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Paraoxonase 1 (PON1) gene -108C>T and p.Q192R polymorphisms and arylesterase activity of the enzyme in patients with dementia

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Abstract

Paraoxonase 1 (PON1) activity was determined using phenylacetate as substrate (arylesterase activity) in 304 individuals with dementia – 136 recognised as probable Alzheimer's disease (AD), 64 as dementia of vascular origin (VaD) and 104 as mixed dementia (MD) and in 129 persons without symptoms of dementia and in a good general health. -108C>T polymorphism in the PON1 gene promoter and p.Q192R polymorphism in the coding region were identified. PON1 activity was significantly lower in demented patients as compared with controls particularly in dementia of a neurodegenerative character (AD and MD). The prevalence of PON1-108T allele carriers was significantly higher in the AD group than in controls. The frequencies of the p.Q192R genotypes did not differ significantly between the investigated groups. An association of the rare T-R haplotype with dementia, particularly with dementia of the neurodegenerative type, was found.

Multivariate regression analysis showed a significant association of PON1 activity with PON1 -108C>T and p.Q192R polymorphisms.

The influence not only of promoter -108C>T, but also of p.Q192R polymorphism on PON1 arylesterase activity was observed. One has to admit that this kind of polymorphism does not preclude interference with the enzyme activity.

It could be concluded that the PON1 gene promoter polymorphism plays an additional role in Alzheimer's disease development. It seems however that PON1 activity has a dominating influence on the dementia risk.

Key words: *paraoxonase 1 activity, dementia, promoter -108C>T polymorphism, Q192R polymorphism.*

Introduction

Alzheimer disease (AD) is the main type of dementia [1]. In AD, senile plaques with accumulated beta-amyloid and neurofibrils with hyperphosphorylated tau protein as the main component are found [11]. Another type is dementia of vascular origin (VaD). There is however no sharp limit between these types and many cases are classified as mixed dementia (MD).

Paraoxonase 1 (PON1) is an enzyme of a very broad specificity. Its action is directed against organophosphates (the name paraoxonase originates from this activity), arylesters and lactones. Its important function is to protect lipoproteins, particularly low density lipoproteins (LDL), from oxidative modification. PON1 is transported in serum in association with high density lipoproteins (HDL) [24].

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PON1 activity is low in atherosclerosis [23,24]. It was also shown to be low in dementia [25,32].

Human *PON1* gene has almost 200 single nucleotide polymorphisms (SNPs). The most important are the following SNPs: -108C>T (rs705379) and -162A>G (rs705381) in the promoter [22] and p.Q192R (rs662) and p.L55M (rs854560) in the coding region of the gene [24]. Paraoxon hydrolytic activity is higher in the case of 192R allele and 55L allele as compared with Q and M ones, respectively [24]. Protection against LDL oxidation is more effective in the case of the 192Q allele [2]. Several authors reported that when PON1 activity was measured using arylester as a substrate, no differences depending on Q192R polymorphism were observed. It was often admitted that paraoxon was a discriminating substrate and phenylacetate was a non-discriminating one [4,9,20,23].

A genetic variability in the promoter has a significant effect on PON1 level and activity of the C allele being the more active one [3,22].

Multiple studies investigated the influence of particular *PON1* alleles on the development of various diseases particularly on cardiovascular disease, however, they did not give definite results [23,31,32].

The results concerning the significance of *PON1* gene polymorphism in dementia were also conflicting [5,7, 15,19,26,29,30].

An interesting observation concerns the existing relationship between the Q192R polymorphism and responsiveness to cholinesterase inhibitors which is the most established treatment strategy in AD. The carriers of R allele were found to be better responders [27]. This influence of the polymorphism could result from the interaction between PON1 and cholinesterase [4,21].

In our study we determined PON1 activity using phenylacetate as a substrate (i.e. its arylesterase activity) and identified both *PON1* -108C>T and Q192R polymorphic forms in subjects with dementia and in controls. We also investigated the effect of both polymorphisms on serum PON1 activity.

