In schizophrenia, PON1 Q192R genotypes and/or lowered paraoxonase 1 (PON1) enzymatic activity are significantly associated with the deficit syndrome, negative symptoms, formal thought disorders, psychomotor retardation, excitation and increased IgA levels to Gram-negative microbiota.

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Abstract

BACKGROUND: Primary deficit schizophrenia (DS) is characterized by enduring negative symptoms and represents a qualitatively different disease entity with respect to non-deficit schizophrenia (NDS). No studies investigated the association between the enzyme paraoxonase 1 (PON1) and DS and its phenomenology.

METHODS: In this case-control study, Thai women and men, aged 18-65 years, were divided in DS (n=40) and NDS (n=40) and were compared to controls (n=40). PON1 activities against 4-(chloromethyl)phenyl acetate (CMPA) and phenylacetate were determined. Moreover, subjects were genotyped for their PON1 Q192R polymorphism and IgA levels responses directed to Gram-negative bacteria were measured.

RESULTS: DS is significantly associated with the QQ genotype and the Q allele as compared with NDS and controls. PON1 activities are significantly and inversely associated with negative symptoms, formal thought disorders, psychomotor retardation, excitation and DS. The presence of the Q allele is associated with increased IgA responses to *Pseudomonas aeruginosa, Morganella morganii,* and *Pseudomonas putida* as compared with RR carriers.

CONCLUSIONS: The PON1 Q allele and lower PON1 activities especially against CMPA are associated with DS, indicating lowered quorum quenching abilities as well as lowered defenses against lipoperoxidation and immune activation. It is suggested that lowered PON1 activity in DS constitutes an impairment in the innate immune system which together with lowered natural IgM may cause lower immune regulation thereby predisposing towards greater neurotoxic effects of immune-inflammatory, oxidative and nitrosative pathways and Gram-negative microbiota.

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Introduction

Schizophrenia (SCZ) is a chronic disabling psychiatric disorder characterized by abnormal perceptions, incoherent or illogical thoughts, disorganized speech and behavior (Wu et al., 2017). Cardinal symptoms of SCZ include positive and negative symptoms, cognitive dysfunction and deterioration in social and occupational functioning. Deficit schizophrenia (DS) is a distinct nosological entity characterized by the presence of negative symptoms, including affective flattening, alogia, anhedonia, avolition, and social inhibition (Kanchanatawan et al., 2018b; 2018c). Negative symptoms are currently conceptualized as behaviors and thought processes which the patients partially lost due to the illness. This contrasts with positive symptoms including delusions, hallucinations, disorganized thinking, and hostile behaviors, which are considered to be new behaviors or thought processes that were not present before the onset of the illness (Berrios and Luque 2003).

SCZ is accompanied by activation of the immune-inflammatory response system (IRS) and IRS biomarkers are significantly associated with negative and psychotic symptoms as well as cognitive impairments (Maes et a., 1994; 1997; Brinholi et al., 2015; Kanchanatawan et al., 2018a; Sirivichayakul et al. 2019a; 2019b; Maes et al., 2019b; 2019d). The current theory is that products of immune activation, including M1 macrophage, T helper (Th)-1 and Th-2 subsets exert neurotoxic effects on neuronal cell in the brain thereby inducing neuroprogression including neurocognitive deficits and symptoms of SCZ and DS (Noto et al., 2019; Roomruangwong et al., 2019). For example, the cytotoxic and neurotoxic properties of increased tryptophan catabolites (TRYCATs) such as picolinic acid, xanthurenic acid, and quinolinic acid are further augmented by increased levels of eotaxin (CLL11), a Th-2-related product, that may contribute to neurocognitive deficits, negative symptoms, and the overall severity of SCZ (OSOS) (Sirivichayakul et al., 2019a;

