BEng Tomasz Krzywkowski
The work was performed in the Departament of Genetics Institute of Psychiatry and Neurology Warsaw, Poland

Paraoxonase 1 Polymorphism p.Q192R in Patients With Dementia

Abstract
PON1 PCR-RFLP polymorphism frequency and enzyme activity were determined in 223 patients with dementia (94 with AD, 55 with VaD and 74 with MD) and in 100 age and sex matched controls without dementia. I found no statistical significance of genotype frequencies between analyzed groups. Paraoxonase 1 activity was lower in patients carrying the R allele in *locus* 192 as compared with the R allele non-carriers. The results showed statistically significant association between *PON1* polymorphism and enzyme activity and thus can suggest an important relationship between different isoforms of *PON1*, enzyme activity and dementia.

Key words: paraoxonase 1, dementia, polymorphism;

Abbreviations: HDL-C: high-density lipoprotein (HDL-cholesterol); LDL: low-density lipoprotein; PON: paraoxonase; AD: Alzheimer disease; VaD: vascular dementia; MD: mixed dementia.

1. Introduction

Paraoxonase 1, PON1(aryldialkylphosphatase, E.C.3.1.8.1) is a calcium dependent esterase present in serum and synthesized by liver. Serum PON1 resides on high-density lipoprotein HDL_3 and it is known to contribute to antioxidant protection of low-density lipoprotein LDL. By playing a protective role against LDL oxidation, PON1 prevents initiation and progression of atherosclerotic lesions and thus development of cardiovascular diseases. PON1 exists in many polymorphic forms. However, aminoacids 192 or/and 55 substitutions along coding region of *PON1* distinguish two most abundant isoforms of *PON1*. The aim of this work was to investigate *PON1* pQ192R polymorphism in Polish patients with various types of dementia, and to study its association with PON1 serum activity.

2. Materials and methods

223 subjects were investigated, 94 with Alzheimer's disease (25 males and 69 females mean age 73±8,56); 55 with dementia of vascular origin (27 males and 28 females mean age 72±7,70); and 74 with mixed dementia (28 males and 46 females mean age 75±7,45). The controls consisted of 100 people without dementia (44 males and 56 females mean age 71±7,75). Dementia was recognized according to ICD-10 criteria using MMSE test (Minimental State Examination), clock drawing test and neuroimaging methods (magnetic resonance, computer tomography). The type of dementia was diagnosed basing on NINCDS-ADRDA scale for AD and NINDS-AIREN scale for VD.

All genotyping was conducted by PCR amplification (with primers described by Humbert et al. 1993), with 2µl of DMSO, and *OptiTaq* polymerase (Eurx) and at an annealing temperature of 58°C for 35 cycles. The PCR was followed by polymorphism-specific restriction digestion and gel electrophoresis. The Q192R polymorphism was detected by *AlwI* endonuclease (New England BioLabs). 20µl of amplified product was digested with 2,5U of enzyme for 40-48 hours at 37°C. When arginine is present at 192, two digested band of 33bp and 99 bp are observed. Serum PON1 activity was determined spectrophotometrically basing on Kitchen et al.(1973) method, using phenyloacetate as substrate.

Statistical analyses were performed using *Statistica 8.0*. *p* values lower than 0,05 were considered to be statistically significant. HDL_3 serum level, MMSE score and *PON1* c.-108C>T promoter polymorphism in all patients (all three features considered in statistical analysis), were determined previously at the department.

Results

Table 1. Distribution of PON1 genotype and allele frequencies in patiens with dementia and controls.

	Genotype								Allele				
	QQ		QR		RR				Q		R		
Group	N	(%)	N	(%)	N	(%)	∑ N	p^a	N	(%)	N	(%)	p^b
AD	49(48,9)	52,13	38(37,6)	40,43	7(7,5)	7,45	94	0,704	136	72,34	52	27,66	0,410
MD	43(43,14)	58,11	27(26,72)	36,49	4(4,14)	5,41	74	0,986	113	76,36	35	23,64	0,939
VD	34(32,07)	61,82	16(19,85)	29,09	5(3,07)	9,09	55	0,589	84	76,36	26	23,64	0,943
Dementia	**126(124,3)**	**56,50**	**81(84,38)**	**36,32**	**16(14,37)**	**7,18**	**223**	**0,919**	**333**	**74,66**	**113**	**25,34**	**0,717**
Controls	58(57,76)	58,00	36(36,48)	36,00	6(5,76)	6,00	100	---	152	76,00	48	24,00	---

Values show in parentheses are expected values calculated from the Hardy-Weinberg equilibrium.
AD: $\chi2$=0,0378, df=2, p=0,9812; MD: $\chi2$= 0,0081; df= 2, p = 0,9959; VD: $\chi2$= 2,0762, df= 2, p = 0,3541;
Dementia overall: $\chi2$= 0,3435, df= 2, p =0,8422; K: $\chi2$= 0,0173, df= 2, p = 0,9914;
p^a: AD,MD or VD vs. controls; p^b: AD,MD or VD vs. controls.

