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Lowered plasma paraoxonase (PON)1 activity is a trait marker of major depression and PON1 Q192R gene polymorphism B smoking interactions differentially predict the odds of major depression and bipolar disorder

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Abstract.

Background: Major depression and bipolar disorder are accompanied by activation of immune-inflammatory and oxidative and nitrosative stress (O&NS) pathways and lowered levels of antioxidants. Paraoxonase (PON)1 (EC 3.1.8.1) is an antioxidant bound to high density lipoprotein (HDL). Polymorphisms in the PON1 Q192R coding sequence determine three functional genotypes, i.e. 192QQ, 192QR and 192RR.

Aims: This study was carried out to delineate the associations of plasma PON1 activity and functional PON1 Q192R genotypes in major depression and bipolar disorder.

Methods: Plasma PON1 abundance and three functional PON1 Q192R genotypes were assayed in 91 major depressed and 45 bipolar patients and compared to 199 normal controls.

Results: Major depression, but not bipolar disorder, was accompanied by lowered PON1 activity. PON1 activity was decreased by smoking and a diagnosis by genotype interaction (i.e. lower PON1 in major depression with the QQ genotype). Logistic regression showed that smoking by QQ genotype significantly increased the odds of bipolar disorder and that major depression was predicted by plasma PON1 activity, serum HDL cholesterol and interactions between genotype X smoking.

Discussion: The results suggest that lowered plasma PON1 activity is a trait marker of major depression and that PONQ192R gene B environment (smoking) interactions differentially predict

the odds of depression and bipolar disorder.

Limitations: Association studies are prone to a risk of false positive findings and replication is essential.

Conclusions: The findings suggest that there are differential PON1 Q192R functional genotype X environment interactions in major depression and bipolar disorder. The effects of lowered PON1 activity may contribute to increased O&NS and immune-inflammatory burden in depression. PON1 status may contribute to the comorbidity between depression and other immune- and O&NS-related disorders, e.g. cardiovascular disorder.

Key words: depression, bipolar disorder, paraoxonase, inflammation, cytokines, oxidative stress, antioxidants, cardiovascular

Introduction

Growing evidence shows that activated immune-inflammatory (Maes et al., 1995; Berk et al., 2011; Leonard and Maes, 2012), oxidative and nitrosative stress (O&NS) pathways (Maes, 2000; Maes et al., 2011; Berk et al., 2011; Moylan et al., 2013) and lowered high density lipoprotein (HDL) cholesterol (Maes et al., 1997) play an important role in major depression and bipolar disorder. Activation of these pathways and lowered levels of key antioxidants, such as zinc, coenzyme Q10 and glutathione play a role in the neuroprogressive processes (that is increased neurodegeneration and apoptosis and lowered neurogenesis) and secondary autoimmune responses directed against oxidatively/nitrosatively modified epitopes that accompany depression and bipolar disorder (Maes et al., 2009; Berk et al., 2011; Leonard and Maes, 2012; Moylan et al., 2013). Increased translocation of gram-negative bacteria is another immune-inflammatory

pathway noted in major depression (Maes et al., 2008). The strong comorbidity between depression and many (auto)immune disorders, including cardiovascular disorder (CVD), chronic obstructive pulmonary disorder (COPD) and inflammatory bowel disease (IBD), may be explained by shared immune-inflammatory and O&NS pathways and lowered levels of antioxidants (Maes et al., 2011).

There are a few reports that major depression and bipolar disorder may be associated with lowered activity of paraoxonase 1 (PON1, EC 3.1.8.1), an enzyme with paraoxonase and arylesterase activity (Barim et al., 2009; Kotan et al., 2011; Ezzaher et al., 2010). PON1 is a hydrolytic antioxidant enzyme with a very wide range of substrates (Litvinov et al., 2012). PON1 is in the plasma almost exclusively bound to HDL and is responsible for most of the antioxidant activity of HDL (Razavi et al., 2012). HDL-associated PON hydrolyzes hydrogen peroxide and contributes to protective effects against lipid peroxidation and formation of oxidized low density lipoproteins (LDL) and consequently CVD (Aviram et al., 1998). PON1 plays a role in the detoxification of organophosphate pesticides (OPs), has hydrolytic activity against some gram negative bacteria and is a strong anti-inflammatory and antioxidative agent (Furlong et al., 2010). The latter effects help explain the role of PON1 in immune- and O&NS-related disorders, e.g. CVD, COPD, lung cancer, IBD, etc. (Furlong et al., 2010; Isik et al., 2005; Rothem et al., 2007; Goswami et al., 2009).

