

Lowered paraoxonase 1 activities may explain the comorbidities between temporal lobe epilepsy or mesial temporal sclerosis and comorbid depression, anxiety and psychosis.

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Abstract

Background: Temporal lobe epilepsy (TLE) is the most common focal epilepsy subtype in adults and is frequently accompanied by depression, anxiety and psychosis. Aberrations in total paraoxonase (PON)1 status may occur in TLE and those psychiatric conditions.

Methods: We examined paraoxonase (PON)1 status, namely Q192R PON1 genotypes and PON1 enzymatic activities, in 40 normal controls and 104 TLE patients, 27 without comorbidities, and 77 with comorbidities including mood disorders (n=25), anxiety disorders (n=27) and psychosis (n=25).

Outcomes: CMPAase and arylesterase activities were significantly lower in TLE and mesial temporal sclerosis (MTS) with and without psychiatric comorbidities than in normal controls. The areas under the ROC curve of CMPAase were 0.893 (0.037) for TLE and 0.895 (\pm 0.037) for MTS. Partial Least Squares (PLS) path analysis showed that there were specific indirect effects of PON1 genotype on TLE severity ($p < 0.0001$) and psychopathology ($p < 0.0001$), which were both mediated by lowered CMPAase activity, while arylesterase activity was not significant. The severity of TLE was significantly associated with psychopathology scores. Furthermore, PON1 CMPAase activity was inversely associated with Mini Mental State Examination scores.

Interpretation: The severity of TLE and comorbidities are to a large extent explained by lowered PON1 enzyme activities and by effects of the Q192R genotype which are mediated by lowered CMPAase activity. Total PON1 status plays a key role in the pathophysiology of TLE, MTS and psychiatric comorbidities by increasing the risk of oxidative toxicity. PON1 enzyme activities are new drug targets in TLE to treat seizure frequency and psychiatric comorbidities.

Key words: temporal lobe epilepsy, antioxidants, oxidative stress, neuroimmune, major depression, schizophrenia

Introduction

Patients with epilepsy suffer recurrent seizures originating from excessive and synchronous firing of groups of neurons in the brain (1,2). Temporal lobe epilepsy (TLE) is the most common focal epilepsy subtype in adults, with a 40% incidence in relation to all types of epilepsy (3). Hippocampal sclerosis or mesial temporal sclerosis (MTS), which is associated with neuronal loss and gliosis, is the most common primary pathology, accounting for 36% of all focal pathologies of epilepsy (4,5).

Neuropsychiatric disorders such as mood, anxiety and psychotic disorders are observed in about 30-70% of TLE patients and these comorbidities have a significant impact on the patient's quality of life (6–8). In TLE, comorbid depression has the highest prevalence (42.9%) followed by anxiety disorders (18.4%), especially generalized anxiety disorder (GAD), while psychosis shows a lower prevalence (around 5-7%) (9,10).

In epilepsy, the first seizure may induce reactive oxygen and nitrogen species (ROS/RNS) and when these reactive species are produced in large quantities and exceed the antioxidant defense mechanisms, they may cause oxidative damage to lipids, proteins, DNA and mitochondria, excitotoxicity and neuroinflammation (11,12). Oxidative neurotoxicity is particularly important in the central nervous system, since the brain is sensitive to oxidative stress due to its high energy and aerobic metabolic demand (13,14). Mitochondrial dysfunctions arising from ROS/RNS and the consequent oxidative lesions are frequently observed after seizures and during epileptogenesis and, additionally, are associated with neurodegeneration (13). During seizures, performant antioxidant defenses are extremely important to protect brain tissues against oxidative damage ensuing from lipid peroxidation and aldehyde formation (15). Experimental studies suggest such oxidative pathways play an important role in the pathophysiology of TLE and TLE progression (16,17). In addition, TLE

is associated with decreases in antioxidant defenses as indicated by lowered superoxide dismutase (SOD) (18) and glutathione (GSH) levels in the hippocampus (19).

The enzyme paraoxonase (PON)1 is of particular importance because it is bound to high density lipoprotein (HDL), and has the ability to catalyze the hydrolysis of organic phosphates and lipid peroxides, protecting lipids, HDL, and low density lipoprotein (LDL) from oxidation (20). The PON1 Q192R single nucleotide polymorphism (SNPs) determines in part the catalytic activity and antioxidant properties of PON1 enzymes (21). The alloenzyme R has a higher efficiency in detoxifying substrates such as paraoxon and 4-(chloromethyl) phenyl acetate (CMPA) and homozygous RR carriers metabolize lipids more efficiently than alloenzyme Q carriers, explaining that they have a stronger protection against lipid peroxidation (22). Nevertheless, there are only few studies that have examined total PON1 status (that is enzymatic activities and PON1 genotypes) in epilepsy. Dönmezdil et al. (23) and Calik et al. (24) found significantly lowered serum PON1 and arylesterase activities in patients with epilepsy, although these authors did not measure total PON1 status, which should include total enzyme activities and PON1 genotypes (25). Moreover, no studies examined the associations between PON1 status and psychiatric comorbidities in TLE, although PON1 status is significantly associated with major depression, anxiety disorders and subtypes of psychosis (25).

Hence, the objective of this study was to evaluate PON1 status, namely CMPAase and arylesterase activities as well as PON1 Q192R genotypes, in patients with TLE and MTS with and without comorbid psychosis, depression and anxiety.

Subjects and methods

Participants

For this case-control study, 104 patients with TLE and 40 normal controls were recruited. Patients with TLE were admitted to the outpatient clinic of the Comprehensive Epilepsy Unit of King Chulalongkorn Memorial Hospital, Bangkok, Thailand, from December 2013 to December 2014. The 104 patients were diagnosed with TLE by a senior neurologist specializing in epilepsy. The latter diagnosis was based on the history of clinical characteristics of seizures, EEG records and MRI scans performed in all patients. The 104 patients with TLE were subdivided into 4 subgroups based on the presence of psychiatric comorbidities according to the criteria established in the DSM-IV-TR, namely: a) Mood disorders due to TLE with depressive characteristics (n = 25); b) Anxiety Disorder Due to TLE with panic attacks, GAD or obsessive-compulsive symptoms (n = 27); c) Psychotic disorder due to TLE with delusions or hallucinations (n = 25); and d) “Pure TLE” when these or other psychiatric comorbidities were diagnosed as absent (n = 27).

Exclusion criteria for healthy controls were a diagnosis of epilepsy, febrile seizures in childhood and any other axis 1 psychiatric disorder, and a positive family history of epilepsy, mood disorders or psychotic disorders. Exclusion criteria for TLE patients were a) any other axis 1 disorder, except mood, anxiety and psychotic disorders due to TLE, and b) interictive dysphoric disorder (IDD) according to the criteria established by Blumer (2004). Exclusion criteria for patients with mood disorders due to TLE were anxiety and psychotic disorders. In the patient group with anxiety disorders due to TLE, we excluded patients with mood disorders or psychosis and in the patient group with psychotic disorder due to TLE we excluded patients with mood and anxiety disorders. In addition, patients with “pure TLE” did not suffer from any of the above-mentioned psychiatric comorbidities. Exclusion criteria for patients and controls were a) (auto)immune diseases including diabetes, psoriasis, chronic obstructive pulmonary disease, inflammatory bowel disease, b) neurodegenerative and neuroinflammatory disorders, such as multiple sclerosis, Parkinson's and Alzheimer's disease, c) immune, inflammatory or

allergic response three months before the start of the study, d) a lifetime history of treatment with immunomodulatory drugs, e) use of therapeutic doses of antioxidants or supplements containing ω 3-polyunsaturated fatty acids three months before inclusion in the study, and f) pregnant or lactating women.

