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APOE genetic variants and apoE, miR-107 and miR-650 levels in Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative dementia in adults. Pathogenesis of AD depends on various factors, including APOE genetic variants, apolipoprotein E (apoE) phenotype and oxidative stress, which may promote both DNA and RNA damage, including non-coding RNA (ncRNA). Among ncRNAs, microRNA (miRNA) is known to contribute to pathologic processes in AD.

The aim of the study was to analyse the plasma concentration of apoE by ELISA as well as the plasma levels of miR-107 and miR-650 by qPCR in relation to APOE genetic variants and clinical features including the age of onset and dementia severity in 64 AD patients and 132 controls.

Our data showed that a low apoE plasma concentration was a risk factor for developing AD (OR = 5.18, p = 6.58E-06) and was particularly pronounced in severe dementia (p < 0.001) and correlated with cognitive functions (R = 0.295, p = 0.020), similarly as the level of miR-650 (R = 0.385, p = 0.033).

The presence of APOE E4 allele in both AD patients and controls led to a reduction in apoE, while APOE E3/E3 genotype was associated with an increased apoE concentration and level of miR-107 in AD (p < 0.05) which was inversely correlated with the number of APOE E4 alleles (R = –0.448, p = 0.009). Additionally, patients with the onset at 60-69 years of age showed a reduced level of miR-107 (p < 0.05, as compared to AD above 80 years of age).

Changed levels of plasma apoE, miR-107 and miR-650 may be a marker of the neurodegenerative process in the course of AD, associated with amyloid β metabolism and inordinate cell cycle.

Key words: *APOE, apoE, miRNA, miR-107, miR-650, Alzheimer's disease.*

Introduction

Alzheimer's disease (AD) is a progressive, age-dependent disease characterized by accumulation of β-amyloid (Aβ) [2] which is quickly cleared with help of apolipoprotein E (apoE). ApoE is encoded by the *APOE* gene, located on chromosome 19. Our previous studies pointed out that the apoE level may vary in healthy individuals before 60 years of age depending on *APOE* genetic status and age or gender of subjects [50]. The *APOE* locus houses

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two well-known polymorphisms, the rs7412 and rs429358 whose variants distinguish three common *APOE* alleles: protective E2, neutral, most common in population E3 and pathogenic E4. *APOE* E4 has a multidimensional impact on pathogenesis of AD, including dysregulation of lipids and lipoproteins, such as apoE plasma level [52], as well as decreased Aβ clearance and glucose metabolism, reduced neuronal signalling, enhanced neuroinflammation and mitochondrial dysfunction [37,40], and additionally elevated oxidative stress [8]. Previous studies have shown that the changes in oxidative stress regulation mechanisms may be strongly associated with the development and onset of AD as well as with the changes in the level of oxidative damage in DNA (such as 8-oxo-2'deoxyguanosine, 8-oxo2dG) or levels of biothiols (such as homocysteine – Hcy, and glutathione – GSH) [9,49] and with alterations in p53 protein activity [38,41], both in blood cells and plasma [47]. The prolonged oxidative stress, besides DNA damage, may cause also RNA damage [5] and affect the expression on the RNA level, including changes of microRNA (miRNA) [48], e.g. hsa-miR-107-5p (miR-107, MIMAT0000104) [73]. MiR-107 is managed by soluble Aβ[35] and engaged in regulation of its production [21,71]. So far, the miR-107 has not been correlated with apoE or other biochemical markers, what is more such studies have not been performed yet in Polish AD patients.

There are ongoing studies searching for specific miRNAs to explain the pathogenesis of AD and to improve the effectiveness of treatment, especially in patients with an increased predisposition to earlier manifestation of the disease, particularly in the *APOE* E4 carriers, in whom the apoE expression is decreased. It remains unknown whether the apoE individual variation results from the genetic predisposition [27] or rather from the posttranslational modulation by miRNA. We applied bioinformatics tools to predict whether the miRNAs might potentially affect the production of apoE and we have found a promising target, hsa-miR-650-5p (miR-650, MIMAT0003320), previously detected in the brain and associated with various neoplasms [75], including glioblastoma [65]. So far, this miRNA has not been studied in degenerative diseases, such as AD.