PON1 is under genetic control and polymorphisms of the PON1 gene may determine the enzymatic activities, e.g. polymorphism Q-->R at the 192 position (Q192R) (Liu et al., 2013). There is evidence that this Q192R polymorphism, i.e. RR genotype and the R allele, is associated with elevated risk for ischaemic stroke (Liu et al., 2013). The Q isoform is associated with a lower paraoxon hydrolyzing activity and decreased protective activity against HDL and LDL oxidation

(Mackness and Mackness, 2013). Individuals with a QQ genotype are more susceptible to genotoxicity, whereas the RR genotype may be more protective against oxidative stress (Singh et al., 2011; Kotani et al., 2012).

Barim et al. (2009) and Kotan et al. (2011) reported lower arylesterase activity in untreated patients with depression, while treatment with antidepressants increased arylesterase levels. Sarandol et al. (2006) and Kodydkova et al. (2009), on the other hand, were unable to find any differences in PON activity between depressed patients and controls. There is also a report on lowered PON1 activity in bipolar disorder (Ezzaher et al., 2010). Lawlor et al. (2007) detected that in elderly women the R allele was significantly associated with depression (odds ratio 1.22). In two independent population-based studies no significant association was found between PON1 Q192R polymorphism and depression (Rice et al., 2009). Statins such as atorvastatin increase PON1 activity and statins are now thought to have a role in both the prevention and reduction of depressive symptoms (Samy and Hassanian, 2011; Pasco et al., 2010).

However, it is advocated that the assay of PON1 status should be based on the assays of both PON1 enzymatic activity and PON1 Q192R functional genotypes (Furlong et al., 2010). In addition, data on PON1 status should consider smoking effects because cigarette smoking decreases PON1 plasma activity (Haj Mouhamed et al., 2012) and genetic variation in O&NS and antioxidant genes and their interaction with smoking may affect inflammatory and oxidative burden (Stephens et al., 2008). Smoking and nicotine dependence are accompanied with the onset of depressive disorders and suicidal behavior (Vargas et al., 2013; Breslau et al., 2004; 2005; Jamal et al., 2012; Malone et al., 2003; Pasco et al., 2008).

The present study was carried out to examine PON1 status (both PON1 activity and functional PONQ192R polymorphism based on a 2-substrate assay) in major depression and

bipolar disorder in relation to nicotine dependence.

Subjects and Methods

Subjects

We recruited normal controls (n=199) and patients with major depression (n=91) or bipolar disorder (n=45) from staff at the Londrina State University (UEL) and an outpatient ambulatory of smoking cessation program, UEL, Parana, Brazil. Participants of Caucasian, Asian, African and mixed ethnicities, aged 18-60 years, were accepted in this study. Diagnoses of bipolar disorder and major depressive disorder were made by senior clinicians using the validated Portuguese version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) interview, Clinical Version (SCID-CV) (Del Ben et al., 2001). In this study, we combined patients in the acute phase of illness and patients in partial or total remission and separated these in two study groups, i.e. major depression versus bipolar disorder.

Based on a SCID-CV interview, normal controls with lifetime or current axis 1 and axis 2 diagnoses were excluded from participating in this study. In addition, we collected data on medical disorders and use of medication by means of a semi-structured interview performed by senior psychiatrists. Patients with axis 1 diagnoses other than major depression or bipolar disorder were excluded, e.g. schizophrenia, substance abuse disorders, psycho-organic syndromes, and anxiety disorders. Patients and controls were excluded a) when they showed increased plasma levels of laboratory tests, such as hemogram, aspartate transaminase (AST), alanine transaminase (ALT), urea and creatinine; b) when they suffered from any major medical illness, e.g. diabetes type 1 and 2, (auto)immune disorders, IBD, COPD, and neuroinflammatory disorders; c) when they were treated with immunomodulatory drugs, including glucocorticoids; and d) when they showed

acute inflammatory or infective reactions the month prior to the study.