All participants in this study gave written informed consent form before participating in the study. This study was approved by the Institutional Review Board of the Faculty of Medicine, King Chulalongkorn Memorial Hospital, Bangkok, Thailand (IRB number 305/56), which is in accordance with the International Guideline for the Protection of Human Research, as established by the Declaration of Helsinki, The Belmont Report, International Conference on Harmonization of Good Clinical Practice (ICH-GCP) and CIOMS Guideline.

Measurements

Semi-structured interviews were conducted by a senior neurologist and a senior psychiatrist specialized in epilepsy. The neurologist collected sociodemographic data and TLE characteristics including family history of epilepsy, age at onset of TLE, type of epilepsy, location of the lesion, seizure frequency, seizure control (seizure free, fairly and poorly controlled seizures); history of post-ictal confusion, type of seizures and use of antiepileptic drugs (AEDs). The diagnosis of TLE was made based on the history of partial seizures and EEG records of epileptiform activities in one or both temporal regions. In addition, the senior neurologist and a radiologist used results of magnetic resonance imaging (MRI) scans to make the diagnosis of MTS or other types of TLE. Patients and controls were evaluated by the senior psychiatrist to identify psychotic symptoms, anxiety and depression, using DSM-IV-TR criteria. The diagnosis of mood disorders due to TLE comprised major depression in an acute episode or in partial remission and ictus-related depression. The diagnosis of anxiety disorder due to TLE comprised patients with panic, generalized anxiety disorder, obsessive-compulsive

symptoms, ictus-related anxiety such as fear and horror. Psychotic disorders due to TLE comprised delusions (persecutors, possessed, paranoid and reference ideas), hallucinations (auditory, taste, visual and olfactory) and ictus-related psychoses, as described by (26). These psychoses can be ictal, pre-ictal, post-ictal, psychotic aura, peri-ictal, interictal or schizophrenic-like psychosis. In this context, fear, déjà vu, déjà vecu, horror, forced thoughts, out-of-body experiences and going crazy were not considered to be psychotic.

The senior psychiatrist (BK) also assessed the Brief Psychiatric Rating Scale (BPRS), the Hamilton Depression (HAM-D) and Anxiety (HAM-A) Rating Scale (27–29) and she also assessed the Mini Mental State Examination (MMSE) (30) in patients and controls. The body mass index was calculated using the ratio between body weight in (Kg) and height (meter²) and tobacco use disorder (TUD) was evaluated using the DSM-IV-TR criteria.

PON1 Assays

Blood samples were collected at 8:00 am, after an overnight fasting, and serum was aliquoted and stored at -80 °C until thawed for PON1 status. Total PON1 activity was determined by the formation of phenyl acetate hydrolysis (22). The rate of phenylacetate hydrolysis was determined on a Perkin Elmer® EnSpire model microplate reader (Waltham, MA, USA) at a wavelength of 270 nm measured over 4 minutes (16 readings at 15 seconds between readings) with the temperature maintained at 25°. Activity was expressed in U/mL based on the phenyl acetate molar extinction coefficient which is 1.31 mMol/Lcm-1. For the stratification of the functional genotypes of the PON1Q192R polymorphism (PON1 192Q/Q, PON1 192Q/R, PON1 192R/R), we used CMPA (CMPA, Sigma, USA) and phenyl acetate (PA, Sigma, USA). PON1 polymorphism confers differences in hydrolysis capacity and this allows to stratify the genotypes after phenotypic analysis of enzyme activity. Isoform R has high hydrolysis activity on CMPA whilst alloenzyme Q has lower hydrolytic activity on CMPA

and both alloforms hydrolyze PA with similar efficacy. Therefore, the reaction with PA is performed with high salt concentrations, which partially inhibits the activity of R allozyme thereby providing a better distinction between the three functional genotypes. The rate of PA hydrolysis in low salt concentration by arylesterase was also measured.

Statistics

We used analysis of variance (ANOVA) to assess differences in scale variables between diagnostic groups and analysis of contingency tables (χ^2 -tests) to assess associations among categorical variables. We used multivariate general linear model (GLM) analysis to ascertain the associations between diagnosis and biomarkers while controlling for possible background variables including sex, age, BMI, smoking and the drug state. Consequently, tests for between-subject effects were employed to examine the associations between diagnosis and each of the biomarkers. Model-generated estimated marginal mean values were computed and protected pair-wise comparisons among treatment means were employed to delineate the differences among the study groups. We used p-corrections for false discovery rate (FDR) to control for multiple statistical tests (31). Automatic binary regression analysis was employed to delineate the best biomarker prediction of TLE (controls as reference group). We employed automatic stepwise (step-up) multiple regression analysis to assess the most significant biomarkers predicting the BPRS, HAM-D, HAM-A and MMSE scores. Regression analysis were double-checked for collinearity and bootstrapped using 5000 samples and the bootstrapped results are shown in case of discrepant results. All tests were two-tailed and a p-value of 0.05 was used for statistical significance. IBM SPSS25 for windows was used to analyze the data.

To examine the causal associations between PON1 genotype and PON1 enzyme activities and TLE characteristics and psychopathology, we have performed Partial Least Squares (PLS) path analysis employing the SmartPLS program (32). SmartPLS is a structural

equation modeling technique which allows to examine causal pathways explaining the effects of input variables (PON1 genotype) on output variables (PON1 activities, and clinical aspects of TLS and comorbidities) whereby variables are entered as single indicator variables (PON1 genotype and enzyme activities) or as latent vectors (LV) extracted from TLE features (TLE; aura; postictal confusion; TLE frequency; seizure free, fairly and poorly controlled seizures; and the three psychopathological rating scale scores (BPRS, HAM-A, HAM-D) (32). We conducted PLS path analysis when the model complied with quality criteria, i.e. model SRMR < 0.080 and when the LVs show adequate reliability validity as indicated by composite reliability > 0.7 , $\rho_A > 0.8$, Cronbach's alpha > 0.7 , and average variance extracted (AVE) > 0.5 ; while the outer model factor loadings are > 0.6 with $p < 0.001$ (32). Consequently, we conducted complete and consistent PLS path analysis using 5.000 bootstrap samples to compute path coefficients (with p-values) and the significance of total, total indirect, and specific indirect effects.

Results.

