

The paraoxonase (*PON1*) Q192R polymorphism is not associated with poor health status or depression in the ELSA or InCHIANTI studies

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Background The human paraoxonase (*PON1*) protein detoxifies certain organophosphates, and the *PON1* Q192R polymorphism (rs662) affects *PON1* activity. Groups with higher dose exposure to organophosphate sheep dips or first Gulf War nerve toxins reported poorer health if they had 192R, and these associations have been used to exemplify Mendelian randomization analysis. However, a reported association of 192R with depression in a population-based study of older women recently cast doubt on the specificity of the higher dose findings. We aimed to examine associations between the *PON1* Q192R polymorphism and self-reported poor health and depression in two independent population-based studies.

Methods We used logistic regression models to examine the associations in men and women aged 60–79 years from the English Longitudinal Study of Ageing (ELSA, $n = 3158$) and InCHIANTI ($n = 761$) population studies. Outcomes included the Center for Epidemiologic Studies Depression (CES-D) scale, self-rated general health status and (in ELSA only) diagnoses of depression.

Results The *PON1* Q192R polymorphism was not associated with self-reported poor health {meta-analysis: odds ratio (OR) = 1.01 [confidence interval (CI) 0.91–1.13], $P = 0.80$ } or depressive symptoms in either study or in meta-analyses [CES-D: OR = 1.01 (CI 0.87–1.17), $P = 0.90$]. There was also no association with histories of diagnosed depression in ELSA [OR = 1.03 (CI 0.82–1.30), $P = 0.80$].

Conclusions We found no evidence of an association between the *PON1* Q192R polymorphism and poor general or mental health in two independent population-based studies. Neither the claimed Q192R association with depression in the general population nor its theoretical implications were supported.

Keywords *PON1* Q192R polymorphism, rs662, organophosphates, detoxification, paraoxonase activity, depression, Mendelian randomization

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Introduction

The paraoxonase 1 (*PON1*) protein contributes significantly to the detoxification of several organophosphates (OPs). The toxicity of many OPs occurs through inhibition of acetylcholinesterase, an enzyme essential for normal nerve impulse transmission. The effect of OPs on neural signalling is the basis of their use as insecticides and nerve gas agents.¹ OPs have been used in sheep dip since the 1960s and were used extensively in the UK between 1976 and 1992 when compulsory sheep dipping was in place. One of the main OPs used in sheep dips is diazinon.² Human serum *PON1* hydrolyses diazoxon, the active metabolite of diazinon, thus limiting the toxicity of the OP.³ *PON1* is also involved in metabolizing oxidized phospholipids and has been linked with systemic oxidative stress and cardiovascular disease risk.⁴

It is widely accepted that people exhibiting low *PON1* activity may be more sensitive to the toxicity of certain OPs.⁵ There are several polymorphisms associated with both the level of expression of *PON1* and its catalytic activity,⁵ but one common variant i.e. Q192R (rs662) in the coding region of the *PON1* gene has received most attention. This non-synonymous polymorphism involves a substitution of glutamine (Q, 'wild' or common type) with arginine (R, variant) at amino acid position 192 of the protein sequence. Early studies indicated that individuals with the 192R genotype demonstrated higher serum levels and activity of *PON1* compared with individuals with the QQ192 genotype.⁶

In 1996, Davies *et al.*¹ identified that the Q192R polymorphism affected the catalytic activity of the *PON1* protein in a substrate-specific manner, suggesting that individuals with the 192R substitution were less efficient at detoxification of diazoxon, soman and sarin, although the opposite may apply to paraoxon. In 2002, Cherry *et al.*⁷ hypothesized that sheep dippers with the 192R genotype might be more vulnerable to toxic effects of the high-dose OPs they were exposed to. More recent evidence has been mixed, with some studies reporting no difference in hydrolysis rates of diazoxon by genotype^{8,9} or even faster detoxification in individuals with the 192R genotype¹⁰ under certain conditions.