The severity of illness was measured using a validated Portuguese version of the 17-item Hamilton Depression Rating Scale (HDRS) which was adapted for use in the Brazilian population (Moreno and Moreno, 1998). Ethnicity (Caucasian, Asian, African, mixed ethnicities), years of education, marital status (single, stable relationship, separated or widowed), use of alcohol (no use, monthly, weekly, daily use), work disabled due to medical reasons, use of statins, and body weight and height were collected using a self-report questionnaire. A clinical diagnosis of nicotine dependence was made using DSM-IV criteria. Body mass index (BMI) was calculated as $\text{weight (kg)} / \text{body height (in meter)}^2$. The study was approved by the Research Ethics Committee at UEL (number 250/2010). All participants gave written informed consent prior to participating in this study.

Laboratory Assessments

Blood for the assay of plasma PON1 enzyme activity and PON1 Q192R genotypes and serum HDL was sampled at 8.00 a.m. after an overnight fast. PON1 status (plasma activity levels and functional position 192 genotype) was determined using assays as described by Richter et al. (2008). The substrates were phenylacetate (PA, Sigma, USA) and 4-(chloromethyl)phenyl acetate (CMPA, Sigma, USA) and the analysis was conducted in a microplate spectrophotometer reader (EnSpire, Perkin Elmer, USA) using ultraviolet transparent 96-well microplates. All assays were carried out in triplicate and replicates that varied by >10% were repeated. Briefly, CMPA hydrolysis was measured at 280 nm for 4 minutes at 25°C using 20 μL of plasma diluted 1:40 in dilution buffer [20 mmol/L Tris-HCl (pH 8.0), 1.0 mmol/L CaCl_2]. PA hydrolysis under high salt conditions was measured at 270 nm for 4 minutes at 25°C using 20 μL of plasma diluted 1:40 in

dilution buffer. High salt media was composed by PA added to 2 mol/L NaCl, 20 mmol/L Tris-HCl (pH 8.0), 1.0 mmol/L CaCl₂. The results obtained with these two assays were used to plot a 2-dimensional enzyme activity graphic that displays rates of arylesterase activity (PA hydrolysis) under high salt conditions versus CMPase activity (CMPA hydrolysis). Since the polymorphism Q192R confers differential catalytic activity against these two substrates, the plot splits the population into the three functional position 192 genotype (QQ, QR and RR). Measurement of PA hydrolysis at low salt concentrations reveals plasma PON1 abundance since at this condition PON1 Q192R polymorphism does not influence PON1 catalytic activity against PA (Richter et al., 2011). For this assay, rates of hydrolysis of PA under low salt conditions were measured at 270 nm for 4 minutes at 25°C using 20 µL of plasma diluted 1:80 in dilution buffer.

Serum HDL cholesterol was determined by an automated method in a clinical chemistry system, Dimension RXL (Siemens Healthcare Diagnostics Inc, Newark, DE, USA). This method measures HDL cholesterol levels directly, without the need for sample pretreatment or specialized centrifugation steps. HDL cholesterol was measured as a surrogate marker of HDL. The inter-assay coefficient of variation values were less than 4.0%.

Statistics.

Analyses of contingency tables (Chi-square test) were employed to check the distribution of variables in different study groups. Univariate analyses of variance (ANOVAs) were used to ascertain differences in PON activity, serum HDL and other continuous variables between study groups, e.g. controls versus major depression and bipolar disorder and QQ, QR and RR genotypes. Power analyses, performed using GPower 3.1, showed that for the examination of differences in PON1 between 3 groups at $\alpha=0.05$, power=0.80 and effect size of 0.20 the

minimum sample size should be around 240. Factorial design analyses of covariance (ANCOVA) were employed to delineate the differences in continuous variables among groups taking into account the effects of different categories and their interactions and while adjusting for relevant covariates. When significant, the Tukey test or Bonferroni's test were used to examine planned comparisons among treatment means. Binary logistic regression analyses were used to examine the relationships between major depression or bipolar disorder (versus controls) as dependent variable and independent variables, including PON1 activity, PON1 genotypes and clinical / socio-demographic data. Relationships between variables were assessed by means of Pearson's correlation coefficients and linear multiple regression analyses. As PON1 is bound to HDL and contributes to the effects of HDL, the PON1 X HDL cholesterol product term was computed to denote possible (antioxidative) potentiating effects between both compounds. Transformations were used to normalize the distribution of PON1 activity, the PON1 X HDL cholesterol product term and years of education in Ln transformation. Data was analyzed using the SPSS windows version to analyze our data. Statistical significance was set at $\alpha=0.05$ (two tailed).