Demographic and clinical data

Table 1 shows the socio-demographic data of the participants in this study. There were no significant differences in age, BMI, marital status, and TUD between the study groups. There was a trend towards more females in TLE patients with depression and anxiety. Subjects with TLE were somewhat less educated than the healthy controls. Therefore, we have statistically controlled for education in regressions with psychopathology ratings and MMSE as dependent variables. There were no significant differences in seizure frequency, age of onset of TLE, a history of aura, postictal confusion and control of seizures (free of seizures, fair and poor control) between the 4 TLE subgroups. Patients with psychotic disorder due to TLE showed a higher incidence of status epilepticus as compared with those with “pure TLE”. Table

1 also shows the rating scale scores and MMSE scores in the 5 subgroups. The BPRS and HAM-A scores were significantly different between the 5 subgroups with the lowest levels in controls and highest values in patients with TLE+psychosis and TLE+anxiety, respectively. The HAM-D score was significantly higher in patients with TLE+depression than in all other study groups, while the MMSE was significantly lower in TLE patients than in controls with the lowest scores being established in TLE+psychosis.

Associations between TLE with and without comorbidities and PON1 genotypes

The total study group (patients and controls) was at Hardy-Weinberg equilibrium ($\chi^2=1.086$, $df=1$, $p=0.297$), while also the control ($\chi^2=1.2013$, $df=1$, $p=0.273$) and the TLE ($\chi^2=0.530$, $df=1$, $p=0.467$) subgroups were at Hardy-Weinberg equilibrium. There was no significant association between PON1 Q192R genotypes and TLE subgroups ($\psi=0.137$, $p=0.251$), namely in controls: 1 / 17 / 22 versus 10 / 51 / 47 in TLE for the QQ, QR and RR genotypes, respectively. Also, there were no significant associations between TLE and different genetic models of the PON1 gene, including allelic, dominant, recessive and overdominant models.

Associations between PON1 activity and diagnosis

We examined the associations between the activities and diagnosis using multivariate GLM analysis while adjusting for sex, age and BMI. We examined 4 PON1 activity indices namely PON1 CMPAase and AREase enzyme activities as measured in this study and their residualized values after covarying for PON1 genotypes. The latter explained 70.0% of the variance in PON1 CMPAase and arylesterase activities ($F=173.88$, $df=2/145$, $p<0.001$) with the lowest CMPAase activities and the highest arylesterase activities in QQ carriers.

Multivariate GLM analysis showed that there was a significant association between PON1 activity and diagnosis (**Table 2**). Tests for between-subjects effects showed significant associations between diagnosis and either CMPAase and the residualized CMPAase activities with an explained variance of around 43.7% - 45.0 %. The associations with arylesterase and the residualized arylesterase activity shared around 24.8% - 33.0% of the variance. **Table 3** shows the model-generated estimated marginal mean values indicating that all PON1 activities were significantly lowered in TLE patients than in controls. These differences were highly significant with a distance of around 1.586 standard deviations between controls and patients with TLE+psychosis in residualized PON1 CMPAase activity. **Figure 1** shows the box-plot of CMPAase activity values in controls, pure TLE and TLE with psychiatric comorbidities.

Binary logistic regression analysis with TLE as dependent variable (controls as reference group) showed that the residualized PON1 CMPAase activity was the most significant biomarker discriminating TLE from controls with a sensitivity of 70.4%, specificity of 90.0% and accuracy of 75.7% ($\chi^2=69.74$, $df=1$, $p<0.001$, Nagelkerke=0.546). The Odds ratio was 0.111 (95% confidence interval: 0.053-0.230; Wald=29.41, $p<0.001$; B=1.515, SE=0.279).

Table 4 shows that CMPAase and arylesterase activities were significantly lower in MTS (with psychiatric comorbidities), “pure” TLE and “pure” MTS (both without psychiatric comorbidities) than in controls. The strongest impact was established for CMPAase activity in MTS. The area under the ROC curve using lowered CMPAase activity as discriminatory variable was 0.893 (0.037) for TLE and 0.895 (± 0.037) for MTS.

Effects of possible confounding variables.

As shown in Table 2 there were no significant effects of possible confounders including sex, age and BMI. There were also no significant effects of smoking ($F=0.48$, $df=4/135$,

$p=0.748$) and the Fagerstrom score ($F=0.16$, $df=4/135$, $p=0.960$). We have also examined the possible effects of treatments with valproate ($n=34$), carbamazepine ($n=61$), phenytoin ($n=38$), levetiracetam ($n=38$), lamotrigine ($n=27$), phenobarbital ($n=26$), clonazepam ($n=10$), clobazam ($n=58$), topiramate ($n=12$), gabapentin ($n=8$), antipsychotics ($n=9$), antidepressants ($n=16$), anxiolytics ($n=10$), CaCo_3 ($n=13$) and folic acid ($n=27$). However, multivariate GLM analyses followed by tests for between-subject effects showed no significant effects of these drugs even without p -correction for multiple testing. There was no significant association (Spearman rank order correlation) between the number of antiepileptic drugs (AEDs) and either CMPAase ($r=-0.086$, $p=0.398$, $n=102$) or arylesterase ($r=0.052$, $p=0.605$, $n=102$) activity.

Prediction of rating scale scores using biomarkers.

In order to examine the effects of biomarkers on the rating scale scores we performed automatic multiple regression analysis with the rating scales as dependent variables and the 4 PON1 measurements (residualized and non-residualized CMPAase and AREase activities), the PON1 genetic models, age, sex as well as education (**Table 5**). Firstly, we examined associations with the BPRS and two symptoms profiles namely psychosis that is sum of BPRS items 4 (conceptual disorganization), 11 (suspiciousness), 12 (hallucinations) and 15 (unusual thought disorders), and negative symptoms namely the sum of BPRS symptoms 3 (emotional withdrawal) and 16 (blunted affect). We found that 29.1% of the variance in the BPRS total score and 11.8% of the variance in psychosis was predicted by PON1 CMPAase activity and education (both inversely). **Figure 2** shows the inverse association between total BPRS score and CMPAase activity (partial regression based on the first regression in table 5). The best predictors of negative symptoms were the residualized CMPAase activity, age and education (all inversely correlated) and male sex. We found that 25.4% of the variance in the HAM-D score was predicted by PON1 CMPAase activity, education (both inversely), female sex and

being a QQ or RR carrier. **Figure 3** shows the partial regression of the total HAM-D score on CMPAase activity. A large of the variance in suicidal ideation (item 3 of the HAM-D) was explained by QQ genotype and residualized CMPAase activity (inversely associated) combined. We also computed the associations between physiosomatic symptoms, namely the sum of the HAM-A items 11 (anxiety somatic), 12 (somatic symptoms GIS), 13 (somatic symptoms general), 14 (genital symptoms) and 15 (hypochondriasis) and found that 12.2% of its variance was explained by PON1 CMPAase activity (inversely associated). Consequently, we have computed an index of psychomotor retardation (PMR) as z values of item 8 of the HAM-D and item 13 of the BPRS (both psychomotor retardation) and found that 29.5% of the variance in PMR was explained by residualized CMPAase activity, age and education (all inversely associated) and male sex. CMPAase activity combined with female sex predicted 15.3% of the variance in the total HAM-A score. We have computed an overall psychopathology index as the sum of the z values of the BPRS, HAM-D and HAM-A. This index was best predicted by PON1 CMPAase activity, education (both inversely), female sex and PON1 genotype. We found that 43.4% of the variance in the MMSE score was predicted by education and CMPAase activity (both positively associated).