In the present study, the authors tried to determine the apoE concentration as well as the levels of miR-107 and miR-650 in plasma of Polish AD patients and controls. The study also investigated whether these biochemical parameters correlate with *APOE* genetic variants. Additionally, the analysis of clinical features including age of onset and severity of dementia symptoms according to MMSE scale in AD patients was performed in relation to *APOE* genotypes, apoE plasma level as well as measured miRNAs.

Material and methods

We recruited 196 subjects, including 64 AD patients diagnosed by a trained neurologist according to NINCDS-ADRDA criteria [16] (mean age 76.0 years, 68.8% females); and 74 control volunteers over 60 years of age with no signs of dementia and other neurological disorders or family history of AD (UC, mean age: 71.4 years, 78.4% females) and 58 volunteers aged over 60 years with a positive family history of AD and no signs of dementia or other neurological diseases, as a comparative group (RC, mean age: 65.5 years, 70.7% females). For all participants we analysed the *APOE* genetic status.

The peripheral blood was drawn from all subjects, fasting for at least 12 hours, by S-Monovette system (Sarstedt, Germany) with K_3EDTA as anticoagulant for plasma isolation and subsequent apoE and miRNA analysis.

ApoE quantification in K_3EDTA plasma was performed by the ELISA method (MABTECH, Sweden) on EPOCH spectrophotometer (Bio-TEK, USA), as previously described [50].

RNA isolation

100 µl of plasma was mixed with 1 ml of RNA Extracol (EURx, Poland) and 5 µl of spike-in miRNA standard (5 nM solution of cel-miR-39-3p, MIMAT0000010; Genesius, Poland) until homogeneity. Next, 200 µl of chloroform (POCH, Poland) was added and mixed. After 5 min incubation, the samples were centrifuged at 4°C and 500 µl of the aqueous phase was mixed with 5 µl of GlycoBlue Coprecipitant (15 µg/ml; ThermoFisher Scientific, USA). RNA was precipitated with 505 µl of isopropanol (POCH, Poland) and incubated at –20°C for 20 min, followed by centrifugation at 4°C. The pellet was washed twice with ice cold 75% EtOH (Merk, Germany), air dried and dissolved in 10 µl of Milli-Q water supplied with 1 U/µl RNase inhibitor (A&A Biotechnology, Poland). The benchtop isolation stages were performed on ice.

MiRNAs analysis

We applied 2 µl of isolated RNA for simultaneous single tube polyadenylation/reverse transcription reaction (*PA-RT*, 0.3 U Poly-A polymerase, EURx; 40 U of TranScriba MMLV reverse transcriptase, 10 U of RNase inhibitor; A&A Biotechnology, Poland) with the mixture of 0.5× Poly-A and 0.5× MMLV reaction buffers, supplemented with $MnCl_2$, dNTPs and 3'RACE adaptor as primer (Genesius, Poland; final concentrations: 1.9 mM, 1 mM and 0.5 µM, respectively) in volume of 10 µl, on ice. The obtained cDNA was diluted to 200 µl with Milli-Q water and 2 µl was used for qPCR assessment of miRNAs relative content using complementary DNA primers (final concentration 0.25 nM) and 1× SsoFast EvaGreen Supermix (BIO-RAD, USA) in final volume of 10 µl on CFX-Connect Real-Time System (BIO-RAD, USA), as previously reported [3]. Oligos' sequences and cycling conditions are shown in Tables I and II. The Ct values and relative abundance of circulating plasma miRNAs were calculated with respect to spike-in RNA standard by ΔCt method, as described before [54].