Results.

Demographic data

Table 1 shows the demographic data of the 335 subjects in this study. There were no significant differences in age, ethnicity, marital status, work disabled, use of statins and years of education between the three diagnostic groups. There were more females in both mood disorder groups than in the normal control group. Smoking frequency was higher in bipolar participants than in normal controls ($\chi^2 = 12.48$, $df=1$, $p<0.001$) and depressed patients ($\chi^2 = 10.36$, $df=1$,

$p=0.001$). BMI was significantly higher in bipolar participants than in depressed participants ($p=0.049$) and controls ($p=0.014$). Bipolar participants had a significantly increased HDRS score than controls ($p<0.001$) and depressed participants ($p<0.001$), while the latter had a higher HDRS score than controls ($p<0.001$).

Table 2 shows that there were no differences in age, gender, marital status, years of education, use of statins, BMI and HDRS between the PON1 Q192R genotypes. There was a significant association between ethnicity and PON1 Q192R genotypes. There was a marginally significant association between smoking and the three genotypes. **Table 3** shows that in individuals with a QQ genotype but not in those with a QR+RR genotype there was an association between smoking and diagnosis.

PON1 activity and PON1 Q192R genotypes in depression

There were weak although significant correlations between PON1 and HDL ($r=0.189$, $p=0.001$, $n=331$), age ($r=-0.156$, $p=0.004$, $n=335$) and years of education ($r=0.282$, $p<0.001$, $n=335$), but not BMI ($r=-0.054$, $p=0.325$, $n=333$) and HDRS ($r=-0.094$, $p=0.085$, $n=334$). PON1 was significantly lower ($F=33.61$, $df=1/333$, $p<0.001$) in smokers (mean $\pm$ SD = 174.68 ± 50.63 U/mL) than in non smokers (mean = 205.91 ± 53.48 U/mL). There were no significant differences in PON1 between men and women ($F=0.61$, $df=1/333$, $p=0.436$), ethnical groups ($F=1.11$, $df=2/332$, $p=0.331$), work disabled or not ($F=0.00$, $df=1/333$, $p=0.991$), marital status ($F=0.47$, $df=2/332$, $p=0.628$), use of statins ($F=2.47$, $df=1/314$, $p=0.12$) or use of alcohol ($F=0.44$, $df=4/329$, $p=0.781$).

Table 1 shows the mean PON1 activities in controls and depressed and bipolar participants. Simple ANOVA showed that there are significant differences between the groups, with lower

levels in major depression than in controls (Tukey test: $p=0.028$), while no significant differences were found between bipolar disorder and depression ($p=0.722$) or controls ($p=0.500$). ANOVA showed no significant differences in HDL concentrations between the three study groups. Table 2 shows that there were no significant differences in PON1 activity and HDL cholesterol between the three PON1 Q192R genotypes.

Table 4 shows the outcome of univariate factorial design ANCOVA with PON1 activity as dependent variable and smoking, PON1 Q192R genotypes (QQ versus QR+RR), diagnosis (all entered as factors), age, gender, BMI, HDRS, and years of education as explanatory variables. We found that PON1 activity was significantly ($F=4.46$, $df=16/315$, $p<0.001$; $R^2=18.5\%$) predicted by age (negative), diagnosis, smoking, and a diagnosis X genotype interaction (i.e. lower PON1 in major depression with the QQ genotype). Bonferroni post hoc tests showed lower PON1 activity in major depression versus controls ($p=0.001$), while there were no significant differences between bipolar disorder and either controls ($p=0.99$) and major depression ($p=0.332$). After entering HDL cholesterol in this analysis, the effects of diagnosis remained significant while HDL was also a significant explanatory variable ($F=4.2$, $df=1/314$, $p=0.041$). Using the same predictor variables, we found that the product term POX X HDL was significantly predicted by diagnosis ($F=10.43$, $df=1$, $p<0.001$), smoking ($F=19.76$, $df=1$, $p<0.001$), gender ($F=18.65$, $df=1$, $p<0.001$) and BMI ($F=6.47$, $df=1$, $p=0.012$). Bonferroni's post hoc tests showed that the product ratio was significantly lower in depression than in controls ($p<0.001$), while there were no significant differences between bipolar participants and controls ($p=0.291$) or depressed subjects ($p=0.354$).