Results of path analysis

Figure 4 shows the results of a consistent and complete PLS path analysis with the PON1 genotype (additive model) as input variable and a LV extracted from the three rating scale scores (BPRS, HAM-D, HAM-A) as final outcome in a multi-step mediation model with PON1 activities (CMPAase and arylesterase) and a LV extracted from TLE features (frequency, aura, controlled epilepsy, postictal confusion) as mediators. There were no significant effects of arylesterase (after considering the effects of CMPAase) and therefore only the latter are shown in this figure. The fit of the model was adequate with SRMR=0.053 while

the construct reliability validity of both LVs was adequate with composite reliability values of 0.890 and 0.855; Cronbach α values of 0.847 and 0.805, rho_A values of 0.898 and 0.810, and AVE values of 0.620 and 0.719 for TLE and psychopathology LVs, respectively. All outer loadings of the indicators of both LVs were > 0.694 (all at $p < 0.0001$). We found that 46.0% of the variance in the psychopathology index was explained by the TLE LV (positively associated) and PON1 CMPAase activity (inversely associated) while 25.3% of the variance in the TLE LV was explained by CMPAase activity. Finally, the PON1 genotype additive model explained 9% of the variance in CMPAase activity. There were specific indirect effects of PON1 genotype on a) the TLE LV, which were mediated by CMPAase activity ($t=4.07$, $p < 0.0001$), and b) the psychopathology LV mediated by CMPAase activity ($t=1.97$, $p=0.048$) and the path from PON1 genotype \rightarrow CMPAase activity \rightarrow TLE LV \rightarrow psychopathology LV ($t=3.74$, $p < 0.0001$). Likewise, the PON1 genotype had significant total (indirect) effects on TLE LV ($t=4.07$, $p < 0.0001$) and psychopathology LV ($t=3.87$, $p < 0.0001$). We have also examined the total effects of the QQ, QR and RR genotypes on the TLE and psychopathology LVs and found that QQ ($t=3.39$, $p=0.001$ and $t=3.20$, $p=0.001$) and RR ($t=-3.35$, $p=0.001$ and $t=-3.26$, $p=0.001$), but not QR had significant total effects on the TLE and psychopathology LVs, respectively.

Discussion

The first major finding of this study is that PON1 CMPAase and arylesterase activities were significantly decreased in patients with TLE, especially MTS, as compared with healthy controls. In our study, lowered levels of CMPAase yielded an area under the ROC curve of around 0.893 for TLE and MTS. These findings extend those of previous publications reporting significantly reduced levels of PON1 and arylesterase in patients with epilepsy when compared to healthy controls (24,33).

The second major finding of this study is that there are no significant differences in PON1 status between TLE without any comorbidities and depression, anxiety or psychosis due to TLE, although severity of depression, psychosis and anxiety were strongly associated with CMPAase activity. As such, CMPAase and to a lower degree also arylesterase activity are important in predicting the severity of psychopathology in TLE. We also observed that the severity of TLE predicts a general index of psychopathology.

Psychiatric comorbidities such as depression and anxiety are prevalent in patients with epilepsy and occur 2 to 3 times more frequently in this group of patients than in people who do not have the disease (34,35). Some authors found a strong association between low levels of PON1 and CMPAase activities and major depression (36,37) whilst reduced activity of CMPAase was additionally associated with lower quality of life, increased disability and staging of illness (36,38) suggesting that reduced total PON1 and CMPAase activities may play a role in the pathophysiology and progression of mood disorders (36). In patients with anxiety disorders, decreased levels of PON1 are accompanied by high levels of lipid hydroperoxides as compared with individuals without anxiety (39,40). CMPAase activity is also inversely associated with symptoms characteristic of (deficit) schizophrenia including psychosis, negative symptoms, and psychomotor retardation (37). The latter authors reported that CMPAase activity was significantly reduced in patients with schizophrenia and that this effect was to a large extent determined by increased frequency of the QQ genotype. Noto et al. (41) reported a significant decrease in PON1 activity in drug-naïve patients with first-episode psychosis.

Our results show that PON1 CMPAase activity is positively associated with the MMSE score, which is significantly lowered in TLE patients, suggesting that PON1 activity protects against cognitive decline in TLE. In this regard, epilepsy per se is accompanied by a neurocognitive decline (42). In patients with schizophrenia reduced PON1 activity is strongly

associated with neurocognitive deficits (37) while in mood disorders lowered PON1 status is associated with staging of the disorder, which is characterized by increased neurocognitive deficits (43).

The third major finding of this study is the significant association between the PON1 genotype and TLE features including seizure frequency, aura, postictal confusion, uncontrolled seizure type, and TLE-associated psychopathology including severity of psychosis, depression and anxiety. Thus, PLS path analysis revealed that the PON1 genotype especially the QQ, but also the QR, genotype increases risk and severity of TLE and TLE-associated psychopathology and that the RR genotype is protective. Our study indicates that genetically determined decreases in PON1 CMPAase activity as well as lowered PON1 enzyme activities, which occur independently from PON1 genotype, may be causally related to TLE and its psychiatric comorbidities. As such, alterations in CMPAase and PON1 activities, which are secondary to oxidative stressors (25,43) or environmental factors including nutritional factors and smoking may also be involved (25). Interestingly, tobacco use, which lowers PON1 activity, is associated with focal or generalized seizures (44,45) indicating that chemicals in tobacco smoke may have pro-convulsive effects (46).

PON1 is a detoxifying enzyme, which is associated with HDL (40) and has anti-inflammatory (25) and antioxidant properties including hydrolyzing lipid peroxides (47). PON1 activity may protect against lipid, LDL and HDL oxidation and increase HDL's ability to increase cholesterol efflux from macrophages (48). Moreover, PON1 protects against the pro-oxidative effects of macrophages, which produce free radicals and myeloperoxidase (MPO), thereby generating the highly toxic peroxynitrite and hypochlorous acid (40). Moreover, Borowczyk et al. (49) reported that PON1 may hydrolyze homocysteine thiolactone, a toxic metabolite that is capable of inducing epileptic seizures in rats and is implicated in neurodegenerative disorders. Importantly, in oxidative stress conditions, PON1 may be

damaged by increased MPO activity and elevated production of peroxynitrite and hypochlorous acid, leading to lowered antioxidant defenses and thus an increased vulnerability to oxidation of LDL and HDL, which in turn will lower the protective effects of HDL (43). Therefore, a well-functioning antioxidant system is important to prevent lipid peroxidation and conditions characterized by PON1 gene-associated decreases in PON1 activity (like TLE and MTS) are accompanied by increased risk to develop lipid oxidation.