Material and methods

The investigated group consisted of 304 individuals with dementia – 108 men and 196 women, mean age 73.3 ± 7.90 years, and a control group of 129 persons – 58 men and 71 women, mean age 71.6 ± 7.26 without symptoms of dementia and in good general health.

Mini Mental State Examination and a clock drawing test were used as screening tests for existing dementia. Dementia was diagnosed using ICD-10 and DSM-IV. Patients and controls were subjected to a general medical and neurological evaluation, CT or MR examinations and neuropsychological tests. The type of dementia was recognized according to NINCDS-ADRDA criteria for Alzheimer's disease (AD) and NINDS-AIREN criteria for vascular dementia (VaD). Significant radiological evidence of cerebrovascular disease in CT or MRI suggested a coexisting cerebrovascular disease in AD and those patients were included into the mixed dementia (MD) group. 136 individuals were recognized as AD (38 men and 98 women, 72.3 ± 8.45 years), 64 as VaD (29 men and 35 women, 71.8 ± 7.46 years) and 104 as MD (41 men and 63 women, 75.5 ± 6.93 years).

Paraoxonase1 activity was determined spectrophotometrically based on the Kitchen *et al.* method [18] using phenyloacetate as a substrate. One unit of activity was 1 μmol of phenol liberated per minute by 1 ml of serum.

PON1 gene polymorphisms were identified in genomic DNA isolated from blood leukocytes using phenol extraction. *PON1* gene promoter -108 C>T polymorphism was identified by the Brophy *et al*., PCR-RFLP method [3] and *PON1* p.Q192R polymorphism was investigated by the PCR-RLFP method of Humbert *et al*. [16] with minor modifications described by Hasselwander *et al*. [13].

High density lipoprotein cholesterol (HDL-C) was determined after removing apoB containing lipoproteins by precipitation. Cholesterol was determined using the enzymatic method.

Linkage disequilibrium (LD) statistics (D' and r^2) for genetic polymorphisms and the haplotype frequency were determined using the SNPAnalyzer v2.0 (ISTECH Inc, Goyang, Korea) software [34]. All other statistical analyses were performed using Statistica version 9. The χ^2 test was used to evaluate the concordance of genotype frequencies with Hardy-Weinberg's equilibrium expectations. Differences in means between groups were tested using Student *t*-test or the variance analysis (ANOVA) followed by Dunnet or Bonfferoni post-hoc tests for multiple comparisons. The means were adjusted for age, sex and HDL-C (and *PON1*-108C>T polymorphism) using the covariance analysis (ANCOVA) with above-mentioned variables as covariates. Statistical significance of the differences in the frequencies of qualitative variables was evaluated using Pearson's χ^2 test. The associations between various types of dementia and different variables were identified using mul-

tiple logistic regression analysis and presented as odds ratios (OR) with 95% confidence intervals (CI). Multivariate stepwise regression analysis was performed to assess the influence of some genetic and non-genetic variables on PON1 activity. The following factors were considered as independent variables: age, sex, HDL-C, presence of *PON1* -108T allele and presence of *PON1* 192R allele. *P*-values lower than 0.05 were considered as statistically significant.

The study was approved by the Ethics Committee of the Institute of Psychiatry and Neurology. Subjects gave their informed consent either directly or through his or her guardian.

Results

PON1 arylesterase activity in dementia and in the control group

The observation that PON1 activity in demented patients, particularly in dementia of a neurodegenerative character, was significantly lower as compared with controls was made before [32] and confirmed in the present study (Table I). In the present study we found that low PON1 activity i.e. below 135 mU/mL (the lowest quartile of control group) adjusted for age, sex and HDL-C significantly increased risk of dementia (OR = 2.12 [95% CI: 1.29-3.48; p = 0.003]), of AD (OR = 2.11 [95% CI: 1.18-3.76; p = 0.012]) and of MD (OR = 2.54 [95% CI: 1.36-4.75; p = 0.004]) (data not shown).