Statistical analyses

Statistical analyses were performed using GraphPad for Windows software and Statistica 12 for Windows software (StatSoft, USA), with the nonparametric tests (Kruskal-Wallis test and Mann-Whitney test), unless stated otherwise. The normality of data distributions was checked using the Shapiro-Wilk test (when the sample size was 50 or less) and Kolmogorov-Smirnov (when the sample size was larger than 50). Analysis of the homogeneity of variance was carried out using the Levene's test. The level of significance was set at $p < 0.05$. The associations were assessed by Spearman's correlation coefficient, with the level of significance set at $p < 0.05$.

Table I. The sequences of primers used for reverse transcription (3'RACE adaptor) and for microRNAs real-time qPCR assessment (cel-miR-39-3p – cel-miR; hsa-miR-107-5p – miR-107; hsa-miR-650-5p – miR-650 and universal reverse primer)

Primers' name	Primers' sequence
3'RACE adaptor	5'-GCGAGCACAGAATTAATACGACTCACTATAGG TTTTTTTTTTTVN-3' where V = A, G, C; N = A, T, G, C
cel-miR	5'-TCACCGGGTGTAAATCAGCTTG-3'
miR-107	5'-AGCAGCATTGTACAGGGCTATCA-3'
miR-650	5'-AGGAGGCAGCGCTCTCAGGAC-3'
universal	5'-GCGAGCACAGAATTAATACGACTCA-3'

Results

The current study showed that in the course of AD, the median apoE concentration in plasma was decreased by 28.7% and 49.2% as compared to UC and RC, respectively. Moreover, the authors observed that 62.5% of AD patients had a reduced apoE concentration (< 1.315 mg/dl – 1st quartile for UC, Table III) as compared to 24.3% of UC and only 10.3% of RC (OR = 5.18, p = 6.58E-06 and OR = 14.4, p < 1.00E-8).

Subsequently, the lowest recorded apoE median concentration was observed in AD patients, homozygous carriers of *APOE* E4/E4 allele while the homozygous E3/E3 carriers had the highest apoE median concentration among all groups, except UC where the highest concentration was observed in single homozygous E2/E2 genotype carrier (Table IV).

The present study has also shown that the median concentration of apoE may be associated with age of onset of AD. The lowest apoE levels were recorded in patients with late onset disease (e.g. 7th decade of life) while in persons with higher apoE levels,

Table II. Cycling conditions for one-tube-polyadenylation-reverse transcription reaction (PA-RT) and real-time qPCR for cel-miR-39-3p (cel-miR), hsa-miR-107-5p (miR-107) and hsa-miR-650-5p (miR-650)

Temperature	Time	No. of repeats
One-tube-polyadenylation-reverse transcription (PA-RT)		
4°C	00:01:00	n.a.
37°C	00:45:00	
42°C	01:00:00	
75°C	00:05:00	
4°C	∞	
Real-time qPCR for cel-miR, miR-107 and miR-650		
95°C	00:01:30	1×
95°C	00:00:10	40×
64°C	00:00:06	
95°C	00:00:30	1×
60→95°C*	↑ 0.3°C every 3 sec*	117×
4°C	∞	n.a.

**Melting curve analysis*

Table III. The concentration of apolipoprotein E (apoE) and relative level of microRNAs: hsa-miR-107-5p (miR-107), hsa-miR-650-5p (miR-650) in plasma of Alzheimer's disease (AD) patients and related controls (RC), and unrelated controls (UC)

Parameters	Unrelated controls (UC)	Related controls (RC)	Alzheimer's disease (AD)	*p*			
				AD vs. UC vs. RC	AD vs. UC	AD vs. RC	UC vs. RC
apoE [mg/dl]	1.628 [1.315-2.097]	**2.285*** ** [1.614-2.846]	**1.161***(***)** [0.8405-1.595]	**0.0053#**	**6.61E-06@**	**6.38E-13@**	**3.80E-05@**
miR-107 [R.U.]	0.9179 [0.6307-1.304]	0.9727 [0.5415-1.255]	0.8827 [0.6146-1.240]	0.8199#	0.7786@	0.7328@	0.5448
miR-650 [R.U.]	0.8135 [0.4130-1.268]	0.5614 [0.4188-0.8701]	0.6152 [0.4031-1.220]	0.5148#	0.7867@	0.5179@	0.2308

*Median [1st-3rd quartile]; #Kruskal-Wallis test; @Mann-Whitney test; ***p < 0.001, as compared to unrelated controls, (***) p < 0.001 as compared to related controls; R.U. – relative units*

the disease developed at even older age (> 80 years), as shown in Table V.

