

Original Research Article

Profile of the Paraoxonase 1 (PON1) Gene 192Q/R Polymorphism and Clinical Associations among Older Singaporean Chinese with Alzheimer's and Mixed Dementia

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Key Words

Paraoxonase 1 · Alzheimer's disease · Mixed dementia · Singaporean Chinese elderly · Neuropsychiatric symptoms

Abstract

Background: To examine the prevalence of the Paraoxonase1 (PON1) gene 192Q/R polymorphism amongst Singaporean Chinese with Alzheimer's disease (AD) and mixed dementia and possible clinical associations. **Methods:** We examined the presence of the PON1 192Q/R polymorphism together with cognitive status, functional status and neuropsychiatric symptoms among 186 older Singaporean Chinese with AD (n = 109) and mixed dementia (n = 77). **Results:** The R allele predominated in 67% of the AD patients and 63.1% of the patients with mixed dementia. Within the mixed dementia subgroup, the R allele was significantly associated with a higher BADLS score, NPI-Q scores and CDR scores. **Conclusion:** Among older Singaporean Chinese with AD and mixed dementia, the R allele was predominant. In particular, within the mixed dementia subgroup, the R allele carrier status was associated with poorer functional status, greater presence of neuropsychiatric symptoms and a more severe stage of dementia. Further studies should be conducted.

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Introduction

Singapore is a multiracial South-East Asian country with a rapidly ageing population. Together with the greying of the nation, the numbers of persons with dementia (PWD) is increasing from an estimated 53,000 by 2020 to 187,000 by 2050. Amongst the older population, late onset Alzheimer's disease (AD) and vascular dementia (VaD) are the predominant forms of dementia. In a community study of older Singaporeans (>70 years), the prevalence of possible AD was 60%; while in a younger age group (50–69 years), the prevalence of possible VaD was 65% [1]. Mixed dementia as a diagnostic entity consisting primarily of AD with the presence of stroke disease is also frequently observed in the local memory clinics [2]. Neuropsychiatric symptoms are frequently observed in about 60–75% of all PWD, most commonly apathy (27–36%), depression (24–32%) and agitation/aggression (24–30%) [3, 4]. They pose enormous burdens of care, but remain less understood in terms of pathogenesis, risk factors and clinical course.

The Paraoxonase Family

The paraoxonase (PON) enzyme family comprises three members, PON1, PON2 and PON3, whose genes are located adjacent to each other on chromosome 7q21–22 [5]. In vivo, the PONs are involved in the reduction of oxidative stress and the prevention of atherosclerosis. PON polymorphisms with decreased levels of PON activity have thus been associated with vascular diseases including coronary artery disease and stroke [6–12]. PON2 is expressed in nearly all human tissues and exerts its anti-oxidative properties through the reduction of reactive oxygen species, low density lipoprotein (LDL) protection from oxidative stress and the enhanced anti-oxidant capacity of high density lipoprotein (HDL) [13]. PON3 is less studied but has been shown to reduce LDL oxidative stress with protection against atherosclerosis, although the specific natural substrates of PON3 have not been well characterized [13].

Paraoxonase 1

Of the 3 PONs, PON1 has been most intensely studied in relation to the risk of cardiovascular disease, stroke, oxidative stress and inflammation. Its encoding gene has also been identified as a longevity gene [14]. PON1 is synthesized in the liver and circulates on the surface of HDL particles, and it is a Ca^{2+} -linked enzyme [15]. It was first studied for its organophosphatase activity which explained its ability to detoxify organophosphate through hydrolysis and thus provide neuroprotection against the effects of environmental neurotoxins and age-related neurodegeneration [16, 17]. Subsequently, PON1 was ascribed to have significant anti-oxidative and anti-inflammatory properties through its enzymatic actions as a lactonase, peroxidase and esterase [13]. These properties account for the ability of HDL to prevent LDL oxidation.