2019b; Maes et al., 2019; 2019b; 2019d). Moreover, patients with DS show a breakdown of the paracellular tight and adherens junctions and vascular barriers coupled with increased translocation of gut-commensal Gram-negative bacteria (Maes et al., 2019a; 2019b; 2019d). Furthermore, this breakdown of the gut tight and adherens junctions is accompanied by increased permeability of the blood-brain barrier allowing neurotoxic immune products including lipopolysaccharide (LPS) to access the brain (Maes et al., 2019a; 2019b; 2019d). LPS is neurotoxic and may lead to neurodegenerative processes through microglial activation thereby explaining that LPS neurotoxicity may contribute to the pathophysiology of DS (Maes et al., 2019a). Furthermore, DS is characterized by a deficit in natural IgM antibodies to oxidative-specific epitopes (OSEs), which indicates an impairment in the innate immune system that serves as a first-line defense against bacterial infections (Maes et al., 2019b; 2019c).

Increased production of reactive oxygen (ROS) and nitrogen (RNS) species coupled with lowered antioxidant defenses may induce damage by oxidative & nitrosative stress (O&NS), which, in turn, may cause degeneration of proteins, lipids, nucleic acids and membrane phospholipids thereby damaging membranes, mitochondria as well as DNA (Anderson and Maes, 2013; Anderson et al., 2013; Davis et al., 2014; 2016). Moreover, activated O&NS pathways may affect signal transduction, structural plasticity and cellular resilience and, therefore, may be associated with neuroprogressive disorders including SCZ (Anderson et al., 2013; Brinholi et al., 2015). High density lipoprotein (HDL) is an important antioxidant and its protective role is attributed mainly to the enzyme paraoxonase 1 (PON1) that has antioxidant and anti-inflammatory activities (Moreira et al., 2018; 2019). Genetic and epigenetic factors may cause significant differences among individuals in terms of PON1 levels and enzymatic activity. The most studied PON1 polymorphism is Q192R, which influences the catalytic activity of PON1 thereby

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modulating PON1 antioxidant properties including lipid oxidation (Bayrak et al., 2016; Atagün et al., 2017). The R allozyme is more efficient to detoxify substrates such as paraoxon, 4-(chloromethyl)phenyl acetate (CMPA) and 5-thiobutil butyrolactone (Richter et al., 2008; Marsillach et al., 2009). R allozyme homozygotes (RR carriers) metabolize lipids more efficiently than QQ carriers and additionally have a stronger defense against lipid peroxidation (Bayrak et al., 2016; Atagün et al., 2017). Moreover, PON1 hydrolyzes N-(3-oxo-dodecanoyl)-homoserine lactone, a quorum-sensing molecule, which regulates virulence and biofilm formation in many bacteria, indicating that PON1 activities have antimicrobial properties (Bar-Rogovsky et al., 2013). However, to date, there are no studies investigating PON1 status (i.e., activity and Q192R polymorphism) in DS as compared with non-deficit schizophrenia (NDS) and the associations between PON1 status and bacterial translocation in SCZ.

Hence, the current study was executed to examine: a) PON1 activity and Q192R polymorphism in DS versus non-DS (NDS) and controls; and b) PON1 status in association with specific symptom domains of SCZ including OSOS and it target subdomains; and c) associations between PON1 status and bacterial translocation.

Methods

Participants and Methods

Participants

In this study 80 outpatients with SCZ were recruited by the Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand and 40 healthy controls were recruited by word of mouth from the same catchment area as the patients. All participants were Thai nationals, aged 18-65 years, both women and men. All patients were in a stable phase of

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illness and complied with the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR) diagnostic criteria for SCZ. 40 patients reached criteria for DS, made using the SDS criteria (Kirkpatrick, 1989) while patients not fulfilling the deficit criteria were classified as patients with NDS (n=40). The exclusion criteria for controls were a family history of psychotic disorders or a lifetime diagnosis of axis I DSM-IV-TR disorders. Exclusion criteria for patients were: axis I disorders other than SCZ (including bipolar disorder, major depressive episode, schizoaffective disorder, autism spectrum disorders, and substance use disorders). Exclusion criteria for all subjects were any neuroinflammatory disorder (including Parkinson's disease, stroke, multiple sclerosis) and medical illness (including psoriasis, diabetes, chronic obstructive pulmonary disease and rheumatoid arthritis); use of immunomodulatory drugs, antioxidant or ω3-polyunsaturated fatty acid supplements. All participants, as well as the guardians of patients (parents or other close family members) provided written informed consent to take part in the study. Approval for the study was obtained from the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (No 298/57), which is in compliance with the International Guideline for Human Research protection as required by the Declaration of Helsinki, The Belmont Report, CIOMS Guideline and International Conference on Harmonization on Good Clinical Practice (ICH-GCP).

