RESEARCH REPORT

The association of the paraoxonase (*PON1*) Q192R polymorphism with depression in older women: findings from the British Women's Heart and Health Study

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Background: The association between the R allele of *PON1* Q192R and symptoms reported by sheep dippers and Gulf War veterans has been used to suggest a biological basis for these symptoms. In the absence of such studies in non-occupational populations, these conclusions may not be valid.
Objective: To examine the association of paraoxonase (*PON1*) Q192R with a report of ever being diagnosed with depression among a random sample of 3266 British women, aged 60–79 years.
Results: The R allele of *PON1* Q192R was associated with depression: per-allele odds ratio 1.22 (95% confidence interval: 1.05 to 1.41) in this population.
Conclusions: These findings suggest that the association of *PON1* Q192R with symptoms of depression in occupationally exposed groups may be driven by exposure to toxins that everyone in the general population is exposed to rather than exposure to toxins specifically used by sheep dippers or Gulf War veterans, or that other mechanisms underlie the association. This is because the study population in which we have found an association consisted of British women aged 60–79 years, few of whom were sheep dippers or Gulf War veterans. When using genotype–outcome associations to infer causality with respect to an environmental exposure modified by the genotype, it is important to examine these associations in general populations and in those specifically exposed to the putative agent. The possible role of *PON1* Q192R in psychiatric morbidity requires further examination.

The toxic effects of organophosphates are believed to lead to a range of non-specific symptoms reported by individuals who are occupationally exposed to large quantities of organophosphates—for example, sheep dippers.[1] The allelic variants arising from the paraoxonase (*PON1*) Q192R polymorphism have been reported to vary in their metabolism of several organophosphate substrates, including paraoxon, methyl-paraoxon and chlorthion-oxon. In 1996, Davies *et al*[2] reported an apparent paradoxical difference between *PON1* Q192R genotypes in their ability to hydrolyse diazoxon and paraoxon. They found that the RR 192 alloform of *PON1* hydrolysed paraoxon more rapidly, but hydrolysed diazoxon more slowly than the QQ alloform. Diazinon is the only organophosphorus insecticide that is currently approved for use in sheep dip in the US or UK, and its slower hydrolysis among individuals with the RR alloform would result in them having a greater risk of any toxic effects of this organophosphate.

Several studies on both sheep dippers and Gulf War veterans have found that possession of the R allele of *PON1* Q192R is associated with a greater likelihood of reporting symptoms, and this association has been used to suggest a biological aetiology for the symptoms reported by sheep dippers and for Gulf War syndrome.[1 3–5] Indeed, the US research advisory committee on Gulf War Veterans' Illness has reported that the *PON1* gene–symptom association is one of the strongest pieces of evidence supporting their conclusion that the syndrome has a biological basis (http://www1.va.gov/rac-gwvi/). As individuals tend to be unaware of their genotype and as genotypes are randomly allocated at conception, these gene–symptom associations are unlikely to be confounded by the usual socioeconomic and environmental exposures that are problematic in observational epidemiology.[5 6] The conclusions of these findings are not that individuals should be genetically screened before being allowed to take up certain occupations, but that the vague symptoms of the Gulf War syndrome, and reported by other groups who are occupationally exposed to organophosphates, are likely to have a biological basis possibly related to exposure to organophosphates.[5]

However, these conclusions may not be valid in the absence of studies on general populations. An association in individuals who are not specifically occupationally exposed to organophosphates (and, by extension, in the specific occupationally exposed groups that have been studied so far) may be related to sensitivity to low levels of organophosphates in general household insecticides, or to the effects of the polymorphism on other substrates, including oxidised lipids and some drugs. Such an association would implicate these alternative pathways as sources of the range of symptoms that have been reported in these specifically exposed groups, rather than their occupational exposure.

This study aimed to examine the association of *PON1* Q192R with depression in a random sample of British women aged 60–79 years. Women of this age are extremely unlikely to be sheep dippers or Gulf War veterans, and therefore any association in this group would suggest that the association in those who were occupationally exposed to organophosphates or other toxins may not be explained by their specific occupational exposure.

METHODS

Data from the British Women's Heart and Health Study were used. This study has been described in detail elsewhere.[7 8] Between 1999 and 2001, 4286 women aged 60–79 years, who were randomly selected from 23 British towns, were interviewed, examined and asked to complete medical questionnaires. Of those invited to participate 60% did so, with participants being similar to the general population of British women of that age with respect to social class distribution, and with participants being similar to non-participants with respect

Table 1 Depression and leg pain by *PON1* Q192R genotype in a general population of older women (aged 60–79 years)

	n/number with information, % (95% CI) by genotype					
	QQ n = 1669	QR n = 1326	RR n = 271	p Value*	OR (95% CI) for per allele effect	p value†
Ever diagnosed with depression	226/1500, 15.1 (13.3 to 17.0)	243/1199, 20.3 (18.0 to 22.7)	43/246, 17.5 (12.9 to 22.8)	0.002	1.22 (1.05 to 1.41)	0.008
Leg pain	643/1553, 41.4 (38.9 to 43.9)	509/1238, 41.1 (38.4 to 43.9)	103/254, 40.6 (34.5 to 46.9)	0.9	0.98 (0.88 to 1.10)	0.8