The PON1 192Q/R Polymorphism and Dementia

PON1 also has cholinesterase-inhibitive properties. Human serum PON1 levels and activity display an up to 40-fold interindividual variability and are genetically associated with a single nucleotide polymorphism (SNP) in the PON1 gene. PON1 polymorphisms include those in the coding (L55M; rs854560 and R192Q; rs662) and promoter region (–107A/G; rs705379 and –161C/T; rs705381) of the gene [18]. Of these, the molecular basis of the PON1 192Q/R gene polymorphism is a glycine (Gln) → arginine (Arg) substitution at residue 192 (NCBI database dbSNP: rs662) that results in three possible genotypes: QQ, QR and RR. The PON1 192Q/R polymorphism is of particular interest in AD because of its possible effects on dementia pathophysiology and response to cholinesterase inhibition [19]. In terms of activity based on genotyping, individuals carrying Arg at residue 192 (R allele) reportedly have a higher PON1-hydrolyzing activity than those carrying Gln (Q allele) [20]. Pola et al. [21] (in

2005) demonstrated that subjects carrying the R allele were more likely to respond to cholinesterase inhibitor therapy. As an endogenous cholinesterase inhibitor, PON1 may thus augment the biological activity of cholinesterase inhibitor drugs, thus improving their efficacy.

Regarding the association between dementia and PON1 192Q/R, clinical research has produced mixed results. A recent meta-analysis of 10 studies on AD patients showed no significant association of the PON1 192Q/R polymorphism on AD susceptibility [22]. To date, 11 case-control studies have observed the relationship between the PON1 polymorphism at 192Q/R and the risk of developing AD or dementia with inconsistent results. Only 2 of 8 studies in which AD patients were selected as cases reported a protective effect of the PON1 192Q/R polymorphism on the risk of developing AD [23, 24], and the other 6 studies showed a lack of association [19, 25–29]. For AD and VaD, an earlier study reported low serum levels of PON1 in AD compared to VaD patients [30]. In three other studies which looked into both AD and VaD patients, one showed that PON1 192Q/R was not significantly associated with AD or VaD, one found no significant difference, and one suggested that the R allele was an independent risk factor for VaD and mixed dementia [31–33]. Furthermore, no previous studies of patients with AD and VaD have examined the effect of the PON1 genetic polymorphism on the clinical manifestations and severity of dementia.

Aims of the Study

The primary aim of our study was to examine the profile of the PON1 gene and its 192Q/R polymorphism amongst PWD attending a Memory disorders clinic in Singapore. The secondary aim was to examine the possible associations of the 192Q/R polymorphism with cognition, physical function and neuropsychiatric symptoms. At the time of the study conception, due to limited resources, the 192Q/R polymorphism was chosen to be studied as there was no local data on its profile among PWD.

Methods

Subjects

A cross-sectional study using convenience sampling among patients attending the outpatient Memory Clinic of a Geriatric Medicine Department in a regional hospital of Singapore was conducted from 2006 till 2009. The inclusion criteria were PWD including AD, VaD, mixed dementia, dementia with Lewy bodies and frontotemporal dementia. Exclusion criteria were: (1) patients with mild cognitive impairment and (2) patients with no caregivers.

Diagnosis of Dementia

The diagnosis of dementia based on DSM-IV (TR) was established by a multidisciplinary consensus approach on the basis of medical history, clinical examination, relevant blood investigations and brain imaging with either CT scan or MRI. The National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [34] and NINDS-AIREN [35] criteria were used to classify the dementias into AD, VaD and AD with CVD (mixed dementia), respectively. The diagnosis of dementia with Lewy bodies was based on the criteria by McKeith et al. [36]. The clinical diagnosis of frontotemporal dementia was based on the criteria by McKhann et al. [37].

Ethics Approval

All patients who fulfilled the inclusion criteria gave written informed consent for participation. The research was approved by the Hospital's Ethics Committee and the domain-specific Review Board of the National Healthcare Group.

Data Collection

Data was collected from reviews of case notes, physical and functional assessments and questionnaire interviews upon first diagnosis by the clinicians and a nurse clinician trained in administering the questionnaires.