The authors observed also a decrease in the apoE median level with advancement of the disease (measured in MMSE scale, $R = 0.295$, $p = 0.020$, Spearman correlation coefficient – SCC). We recorded a 14.6% decrease in preclinical AD (mild cognitive impairment – MCI) as well as a 20.8% and 23.8% reduction in mild and moderate dementia, respectively. The patients with severe dementia showed even further reduction in the apoE level (48.2%, as compared to UC and 39.4% as compared to MCI), as shown in Table VI.

In the present study, the authors observed a tendency for a decreased median concentration of miR-107 in AD patients as compared to both RC and UC. Interestingly, the difference was more pronounced as compared to related controls (Table III).

In the current work, for the first time, it has been shown that miR-107 level may be associated with the *APOE* genotype. In AD, a reverse correlation between the number of *APOE* E4 alleles and the level of miR-107 ($R = –0.448$, $p = 0.009$, SCC) was observed. In *APOE* E3/E3 AD carriers a significantly increased miR-107 level was measured as compared to both related and unrelated controls (Table IV).

Moreover, the present study showed that the level of miR-107 was significantly associated with age of onset of AD. The authors observed a significant reduction in miR-107 in patients who developed AD at the age of 60-69 years old ($p = 0.036$) and reverse tendency in patients who developed AD at old age ($p > 0.05$; age > 80 years, Table V). Additionally, the most reduced median level of miR-107 was observed in severe dementia ($p > 0.05$, Table VI).

The present data revealed a tendency for a decreased median miR-650 level in AD patients as well as in RC as compared to UC ($p > 0.05$; Table III).

The authors observed a significant correlation between the severity of dementia and the level of miR-650 ($R = 0.385$, $p = 0.033$, SCC). The lowest miR-650 levels were observed in patients with severe dementia, with a 58.6% reduction of median as compared to UC ($p = 0.091$, Table VI).

Discussion

The pathogenesis of AD is currently explained by various hypotheses, including mitochondrial dysfunction [40], immunological disturbances, augmented oxidative stress, and amyloid cascade associated with Aβ deposition [14]. Due to years of research, which did not contribute to development of new drugs, recently the Aβ hypothesis grew less popular in favour of a multidirectional approach [6]. Supporters of the central role of amyloid cascade claim that in the AD brain the formation of the Aβ plaques precedes the NFT formation and is especially marked in people with unfavourable *APOE* variants, coding for different apoE isoforms [14].

In 2018, the National Institute on Aging and the Alzheimer's Association presented the research framework [44] that similarly as the Erlangen Score Algorithm [19,28] did not officially include apoE as AD biomarker, probably due to contradicting results regarding its function in animal models [4,24] and diverse data on apoE level in human AD pathology [27]. There are studies performed on peripheral blood drawn from AD patients, demonstrating elevated [26,62,66], unchanged [11,32,39,46,53,57,61] and reduced apoE levels [18,20,29,30,36,52,55,60,68] in

Table IV. The concentration of apolipoprotein E (apoE) and relative level of microRNAs: hsa-miR-107-5p (miR-107), hsa-miR-650-5p (miR-650) in plasma of Alzheimer's disease (AD) patients, related (RC) and unrelated controls (UC) stratified according to the APOE genotype