In the patient groups we were unable to find any significant difference in PON1 activity ($F=0.13$, $df=1/132$, $p=0.720$, results of factorial ANOVA with diagnosis as second factor) between patients who were drug free for at least 2 months (mean=184.44 $\pm$ 60.21 U/mL, $n=105$) and those

who had taken psychotropic drugs (mean=189.55.97 U/mL, n=31). In drug free depressed participants (mean=181.18 U/mL, n=76) PON activity was significantly lower ($F=8.05$, $df=1/273$, $p=0.005$) than in controls (mean=197.16 $\pm$ 49.01 U/mL, n=199).

Logistic regression of odds of major depression compared to normal controls

Table 5 shows the results of logistic regression analysis with depression versus controls as dependent variable. Entering PON1 Q192R genotypes (RR is used as reference group; Wald=0.54, $df=2$, $p=0.76$) and smoking (Wald=0.20, $df=1$, $p=0.888$) separately showed that both variables did not increase the odds of depression. We found that depression was significantly distinguished from controls ($\chi^2=105.42$, $df=10$, $p<0.001$; Nagelkerke=0.432); the number of correctly classified cases was 81.1% with a sensitivity of 53.8% and a specificity of 93.8%. The HDRS and an interaction between PONQ192R genotypes and smoking increased the odds of major depression, while PON1 activity decreased the odds of depression. The interaction pattern between PON1 Q192R genotypes and smoking shows that in non smokers the QQ and QR genotypes increase the odds of depression versus the RR phenotype. Logistic regression performed on the drug free depressed patients showed similar main and interaction effects. Ethnicity (Wald=0.80, $df=2$, $p=0.961$) or ethnic groups by smoking (Wald=0.301, $df=2$, $p=0.860$) did not show significant relationships with major depression.

Logistic regression of odds of bipolar disorder compared to normal controls

We performed a similar logistic regression analysis (without using HDRS as independent variable) with bipolar disorder and normal controls as dependent variables. After adjusting for the effects of all other explanatory variables, we observed that bipolar participants significantly

differed from controls ($\chi^2=26.53$ df=9, $p=0.002$) using female gender and smoking by genotype QQ as independent variables. PON1 activity and HDL were not significant in distinguishing the bipolar and control groups.

Discussion

The first major finding of this study is that plasma PON1 activity was significantly decreased in patients with major depression, but not in those with bipolar disorder. These results correspond with those of Barim et al. (2009) who found lowered arylesterase activity in patients with depression using assays with arylesterase with phenylacetate as substrates. Kotan et al. (2011) also reported lowered arylesterase activity of PON1 in depressed patients. Using a comparable method, two other studies were unable to find lowered PON1 activity in depression (Kodykova et al. 2009; Sarandol et al., 2006). ~~It should be stressed however that these studies in depression did not measure PON1 status according to the current state-of-the-art method used in our study (Richter et al., 2011).~~ Our study failed to replicate the findings of Ezzaher et al. (2010) who reported decreased PON1 activity in bipolar disorder. ~~Other factors that were inversely associated with PON1 activity were increasing age and years of education. Interestingly, we found also a positive relationship between PON1 activity and HDL cholesterol, which may indicate that PON1 is bound to HDL.~~

The reductions in PON1 activity may in theory contribute to the activated O&NS (including lipid peroxidation) and immune-inflammatory processes in depression. For example, PON1 hydrolyzes hydrogen peroxide and inhibits LDL oxidation and lipid peroxidation and the consequent inflammatory responses to LDL oxidation, ~~showing that PON1 has antiinflammatory and antioxidative properties~~ (Mackness and Mackness, 2010). PON^{-/-} mice show a significant

increase in vascular inflammation and aortic superoxide and consequently CVD (Ng et al., 2007; Shih et al., 1998). Inflammatory responses in depression, including increased interleukin-1 (IL-1) and tumor necrosis factor (TNF) α , may lower PON1 mRNA production and plasma PON1 activity thereby modulating the antiinflammatory and antioxidative properties of HDL (Kumon et al., 2003). Thus, both cytokines could play a role in the comorbidity between depression and atherosclerosis (Maes et al., 2011) via alterations in PON1 mRNA (Kumon et al., 2003). Nevertheless, IL-6, another cytokine that is increased in depression (Maes et al., 1993), enhances PON1 gene expression in hepatocytes (Cheng et al., 2013).