Cognitive Status, Neuropsychiatric Symptoms and Functional Status

Cognitive status was assessed using the Mini-Mental State Examination (MMSE), a validated and widely used measure to determine global cognitive functioning on domains of memory, attention, language, praxis and visual-spatial ability, with summed scores ranging from 0 to 30, higher values denoting better cognitive performance [38]. Global rating scales utilized in the assessment of patients included the Clinical Dementia Rating Scale (CDR) and the Global Deterioration Scale (GDS). The CDR clinically stages the severity of dementia by assessing domains of memory, orientation, judgment and problem-solving, community affairs, home & hobbies and personal care, with global scores ranging from 0 to 3 [39, 40]. The GDS provides caregivers and clinicians with an overview of the stages of cognitive function for those suffering from a primary degenerative dementia such as AD [41]. It has 7 stages of which 1–3 are pre-dementia stages and 4–7 are dementia stages. In this study, the patients had a minimum CDR (global) of ≥ 0.5 and GDS of ≥ 4 .

Neuropsychiatric symptoms were evaluated using the Neuropsychiatric Inventory Questionnaire (NPI-Q), a brief clinical form of the Neuropsychiatric Inventory (NPI) [42], which had previously been validated and highly correlated with NPI and found to be useful in general clinical practice. The NPI-Q is made up of 12 symptom domains derived from the original NPI [43]. Neuropsychiatric symptoms are assessed on a 3-point scale for each symptom domain, and the total NPI severity score represents the sum of the individual symptom scores, it ranges from 0 to 36. Caregiver distress is anchored on a 0- to 5-point scale with the total NPI-Q distress score of 0–60.

Physical functional status was defined by the Bristol Activities of Daily Living Scale (BADLS), a 20-item carer-rating instrument specifically designed for dementia patients, giving a total score range of 0 to 60, a lower score indicating better daily function [44]. Functional assessment staging (FAST) was also utilized in evaluating the functional deterioration of AD and mixed dementia patients during the course of their illness. It is a functional ordinal scale ranging from 1 (no disability) to 7 (highest level of disability) with information based on caregiver input.

Other Biodata

Other demographic and clinical variables included ethnicity, gender, age, birthplace, education, housing type and a history of diabetes mellitus, hypertension, hyperlipidemia and stroke.

Genotyping

Whole-blood DNA was extracted using a commercial column (Qiagen-QIAamp DNA Blood mini kit). Real-time polymerase chain reaction was used for genotyping of SNP rs662 (chromosome 7; Applied Biosystems, TaqMan Pre-Designed SNP Genotyping Assay). Multiplex polymerase chain reaction (more than one primer/probe pair per reaction), which allows genotyping of the two possible variants at the single SNP site in a target template sequence, was performed. In each assay, there were two fluorescent dye detectors (one for the wild type, and the other for the mutation). The allelic discrimination assay classified samples as homozygotes (samples with only allele 1 or 2) and heterozygotes (samples with both alleles). TaqMan[®] MGB probe for SNP rs662 (labeled with VIC[®] dye) was used: (20X)TAAACCCAAA-TACATCTCCCAGGAT[C/T]GTAAGTAGGGTCAAGAAAATAGTG

Table 1. Demographic characteristics of the study population (n = 186)

Mean age ± SD, years	77.3 ± 7.1
Female gender	126 (67.7%)
AD	109 (58.6%)
Mixed dementia	77 (41.4%)
Diabetes	52 (28.0%)
Hypertension	121 (65.1%)
Hyperlipidemia	88 (47.3%)
Stroke	20 (10.8%)

Based on the Gln (for the Q allozyme) → Arg (for the R allozyme) substitution at residue 192, three genotypes are derived: QQ, QR and RR.