APOE / Parameters	Unrelated controls (UC)				Related controls (RC)				Alzheimer's disease (AD)			
	E2/E2	E2/E3	E2/E4	E3/E3	E3/E4	E2/E3	E2/E4	E3/E3	E3/E4	E4/E4	E2/E3	E2/E4	E3/E3	E3/E4	E4/E4
apoE [mg/dl]	(2.528)	1.566 [1.356-2.504]	(0.5650-1.620)	1.741 [1.320-2.097]	1.326 [1.176-1.883]	2.027 [1.819-2.144]	(1.792)	**2.830***** [2.188-3.346]	**2.106*** [1.504-2.582]	(1.328-1.544)	(0.4790-1.592)	1.025 [0.8920-1.157]	**1.252*(***)** [0.8850-1.735]	**1.147(***)** [0.8430-1.493]	1.008 [0.6980-1.284]
miR-107 [R.U.]	–	0.9266 [0.7819-1.304]	(1.004)	0.8736 [0.6095-1.235]	1.173 [0.7284-1.306]	0.9727 [0.4572-1.028]	–	0.7153 [0.3579-1.324]	1.005 [0.5978-1.381]	(0.7983)	(0.8015)	0.5509 [0.2579-0.8438]	**1.400*(*)** [1.039-1.809]	0.7567 [0.5322-1.206]	0.8827 [0.6029-0.9368]
miR-650 [R.U.]	–	0.6960 [0.4017-1.745]	(0.2312)	0.9102 [0.4335-1.268]	0.6640 [0.4853-0.9087]	0.5498 [0.5450-0.8701]	–	0.6764 [0.2169-0.9325]	0.5613 [0.3768-0.8219]	(0.5943)	(1.220)	0.9375 [0.8581-1.017]	1.256 [0.4473-3.276]	0.4980 [0.3577-0.6369]	0.6594 [0.5412-0.8346]

*Median [1st–3rd quartile] or (single/double result); Mann-Whitney test, *p < 0.05, **p < 0.01, as compared to unrelated controls; (*/**) p as compared to related controls; R.U. – relative units*

the course of this neurodegenerative disease. The present study showed a reduction in plasma apoE concentration in AD patients, associated with disease advancement. The similar results were presented by Gupta *et al.* [22], Rasmussen *et al.* [51] and Wolters *et al.* [72].

At the same time, it was shown that a reduced level of apoE may result from the *APOE* polymorphisms [36] and oxidative modifications in the course of AD [1]. Moreover, the reduced apoE concentration may impair Aβ removal from the central nervous system (CNS) and may decrease its level in cerebrospinal fluid (CSF) [64], as shown by Hu *et al.* [25] who demonstrated that a reduction in Aβ levels was associated with an increase in T-tau/Aβ ratio and *APOE* E4 allele. A reduced level of Aβ in CSF is associated with the accumulation of Aβ plaques [17] and oligomers, which may generate reactive oxygen species (ROS) [23]. Increased production of ROS leads to damage of macromolecular compounds, among others, DNA in the form of 8-oxo2dG [10] and impaired efficiency of the repairing enzyme 8-oxoguanine glycosylase (OGG1). This dependence seems to be confirmed by the correlation obtained in our study, between the plasma concentrations of apoE and the marker of oxidative damage (8-oxo2dG) and its repair enzyme (OGG1) observed only in physiological conditions (UC, $R = 0.314$, $p = 0.008$ and $R = 0.328$, $p = 0.005$, respectively, SCC) and most likely impaired in AD patients and in persons with a positive family history of AD ($p > 0.05$) [49].