On the basis of early evidence of 192R carriers being poorer detoxifiers of the relevant OPs, the Q192R polymorphism has been used in Mendelian randomization studies to determine whether there is a causal link between OPs and neurological impairment.^{11,12} If exposure to OPs (especially diazinon) causes ill-health, then in farmers exposed to higher dose OPs from sheep dips, those carrying the 192R isoform, would be more likely to report poorer general health and greater morbidity; this association was observed in studies by Mackness *et al.* and Cherry *et al.*^{3,7} Similarly, first Gulf War veterans (who are reported to have been exposed to OP nerve agents including sarin) described

more neurological impairment compared with controls. These neurologically impaired veterans were more likely to have the 192R genotype.¹³

Recently, Lawlor *et al.*¹⁴ aimed to extend the associations between the *PON1* Q192R polymorphism and poor health and neurological impairment to a more general population. Health outcomes in women aged 60–79 years in the British Women's Heart and Health (BWHH) study were examined and the presence of the 192R genotype was found to be associated with respondent reports of doctor-diagnosed depression [per-allele odds ratio (OR) = 1.22; 95% confidence interval (CI) 1.05–1.41]. It was argued that this result had important implications, casting doubt on the attribution of specific causation in the previous sheep dip and Gulf War veterans' studies. The apparent association of the *PON1* polymorphism with depression in this group of older women, who were unlikely to have had recent occupational or high-dose exposure to OPs, was argued to suggest that ill health in the sheep dip and war exposure groups may be part of a general vulnerability and not necessarily specific to their exceptional (high-dose) exposures. Implications for psychiatric causation were also claimed. However, the analysis was based on a single question to respondents about diagnosed depression, and no independent replication has been available.

Given the potential methodological and causal importance of the previous report, we aimed to examine associations between the Q192R polymorphism in *PON1* in two independent older population samples (the ELSA and InCHIANTI studies). These studies are not gender specific and also have the advantage of having data on overall health status and from validated scales of depression symptoms.

Methods

The English Longitudinal Study of Ageing (ELSA) study

ELSA¹⁵ is a follow-up study of respondents to the UK Government's Health Survey for England (HSE) (at <http://www.dh.gov.uk/>), an annual cross-sectional survey designed to be representative of the community-living population. The ELSA sample included those aged ≥ 50 years seen originally either in HSE 1998, 1999 or 2001, and is described in detail elsewhere.¹⁵ ELSA follow-up of HSE respondents occurred in 2002 and 2004, with data collected by face-to-face interviewing in respondents' own homes. Blood samples were taken for DNA analysis from those respondents who were willing and eligible to donate samples during a nurse visit following the 2004 questionnaire. There were 9432 interviewed respondents in ELSA 2004, of which 7666 volunteered for the nurse-led clinical visit, during which 6231 donated blood specimens. Of these, 3676 were aged between 60 and 79 years inclusive.

The InCHIANTI study

The InCHIANTI study¹⁶ is a population study of decline of mobility in later life. The sample is representative of the population of two small towns in Tuscany, Italy, and all participants were of White European origin. The study includes 1453 respondents, of whom 1343 donated blood samples at baseline. Of these individuals, 761 were aged between 60 and 79. The Italian National Institute of Research and Care of Aging Institutional Review Board approved the study protocol. As in ELSA, data were collected on self-reported health and depression by face-to-face interviewing.

Genotyping methods

The *PONI* Q192R polymorphism (rs662) was genotyped in ELSA as part of a 1536 Goldengate custom SNP panel by Illumina, using high-throughput BeadArray™ technology. Genotyping was successfully completed in 3666 individuals aged between 60 and 79 years, with a call rate of 99.7%. Of the genotyped population, 3158 were of White European origin and formed the sample group for our analysis.