PON1 polymorphism in dementia and in the control group

The frequencies of *PON1* -108C>T and *PON1* p.Q192R genotypes and alleles are presented in Table I. In all investigated groups the distributions of genotypes were in Hardy-Weinberg equilibrium.

In the whole dementia group, the prevalence of the T allele was slightly higher than in controls (73.3% vs. 64.4%, p = 0.062). In the AD group, the prevalence of *PON1*-108T allele carriers was significantly higher than in controls (75.7% vs. 64.4%, p = 0.043). The frequencies of the variants did not differ significantly between other investigated groups. These observations were confirmed by logistic regression analysis. It can be summarized that the dementia risk was 1.52 (OR) [95% CI: 0.98-2.37; p = 0.064] and AD risk 1.73 (OR) [95% CI: 1.01-2.75; p = 0.045] times greater in carriers of the PON1 -108T allele as compared with non-carriers of this allele. The risk was still greater after adjustment of the results for age, sex and HDL-C, i.e. OR = 1.73 [95% CI: 1.09-2.75; p = 0.020] in the whole dementia group and 2.13 [95% CI: 1.20-3.81; p = 0.018] in AD (data not shown).

The frequencies of the p.Q192R genotypes and alleles did not differ significantly between the investigated groups.

No significant linkage disequilibrium (LD) was observed among the studied *PON1* coding and promoter polymorphisms (D' = 0.096 and r^2 = 0.0029; p = 0.117).

The results of the haplotype analysis for combination of both *PON1* polymorphisms in various groups of demented patients and controls are shown in Fig. 1. The frequency of rare T-R haplotype was significantly higher in the whole dementia group and AD and MD groups as compared with controls. This haplotype was associated with the whole dementia group, as well as AD and MD with odds ratios of 1.72, 1.87 and 1.93, respectively.

PON1 polymorphism and activity

The relationship of PON1 activity with *PON1* gene polymorphisms in dementia and in the control group is shown in Table II. Both in the whole dementia group and in controls, serum PON1 activity was significantly (p < 0.00001) associated with *PON1* -108C>T promoter polymorphism. The highest activities were found in CC and the lowest in TT homozygotes. The statistically significant differences were observed also after adjustment of the means for factors known to affect PON1 activity, i.e. age, sex and HDL-C. A weaker association of PON1 activity concerned *PON1* p.Q192R polymorphism. There was a tendency to higher PON1 activity in QQ in comparison with QR and RR genotypes both in the whole dementia group (p = 0.064) and in the control group (p = 0.095). The statistically significant differences were observed after adjustment of the means for age, sex, HDL-C and presence of PON-108T allele, both in dementia and controls (p = 0.0005 and p = 0.0003, respectively).

The simultaneous effect of both *PON1* polymorphisms on enzyme activity in dementia and in controls is illustrated in Fig. 2. In both groups, the -108CC/192QQ genotype was associated with the highest, whereas the -108TT/192RR genotype in dementia and 108TT/192QR genotype in controls (-108TT/192RR genotype was absent in the control group) with the lowest arylesterase activity. A comparison of subjects – carriers of these two genotypes revealed an almost two-fold difference in

Table I. PON1 activity and polymorphism in various types of dementia and the control group