Methods

Clinical assessments

Socio-demographic and clinical data were collected from all participants. We used a semi-structured interview to collect data on socio-demographics, family history of psychosis, duration of illness, psychiatric and medical history. SCZ was diagnosed using the Mini-International

 doi:10.20944/preprints201909.0095.v1

Neuropsychiatric Interview (M.I.N.I.) in a validated Thai translation (Kittirathanapaiboon and Khamwongpin, 2005), while the diagnosis of DS was made using the SDS criteria (Kirkpatrick, 1989) and, consequently, SCZ outpatients were divided into two groups, DS and NDS. The Scale for the Assessment of Negative Symptoms (SANS) was used to measure negative symptoms in all patients (Andreasen, 1989). In addition, we assessed the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). As explained previously, based on selected items of those scales and other ratings scales including the Brief Psychiatric Rating Scale (Overall and Gorham, 1962) and the Hamilton Depression Rating Scale (Hamilton, 1960) we computed z unit weighted composite scores refecting psychosis, hostility, mannerism, formal thought disoders (FTD), and psychomotor retardation (PMR) (Sirivichayakul et al., 2019a; 2019b; Maes et al., 2019c; 2019d). The diagnosis of tobacco use disorder (TUD) was made using DSM-IV-TR criteria. We also measured body mass index (BMI) as body weight (kg) / length (m^2).

PON1 assay

A blood sample of 10 mL was withdrawn from all individuals. Blood was immediately centrifuged, and the serum was aliquoted and stored at −80 °C until thawed for PON1 assays. To stratify individuals in the functional genotypes of the PON1 Q192R polymorphism (QQ, QR, and RR), the substrates used were phenyl acetate (PA, Sigma, USA) under high salt condition and 4-(chloromethyl)phenyl acetate (CMPA, Sigma, USA), which is an alternative to the use of the toxic paraoxon. PON1 activities were determined by the rate of hydrolysis of CMPA (CMPAase, which is influenced by the PON1 Q192R polymorphism) as well as by the rate hydrolysis of phenyl acetate under low salt condition (AREase, which is less influenced by the PON1 Q192R polymorphism). Analysis were conducted in a microplate reader (EnSpire, Perkin Elmer, USA)

 doi:10.20944/preprints201909.0095.v1

(Richter et al., 2008). We previously described the ELISA assay of IgA responses to Gram-negative bacteria using ELISA including *H. alvei, P. aeruginosa, M. morganii, P. putida,* and *K. pneumoniae* (Geffard et al., 2002; Maes et al., 2008). The inter-assay coefficients of variation (CV) were < 10%.

Statistics

Analysis of variance (ANOVA) was employed to check differences in continuous variables between diagnostic groups. We used analysis of contingency tables (χ^2-tests) to check associations among nominal variables. P-corrections for false discovery rate (FDR) were employed to adjust for multiple comparisons (Benjamini and Hochberg, 1995). We used multiple regression analysis to check the most significant explanatory variables (including the biomarkers, age, sex, BMI, education and nicotine dependence) predicting symptom domains. All regression analysis were checked for multicollinearity. We employed multivariate general linear model (GLM) analysis to check the effects of diagnosis on biomarkers while controlling for sex, age, education, BMI and nicotine dependence. We used tests for between-subject effects to examine the effects of significant independent variables on the biomarkers. Subsequently, model-generated estimated marginal mean values were computed after z transformation of the biomarkers. Protected pair-wise comparisons among treatment means were used to examine the differences in biomarkers between three study groups. We bootstrapped (5000 samples) all results and show the bootstrapped results in case they differ from the non-bootstrapped results. Principal component analysis (PCA) followed by varimax rotation was performed on the biomarkers (which show a high degree of collinearity) in order to obtain 2 orthogonal PCs, which explain most of the variance and

 doi:10.20944/preprints201909.0095.v1

subsequently we use the rotated PC scores in statistical analysis. All tests were two-tailed and a p-value of 0.05 was used for statistical significance.