In InCHIANTI, genome-wide genotyping was performed using the Illumina Infinium HumanHap550 genotyping chip (chip versions 1 and 3) as previously described.¹⁷ All single-nucleotide polymorphisms (SNPs) on the chip were fully quality controlled, and SNPs were only used where call rates were >98% and had minor allele frequencies >1%. The *PONI* Q192R polymorphism (rs662) that was on the chip was genotyped in 782 individuals aged between 60 and 79 years.

In both studies, the Q192R polymorphism did not deviate appreciably from the expected population distribution, i.e. it was in Hardy Weinberg equilibrium ($P > 0.05$), and there were no duplicate errors.

Measures of depression

The Center for Epidemiologic Studies Depression scale (CES-D) has been extensively validated in older populations.¹⁸ In InCHIANTI the full 60-point scale was used, with a cut-off point of 16^{18,19} being indicative of depression. In ELSA, an abridged 8-point CES-D scale, with a cut-off point of three or more being symptomatic of depression in line with previous studies that have used this abridged version of the scale,²⁰ was calculated from responses to the questions: 'Which of the following was true for you much of the time during the past week: felt depressed; everything you did was an effort; sleep was restless; were happy (reversed); felt lonely; enjoyed life (reversed); felt sad; could not get going?'

Results

In the InCHIANTI cohort, 36.6% (95% CI 33.4–39.8%) of respondents aged between 60 and 79 reported

having fair to very poor general health. The equivalent prevalence in the ELSA cohort was 28.5% (26.9–30.1%). We found no association between *PONI* Q192R and fair to very poor self-reported general health in either study or in the meta-analysis (MA); in MA, 31.0% of the sample reported having fair to very poor general health in the homozygous 'RR' variant group compared with 29.9% in the common homozygous 'QQ' genotype (OR=1.01; 95% CI 0.91–1.13, $P=0.795$ in the additive genotype logistic regression model adjusting for age, sex and study; Table 1).

In the InCHIANTI cohort, the prevalence of depression in individuals aged between 60 and 79 based on a cut-off point of 16 on the full CES-D scale was 18.6% (95% CI: 16.0–21.2%). In the ELSA cohort, based on the abridged 8-point CES-D scale using a cut-off point of three or more depressive symptoms, the prevalence of depression was 19.4% (18.0–20.8%). In ELSA, we also considered a cut-off point of four or more depressive symptoms, yielding a prevalence of 12.5% (11.3–13.6%). We found no association between depressive symptoms measured by the CES-D scale and the genotype in the individual studies or in the meta-analysis (MA: OR=1.01; 95% CI 0.87–1.17, $P=0.90$ in the additive genotype model; Table 1).

Data on diagnoses of depression were only available from ELSA respondents. Again, we found no difference in the prevalence of this outcome by genotype (OR=1.03; 95% CI 0.82–1.30, $P=0.80$). These results remain consistent in sex-specific analyses (data available from authors).

Discussion

In this analysis we have used two population-based studies covering the same age range as the original report by Lawlor *et al.*¹⁴ We have found that the *PONI* Q192R polymorphism (rs662) is not associated with current depressive symptoms or history of diagnosed depression in either study independently, or across our combined sample of 3919 people aged 60–79 years.

In addition to a simple reporting of diagnosed mental illness, we have examined data from a validated depression scale, the CES-D. We have also examined the broader concept of self-rated health status. In neither women nor men did we find the previously reported association between the *PONI* Q192R polymorphism and health status.

It has also been suggested that the *PONI* Q192R genotype might predispose to chronic neurodegenerative disease in a study that reported a higher prevalence of Parkinson's disease in people with the R192 isoform than in those with the Q192 isoform.²¹ However, more recent genome-wide association studies on specific neurological conditions including Parkinson's disease,²² major depressive disorder,²³ neuroticism²⁴ and bipolar disorder²⁵ have reported

Table 1 General health status, diagnosed depression and CES-D scores by the PON1 Q192R genotype in individuals aged between 60 and 79 years, with additive and per-allele age and sex-adjusted logistic regression results