		Dementia all (n = 304)	p-value[&]	AD (n = 136)	VaD (n = 64)	MD (n = 104)	Controls (n = 129)	p-value[$]
PON1 activity (U/mL)	Mean ± SD	146.9 ± 40.57 [147.2]^	< 0.00001 [< 0.00001]^	148.4 ± 41.74[a] [144.6]^	153.9 ± 38.23[b] [156.8]^	140.7 ± 39.90[c] [142.4]^	168.0 ± 43.33 [168.3]^	0.00001 [< 0.00001]^
PON1 -108C>T								
genotypes	n (%)		0.102					
CC		81 (26.7)		**33 (24.3)**	20 (31.3)	28 (27.2)	46 (35.6)	**0.319[d]**
CT		156 (51.5)		**72 (52.9)**	34 (53.1)	50 (48.5)	53 (41.1)	
TT		66 (21.8)		**31 (22.8)**	10 (15.6)	25 (24.3)	30 (23.3)	
alleles	n (%)		0.315					
C		318 (52.5)		138 (50.7)	74 (57.8)	106 (51.5)	145 (56.2)	0.408
T		288 (47.5)		134 (49.3)	54 (42.2)	100 (48.5)	113 (43.8)	
T allele carriers	n (%)	**222 (73.3)**	0.062	**103 (75.7)[e]**	44 (68.7)	75 (72.8)	83 (64.4)	0.211
PON1p.Q129R								
genotypes	n (%)		0.673					
QQ		164 (54.0)		67 (49.3)	40 (62.5)	57 (54.8)	75 (58.1)	0.551
QR		116 (38.1)		56 (41.2)	19 (29.7)	41 (39.4)	46 (35.7)	
RR		24 (7.9)		13 (9.5)	5 (7.8)	6 (5.8)	8 (6.2)	
alleles	n (%)		0.673					
Q		444 (73.0)		190 (69.8)	99 (77.3)	155 (74.5)	196 (76.0)	0.294
R		164 (27.0)		82 (30.2)	29 (22.7)	53 (25.5)	62 (24.0)	
R allele carriers	n (%)	140 (46.0)	0.422	69 (50.7)	24 (37.5)	47 (45.2)	54 (41.9)	0.287

[&] – p-value vs. controls (Student t-test or χ^2 test); [$] – ANOVA or χ^2 test, ^ – values in square parentheses are adjusted for age, sex and HDL-C [ANCOVA];

[a] – p = 0.0004 vs. controls (ANOVA Dunnet post-hoc test) [p = 0.0003 – adjusted for age and sex; p = 0.00009 – adjusted for age, sex and HDL-C]

[b] – p = 0.066 vs. controls (ANOVA Dunnet post-hoc test) [p = 0.058 – adjusted for age and sex; p = 0.033 – adjusted for age, sex and HDL-C]

[c] – p = 0.00003 vs. controls (ANOVA Dunnet post-hoc test) [p = 0.00003 – adjusted for age and sex; p = 0.00003 – adjusted for age, sex and HDL-C]

[d] – p = 0.088 AD vs. controls (χ^2 test)

[e] – p = 0.043 vs. controls (χ^2 test)

The distributions of genotypes were in Hardy-Weinberg equilibrium – PON1-108 polymorphism: dementia all – p = 0.854; AD – p = 0.789; VaD – p = 0.439; MD – p = 0.959 and controls – p = 0.171 and PON1p.Q192R polymorphism: dementia all – p = 0.861; AD – p = 0.967; VaD – p = 0.476; MD – p = 0.928 and controls – p = 0.965)

Table II. Relationship of *PON1* activity with PON1 -108C>T and p.Q192R genotypes in dementia and in the control group. Values are presented as mean ± SD

	Dementia all				Controls			
	CC (*n* = 81)	CT (*n* = 156)	TT (*n* = 66)	*p*	CC (*n* = 46)	CT (*n* = 53)	TT (*n* = 30)	*p*
PON1 activity (U/mL)	182.1 ± 34.82	144.3 ± 32.40	109.8 ± 26.68	< 0.00001[a]	197.9 ± 36.30	165.2 ± 36.14	127.2 ± 27.33	< 0.00001[c]
adjusted for age, sex and HDL-C[&]	(184.2)	(142.9)	(110.2)	(< 0.00001[b])	(199.5)	(168.7)	(124.0)	(< 0.00001[d])
	QQ (*n* = 164)	QR (*n* = 116)	RR (*n* = 24)	*p*	QQ (*n* = 75)	QR (*n* = 46)	RR (*n* = 8)	*p*
PON1 activity (U/mL)	152.0 ± 41.02	141.2 ± 40.51	140.3 ± 34.04	0.064[e]	174.5 ± 45.43	160.9 ± 35.37	147.6 ± 51.31	0.095
adjusted for age, sex, HDL-C and PON1 -108C>T[&]	(154.4)	(143.1)	(135.6)	(0.0005[f])	(179.2)	(160.7)	(145.1)	(0.0003[g])