The second major finding of this study is that PON1 Q192R functional genotypes may play a role in depression and bipolar disorder. As with Rice et al. (2009), we were unable to validate a previous report that the R allele is significantly associated with depression (Lawlor et al., 2007). We could similarly not validate the findings of Ezzaher et al. (2012) that there is an association between bipolar disorder and the QR and RR genotypes. Nevertheless, we found different interactions between PON1 Q192R genotypes and smoking and diagnosis. Firstly, major depressed subjects with a QQ genotype (in contrast to QR and RR) showed lowered PON1 activity. Secondly, the odds of major depression was significantly increased by an interaction between PON1 Q192R genotype and smoking, i.e. in non smokers the QQ and QR genotypes increased the odds to depression. The odds of bipolar disorder were increased by the QQ genotype in smokers, suggesting that the effects of smoking on bipolar disorder are mediated by PON1 Q192R polymorphisms.

This interaction may be explained by the effects of functional PON1 Q192R genotypes and smoking on immune-inflammatory and O&NS pathways. Firstly, smoking increases risk for mood disorders through activation of immune-inflammatory and O&NS pathways, including effects on

proinflammatory cytokines, microglial activation, increased lipid peroxidation, decreased levels of antioxidants and neuroprogressive pathways (Vargas Nunes et al., 2012; Edirisinghe and Rahman, 2010; Vargas et al., 2013). Smoking-induced activation of these pathways including suppression of PON1 activity may explain the increased incidence of immune- and O&NS-related degenerative disorders in smokers, e.g. CVD, COPD, lung cancer, IBD, ect. (Minicucci et al., 2012; Milara and Cortijo, 2012; Leone, 2011). Secondly, individuals with the Q isoform have also less protective activity against oxidative stress, including HDL and LDL oxidation, lower paraoxon hydrolyzing activity and are more susceptible to genotoxicity (Mackness and Mackness, 2013). ~~PON1 gene polymorphisms determine PON1 enzymatic activities and differently regulate hydrolysis of different toxins (Furlong et al., 2010; Liu et al., 2013). QR and RR individuals have a better survival advantage to become centenarians than Q allele carriers (Bonafe et al., 2002). Patients with a QQ genotype have a worse diabetes control than those with a RR genotype (Flekae et al., 2008).~~

A third major finding of this study is that smoking not only interacts with the functional PON1 genotypes to predict bipolar disorder but also decreases plasma PON1 activity. We replicated previous findings that cigarette smoking lowers PON1 plasma activity in all three PON1 Q192R genotypes (Mouhamed et al., 2012). In CVD patients and controls, smoking was associated with lowered PON activity an effect that was readily reversed upon cessation (James et al., 1999). In vitro, cigarette smoke extracts inhibit PON1 activity in a dose- and time dependent manner by modification of free thiols (Nishio and Watanabe, 1997). Evidently, the PON1 lowering effects of smoking may contribute to inflammatory and oxidative burden and thus to the increased incidence of degenerative disorders (Nishio and Watanabe, 1997; James et al., 1999). Likewise, the same pathways may in part account for the association between smoking and bipolar

disorder. It is possible that the smoking status of patients and controls could explain some of the discrepancies in the literature on plasma PON1 in depression. Accordingly, some (e.g. Barin et al., 2009), but not all studies (e.g. Kodykova et al., 2009; Kotan et al., 2011) controlled their data for smoking.

Not all catalytic specifications and substrates of PON1 are known and therefore other putative pathways may explain the association between depression and functional PON1 Q192R genotypes and lowered PON1 activity. For example, PON1 alters bacterial colonization and displays lactonase activity hydrolyzing acyl homoserine lactones, which function as quorum sensing signals in gram negative bacteria (Pezzulo et al., 2012; Camps et al., 2011; Teiber and Draganov, 2011). Thus lowered PON1 activity could play a role in the immune effects directed against the increased translocation of gram negative bacteria in depression (Maes et al., 2008).