It has been shown that in AD, not only DNA, but also RNA and miRNA may undergo oxidation, and form of 8-oxo-Gua [5], which could be subsequently repaired by base excision (BER) enzymes [31]. In addition, oxidized miRNA may lose or acquire new functions, e.g. proapoptotic activity by miR-184 [69]. It would seem that the oxidative damage could also affect other miRNAs in AD. The literature data and our study showed that the levels of miR-107 were significantly reduced in the course of AD [34,70,74]. The changes of peripheral miR-107 probably reflect its fluctuations in the brain [71] where the reduced content of miR-107 augments the activity of beta-site cleaving enzyme (BACE1, an active subunit of β-secretase) involved in amyloid cascade, and thus promotes Aβ production [43]. MiR-107 may be also associated with regulation of cell cycle via p53 protein. According to Chen *et al.* [7], the level of miR-107 is reduced in p53 mutated cell lines. Moreover, muta-

Table V. The concentration of apolipoprotein E (apoE) and relative level of microRNAs: hsa-miR-107-5p, hsa-miR-650-5p in plasma of Alzheimer's disease (AD) patients stratified according to the age of onset (years)

Parameter	Unrelated controls (UC)	Related controls (RC)	Alzheimer's disease (AD) Age of onset [years]				*p* values			
			< 60	60-69	70-79	> 80		vs. UC@	vs. RC@	vs. > 80@
apoE [mg/dl]	1.628 [1.315 -2.097]	2.285 [1.614 -2.846]	(1.592-2.894)	1.234**(***) [0.795-1.498]	1.190***(***) [0.838-1.596]	1.396(*) [1.119-2.204]	K-W	$p < 0.0001^{\$}$		$p = 0.1172^{\#}$
							60-69	**0.0021**	**1.00E-06**	0.1747
							70-79	**6.00E-05**	**1.00E-07**	0.0955
							> 80	0.6070	0.0149	-
miR-107 [R.U.]	0.9179 [0.6307 -1.304]	0.9727 [0.5415-1.255]	(0.8015-1.809)	0.7449[*] [0.4031-1.373]	0.9969 [0.5885-1.324]	1.263 [1.161-1.559]	K-W	$p = 0.4632^{\$}$		$p = 0.2248^{\#}$
							60-69	0.1579	0.4053	**0.0360**
							70-79	0.9777	0.7346	0.4940
							> 80	0.2902	0.1957	–
miR-650 [R.U.]	0.8135 [0.4130 -1.268]	0.5614 [0.4188-0.8701]	(0.2706-1.220)	0.6369 [0.6029-0.8827]	0.5917 [0.4980-1.008]	0.7179 [0.3437-2.892]	K-W	$p = 0.9166^{\$}$		$p = 0.9833^{\#}$
							60-69	0.9710	0.6266	0.8639
							70-79	0.7862	0.4764	0.8411
							> 80	1.0000	0.8153	–

*Median [1st-3rd quartile] or (double result), $^{\$}$Kruskal-Wallis test (all groups), $^{\#}$Kruskal-Wallis test (AD groups), @Mann-Whitney test, *p < 0.05, **p < 0.01, as compared to UC, (*/**) p as compared to RC; [*] p as compared to AD with onset > 80 years; R.U. – relative units*

Table VI. The concentration of apolipoprotein E (apoE) and relative level of microRNAs: hsa-miR-107-5p, hsa-miR-650-5p in plasma of Alzheimer's disease (AD) patients stratified according to dementia severity according to MMSE scale [0-30 points] and related (RC), and unrelated controls (UC)