[&] – *values in parentheses are means adjusted on age and sex and HDL-C (and PON1-108C>T polymorphism) (ANCOVA)*

[a] – *p < 0.000001 CT vs. CC and TT vs. CC; p = 0.00008 CT vs. TT (Bonfferoni post-hoc test)*

[b] – *p < 0.000001 CT vs. CC and TT vs. CC; p = 0.00001 CT vs. TT (Bonfferoni post-hoc test)*

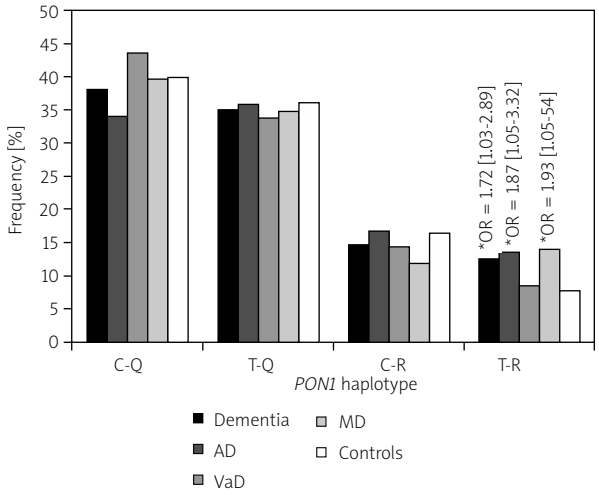
[c] – *p = 0.00002 CT vs. CC; p < 0.000001 TT vs.CC; p = 0.00001 CT vs. TT (Bonfferoni post-hoc test)*

[d] – *p = 0.000008CT vs. CC; p < 0.000001 TT vs.CC; p < 0.000001 CT vs. TT (Bonfferoni post-hoc test)*

[e] – *p = 0.085 QR vs. QQ (Bonfferoni post-hoc test)*

[f] – *p = 0.002 QR vs. QQ (Bonfferoni post-hoc test)*

[g] – *p = 0.029 QR vs. QQ and p = 0.025 RR vs. QQ (Bonfferoni post-hoc test)*



**p < 0.05 (order of polymorphisms in haplotype is: -108C>T and Q192R)*

Fig. 1. *PON1* haplotype frequencies in various types of dementia and control group.

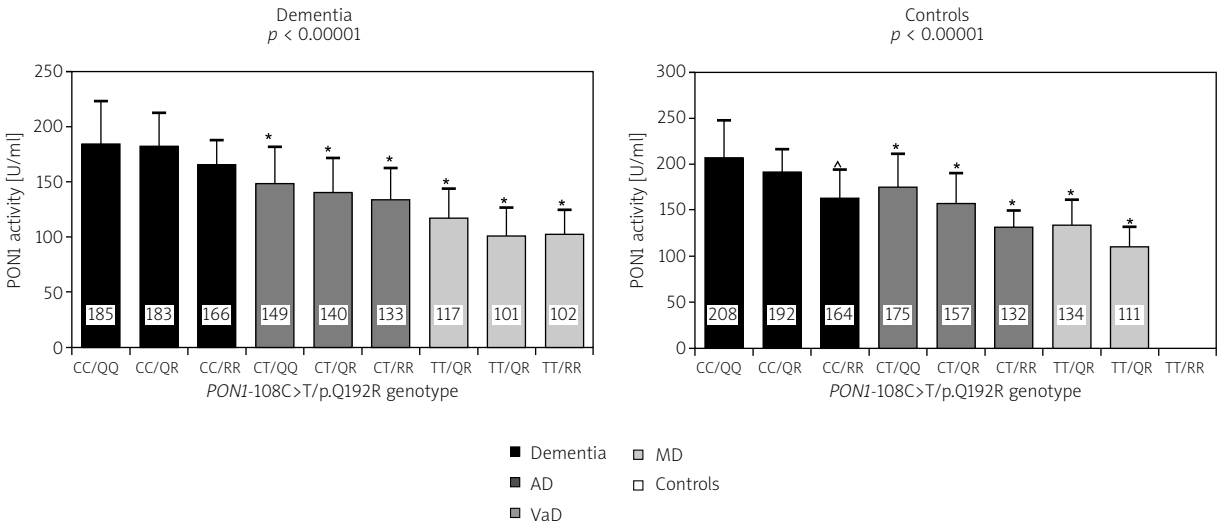
mean arylesterase activity (185 vs. 102 U/mL in the dementia group and 208 vs. 111 in controls).

