 1.04; 0.35–3.04)	0.43 (0.87; 1.10; 0.57–2.13)	0.42 (0.87; 1.09; 0.58–2.04)
GG	0.07	0.12 (0.61; 1.78; 0.32–10.11)	0.12 (0.40; 1.83; 0.58–5.74)	0.12 (0.43; 1.82; 0.60–5.50)
APOE4+				
rs9278	freq.	freq. (*P*; OR; 95% CI)	freq. (*P*; OR; 95% CI)	freq. (*P*; OR; 95% CI)
GG	0.48	0.29 (0.43; 0.44; 0.07–2.73)	0.68 (0.10; 2.29; 0.93–5.62)	0.66 (0.11; 2.09; 0.85–5.10)
GA	0.43	0.71 (0.40; 3.25; 0.52–20.38)	0.28 (0.14; 0.49; 0.20–1.23)	0.30 (0.23; 0.55; 0.22–1.37)
AA	0.09	0 (1.00; 0.57; 0.02–13.36)	0.05 (0.35; 0.52; 0.10–2.76)	0.04 (0.33; 0.09; 0.38–2.60)
rs2069454	freq.	freq. (*P*; OR; 95% CI)	freq. (*P*; OR; 95% CI)	freq. (*P*; OR; 95% CI)
GG	0.91	1.0 (1.00; 1.74; 0.07–40.66)	0.94 (0.63; 1.63; 0.32–8.41)	0.95 (0.62; 1.73; 0.34–8.89)
GC	0.09	0 (1.00; 0.57; 0.02–13.36)	0.06 (0.63; 0.61; 0.12–3.15)	0.05 (0.62; 0.58; 0.11–2.98)
CC	0	0 (n.a.)	0 (n.a.)	0 (n.a.)
rs2069442	freq.	freq. (*P*; OR; 95% CI)	freq. (*P*; OR; 95% CI)	freq. (*P*; OR; 95% CI)
CC	0.65	1.0 (0.14; 8.23; 0.42–162.5)	0.50 (0.18; 0.53; 0.21–1.33)	0.52 (0.27; 0.58; 0.23–1.47)
GC	0.35	0 (0.14; 0.12; 0.01–2.40)	0.39 (0.82; 1.22; 0.48–3.08)	0.37 (1.00; 1.12; 0.44–2.82)
GG	0	0 (n.a.)	0.11 (0.13; 6.00; 0.35–104.3)	0.10 (0.23; 5.66; 0.33–98.20)

n.a. – data not analyzed

Table II

Frequencies of *CDK5* genotypes in APOE4 carriers and non-carriers

	CONTROL	EOAD	LOAD	AD (EO+LO)
	APOE4-/APOE4+	APOE4-/APOE4+	APOE4-/APOE4+	APOE4-/APOE4+
	freq./freq. (*P*;OR;95% CI)	freq./freq. (*P*;OR;95% CI)	freq./freq. (*P*;OR;95% CI)	freq./freq. (*P*;OR;95% CI)
rs9278				
GG	0.54/0.48 (0.64; 1.29; 0.50-3.30)	0.59/0.29 (0.37; 3.57; 0.53-23.96)	0.57/0.68 (0.17; 0.64; 0.36-1.16)	0.58/0.66 (0.26; 0.71; 0.41-1.23)
GA	0.38/0.43 (0.63; 0.78; 0.30-2.02)	0.35/0.71 (0.18; 0.22; 0.03-1.49)	0.41/0.28 (0.06; 1.85; 1.01-3.38)	0.40/0.30 (0.12; 1.58; 0.90-2.76)
AA	0.08/0.09 (1.00; 0.95; 0.18-5.09)	0.06/0 (1.00; 1.36; 0.05-37.56)	0.01/0.05 (0.26; 0.27; 0.03-2.31)	0.02/0.04 (0.48; 0.47; 0.09-2.40)
rs2069454				
GG	0.90/0.91 (1.00; 0.88; 0.17-4.59)	0.94/1.0 (1.00; 0.73; 0.03-20.20)	0.92/0.94 (0.55; 0.67; 0.22-2.08)	0.92/0.95 (0.58; 0.67; 0.23-1.98)
GC	0.10/0.09 (1.00; 1.13; 0.22-5.87)	0.06/0 (1.00; 1.36; 0.05-37.56)	0.08/0.06 (0.56; 1.49; 0.48-4.62)	0.08/0.05 (0.58; 1.49; 0.51-4.41)
CC	0/0 (n.a.)	0/0 (n.a.)	0/0 (n.a.)	0/0 (n.a.)
rs2069442				
CC	0.53/0.65 (0.34; 0.60; 0.22-1.58)	0.47/1.0 (0.02; 0.06; 0.003-1.21)	0.45/0.50 (0.56; 0.84; 0.48-1.49)	0.46/0.52 (0.35; 0.79; 0.45-1.31)
GC	0.40/0.35 (0.81; 1.27; 0.48-3.37)	0.41/0 (0.06; 10.71; 0.53-218.0)	0.43/0.39 (0.66; 1.15; 0.64-2.05)	0.42/0.37 (0.49; 1.24; 0.72-2.13)
GG	0.07/0 (0.33; 3.83; 0.20-71.98)	0.12/0 (1.00; 2.42; 0.10-57.02)	0.12/0.11 (0.82; 1.10; 0.45-2.68)	0.12/0.10 (0.83; 1.16; 0.50-2.69)