Partial Least Squares (PLS) path modeling analysis (SmartPLS; Ringle et al., 2015) was used to examine causal paths from the biomarkers to symptom dimensions. SmartPLS uses structural equation modeling and models pathways whereby single indicators or a set of indicator variables (reflected by latent vectors (LV)) may be entered in the analysis. In the current study, a LV extracted from different symptom domains (reflecting the latent construct OSOS) was entered as output variable (response variable) and the biomarkers, sex and education were entered as input variables (single indicators). Consistent and complete PLS analysis (5000 bootstraps) was performed when the LV showed good reliability as indicated by composite reliability > 0.7, Cronbach's alpha > 0.7, rho_A > 0.8 and when the average variance extracted (AVE) was > 0.500. Moreover, the indicators in the LV should have factor loadings > 0.500 (at $p<0.001$) and the construct crossvalidated redundancies should be adequate (Ringle et al., 2015). Adequacy of model fit was evaluated using the SRMR which should be < 0.080. Path coefficients with exact p-values and factor loadings with p-values were computed for the inner and outer models, respectively.

Results

Demographic and clinical data

Table 1 shows the socio-demographic and clinical data in subjects divided into QQ, QR and RR PON1 polymorphism. There were no significant differences in age, sex, BMI, tobacco use disorder, employment and education among the study groups. The total SANS score, PANSS negative test score and psychomotor retardation were significantly higher in QQ carriers than in the two other groups. There were no significant differences in psychosis, hostility, mannerism,

 doi:10.20944/preprints201909.0095.v1

formal thought disorders between the three study groups, although there was a trend towards higher scores in QQ carriers. As expected, PON1 CMPAase activity was significantly different among the three genotypes and increased from QQ → QR → RR carriers. PON1 activity towards phenylacetate (i.e., AREase activity) was significantly lower in RR carriers than in QR carriers.

Associations between PON1 genotype and diagnostic classification

Table 2 shows the associations between diagnostic groups and different genotype models. The total study group was at Hardy-Weinberg equilibrium (χ^2=1.20, df=1, p=0.273) and also, the SCZ sample was at Hardy-Weinberg equilibrium (χ^2=2.55, df=1, p=0.110). We found a significant association between the genotypes and the diagnostic groups indicating a difference between DS versus controls and NDS. Thus, there was a significantly increased QQ frequency in DS as compared with controls and NDS. The allelic distribution was significantly different between the three diagnostic groups with increased Q allele frequency in DS versus controls, whereas those with NDS occupied an intermediate position. Table 2 also shows the outcome of dominant, codominant and recessive models. The dominant model showed significant differences between the three diagnostic groups with a highly increased QQ frequency in patients with DS versus the other two study groups.

Since there are significant associations between the genotypes and the diagnostic groups as well as PON1 AREase and CMPAase activities there could be issues with multicollinearity when examining the effects of genotypes and enzymatic activities on clinical variables including rating scale scores. Of all gene models, an additive model (0= no Q allele; 1= one Q allele; 2= two Q alleles) performed best in subsequent analyses. Therefore, we have examined, using PCA, whether the data structure of the additive model, and PON1 AREase and CMPAase activities could be

 doi:10.20944/preprints201909.0095.v1

restructured in interpretable rotated factors that consequently could be used in regression analysis. We found that, after varimax rotation, 2 PCs explained 96.71% of the variance in those three PON1 data, whereby the first PC (explaining 52.13% of the variance) loaded highly on CMPAase (-0.808) and the additive genotype (0.953) and that PON1 activity did not load on this PC (0.056). The second PC (explaining another 44.57% of the variance) loaded highly on PON1 AREase activity (0.986) and CMPAase activity (0.553), whereas the additive genotype did not load significantly (0.245). As such, the first PC reflects the Q allele and associated lowered CMPAase activity (named GENZA PC, indicating gene and enzyme activity), while the second PC reflects PON1 AREase plus CMPAase activities relatively independent from the genotypes (named ENZA PC, indicating combined enzymatic activities). Subsequently, we examined the associations of both the GENZA and ENZA PCs, and PON1 AREase and CMPAase activities on the clinical variables.