There is now some evidence of increased oxidation of lipids (50,51) and lipid peroxidation occur during TLE progression (15). Moreover, experimental epilepsy/TLE models show increased hydroxyl radicals in the CNS (52), increased lipid peroxidation and lowered antioxidant defenses (15,16,53). For example, Mojarad and Roghani found increased lipid peroxidation and decreased superoxide dismutase (SOD) activity in a kainic acid induced TLE model. The latter model is also accompanied by a decrease in the reduced form of glutathione (GSH) in the hippocampus (19) and increased malondialdehyde (MDA), the end product of lipid peroxidation, in the piriform cortex (17). Moreover, elevated MDA is associated with a greater vulnerability of the piriform cortex to seizure-induced damage. Lipid peroxidation can alter the permeability of the mitochondrial membrane and enzymes present in the membrane possibly leading to neurodegenerative processes (12). Therefore, the results of the current study suggest that lowered PON1 CMAAase activity, which is in part genetically determined, participates in the pathophysiology of TLE and MTS and the onset of comorbid psychopathology through increased oxidative stress. This mechanistic explanation may, at least in part, underpin the strong comorbidity between TLE and psychiatric symptoms. Moreover, the decrease in serum PON1 activity may explain the cognitive impairments in TLE/MTS. Previously, it was reported that lowered PON1 CMAAase activity is associated with cognitive deficits in schizophrenia and dementia (37). For example, increased ROS levels may cause loss of inhibitory neurons in the hippocampus in patients with epilepsy and induce a

hyperexcitability state, which can initiate reactive gliosis and consequently mitochondrial dysfunction leading to neurodegeneration (12).

Limitations

Our results were adjusted for possible effects of sex, age, BMI, and smoking which may all affect PON1 enzymatic activity and oxidative biomarkers (25). All patients in the current study were medicated (AEDs or psychotropic drugs). Some, but not all, studies showed a possible effect of treatment with AEDs on lipid peroxidation (54,55). For example, treatment with levetiracetam is accompanied by a decrease in serum PON1 and arylesterase activity and a significant increase in oxidized LDL (56). Nevertheless, in our study, there were no significant effect of AEDs, antipsychotics or mood-stabilizing drugs on PON1 activity, and we found no associations between the number of AEDs patients were taking and PON1 enzymatic activity. Moreover, other studies reported that there are no significant differences in indicators of oxidative stress in treated versus untreated epilepsy patients (57,58). Arylesterase activity is not significantly different between chronically polymedicated psychiatric patients and controls suggesting that treatment with psychotropic medications does not induce changes in arylesterase activity (25).

Conclusions

The activities of CMPAse and arylesterase enzymes are significantly decreased in patients with TLE, especially MTS, as compared with healthy controls. The detrimental effects of the PON1 genotype on severity of TLE, depression, psychosis and anxiety are mediated by lowered CMPAase. The aberrations in PON1 status may play a key role in the oxidative pathophysiology of TLE, MTS and psychiatric comorbidities. PON1 enzymatic activity is a new drug target in TLE to treat seizure frequency and psychiatric comorbidities.

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Conflict of interest

The authors have no conflict of interest with any commercial or other association in connection with the submitted article.

Author's contributions

All the contributing authors have participated in the manuscript. BK and MM designed the study. BK and CL recruited patients and completed diagnostic interviews and rating scale measurements. MM carried out the statistical analyses. All authors contributed to interpretation of the data and writing of the manuscript. All authors approved the final version of the manuscript.

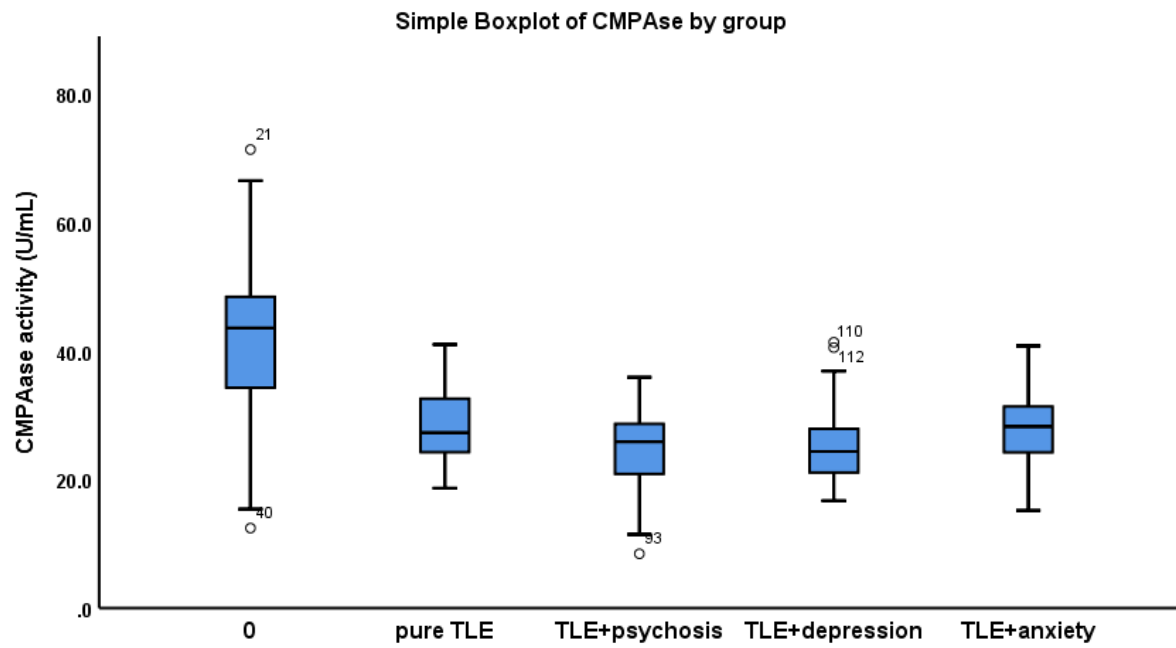


Figure 1. Box plot of CMPAase activity in controls (0), pure temporal lobe epilepsy (TLE: no comorbidities are present) and TLE with psychiatric comorbidities.