Statistical Analysis

Data were analyzed with the independent t test for continuous variables and the χ^2 test for categorical variables. The Hardy-Weinberg equilibrium was confirmed for all patients. Allelic frequencies were estimated by the allele counting method. The χ^2 test was used (1) to compare genotype and allele frequencies and (2) to compare the differences in prevalence of neuropsychiatric symptoms by the PON1 192Q/R genotypes: RR versus QR, RR versus QQ and QR versus QQ. Further on, the χ^2 test was applied to evaluate the differences in prevalence of neuropsychiatric symptoms in PWD by the PON1 192Q/R allele status (QR or RR, QQ) and among dementia subtypes (AD and mixed dementia). All analyses were conducted using SPSS statistical software version 15.0 (SPSS, Inc., Chicago, Ill., USA). A two-sided p value of <0.05 was considered significant.

Results

Predominant Dementia Subtypes

AD and mixed dementia were the predominant dementia subtypes. There were a total of 186 Chinese patients with AD and mixed dementia who had genotyping done. For the other dementia subtypes, there were 7 VaD patients, 1 Parkinson's disease with dementia and 2 dementia with Lewy bodies. As their numbers were too small, these were excluded from the analysis.

Within the AD and mixed dementia group, 109 were AD patients and 77 were mixed dementia patients (mean age 77.3 years, 67.7% women). More than half had hypertension (65.1%) and 47.3% had hyperlipidemia (table 1). By stage of dementia, 85 patients were at a mild, 90 at a moderate and 11 at a severe stage of dementia.

Distribution of Allele Genotypes

The distribution of PON1 polymorphisms in the study population is presented in table 2. The genotypes were in Hardy-Weinberg equilibrium and their distribution was not significantly different between the AD patients and the mixed dementia patients (9 QQ, 54 QR, 46 RR; 9 QQ, 39 QR, 29 RR, respectively, $p = 0.673$). In addition, the presence of at least one R allele (QR or RR) and the presence of at least one Q allele (QR or QQ) were not significantly different between the AD patients and the mixed dementia patients (91.7 and 88.3%, $p = 0.436$; 57.8 and 62.3%, $p = 0.534$, respectively). Likewise, the allele distribution was not significantly different between the two groups: the Q allele frequency was 33.0% in the AD patients and 37.9% in the mixed dementia patients, while the R allele frequency was 67.0% in the AD patients and 63.1% in the mixed dementia patients ($p = 0.426$). The

Table 2. PON1 genotype distribution and allele frequencies in the study population

Group	n	Genotype, n (%)			p ₁	p ₂	p ₃	Allele, n (%)			p ₄
		QQ	QR	RR				Q	R	Q/R	
AD	109	9 (8.3)	54 (49.5)	46 (42.2)	0.673	0.436	0.534	72 (33.0)	146 (67.0)	0.493	0.426
Mixed dementia	77	9 (11.7)	39 (50.6)	29 (37.7)				57 (37.9)	97 (63.1)	0.588	
Total	186	18 (9.7)	93 (50.0)	75 (40.3)				129 (34.7)	243 (65.3)	0.531	

p₁ value for χ^2 test of differences in PON1 genotype frequencies between AD and mixed dementia patients.

p₂ value for χ^2 test of differences in (QR + RR) and QQ genotype between AD and mixed dementia patients.

p₃ value for χ^2 test of differences in (QR + QQ) and RR genotype between AD and mixed dementia patients.

p₄ value for χ^2 test of differences in PON1 allele frequencies between AD and mixed dementia patients.

Table 3. Clinical manifestations and severity of dementia in PWD by PON1 allele status

	RR (n = 75)	QR (n = 93)	QQ (n = 18)	p values (from c^2 test)		
				RR vs. QR	RR vs. QQ	QR vs. QQ
MMSE score	15.8±6.30	15.6±5.81	16.9±5.06	0.829	0.508	0.390
Global CDR score	1.52±0.67	1.41±0.59	1.47±0.56	0.254	0.780	0.673
Total CDR score	8.47±3.86	8.07±3.49	7.50±3.15	0.480	0.327	0.526
GDS/FAST score	4.71±0.90	4.74±0.85	4.50±0.62	0.794	0.358	0.251
NPI-Q (S) score	8.85±8.20	8.76±8.42	4.89±4.51	0.945	0.008	0.007
NPI-Q (CD) score	8.65±9.89	9.13±9.36	3.94±5.22	0.750	0.007	0.002
BADLS score	7.29±10.2	6.24±7.56	4.50±6.70	0.455	0.271	0.366

Data are expressed as mean ± SD.

ratio between Q and R allele frequencies was 0.493 in the AD patients and 0.588 in the mixed dementia patients.