	PON1 genotype			Additive logistic regression results, age, sex adjusted		Per-allele logistic regression results, age, sex adjusted (RR compared with QQ=1)	
	QQ	QR	RR	OR (95% CI)	P-value	OR (95% CI)	P-value
ELSA	<i>n</i> = 1582 (50.1%)	<i>n</i> = 1302 (41.2%)	<i>n</i> = 274 (8.7%)				
Self-reported general health (% reporting fair to very poor)	28.3	27.93	32.35	1.05 (0.93–1.18)	0.42	1.21 (0.92–1.60)	0.18
Any history of diagnosed depression (% diagnosed)	5.75	5.45	6.57	1.03 (0.82–1.30)	0.80	1.17 (0.69–1.31)	0.55
CES-D score (8-point scale) [mean (SD)]	1.36 (1.80)	1.39 (1.86)	1.36 (1.68)				
% depressed (using CES-D ≥ 3 as symptomatic of depression)	19.2	19.7	19.8	1.04 (0.90–1.19)	0.61	1.06 (0.77–1.48)	0.71
% depressed (using CES-D ≥ 4 as symptomatic of depression)	12.4	12.4	13.2	1.03 (0.87–1.21)	0.74	1.09 (0.74–1.61)	0.65
InCHIANTI	<i>n</i> = 368 (48.4%)	<i>n</i> = 337 (43.3%)	<i>n</i> = 56 (7.4%)				
Self-reported general health (% reporting fair to very poor)	36.5	37.8	24.1	0.86 (0.67–1.10)	0.23	0.55 (0.28–1.08)	0.08
CES-D (60-point scale) [mean (SD)]	11.70 (8.50)	11.98 (8.12)	9.85 (8.32)				
% depressed (CES-D ≥ 16)	17.3	17.7	14.8	0.94 (0.68–1.30)	0.71	0.86 (0.38–1.96)	0.72
Meta-analysis	<i>n</i> = 1950 (49.8%)	<i>n</i> = 1639 (41.8%)	<i>n</i> = 330 (8.4%)				
Self-reported general health (% reporting fair to very poor)	29.9	30.0	31.0	1.01 (0.91–1.13)*	0.80*	1.06 (0.82–1.37)*	0.63*
% above study specific CES-D cut-off points	13.3	13.5	13.5	1.01 (0.87–1.17)*	0.90*	1.04 (0.73–1.47)*	0.84*

*Adjusting for study also in meta-analysis. SD = standard deviation.

no associations at genome-wide significance levels between these neurological conditions and the Q192R polymorphism or any other polymorphism in the *PON1* gene.

Our findings are not consistent with those of Lawlor *et al.*, who reported an association between the *PON1* Q192R polymorphism and symptoms of depression in a population-based sample of elderly women. Although the absence of an association is difficult to prove, it is clear that if such an association does exist, it is likely to be small. We also note that whilst our two populations studied are representative samples of community-dwelling individuals, like Lawlor *et al.*, we have no direct measure of the level of exposure to relevant OPs within these subjects. Given the mixed evidence on the biological effect of the *PON1* 192R variant, future work is needed to clarify the effect of this variant on *in vivo* *PON1* activity in relevant exposures and circumstances. Work is also needed on quantifying the relevant OP exposures at the individual level. Given the paucity of support for the association of the studied health effects with the 192R variant in the general population, however, the case for this area being a research priority can be doubted.

Conclusion

In two independent cohorts, we found no evidence of an association between the *PON1* Q192R

polymorphism and general health status or depression in the general older population. Neither the claimed 192R association with depression in the general older population nor its theoretical implications are supported.

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Conflict of interest: None declared.

KEY MESSAGES

- The *PON1* Q192R polymorphism has been associated with more diagnoses of disease and depression in groups exposed to high-dose OPs.
- In 2007, this association was extended to a general population-based cohort of elderly women, casting doubt on the specificity of the genetic association.
- We found no association between the *PON1* Q192R polymorphism and poor general or mental health in two independent population-based studies of older people.

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