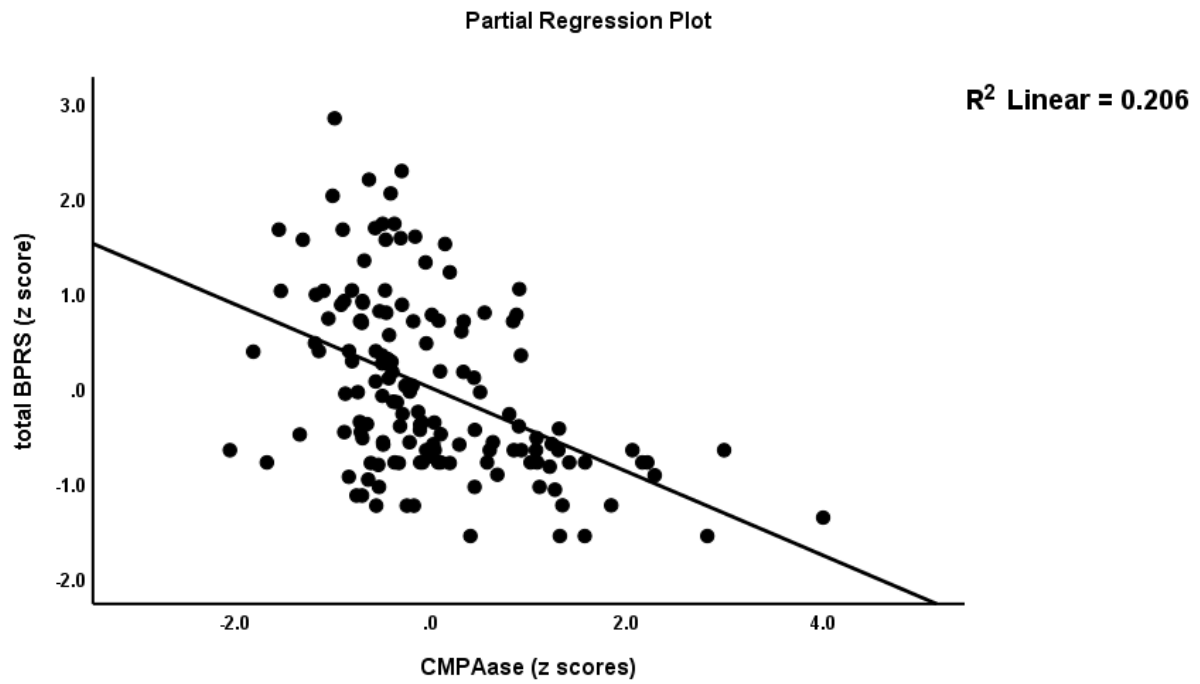


Figure 2. Inverse association between the Brief Psychiatric Rating Scale (BPRS) score and CMAAase activity in 104 patients with temporal lobe epilepsy with and without comorbidities and 40 healthy controls.

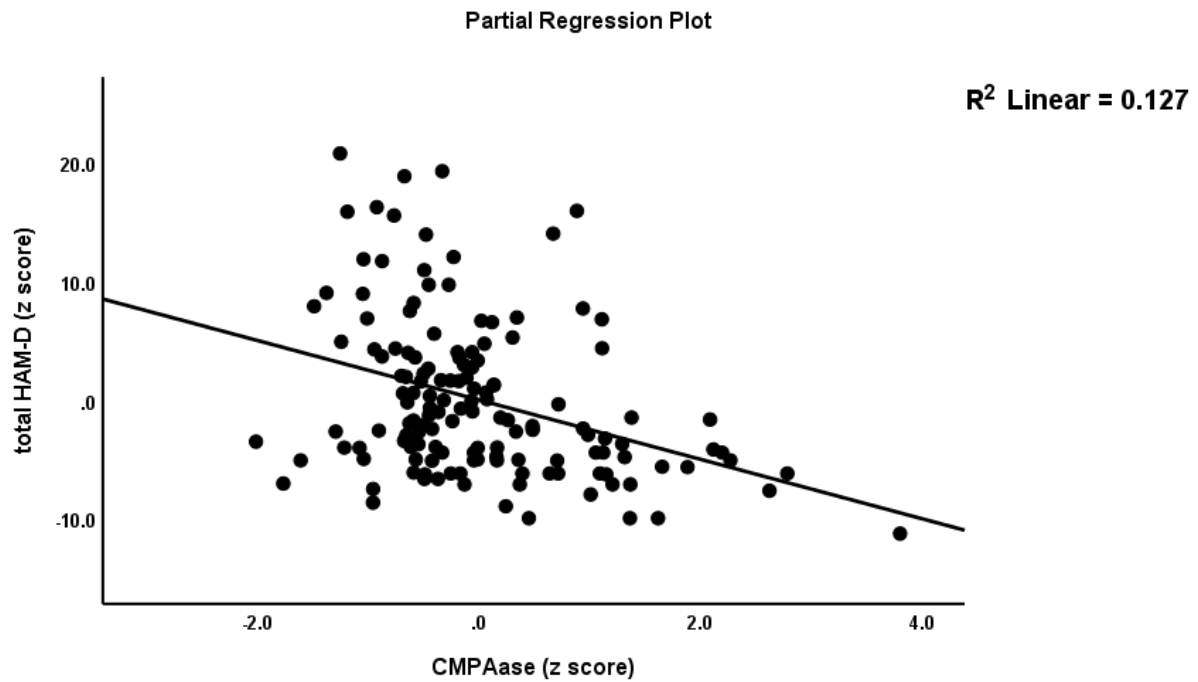


Figure 3. The partial regression of the total Hamilton Depression Rating Scale (HAM-D) score on CMPAase activity in 104 patients with temporal lobe epilepsy with and without comorbidities and 40 healthy controls.

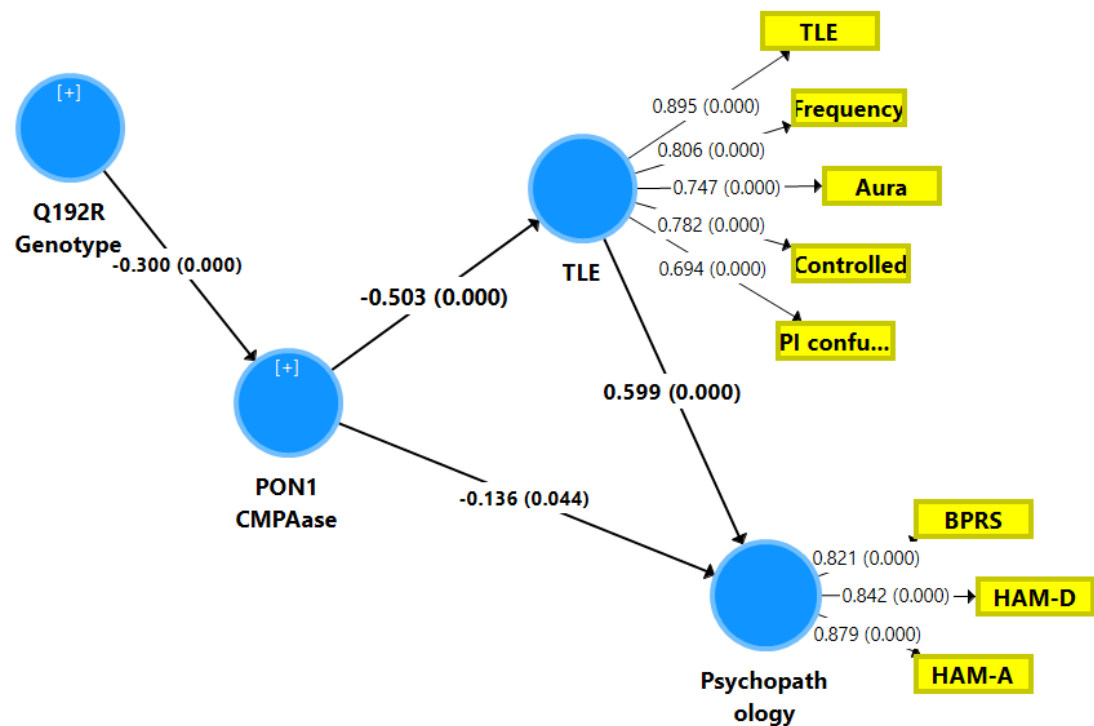


Figure 4. Results of Partial least Squares (PLS) path analysis with a latent vector (LV) extracted from three psychopathology dimensions as outcome variable and a LV extracted from temporal lobe epilepsy (TLE) features, paraoxonase (PON)1 activity and the Q19R PON1 genotype (additive model) as input variables. Shown are path coefficient with p-values (between brackets).