Allele Associations with Cognitive Status, Neuropsychiatric Symptoms and Function

In the total group, R allele carriers, compared with non-R allele carriers, showed higher mean NPI-Q (S) scores and NPI-Q (CD) scores (8.80 vs. 4.89, $p = 0.004$; 8.92 vs. 3.94, $p = 0.002$, respectively; tables 3, 4). There were no significant differences in MMSE score ($p = 0.426$), global CDR score ($p = 0.928$), total CDR score ($p = 0.407$), GDS/FAST score ($p = 0.283$) and BADLS score ($p = 0.303$). The same relationships were examined in patients by AD and mixed dementia subtypes. In the mixed dementia group, the presence of the R allele was significantly associated with pronouncedly higher mean NPI-Q (S) scores (9.01 vs. 3.11, $p = 0.039$) and NPI-Q (CD) scores (9.09 vs. 2.33, $p = 0.006$), as well as with total CDR scores (8.57 vs. 5.89, $p = 0.042$), GDS/FAST scores (4.84 vs. 4.11, $p = 0.007$) and BADLS scores (6.99 vs. 1.00, $p < 0.001$). In contrast, among patients in the AD subgroup, no significant differences in any of these measures between R allele carriers and non-carriers were found.

Prevalence of Various Neuropsychiatric Symptoms by the PON1 192Q/R Genotypes and by the Genotype and Dementia Subgroups

For the total group, the prevalence of delusion was significantly higher among patients with the QR genotype than among those with the QQ genotype (47.3 and 22.2%, respectively,

Table 4. Clinical manifestations and severity of patients with dementia by PON1 polymorphism status and among dementia subtypes

	Whole sample			AD			Mixed Dementia		
	QR or RR (n = 168)	QQ (n = 18)	p value	QR or RR (n = 100)	QQ (n = 9)	p value	QR or RR (n = 68)	QQ (n = 9)	p value
MMSE score	15.7±6.01	16.9±5.06	0.426	15.7±5.77	14.7±3.57	0.596	15.7±6.39	19.1±5.53	0.134
Global CDR score	1.46±0.63	1.47±0.56	0.928	1.42±0.595	1.78±0.441	0.043	1.52±0.672	1.17±0.500	0.079
Total CDR score	8.24±3.66	7.50±3.15	0.407	8.03±3.36	9.11±2.26	0.214	8.57±4.06	5.89±3.19	0.042
GDS/FAST score	4.73±0.87	4.50±0.62	0.283	4.65±0.809	4.89±0.333	0.099	4.84±0.940	4.11±0.601	0.007
NPI-Q (S) score	8.80±8.30	4.89±4.51	0.004	8.66±8.39	6.67±3.78	0.206	9.01±8.23	3.11±4.68	0.039
NPI-Q (CD) score	8.92±9.58	3.94±5.22	0.002	8.80±9.69	5.56±4.90	0.109	9.09±9.47	2.33±5.29	0.006
BADLS score	6.71±8.80	4.50±6.70	0.303	6.52±8.06	8.00±7.98	0.598	6.99±9.85	1.00±2.00	<0.001

Data are expressed as mean ± SD.