Associations of the biomarkers with the clinical diagnosis

Table 3 shows the results of multivariate GLM analysis with GENZA PC, ENZA PC, PON1 CMPAase and AREase activities as dependent variables and diagnosis, sex, tobacco use disorder, age and BMI as explanatory variables. We found that there was a significant effect of diagnosis on the 4 biomarkers with an effect size of 0.078 (partial eta squared) and that the other 4 background variables were non-significant. Tests for between-subject effects showed that there were significant associations between diagnosis and CMPAase (impact size: 0.105) and GENZA PC (effect size: 0.102), whereas AREase and ENZA PC were not significant.

Table 4 shows the model-generated estimated marginal mean values of those 4 biomarkers in the three study groups. We found that DS is accompanied by significantly decreased CMPAase activity as compared with healthy controls (difference of 0.73 standard deviations) and NDS

 doi:10.20944/preprints201909.0095.v1

(difference of 0.675 standard deviations). The GENZA PC was significantly higher in DS as compared with controls (difference of 0.72 standard deviations) and NDS (difference of 0.63 standard deviations). There was a trend towards lower values in patients with DS.

We have also examined the effects of the drug state of the patients on the 4 biomarkers in this multivariate GLM analysis. We could not find any significant effects of treatment with risperidone (F=0.06, df=3/103, p=0.982), olanzapine (F=0.79, df=3/103, p=0.500), quetiapine (F=0.48, df=3/103, p=0.697), haloperidol (F=1.52, df=3/103, p=0.213), perphenazine (F=0.83, df=3/103, p=0.482), chlorpromazine (F=0.20, df=3/103, p=0.897), antidepressants (F=0.58, df=3/103, p=0.631), mood stabilizers (F=0.23, df=3/103, p=0.875), and anxiolytics (F=0.43, df=3/103, p=0.735). There was, however, a significant effect of clozapine on the biomarkers (F=2.93, df=3/103, p=0.037) (without p-correction for multiple testing), while tests for between-subjects effects showed significant effect on PON1 AREase activity only (F=4.68, df=1/104, p=0.033) with a very small effect size (0.043). Model-generated estimated marginal means showed that AREase activity was significantly higher in subjects who were treated with clozapine as compared with those without clozapine (means ±SDs: 0.746 ±0.687 versus -0.14 ±0.201 in z scores).

Associations between biomarkers and clinical scores

Table 5 shows the results of automatic regression analyses with symptom or rating scale scores as dependent variables and the 4 biomarkers, age, sex, education, BMI and tobacco use disorder as explanatory variables. We found that 22.4% of the variance in the total SANS score was explained by the regression on the GENZA PC (positive association), education (negative association) and sex. Up to 21.1% of the variance in PANSS negative subscale score was explained

 doi:10.20944/preprints201909.0095.v1

by the regression on CMPAase activity and education (both negatively associated) and sex, and 18.9% of the variance in the excitement score was explained by CMPAase activity and education (both negatively) and sex. We found that a large part of the variance in mannerism (20.5%) and FTD (19.0%) was explained by the regression on ENZA PC and education (both negatively) and sex. A large part of the variance in PMR (20.8%) was explained by GENZA PC (positively), and AREase activity and education (both negatively). We found that 24.7% of the variance in the OSOS index was predicted by CMPAase activity (negatively), education (negatively) and sex.