Frequency: seizure frequency; Aura: aura present or not; Controlled: seizure free and fairly and poorly controlled seizures; PI confu...: history of post-ictal confusion.

BPRS: Brief Psychiatric Rating Scale; HAM-D/HAM-A: Hamilton Depression and Anxiety Rating Scale scores

Table 1. Sociodemographic and clinical data of healthy controls (HC) and patients with temporal lobe epilepsy (TLE) and patients with psychosis (TLE+PSY), depression (TLE+DEP) and anxiety (TLE+ANX) due to TLE

| Variables | HC ^a | TLE ^b | TLE+PSY ^c | TLE+DEP ^d | TLE+ANX ^e | F/X ² | df | p |
|---------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|------------------|-------|--------|
| Age (years) | 37.4 (12.8) | 40.0 (12.8) | 37.9 (10.5) | 39.0 (10.7) | 37.0 (8.2) | 0.34 | 4/141 | 0.849 |
| Sex (♂/♀) | 10/30 | 11/16 | 13/14 | 4/21 | 5/22 | 10.31 | 4 | 0.036 |
| BMI (kg/m ²) | 24.0 (4.3) | 24.1 (4.0) | 23.5 (3.7) | 23.9 (4.3) | 22.4 (4.3) | 0.79 | 4/140 | 0.535 |
| Married (No/Yes) | 26/14 | 18/9 | 20/7 | 20/5 | 15/11 | 3.58 | 4 | 0.466 |
| Education (years) | 14.2 (4.9) ^{b,c,d,e} | 11.4 (4.7) ^a | 9.4 (4.4) ^a | 10.3(5.4) ^a | 10.8 (4.5) ^a | 5.14 | 4/141 | 0.001 |
| TUD (No/Yes) | 38/2 | 24/3 | 23/4 | 21/4 | 23/4 | Ψ=0.136 | - | 0.607 |
| Frequency seizures | - | 29.1 (84.7) | 19.1 (40.7) | 8.0 (17.0) | 9.7 (11.0) | 0.99 | 3/89 | 0.402 |
| Age onset TLE (years) | - | 17.8 (12.6) | 12.2 (10.1) | 17.6 (8.9) | 16.1 (8.8) | 1.75 | 3/100 | 0.162 |
| Hx Aura (No/Yes) | - | 6/21 | 5/22 | 7/18 | 8/19 | 1.15 | 3 | 0.766 |
| Hx Postictal confusion (No/Yes) | - | 8/19 | 10/16 | 9/16 | 11/15 | 0.97 | 3 | 0.808 |
| Hx Status epilepticus (No/Yes) | - | 24/3 ^c | 14/11 ^b | 21/4 | 13/9 | 10.75 | 3 | 0.013 |
| Seizure control | - | 7/8/8 | 7/8/8 | 5/4/0 | 5/5/10 | Ψ=0.309 | - | 0.307 |
| BPRS | 18.3 (1.1) ^{b,e} | 23.6 (3.3) ^{a,c,d,e} | 41.3 (5.9) ^{a,b,d,e} | 32.9 (6.7) ^{a,b,c,e} | 29.4 (5.0) ^{a,b,c,d} | 115.64 | 4/141 | <0.001 |
| HAM-D | 0.6 (2.0) ^{b,e} | 4.8 (2.5) ^{a,d,e} | 5.8 (2.9) ^{a,e} | 19.8 (4.9) ^{a,b,c,e} | 10.3 (3.8) ^{a,b,c,d} | 145.21 | 4/140 | <0.001 |
| HAM -A | 2.6 (5.4) ^{b,e} | 7.8 (3.9) ^{a,c,d,e} | 11.6 (6.7) ^{a,b,c,e} | 18.9 (8.8) ^{a,b,c,e} | 23.8 (5.4) ^{a,b,c,d} | 59.69 | 4/141 | <0.001 |
| MMSE | 28.3 (2.4) ^{b,e} | 25.1 (4.4) ^{a,c} | 22.4 (5.4) ^{a,b,d,e} | 25.7 (2.4) ^{a,c} | 25.8 (8.9) ^{a,c} | 11.06 | 4/140 | <0.001 |

All values are shown as mean (SD); BMI: body mass index; TUD: tobacco use disorder; Hx: a history of; control seizures: seizure free and fairly and poorly controlled seizures; BPRS: Brief Psychiatric Rating Scale; HAM-D: Hamilton Depression Rating Scale; HAM-A: Hamilton Anxiety Rating Scale; MMSE: Mini Mental State Examination; HC: healthy controls TLE: TLE without ant psychiatric comorbidities; TLE+PSY: Psychotic Disorder due to TLE; TLE+DEP: Mood Disorder due to TLE with depressive features; TLE+ANX: Anxiety Disorder due to TLE.

Table 2. Results of multivariate GLM analysis examining the differences between diagnostic groups (diagnosis), namely healthy controls, temporal lobe epilepsy with and without comorbidities including depression, psychosis, and anxiety.

| Tests | Dependent variables | Exploratory variables | F | df | p | Partial Eta Squared |
|-------------------------|------------------------|-----------------------|-------|--------|--------|---------------------|
| Multivariate | All 4 biomarkers: | Diagnosis | 6.49 | 16/410 | <0.001 | 0.158 |
| | CMPAase, Arylesterase, | Sex | 1.22 | 4/134 | 0.306 | 0.035 |
| | Res CMPAase, Res | Age | 0.61 | 4/134 | 0.654 | 0.018 |
| | Arylesterase | BMI | 1.07 | 4/134 | 0.375 | 0.031 |
| Between-subject effects | CMPAase | Diagnosis | 28.06 | 4/137 | <0.001 | 0.450 |
| | Res CMPAase | Diagnosis | 26.60 | 4/137 | <0.001 | 0.437 |
| | Arylesterase | Diagnosis | 11.31 | 4/137 | <0.001 | 0.248 |
| | Res Arylesterase | Diagnosis | 16.90 | 4/137 | <0.001 | 0.330 |

Diagnosis: five diagnostic groups, namely Psychotic Disorder due to temporal lobe epilepsy (TLE), Mood Disorder due to TLE with depressive features, Anxiety Disorder due to TLE, "Pure TLE" (without any comorbidities) and healthy controls

PON1: paraoxonase; CMPAase: 4-(chloromethyl)phenyl acetate hydrolysis; Res: residualized (after regression on PON1 genotype)