One limitation of our study is that we did not control for all possible dietary effects. PON1 activity is increased by polyphenol-rich diets and moderate alcohol consumption, while high fat diets may decrease PON1 activity (Thomas-Moya et al., 2007; Gouedard et al. 2004; Costa et al., 2011). Nevertheless, the subjects included in this study were largely on a traditional Brazilian diet and subjects with alcohol abuse were excluded from this study, while there was no association between moderate alcohol consumption and plasma PON1 activity. There were no effects of treatment with psychotropic drugs on our results because a) there were no significant differences in PON1 activity between patients who were drug free for at least two months and those who took psychotropic drugs; and b) drug free major depressed subjects showed significantly lowered PON1 activity than controls. In addition, Kotan et al. (2011) found that treatment with antidepressants in standard doses for 24 weeks normalized decreased baseline PON activity in depressed patients. Another limitation of the study is that reports from Association studies

constitute tentative knowledge and must be interpreted with caution@ (Sullivan, 2007). Strengths of our study are that we adjusted our results for many potential confounders, including age, sex, ethnicity, BMI, use of statins, education and excluded subjects with abnormal liver and kidney tests and with illnesses that affect plasma PON1 activity, e.g. liver cirrhosis, renal disease, increased uremia, diabetes, etc.

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Contributions

All authors contributed equally to the work

Table 1. Demographic and clinical data of the controls and major depressed (MDD) and bipolar patients (BP) included in this study.

Variables	Controls	MDD	BP	F	df	p
Age (years)	46.4 (∓8.3)	47.0 (∓8.2)	44.5 (∓8.9)	1.34	2/332	0.265
Gender				9.45	2	0.009
Female	117	69	33			
Male	82	22	12			
Ethnicity				7.38	4	0.117
Caucasians	136	67	26			
Asian + African	34	10	6			
Mixed	29	14	13			
Marital status				1.26	4	0.868
single	27	15	8			
stable	136	57	29			
separated or widow	36	19	8			
Work disabled				4.52	2	0.105

No Yes	184 15	81 10	37 8			
Years of education	13.27 ($\sqrt{5.29}$)	14.60 ($\sqrt{7.71}$)	11.96 ($\sqrt{5.82}$)	1.98	2/332	0.140
Smoking No Yes	122 77	55 36	14 31	14.25	2	0.001
BMI (kg) / body height (in meter) ² .	26.84 ($\sqrt{4.80}$)	26.27 ($\sqrt{4.35}$)	28.76 ($\sqrt{5.77}$)	4.16	2/330	0.017
HDRS	2.77 ($\sqrt{3.67}$)	8.96 ($\sqrt{7.11}$)	14.20 ($\sqrt{12.43}$)	69.53	2/331	<0.001
plasma PON1 (U/mL)	197.16 ($\sqrt{50.64}$)	183.74 ($\sqrt{58.91}$)	189.52 ($\sqrt{59.99}$)	3.45	2/332	0.033
serum HDL (mg/dL)	49.01 ($\sqrt{14.51}$)	45.86 ($\sqrt{14.95}$)	43.98 ($\sqrt{11.64}$)	2.99	2/328	0.051
PON1 X HDL	9871 ($\sqrt{4613}$)	8531 ($\sqrt{4250}$)	8109 ($\sqrt{3437}$)	5.44	2/328	0.005

HDRS: total score on the Hamilton Depression Rating Scale

BMI: body mass index

PON1: paraoxonase 1 plasma activity

HDL: high density lipoprotein cholesterol

Table 2. Demographic and clinical data in subjects divided according to paraoxonase (PON)1 Q192R genotypes into QQ, QR and RR carriers.

Variables	QQ	QR	RR	F	df	p
Age (years)	46.1 (√8.5)	46.6 (√8.0)	46.2 (√9.0)	0.128	2/332	0.879
Gender				5.03	2	0.081
Female	89	94	36			
Male	62	39	15			
Ethnicity				22.63	4	<0.001
Caucasians	121	83	25			
Asian + African	11	27	12			
Mixed	19	23	14			
Marital status				6.13	4	0.189
single	19	19	12			
stable	102	93	27			
separated or widow	30	21	12			
Years of education	14.38 (√7.19)	12.84 (√4.48)	12.29 (√6.21)	2.02	2/332	0.135
Smoking				7.67	2	0.022
No	97	72	22			
Yes	54	61	29			
BMI (kg) / body height (in meter) ²	26.47 (√4.71)	27.38 (√4.97)	27.20 (√5.04)	1.33	2/330	0.266
HDRS	5.77 (√7.64)	5.68 (√7.69)	7.49 (√8.04)	1.13	2/331	0.324
plasma PON1 (U/mL)	201.7 (√61.4)	185.7 (√47.9)	183.1 (√44.5)	2.73	2/332	0.067
serum HDL (mg/dL)	47.89 (√14.34)	47.48 (√14.18)	46.29 (√15.28)	0.228	2/328	0.796
PON1 X HDL	9838 (√4890)	8935 (√4101)	8456 (√3567)	1.95	2/328	0.144
Diagnosis				2.44	4	0.656
controls	90	78	31			
MDD	45	34	12			
BD	16	21	8			