Table 5. Frequencies of neuropsychiatric symptoms in PWD by PON1 192 Q/R genotypes

	RR (n = 75)	QR (n = 93)	QQ (n = 18)	p values (from c ² test)		
				RR vs. QR	RR vs. QQ	QR vs. QQ
Delusions	26 (35.1)	43 (47.3)	4 (22.2)	0.117	0.295	0.050
Hallucinations	15 (20.3)	22 (24.2)	3 (16.7)	0.550	1.000	0.759
Agitation/aggression	36 (48.6)	50 (54.9)	9 (50.0)	0.421	0.918	0.700
Depression/dysphoria	35 (47.3)	37 (40.7)	6 (33.3)	0.393	0.285	0.561
Anxiety	29 (39.2)	40 (44.0)	5 (27.8)	0.537	0.368	0.203
Elation/euphoria	9 (12.2)	8 (8.8)	2 (11.1)	0.479	1.000	0.669
Apathy/indifference	39 (52.7)	51 (56.0)	9 (50.0)	0.668	0.837	0.638
Disinhibition	30 (40.5)	31 (34.1)	5 (27.8)	0.392	0.317	0.604
Irritability/liability	34 (45.9)	40 (44.0)	7 (38.9)	0.798	0.589	0.692
Aberrant motor behavior	22 (29.7)	32 (35.2)	7 (38.9)	0.459	0.453	0.763
Nighttime behaviors	38 (51.4)	41 (45.1)	6 (33.3)	0.421	0.170	0.359
Appetite/eating change	23 (31.1)	36 (39.6)	4 (22.2)	0.258	0.459	0.163

Figures are numbers with percentages in parentheses.

p = 0.050; table 5). Within the mixed dementia subgroup, R allele carriers, compared with non-R allele carriers, showed a significantly lower prevalence of elation/euphoria (11.9 vs. 100.0%, p < 0.001) and a higher prevalence of nighttime behaviors (49.3 vs. 11.1%, p = 0.037; table 6).

Discussion

This study provides unique pilot data on the PON1 192Q/R polymorphism and its distribution among older Chinese patients with AD and mixed dementia in Singapore. We found that 67% of the AD patients and 63.1% of the patients with mixed dementia carried at least one R allele. In contrast to populations of European descent where the Q allele is predominant, the R allele is more frequent among Chinese PWD in Singapore. This finding is similar to that in Japan [29]. In a study on a mainland Chinese Han ethnic population, He et al. [23] reported that the PON1 R allele frequencies in AD patients and healthy controls were 0.607

Table 6. Frequencies of neuropsychiatric symptoms in PWD by PON1 192 Q/R allele status and among dementia subtypes

	Whole sample			AD			Mixed dementia		
	QR or RR (n = 168)	QQ (n = 18)	p value	QR or RR (n = 100)	QQ (n = 9)	p value	QR or RR (n = 68)	QQ (n = 9)	p value
Delusions	69 (41.8)	4 (22.2)	0.107	41 (41.8)	2 (22.2)	0.309	28 (41.8)	2 (22.2)	0.469
Hallucinations	37 (22.4)	3 (16.7)	0.767	23 (23.5)	1 (11.1)	0.680	14 (20.9)	2 (22.2)	1.000
Agitation/aggression	86 (52.1)	9 (50.0)	0.864	50 (51.0)	5 (55.6)	1.000	36 (53.7)	4 (44.4)	0.728
Depression/dysphoria	72 (43.6)	6 (33.3)	0.401	42 (42.9)	2 (22.2)	0.302	30 (44.8)	4 (44.4)	1.000
Anxiety	69 (41.8)	5 (27.8)	0.249	44 (44.9)	1 (11.1)	0.076	25 (37.3)	4 (44.4)	0.725
Elation/euphoria	17 (10.3)	2 (11.1)	1.000	9 (9.2)	2 (22.2)	0.232	8 (11.9)	9 (100)	<0.001
Apathy/indifference	90 (54.5)	9 (50.0)	0.713	53 (54.1)	6 (66.7)	0.728	37 (55.2)	3 (33.3)	0.294
Disinhibition	61 (37.0)	5 (27.8)	0.441	39 (39.8)	3 (33.3)	1.000	22 (32.8)	2 (22.2)	0.711
Irritability/liability	74 (44.8)	7 (38.9)	0.629	41 (41.8)	3 (33.3)	0.734	33 (49.3)	4 (44.4)	1.000
Aberrant motor behavior	54 (32.7)	7 (38.9)	0.598	33 (33.7)	5 (55.6)	0.275	21 (31.3)	2 (22.2)	0.715
Nighttime behaviors	79 (47.9)	6 (33.3)	0.240	46 (46.9)	54 (55.6)	0.734	33 (49.3)	1 (11.1)	0.037
Appetite/eating change	59 (35.8)	4 (22.2)	0.251	38 (38.8)	3 (33.3)	1.000	21 (31.3)	1 (11.1)	0.271