The significant association of *PON1* activity with PON1 -108C>T and p.Q192R polymorphisms was confirmed in multivariate regression analysis (Table III), in which other factors known to affect PON1 activity, i.e. age, sex and HDL-C were also taken into account. It was shown that the *PON1* -108 T allele explained about 40% of the variance of the PON1 activity, both in the whole dementia and control groups (38.6% and 38.0%, respectively). The influence of *PON1* 192R allele on enzyme activity was considerably lower (ΔR^2 = 2.6% in dementia and 6.1% in controls) and was comparable with influence of age and HDL cholesterol (2.6% to 10.8%).

Discussion

The present study confirms our earlier results (performed on smaller groups) that PON1 activity is significantly lower in demented patients, particularly in dementia of a neurodegenerative type as compared with controls [32]. A significant difference was also found in vascular dementia after adjustment of the means for age, sex, and HDL-C. In addition, multiple linear regression analysis demonstrates low arylesterase activity as an independent predictive risk factor for the whole group with dementia, AD and MD.

The comparison of *PON1* gene alleles frequency in subjects with dementia and in controls was studied by several authors and showed various results. Dantoine [7] found that the frequency of 192Q allele was lower in dementia of vascular origin (VaD) as compared with the general population but not in Alzheimer's disease (AD). Pola *et al*. [26] and also Shi *et al*. [30] in the Chinese population did not find differences between



*^p = 0.085 vs. CC/QQ group (ANOVA Dunnet post-hoc test), *p < 0.001 vs. CC/QQ group (ANOVA Dunnet post-hoc test)*

Fig. 2. Combined influence of PON1 -108C>T and p.Q192R genotypes on PON1 arylesterase activity in dementia and control group.

Table III. Factors affecting PON1 activity based on multivariate stepwise regression analysis. Variables included in the model: age, sex, HDL cholesterol (HDL-C), *PON1*-108C>T polymorphism, *PON1p*.Q192R polymorphism

Variables	Dementia all (n = 304)				Controls (n = 129)			
	β	p	ΔR^2	p	β	p	ΔR^2	p
PON1 -108T	–0.636	< 0.00001	0.386	< 0.00001	–0.685	< 0.00001	0.380	< 0.00001
HDL-C	0.233	< 0.00001	0.065	< 0.00001	0.267	0.00004	0.108	< 0.00001
age	–0.167	0.00008	0.026	0.0002	–0.217	0.0005	0.045	0.0004
PON1 192R	–0.146	0.0005	0.021	0.0006	–0.247	0.00006	0.061	0.0001
sex	–0.103	0.021	0.009	0.021	–0.111	0.081	0.011	0.081
	R^2adj = 0.498 p < 0.00001				R^2adj = 0.587 p < 0.00001			

β – the standard regression coefficient; R^2adj – the multiple coefficient of determination (adjusted)
PON1: 108T – PON1 -108C>T polymorphism (CC genotype = 0; CT genotype = 1; TT genotype = 2)
PON1 192R – PON1 p.Q192R polymorphism (QQ genotype = 0; QR genotype = 1; RR genotype = 2)
Sex (female = 0; male = 1)

AD patients and controls. Scacchi *et al*. [29] as well as He *et al.* [14] suggested that the RR genotype being less frequent in AD could have a protective role for AD development. Helbecque *et al*. [15] and Cellini *et al*. [5] underlined the importance of promoter -108C>T polymorphism as the less active T allele could cause a greater risk for AD. In the study of a Polish population group supplemented with a meta-analysis including 1266 patients and 1286 controls, the association of three polymorphisms: the -161C/T in the promoter, the Q192R and the L55M ones with the AD risk was not confirmed [19].