HDRS: total score on the Hamilton Depression Rating Scale

BMI: body mass index

PON1: paraoxonase 1 plasma activity

HDL: high density lipoprotein cholesterol

MDD: major depression

BD: bipolar disorder

Table 3. Differences in the association between smoking and diagnosis in paraoxonase (PON)192 QQ versus RR or QR carriers

genotype		controls	MDD	BD	χ^2	df	p
QQ	no smoking smoking	63 27	30 15	4 12	12.14	2	0.002
QR+RR	no smoking smoking	59 50	25 21	10 19	3.80	2	0.150

MDD: major depression

BD: bipolar disorder

Table 4. Results of univariate analysis of covariance with plasma paraoxonase (PON)1 activity as dependent variable and diagnosis, PON1 Q192R genotypes, smoking, body mass index (BMI), the Hamilton Depression Rating Scale (HDRS), years of education, gender and age as explanatory variables (entered as factors or covariates).

Explanatory variables	F	df	p
Diagnosis*	6.69	2	0.001
Genotype* (i.e. QQ versus QR+RR)	0.03	1	0.856
Smoking*	15.30	1	0.000
Diagnosis* X genotype*	3.35	2	0.036
Diagnosis* X smoking*	0.90	2	0.408
Genotype* X smoking*	0.20	1	0.655
Diagnosis* X genotype* X smoking*	2.08	2	0.126
BMI	0.03	1	0.860

HDRS	3.17	1	0.076
Years of education	3.75	1	0.054
Age	4.41	1	0.036
Gender	0.21	1	0.645

* These variables were entered as factors

Table 5. Results of logistic regression analysis with major depression versus controls as dependent variable and plasma paraoxonase (PON)1 activity, PON1 Q192R genotypes, body mass index (BMI), the Hamilton Depression Rating Scale (HDRS), age, gender and high density lipoprotein (HDL) cholesterol as independent variables.

Variables	Wald	df	p	Odds ratio	lower 95% CI	Upper 95% CI
plasma PON1	6.40	1	0.011	0.216	0.066	0.708
serum HDL	9.04	1	0.003	0.961	0.936	0.986
age	0.026	1	0.871	1.003	0.966	1.041
gender	2.95	1	0.086	0.535	0.262	1.092
BMI	0.862	1	0.353	0.968	0.903	1.037
HDRS	49.10	1	<0.001	1.329	1.227	1.439
QQ genotype*	0.316	1	0.574	0.701	0.203	2.421
QR genotype*	0.400	1	0.527	0.680	0.206	2.245
genotype* X smoking	15.27	2	<0.001	-	-	-
QQ genotype* x smoking (0)	11.01	1	0.001	7.890	2.33	26.70
QR genotype* x smoking (0)	8.24	1	0.004	5.545	1.721	17.86

CI: confidence intervals

*The RR genotype was used as the reference group

Table 6. Results of logistic regression analysis with bipolar disorder versus controls as dependent variable and paraoxonase (PON)1 activity, PON1 Q192R genotypes, body mass index (BMI), age, gender and serum high density lipoprotein (HDL) cholesterol as independent variables.

Variables	Wald	df	p	Odds ratio	lower 95% CI	Upper 95% CI
plasma PON1	0.558	1	0.455	0.566	0.127	2.518
serum HDL	1.66	1	0.198	0.980	0.950	1.011
age	2.95	1	0.086	0.964	0.925	1.005
gender (female)	4.18	1	0.041	2.383	1.036	5.481
BMI	2.90	1	0.089	1.058	0.992	1.129
QQ genotype*	1.51	1	0.219	0.417	0.103	1.684
QR genotype*	0.00	1	0.973	0.980	0.310	3.101
genotype* X smoking (1)	7.48	2	0.024	-	-	-
QQ genotype* x smoking (1)	6.86	1	0.009	5.647	1.547	20.619
QR genotype* x smoking (1)	0.828	1	0.363	1.631	0.569	4.675

CI: confidence intervals

*The RR genotype was used as the reference group

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