Table 3. Model-generated estimated marginal means of paraoxonase 1 levels in healthy controls (HC) and patients with temporal lobe epilepsy (TLE) and psychosis (TLE+PSY), depression (TLE+DEP) and anxiety (TLE+ANX) due to TLE.

| Variables | HC^a | TLE^b | TLE+PSY^c | TLE+DEP^d | TLE+ANX^e |
|-----------------------------|------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| CMPAase (U/mL) | 42.1 (1.3) ^{b-c} | 28.5 (1.6) ^a | 24.5 (1.6) ^a | 24.8 (1.7) ^a | 27.3 (1.7) ^a |
| Res CMPAase (z scores) | 1.041 (0.128) ^{b-e} | -0.196 (0.150) ^a | -0.545 (0.163) ^a | -0.545 (0.163) ^a | -0.375 (0.159) ^a |
| Arylesterase (U/mL) | 212.4 (9.0) ^{b-e} | 156.4 (10.5) ^a | 144.2 (10.4) ^a | 143.7 (11.4) ^a | 137.3 (11.2) ^a |
| Res Arylesterase (z scores) | 0.920 (0.140) ^{b-e} | -0.193 (0.163) ^a | -0.425 (0.162) ^a | -0.400 (0.177) ^a | -0.434 (0.174) ^a |

TLE: TLE without psychiatric comorbidities; TLE+PSY: Psychotic Disorder due to TLE; TLE+DEP: Mood Disorder due to TLE with depressive features; TLE+ANX: Anxiety Disorder due to TLE; HC: healthy controls.

CMPAase: 4-(chloromethyl)phenyl acetate hydrolysis; Res: residualized values after regression on PON1 genotype

Table 4. Measurement of paraoxonase (PON1) enzymatic activity in healthy controls (HCs) and subgroups of temporal lobe epilepsy (TLE) patients.

| PON1 activities | HC | Pure TLE | F | df | P | Partial eta squared |
|------------------------|--------------|-----------------|----------|-----------|----------|----------------------------|
| CMPAase (U/mL) | 42.8 (1.7) | 28.8 (1.9) | 30.61 | 1/62 | <0.001 | 0.331 |
| Arylesterase (U/mL) | 215.4 (10.8) | 156.4 (12.2) | 13.47 | 1/62 | 0.001 | 0.178 |
| | HC | MTS | F | df | P | Partial eta squared |
| CMPAase (U/mL) | 41.7 (1.5) | 25.6 (1.2) | 81.44 | 1/100 | <0.001 | 0.449 |
| Arylesterase (U/mL) | 209.8 (9.4) | 139.4 (7.5) | 36.98 | 1/100 | <0.001 | 0.270 |
| | HC | Pure MTS | F | df | P | Partial eta squared |
| CMPAase (U/mL) | 42.2 1.8 | 27.3 2.6 | 22.61 | 1/52 | <0.001 | 0.303 |
| Arylesterase (U/mL) | 213.0 10.8 | 139.5 | 14.65 | 1/52 | <0.001 | 0.220 |

Data are results of univariate GLM analysis and are shown as model-generated marginal estimated mean (SE) values after covarying for age, sex and body mass index.

Pure TLE: temporal lobe epilepsy without any psychiatric comorbidity

MTS: established mesial temporal lobe sclerosis (thus including psychiatric comorbidities)

Pure MTS: established mesial temporal lobe sclerosis without any psychiatric comorbidity

Table 5. Results of multiple regression analysis with rating scales as dependent variables and PON1 status as explanatory variables.

| Dependent variables | Explanatory variables | β | t | p | F model | Df | p | partial Eta Squared |
|-------------------------------|-----------------------|---------|-------|--------|---------|-------|--------|---------------------|
| BPRS | Model | | | | 29.35 | 2/143 | <0.001 | 0.291 |
| | CMPAase | -0.444 | -6.09 | <0.001 | | | | |
| | Education | -0.213 | -2.93 | 0.004 | | | | |
| Psychosis | Model | | | | 9.56 | 2/143 | <0.001 | 0.118 |
| | CMPAase | -0.260 | -3.20 | 0.002 | | | | |
| | Education | -0.167 | -2.06 | 0.041 | | | | |
| Negative symptoms | Model | | | | 12.75 | 4/141 | <0.001 | 0.226 |
| | Education | -0.329 | -4.52 | <0.001 | | | | |
| | Sex | -0.329 | -4.29 | <0.001 | | | | |
| | Res CMPAase | -0.189 | -2.56 | <0.011 | | | | |
| | Age | -0.172 | -2.30 | 0.023 | | | | |
| HAM-D | Model | | | | 11.95 | 4/140 | <0.001 | 0.254 |
| | CMPAase | -0.347 | -4.51 | <0.001 | | | | |
| | Education | -0.231 | -3.02 | 0.003 | | | | |
| | Overdominant model | -0.227 | -3.06 | 0.003 | | | | |
| | Sex | 0.170 | 2.31 | 0.023 | | | | |
| Suicidal ideation | Model | | | | 13.21 | 2/143 | <0.001 | 0.156 |
| | Dominant model | 0.354 | 4.61 | <0.001 | | | | |
| | Res CMPAase | -0.173 | -2.26 | 0.025 | | | | |
| Physiosomatic symptoms | Model | | | | 19.94 | 1/144 | <0.001 | 0.122 |
| | CMPAase | -0.349 | -4.47 | <0.001 | | | | |
| PMR | Model | | | | 14.75 | 4/141 | <0.001 | 0.295 |
| | Education | -0.386 | -5.13 | <0.001 | | | | |
| | Sex | -0.252 | -3.54 | 0.001 | | | | |
| | Res CMPAase | -0.243 | -3.36 | 0.001 | | | | |
| | Age | -0.186 | -2.53 | 0.013 | | | | |
| HAM-A | Model | | | | 12.93 | 2/143 | <0.001 | 0.153 |

| | | | | | | | | |
|------------------------------|--------------------|--------|-------|--------|-------|-------|--------|-------|
| | CMPAase | -0.350 | -4.80 | <0.001 | | | | |
| | Sex | 0.163 | 2.10 | 0.037 | | | | |
| Psychopathology index | Model | | | | 19.94 | 3/141 | <0.001 | 0.298 |
| | CMPAase | -0.430 | -5.83 | <0.001 | | | | |
| | Education | -0.250 | -3.41 | 0.002 | | | | |
| | Overdominant model | -0.148 | -2.07 | 0.040 | | | | |
| MMSE | Model | | | | 54.48 | 2/142 | <0.001 | 0.434 |
| | Education | 0.593 | 9.09 | <0.001 | | | | |
| | CMPAase | 0.175 | 2.69 | 0.008 | | | | |

BPRS: Brief Psychiatric Rating Scale; HAM-D: Hamilton Depression Rating Scale; HAM-A: Hamilton Anxiety Rating Scale; MMSE: Mini Mental State Examination; PMR: psychomotor retardation; Res CMPAase: residualized (after regression on PON1 genotype); CMPAase: 4-(chloromethyl)phenyl acetate hydrolysis

Dominant and overdominant model: PON1 Q192R genotype models

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