In our results, the -108 polymorphism showed a statistically significant difference in the distribution as compared with controls in the AD group (T allele was more frequent), whereas the Q192R polymorphism did not show any differences between the investigated groups.

We have found an association of the rare T-R haplotype with dementia, particularly with Alzheimer's disease and mixed dementia. The same haplotype including both -108T and 192R alleles was revealed to be an 'at-risk haplotype' by Helbecque *et al.* in demented non-AD patients [15].

Brophy *et al*. [3] have shown the existence of considerable linkage disequilibrium between the -108C>T polymorphism and the p.Q192R and p.L55M ones. This fact was confirmed neither by other authors [6,12] nor in our study.

Based on our results, the main conclusion is that PON1 activity has a prevailing influence on the dementia risk. A similar view was expressed by Jarvik *et al*. [17] in the case of factors predicting vascular disease. As it was shown that *PON1* gene promoter polymorphism plays a considerable role in PON1 activity, it is obvious that its influence in dementia development particularly in Alzheimer's disease is also apparent. The p.Q192 polymorphism could play an additional role.

The exact mechanism of paraoxonase1 influence on dementia and AD risk is not fully explained. It was shown that low enzyme activity was associated with the increased oxidative stress, increased risk of cardiovascular disease, stroke and type 2 diabetes [2,17, 23], known dementia risk factors [8]. Recently in autopsy-confirmed AD study of Leduc *et al.* [21], *PON1* p. L55M and p.Q192R genetic variants were significantly associated with β-amyloid levels, senile plaque accumulation and choline acetyltransferase activity in different brain areas. These observations could suggest an involvement of the PON1 in AD pathogenesis and response to treatment.

In the present study it was seen that both investigated kinds of *PON1* polymorphism: the -108C>T and the Q192R one exerted a considerable influence on PON1 arylesterase activity. PON1 activity was the highest in CC and the lowest in TT genotype (CC>CT>TT) of promoter -108C>T polymorphism. The same results were obtained by other authors in population-based studies [3,28]. It was suggested that -108 polymorphism is localized within a probable binding site for Sp1, a ubiquitous transcription factor common in TATA-less genes such as *PON1* [3].

We have noticed some differences of PON1 activity depending on Q192R polymorphism though the substrate named by some authors as a "non-discriminating" one was used. The influence of coding p.Q192R polymorphism on enzyme activity was considerably weaker than influence of promoter variants. The highest activity was shown in QQ and the lowest in RR genotype (QQ>QR>RR). The difference was particularly pronounced when the influence of promoter polymorphism was taken into account.

The multivariate regression analysis performed in our study confirmed that *PON1* polymorphisms at positions -108 and 192 are the independent determinants of PON1 arylesterase activity. The most prominent contribution is made by the -108 T allele (it explained about 40% of the variance of the PON1 activity both in dementia and controls). A smaller but significant influence of 192R allele on enzyme activity is also observed (2.6% in dementia and 6.1% in controls).

The influence of the p.Q192R polymorphism on PON1 arylesterase activity was also stated by other authors [3,10,23]. One has to admit that this kind of polymorphism does not preclude interference with the activity of the enzyme.

Conclusions

1. Low PON1 activity has a dominant influence on the dementia risk.
2. *PON1* gene promoter polymorphism plays a considerable role in PON1 activity, therefore, it could have an additional role in dementia development, particularly in Alzheimer's disease. The p.Q192 polymorphism could have less influence.
3. The results of the haplotype analysis for combination of both *PON1* polymorphisms suggest that the rare T-R haplotype including both -108T and 192R alleles could be an 'at-risk haplotype' of dementia of the neurodegenerative type (AD and MD).

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