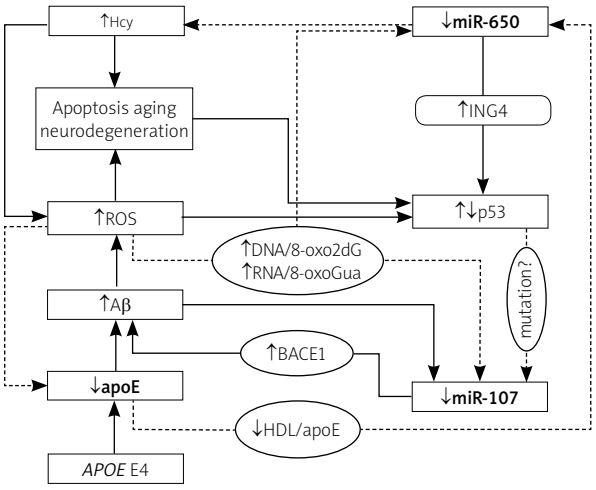
Group [MMSE] / Parameter	UC [30-27]	RC [30-27]	MCI [26-24]	Mild [23-19]	Mod [18-11]	Sev [10-0]	*p* values			
							K-W	Group	vs. UC@	vs. RC@
apoE [mg/dl]	1.628 [1.315-2.097]	2.285 [1.614-2.846]	**1.391(*)** [0.7530-1.592]	**1.290*(***)** [0.944-1.794]	**1.240**(***)** [0.9775-1.595]	**0.843***(***)** [0.6460-1.161]	< 0.0001$^{\$}$ 0.0599$^{\#}$	MCI	0.1943	**0.0199**
								Mild	**0.0314**	**4.40E-06**
								Mod	**0.0017**	**7.97E-08**
								Sev	**1.80E-05**	**2.18E-07**
miR-107 [R.U.]	0.9179 [0.6307-1.304]	0.9727 [0.5415-1.255]	(0.6763-0.8015)	1.1833 [0.8633-1.442]	0.8586 [0.2975-1.093]	0.7647 [0.6385-1.297]	0.5251$^{\$}$ 0.1257$^{\#}$	MCI	–	–
								Mild	0.2878	0.1724
								Mod	0.2554	0.4736
								Sev	0.7210	0.9822
miR-650 [R.U.]	0.8135 [0.4130-1.2675]	0.5614 [0.4188-0.8701]	(1.220-1.373)	0.7470 [0.4109-2.769]	0.6152 [0.5412-1.008]	0.3369 [0.2642-0.4856]	0.1763$^{\$}$ 0.2684$^{\#}$	MCI	–	–
								Mild	0.8162	0.4035
								Mod	0.8176	0.4916
								Sev	0.0905	0.0740

*Median [1st-3rd quartile] or (single/double result), $^{\$}$Kruskal-Wallis test (all groups), $^{\#}$Kruskal-Wallis test (AD groups), @Mann-Whitney test, *p < 0.05, **p < 0.01, ***p < 0.001 as compared to unrelated controls; (*/**/***) p values as compared to related controls*
UC – unrelated controls, RC – related controls, MCI – mild cognitive impairment, Mild – mild AD, Mod – moderate AD, Sev – severe AD, R.U. – relative units

tions in *TP53* gene which are common in cancer [45] have been previously reported in the AD animal model [13], and in AD patients may lead to accumulation of damaged DNA [15].

Furthermore, the homo- or heterozygous AD patients with *APOE* E4 allele and low apoE levels are most exposed to oxidative modifications and changes in the miR-107 level. The genotypic-phenotypic dependence between *APOE* E4 variant, apoE concentration and miR-107 level may be confirmed by a negative correlation of the number of E4 alleles and the level of miR-107 in AD patients ($p = 0.009$, $R = -0.448$, SCC). Similarly to data by Müller *et al.* [42], in our AD patients, the median miR-107 level decreased with the progression of the disease, most likely due to accumulation of Aβ.

However, dissimilar results were obtained in AD patients with the *APOE* E3/E3 genotype. In those patients, the level of miR-107 increased in the course of AD, probably as the response to oxidative stress,



8-oxo2dG – 8-oxo-2'-deoxyguanosine, 8-oxoGua – 8-oxoguanosine, Aβ – amyloid β, APOE – apolipoprotein E (gene), apoE – apolipoprotein E, BACE1 – β-site amyloid precursor protein cleaving enzyme 1, Hcy – homocysteine, HDL/apoE – apoE enriched high-density lipoproteins, ING4 – inhibitor of growth protein 4, miR-107 – hsa-miR-107-5p, miR-650 – hsa-miR-650-5p, p53 – tumour protein 53, ROS – reactive oxygen species; dotted lines – putative interaction