According to table 1, no difference was observed in the allele/genotype distribution between demented and controls, however frequency of Q allele was higher in both groups. Stepwise regression analysis (table 2), revealed statistically relevant association between PON1 192R allele possession and PON1 lower enzyme activity. Presented in table 2 model at the level of 32,6% (R^2=0,326) explains influence of six non-dependent variables on PON1 enzyme activity, where the R allele, explains it by 1,43% (ΔR^2=0,0143).Negative β value means negative influence of the variable.

According to data displayed in table 3, the influence of p.Q192R polymorphism on PON1 enzyme activity is observed. The highest PON1 activity was in QQ homozygotes, both in patients and in controls. In demented QQ homozygotes patients, the level of enzyme activity was equal to 149,7 µmol/min/ml, while the enzyme activity in the carriers of the R allele was equal to 138,6 µmol/min/ml. The 7,25% activity drop was statistically relevant after adjusting for age, sex, HDL-C concentration and promoter *PON1* -108 CC polymorphism.

Table 2. Influence of both genetics and non-genetics factors on PON1 activity (stepwise regression).

	PON1-activity regression summary		Stepwise regression summary	
Non-dependent variables	β#	*p*	ΔR^2	*p*
PON1 pC-108 CC	0,50	$<1*10^{-6}$	0,24	$<1*10^{-6}$
HDL-CH	0,19	0,0012	0,04	0,0009
sex	- 0,16	0,0090	0,02	0,0203
age	- 0,14	0,0204	0,02	0,0271
MMSE	0,12	0,0396	0,01	0,0619
PON1 pQ192R-R	- 0,12	0,0409	0,01	0,0409
R^2=0,346; R^2 adjusted=0,326; p<0,000001				

Variables account in analysis:
PON1 -108 CC (1= homozygous CC genotype of the promoter region, 0= CT or TT genotype); HDL-C (HDL serum concentration); sex (1=M, 0=F); age (years); MMSE (MMSE score); PON1 p192 RR+QR (1= R carrier, 0= QQ genotype)
β -standardized partials of regression coefficient;
R^2 -determination coefficient; ΔR^2- determination coefficient alter;
-positive β value means a positive while negative values a negative influence of variable on PON1 (female sex, age and R allele result in decreasing of enzyme activity).

3. Discussion

Genotype and allele frequencies in Polish patients were similar to patients of Ireland, Italy and France. It was observed in this work, that PON1 enzyme activity was lower in R carrier patients. Presented data suggest advantageous role of Q allele. On the contrary, Pola et al.(2005) demonstrated that subjects carrying "disadvantageous" R allele, are more likely to respond to cholinesterase inhibitors (Alzheimer form of treatment). Interestingly, Lescai et al.(2009) and Rea et al.(2004) demonstrated, that PON1 192RR homozygotes were mostly abundant among Italian centenarians and nonagenarians.

Table 3. Influence of allele R in *locus* 192 on PON1 enzyme activity (t-Student test, analysis of covariance in parentheses).

Group	Genotype	N	PON1 activity(µmol/min/ml)	Standard deviation SD	*P* t-Student test (ANCOVA)
Dementia	R carrier (RR+QR)	88	140,1 (138,6)	38,07	0,1321 (0,0157)
	R non-carrier (QQ)	116	149,7 (149,7)	38,48	
Controls	R carrier (RR+QR)	37	158,4 (158,0)	40,89	0,0683 (0,0157)
	R non-carrier (QQ)	52	174,0 (175,1)	42,42	

Values adjusted for age, sex, promoter *PON1* -108 CC homozygous genotype, HDL-C concentration, age and sex are shown in parentheses.

In conclusion, categorizing a patient to a risk group, basing on his/her *PON1* p.Q192R polymorphism is currently impossible. A significant influence of environmental factors on PON1 enzyme activity suggest that PON1 activity measurement could be an additional indicator of vascular and neurodegenerative diseases.