*For heterogeneity across genotype categories.
†For per-allele linear trend.

to the prevalence of general practitioner records of coronary heart disease and occurrence of cancer.[7] Blood samples were assessed after a minimum of 6 h of fasting. DNA was extracted by the salting out procedure[9] from K-EDTA whole blood or red and white cell residues, which had been stored at −80℃ for 1–2 years. The *PON1* Q192R genotype was determined using fluorescence-labelled oligonucleotide melting from matched or mismatched targets, monitored in an Idaho Technology (Salt Lake City, Utah, USA) 384-well Odyssey. Full details of this genotyping have been reported previously.[8]

Gulf War veterans and sheep dippers report a wide range of mental and physical health-related symptoms, although in studies of occupationally exposed groups the main problems that distinguished people with symptoms from those without are symptoms of depression (in particular difficulty in concentrating) and muscle spasms.[4] In the British Women's Heart and Health Study, we did not specifically ask about concentration or specific symptoms of depression, nor did we ask about leg spasms as the study was a general cohort study primarily concerned with the causes and consequences of cardiovascular disease in older women. We used responses to the questions ''Have you ever been diagnosed by a doctor as having depression? (yes/no)'' and ''Do you ever get pain in your leg, thighs or buttocks when you walk (yes/no)'' as indicators for the specific symptoms that have been commonly reported by previously studied occupational groups. These questions were asked in the self-completed questionnaire given to the women.

Hardy–Weinberg equilibrium was tested on a contingency table of observed-versus-predicted genotypic frequencies using an exact test.[10] The prevalence of depression and leg pain by genotype is presented. We used logistic regression to assess the association between *PON1* Q192R and depression and leg pain. On the basis of previous studies showing an additive increased risk per R allele, our a priori model was of a per allele (1 df) effect. We do, however, also present the p value for heterogeneity between the three possible genotypes (2 df).

Local ethics committee approval was obtained for the British Women's Heart and Health Study. Participants were asked for informed consent to review their medical records and for permission to perform anonymised genetic tests on stored blood. Eight women declined to give consent and have not been included in this study.

RESULTS

Of the 4278 participants who gave consent for genetic testing, 15 (5 Afro-Caribbean, 8 South Asian and 2 other) were defined as not being ''white'' by the examining nurse, and have been excluded from further analysis. Of the remaining 4263 women, 3545 (83%) had DNA available for genotyping, and for 3266 (92%) of these women genotypic data were available. There was no difference in mean (standard deviation) age (68.8 (5.5) *v* 69.0 (5.6) years, p = 0.23) and no difference in the prevalence of depression (17.4% *v* 18.0%, p = 0.5) or leg pain (41.2% versus 41.6%, p = 0.8) between those with and without genotypic data. The genotype frequencies (QQ: 1669 (51.1%); QR: 1326 (40.6%); RR: 271 (8.3%)) were in Hardy–Weinberg equilibrium (p = 0.7). In a previous publication, we showed that, as expected, genotype was not related to socioeconomic or lifestyle (such as smoking and physical activity) characteristics.[8]

The odds of ever being diagnosed with depression were highest in those who were heterozygotic and showed a linear trend of increasing odds with each additional R allele (table 1). When we repeated the analyses excluding women with clinical coronary heart disease (diagnosis of a myocardial infarction or angina) and those with diabetes, both of which have been reported to be associated with *PON1* and are associated with depression, the results were essentially the same, although less precise than those in the whole cohort. However, we found no association with leg pain.

DISCUSSION

Our results suggest that the R allele of the *PON1* Q192R polymorphism may be associated with depression in older British women. To our knowledge, this is the first study to show an association between *PON1* Q192R and depression in a non-occupational population. An association in individuals who are not specifically occupationally exposed to organophosphates (and, by extension, in the specific occupationally exposed groups that have been studied so far) may be related to sensitivity to low levels of organophosphates in general household insecticides, or to the effects of the polymorphism on other substrates, including oxidised lipids and some drugs. A recent study of the physiological effects of PON1 also questions whether the association between the R allele of *PON1* Q192R and symptoms in Gulf War veterans and other occupationally exposed groups is indicative of these symptoms being due to exposure to organophosphates.[11] Contrary to previous studies that have suggested that the R allele of *PON1* Q192R is associated with slower hydrolysis of the organophosphate diazinon,[2] a recent study found that with carefully controlled physiological conditions the opposite was true—that is, the R allele was associated with the fastest hydrolysis of diazinon.[11] If these recent findings are true, then it suggests that individuals with the R allele will clear the toxin diazinon more rapidly than those without this allele and will, therefore, have less exposure to this toxin. The finding of an association between the R allele and depression in our study and in those who are occupationally exposed suggests that organophosphate toxins are unlikely to be responsible for this association. *PON1* is involved in several metabolic pathways, and although our findings cannot determine the mechanism, they do suggest that the association of *PON1* Q192R with symptoms of depression are not restricted to sheep dippers and Gulf War veterans.