Fig. 1. Interaction network between apoE, miR-107, miR-650, oxidative stress and cell cycle in Alzheimer's disease.

most likely due to other damage mechanisms than the generation of Aβ. The increase in miR-107 in E3/E3 AD patients may be explained by relatively high apoE plasma content in this group, probably involved in transport of miRNAs via high density lipoproteins (HDL) [67]. It has been shown that HDL molecules, enriched in apoE, may pass through the blood-brain barrier [63], thus facilitating miRNA flux from the brain to periphery and elevate the miRNAs levels in patients plasma, affecting thereby the levels of miR-107 as well as different miRNAs, e.g. miR-650.

So far, miR-650 has not been analysed in AD pathology. In the present study the authors performed an *in silico* analysis to predict whether apoE expression could be managed by miRNAs interference. According to miRWalk 2.0 algorithm, miR-650 could bind to *APOE* regulating sequence. Moreover, this miRNA was identified in the brain and was shown to affect clinical features of glioblastoma [65].

MiR-650 was previously reported to regulate expression of tumour suppressor protein inhibitor of growth 4 (ING4) in various cancers [75-77]. The ING4 protein is involved in the cell cycle, gene transcription, DNA repair and apoptosis [56] via p53 protein [59]. The overexpression of miR-650 in brain tumours promotes cellular proliferation [65]. Furthermore, the deregulated cell cycle in neurons may be promoted by p38 mitogen activated protein kinase (MAPK), which is another downstream target of miR-650, mediated by inactivation of ING4 [75]. Interestingly, p38 MAPK has been recently proposed as a new target for therapy of AD [33,58].

The role of miR-650 in AD is unknown. MiR-650 could contribute to development of dementia by regulating the level of biothiols, such as Hcy [12]. Our data indicate that in physiological conditions miR-650 is associated with a decrease in Hcy plasma content (R = –0.384, p = 0.012). The neurodegenerative process possibly disturbs these correlations ($p > 0.05$) [49].

It remains unclear whether miR-650 controls apoE production by miRNA interference. The association is disturbed under conditions of oxidative stress in AD, perhaps due to their oxidative modifications (Fig. 1). The present research shows that the decrease in plasma apoE concentration is followed by the decrease in miR-650 levels, as a possible effect of this miRNA, similarly to miR-107, being transported in plasma by apoE-enriched HDL molecules.

In AD, the relation between the genotype and phenotype of *APOE* gene and miR-650 expression requires further studies.

Conclusions

Under physiological conditions, apoE concentration may be regulated by susceptibility for developing AD, as confirmed by higher concentration of apoE in controls with a positive family history of AD. Due to diversity of results, further studies are required to provide the final answer on the role of peripheral apoE in the pathogenesis of AD. As in majority of studies, we point to a reduced apoE plasma concentration in the ongoing neurodegenerative disease. Subsequently, in the pathogenesis of AD, miRNAs may be also involved. In physiological conditions, both the miR-107 and apoE may regulate the metabolic pathways of Aβ synthesis and clearance, while miR-650 may affect the level of Hcy. These affected metabolic pathways have been reported to be disturbed in the course of development of the neurodegenerative disease (AD). The age of onset in AD patients, the severity of symptoms and the *APOE*

genetic variants may influence the regulation of the apoE, miR-107 and miR-650 levels. The strongest relationship between the level of apoE and miRNA seems to occur in patients with disease developing at an early age (60-69 years) and in patients with the most common genotype in population (*APOE* E3/E3). Moreover, it seems that miR-107 and miR-650 are involved in the pathogenesis of AD in various mechanisms of central lesions, although it is possible that both miRNAs pathways may cross via p53 protein.

Ethics approval and consent to participate

Written consent was obtained from all participants or their legal guardians. The Bioethical Committee at the Poznan University of Medical Sciences approved the research project by decision no. 1031/13, dated 5 May 2013, with subsequent supplements.

Disclosure

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The authors report no conflict of interest.

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