Table III

Distribution of *CDK5* alleles among controls and AD cases with Odds Ratios in the studies included in the meta-analysis

SNP	Study	Population	G-allele (frequency) CTR / AD	C-allele (frequency) CTR / AD	OR (95% CI)	% Weight
Rs2069442	Rademakers et al. (2005)	Netherlands	116/55 (0.28/0.26)	296/153 (0.72/0.74)	0.917 (0.630–1.335)	7.51
	Rademakers et al. (2005)	Sweden	143/60 (0.32/0.35)	303/110 (0.68/0.65)	1.156 (0.797–1.677)	7.65
	Arias-Vasquez et al. (2008)	Netherlands	2903/289 (0.26/0.27)	8407/791 (0.74/0.73)	1.058 (0.919–1.218)	53.18
	Vazquez-Higuera et al. (2009)	Spain	192/173 (0.23/0.23)	634/565 (0.77/0.77)	1.011 (0.800–1.279)	19.24
	present study	Poland	105/163 (0.29/0.30)	251/387 (0.71/0.70)	1.007 (0.752–1.349)	12.41
	pooled	pooled	3459/740 (0.26/0.27)	9891/2006 (0.74/0.73)	1.038 (0.937–1.151)	
Rs2069454	Rademakers et al. (2005)	Netherlands	391/191 (0.95/0.92)	21/17 (0.05/0.08)	1.657 (0.854–3.214)	10.27
	Rademakers et al. (2005)	Sweden	434/160 (0.97/0.94)	12/10 (0.03/0.06)	2.260 (0.958–5.334)	7.11
	Reiman et al. (2007)	Netherlands	1042/1633 (0.95/0.96)	58/69 (0.05/0.04)	0.759 (0.531–1.086)	19.13
	Li et al. (2008)	Canada	1275/1308 (0.95/0.96)	71/52 (0.05/0.04)	0.714 (0.495–1.029)	18.82
	Arias-Vasquez et al. (2008)	Netherlands	10779/1033 (0.95/0.95)	567/55 (0.05/0.05)	1.012 (0.762–1.345)	22.01
	Vazquez-Higuera et al. (2009)	Spain	813/762 (0.96/0.97)	37/22 (0.04/0.03)	0.634 (0.371–1.085)	13.24
	present study	Poland	341/533 (0.96/0.97)	15/17 (0.04/0.03)	0.725 (0.357–1.471)	9.41
	pooled	pooled	15075/5620h (0.95/0.96)	781/242 (0.05/0.04)	0.910 (0.700–1.471)	
SNP	Study	Population	G-allele	A-allele	OR (95% CI)	% Weight
Rs9278	Rademakers et al. (2005)	Netherlands	332/157 (0.81/0.75)	80/51 (0.19/0.25)	1.348 (0.904–2.010)	14.43
	Rademakers et al. (2005)	Sweden	370/141 (0.83/0.83)	76/29 (0.17/0.17)	1.001 (0.626–1.602)	11.53
	Arias-Vasquez et al. (2008)	Netherlands	8856/882 (0.80/0.82)	2276/190 (0.20/0.18)	0.838 (0.712–0.987)	31.63
	Vazquez-Higuera et al. (2009)	Spain	746/670 (0.84/0.82)	140/146 (0.16/0.18)	1.161 (0.900–1.497)	23.59
	present study	Poland	272/436 (0.76/0.79)	84/114 (0.24/0.21)	0.847 (0.615–1.166)	18.83
	pooled	pooled	10576/2286 (0.80/0.81)	2656/530 (0.20/0.19)	0.991 (0.822–1.196)	