STUDY LIMITATIONS

Our results should be treated with some caution as depression was one of two a priori outcomes that we assessed, and the association with leg pain was null. Our null result with leg pain on walking may be because this is a poor proxy for muscle

What this paper adds

- Previous studies on groups occupationally exposed to organophosphates (eg, sheep dippers and Gulf War veterans) have found an association between *PON1* and symptoms of depression.
- These results have been interpreted as showing a biological basis for the symptoms experienced by these occupationally exposed groups.
- This is the first study to examine the association between *PON1* and depression in a population that is not exposed to large quantities of organophosphates (older British women).
- We found the R allele of *PON1* Q192R to be associated with increased odds of depression in a study on British women aged 60–79 years. Few of these women were occupationally exposed to organophosphates and therefore these findings suggest that the previous associations found in occupationally exposed groups may reflect pathways that are unrelated to their occupation, but that exist in the population as a whole.

Policy implications

When using genotype–outcome associations to infer causality with respect to an environmental exposure modified by the genotype, it is important to examine these associations in the general population and in those specifically exposed to the putative environmental agent.

spasms, which is one of the specific symptoms reported by Gulf War veterans. Further, it is well established that the initial positive findings in genetic association studies often fail to be replicated in future analyses.[12] Thus, our finding may be due to chance, and needs to be replicated in other studies.

Although the use of a population sample of community-dwelling older British women is useful for examining this association, as this group is unlikely to compromise Gulf War veterans or those occupationally exposed to large quantities of organophosphates, our findings may not be generalisable to other populations, including men. Our outcome of depression is based on a retrospective self-report of ever being diagnosed by a doctor, and as many individuals with depression remain undiagnosed, particularly those of older age, there is likely to be a measurement error here. Individuals probably do not know their genetic status and therefore this error will be non-differential, and would as a result, most probably lead to an underestimation of any effect.

CONCLUSIONS

Our findings suggest that *PON1* Q192R is associated with depression in older British women and they therefore question whether associations of *PON1* with depression-type symptoms in occupationally exposed groups (sheep dippers and Gulf War veterans) can be used as strong evidence for the biological basis of symptoms in these groups. This does not imply that there is no biological basis to these symptoms, but that the reliance on genetic association studies for this assertion is perhaps misplaced. Our findings highlight that when using genotype–outcome associations to infer causality with respect to an environmental exposure modified by the genotype,[5 6] it is important to examine these associations in general populations and in those specifically exposed to the putative environmental agent. The recent findings that question the nature of the association between the R allele of *PON1* Q192R and the metabolism of diazinon[11] also highlight the importance of having sound knowledge of the function of genes when using gene–outcome associations to infer causality about exposure–outcome associations using the principles of mendelian randomisation.[5 6]

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REFERENCES

1 **Cherry N**, Mackness M, Durrington P, *et al*. Paraoxonase (PON1) polymorphisms in farmers attributing ill health to sheep dip. *Lancet* 2002;**359**:763–4.
2 **Davies HG**, Richter RJ, Keifer M, *et al*. The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. *Nat Genet* 1996;**14**:334–6.
3 **Mackness B**, Durrington P, Povey A, *et al*. Paraoxonase and susceptibility to organophosphorus poisoning in farmers dipping sheep. *Pharmacogenetics* 2003;**13**:81–8.
4 **Haley RW**, Billecke S, La Du BN. Association of low PON1 type Q (type A) arylesterase activity with neurologic symptom complexes in Gulf War veterans. *Toxicol Appl Pharmacol* 1999;**157**:227–33.
5 **Davey Smith G**, Ebrahim S. ''Mendelian randomisation'': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 2003;**32**:1–22.
6 **Davey Smith G**, Ebrahim S. What can mendelian randomisation tell us about modifiable behavioural and environmental exposures? *BMJ* 2005;**330**:1076–9.
7 **Lawlor DA**, Bedford C, Taylor M, *et al*. Geographic variation in cardiovascular disease, risk factors and their control in older women: British Women's Heart and Health Study. *J Epidemiol Community Health* 2003;**57**:134–40.
8 **Lawlor DA**, Day INM, Gaunt TR, *et al*. The association of the *PON1* Q192R polymorphism with coronary heart disease: findings from the British Women's Heart and Health Study and a meta-analysis. *BMC Genet* 2004;**5**:17.
9 **Miller SA**, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;**16**:1215.
10 **Guo SW**, Thompson EA. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 1992;**48**:361–72.
11 **O'Leary KA**, Edwards RJ, Town MM, *et al*. Genetic and other sources of variation in the activity of serum paraoxonase/diazoxonase in humans: consequences for risk from exposure to diazinon. *Pharmacogenet Genomics* 2005;**15**:51–60.
12 **Colhoun HM**, McKeigue PM, Davey Smith G. Problems of reporting genetic associations with complex outcomes. *Lancet* 2003;**361**:865–72.