Figures are numbers with percentages in parentheses.

and 0.647, respectively. Our study confirms the predominance of the R allele amongst the Chinese with concordant frequencies between the Han and Singaporean Chinese dementia subpopulations of AD and mixed dementia. It also adds to the findings of previous studies which suggest ethnic variations in the distribution of R and Q alleles, which in turn confer different AD risk associations in different ethnic populations.

The role of PON1 in AD and non-AD dementia has been explored in various studies. Helbecque et al. [32] studied both AD and non-AD demented patients (VaD and mixed dementia) and showed that the R allele was an independent risk factor for non-AD dementia. Within the PON1 gene itself, the differences in importance between the promoter and coding regions of PON1 on dementia have been clearly defined. In a more recent paper by Bednarska-Makaruk et al. [45], the significant association of PON1 activity with PON1 -108C>T and the PON1 Q192R polymorphisms was confirmed in a multivariate regression analysis, and the PON1 -108T allele had an effect on PON1 activity compared to the PON1 192R allele. This study also showed that PON1 activity was significantly lower in demented patients when compared with controls, particularly in AD and mixed dementia patients [45].

At the pathophysiological level, recent research has shown the contributing role of the PON1 192Q/R polymorphism towards the pathogenesis and risk of AD. While research has shown that PON1 gene polymorphisms may be limited in the pathogenesis of AD, a recent paper by Erlich et al. [46] (in 2010) showed that while the mechanisms by which PON1 influences AD is unknown, the risk-conferring effect of PON1 on AD is nevertheless significant. By measuring the arylesterase and lactonase activity, the odds of AD (adjusted for age, gender and ethnicity) increased 20% for each standard deviation decrease in arylesterase or lactonase activity. This study also showed association signals with activity across all 3 PON genes (i.e. PON1, PON2 and PON3). Haplotypes including SNPs spanning the PON genes were noted to be more significant than haplotypes comprising SNPs from 1 gene with significant interactions between SNP pairs located across the PON cluster. It concluded that low serum PON activity is a risk factor for AD with the further explanation that multiple variants in PON influence serum PON activity and their effects may be synergistic.

A recent study also demonstrated the positive correlation of PON1 activity with serum insulin level and homeostatic model assessment index (HOMA-IR) [47]. In another paper by Leduc and Poirier [19], the M55M genotype was significantly associated with AD risk, whereas PON1 192Q/R was not. In addition, the PON1 192Q/R polymorphism had no effect on choline acetyltransferase activity and nicotinic receptor density in the temporal cortex of AD patients compared to the met allele. However, it was found that AD subjects carrying at least one Arg allele at the Q192 locus had significantly lower A β 42/A β 40 ratios relative to AD Q192Q homozygous patients. These results highlight the role of PON1 as an anti-oxidant HDL-associated enzyme in lipoprotein complexes which function as scavengers of normally secreted extracellular lipophilic/ nonaggregated A β peptides in vivo.

The findings of this current study which suggest that Singaporean Chinese patients with mixed dementia (in particular those carrying the R allele) presented with greater numbers of neuropsychiatric symptoms and at more severe stages of dementia and AD invites interesting considerations. Low serum levels of PON1 activity were reported in one study of AD patients compared to VaD [30]. The R allele has been associated with an increased risk of stroke in Asians [48–51].

The presence of cerebrovascular disease in AD patients resulting in neuropsychiatric symptoms has been studied. A previous paper established that cerebral white matter disease is independently associated with behavioral and psychological symptoms of dementia in AD in Singapore [52]. Other studies, e.g. the Cache County Study in the USA, reflect the same association [53, 54].

Regarding the role of PON1 in enhancing the risk of cerebrovascular disease which in turn leads to greater disease burden in AD patients in the form of neuropsychiatric symptoms and severity, this has not been examined before. PON1 inhibits LDL oxidation, which in turn drives HDL to exert its anti-atherogenic activity. It has been established that different PON polymorphisms, which affect lipid oxidation activity, are risk factors for different neurological diseases [26, 55]. Another study has postulated that enhanced lipid peroxides target specific cells in different populations of patients, e.g. endothelial cells in stroke patients and cortical neurons in AD patients [56]. We hypothesize that the PON1 R allele does play a role in increasing cerebrovascular disease burden in AD patients and in turn neuropsychiatric symptoms, the mechanisms of which need to be further explored and understood.

The study has several limitations. (1) The sample size of the patient groups with the PON1 QQ genotype was small (9 QQ in the AD group and 9 QQ in the mixed dementia group). Although highly significant, the use of multiple testing could generate spurious results by chance, hence, the results suggesting an effect of the PON1 192Q/R polymorphism on neuropsychiatric symptoms would have to be replicated in studies with larger numbers of the QQ genotype. (2) The effect of the APOE status on disease severity and neuropsychiatric symptoms in AD patients has been well-documented. Subanalyses (data not shown) did not demonstrate an association of APOE with the PON1 status, or a confounding effect on dementia severity and neuropsychiatric symptoms. However, an interaction effect of APOE and PON1 is still possible and cannot be excluded. (3) Inadequate control of confounding by vascular and other risk factors (including age, smoking, lifestyle and education status) may also have contributed to the findings. (4) The influence of other polymorphisms in the PON1 gene on AD and mixed dementia could not be excluded. Other PON1 polymorphisms including pL55M and -108C>T have been shown to play a significant role in AD risk and pathogenesis. (5) The number of VaD patients was too small to examine the role of the PON1 Q/R genotype on this dementia subtype. (6) While the assessments were conducted by an experienced geriatrician and a trained nurse, it is still possible that there was an element of human subjectivity during the assessments of cognitive status and neuropsychiatric symptoms. This could potentially reduce the credibility of the results. (7) Analyses into possible associations of allele and

genotype frequencies with diabetes mellitus, hypertension, hyperlipidemia and stroke were not conducted. (8) Associations of the R allele status with age of onset, clinical course and stage of dementia together with neuroimaging evidence of cerebrovascular disease were not studied.

Conclusion

This study provides significant pilot data on the PON1 192Q/R polymorphism and its prevalence amongst Singaporean Chinese patients with AD and mixed dementia which has hitherto not been demonstrated locally. It concurs with previous studies demonstrating the predominance of the R allele amongst Chinese dementia patients. It also provides interesting data on the possible association of the R allele with disease severity, functional status and manifestation in the mixed dementia subgroup. These findings should be replicated in other studies. Future study directions include: (1) a comparison of the distribution of the PON1 192Q/R polymorphism among PWD and controls in Singapore; this would enable us to assess the postulated protective effect of the R genotype against AD, (2) association studies between the different PON gene polymorphisms (PON1, PON2 and PON3), vascular risk factors (including lifestyle risk factors e.g. smoking) and dementia across different ethnic groups and (3) association studies of PON1 polymorphisms with neuroimaging markers of cerebrovascular disease and AD in light of the high burden of cerebrovascular white matter disease in the local population and its association with behavioral and psychological symptoms of dementia in AD [57]. These will help further our understanding of the association and clinical significance of PON and dementia in Singapore.

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Disclosure Statement

There is no conflict of interest.

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