Results of PLS analysis

Figure 1 shows the results of a PLS analysis with the OSOS score as an output variable and both the GENZA and ENZA PCs as direct input variables and genotype (additive model) and CMPAase activity as explanatory variables for the GENZA PC. All variables were entered as single indicator variables, whereas OSOS LV was extracted from 9 symptom domains (FTD, PMR, total SDS, PANSS negative subscore, total SANS score, psychosis, hostility, excitement, and mannerism) in a reflective model. The model quality data were adequate with SRMR=0.056 and with adequate reliability data for the outer model namely Cronbach α=0.948 (±0.008), rho_A=0.960 (±0.009), composite reliability=0.946 (±0.009) and average variance extracted=0.669 (±0.035). The discriminant validity was adequate as examined using the Heterotrait-Monotrait ratio (except for the PON1 genotype and GENZA PC). The loadings on the outer model were all significant ($p<0.0001$) and were more than adequate (all > 0.707). We found that 26.4% of the variance in the OSOS LV was explained by the regression on education, sex, and both the GENZA and ENZA PCs. Moreover, the GENZA PC was significantly predicted by both CMPAase activity and the PON1 genotype. There were significant total indirect effects of

 doi:10.20944/preprints201909.0095.v1

CMPAase activity on the OSOS LV (t=-2.14, p=0.013) and of CMPAase activity on the OSOS LV (t=+2.42, p=0.016). Confirmatory Tretrad Analysis showed that the OSOS LV fitted a reflective model. Blindfolding showed that the OSOS LV has a significant cross-validated predictive relevance with a construct crossvalidated redundancy of 0.176.

Associations between PON1 genotype and IgA responses to Gram-negative bacteria

Table 6 shows the results of ANOVAs with the IgA responses to 5 Gram-negative bacteria as dependent variables and the PON1genotype (recessive model) as the explanatory variable. RR carriers showed significantly lower IgA responses to *Pseudomonas aeruginosa*, *Morganella morganii*, *Pseudomonas putida* and the sum of the 5 gut commensal bacteria as compared to non-RR carriers.

Discussion

The first major finding of this study is that DS is significantly associated with the QQ genotype and the Q allele as compared with NDS patients and controls. Previously, a higher prevalence of the QQ genotype was found in SCZ (Atagün et al., 2017) although not all authors were able to observe this association (Kucukalia et al., 2008). Nevertheless, the lack of differentiation of SCZ patients into DS and NDS in previous studies could explain the contradictory results. The R allozyme is more efficient to metabolize 4-(chloromethyl) phenylacetate (CMPA) and to hydrolyze peroxide lipids and, therefore, is more protective against O&NS than the Q alloenzyme (Mackness et al., 1998). In our study, RR individuals showed as expected the highest CMPAase activity, while QQ carriers had much lower CMPAase activity.

 doi:10.20944/preprints201909.0095.v1

The genotypic Q192R distribution obtained in Thai nationals in the current study and in Roomruangwong et al. (2017) is quite different from that in Western countries which reported a higher PON1 192Q allele frequency (Nielsen et al., 2005; Coombes et al., 2011). Nevertheless, studies performed in Asia show a similar genotypic distribution with higher frequencies of the RR genotype as detected here in Thai nationals (Suehiro et al., 2000; Zhou et al., 2000; Seow et al., 2016; Roomruangwong et al., 2017). The predominance of the QQ genotype in Asian DS patients may suggest that part of these patients may be prone to develop cardio-vascular disease (CVD) and certain cancers. For example, SCZ patients show an increased mortality due to CVD (Ringen et al., 2014) while the R allele decreases risk to develop coronary heart disease and myocardial infarction (Hernández-Diaz et al., 2016). Moreover, the presence of the R allele is associated with decreased risk of breast cancer (Saadat 2012; Zhang et al., 2015) while a recent meta-analysis shows that the incidence of breast cancer is higher in women with SCZ than in the general population (Zhuo and Triplett, 2018). There is also a significant comorbidity between type 2 diabetes mellitus (T2DM) and SCZ (Schoepf et al., 2012) and a significant association between PON1 Q192R genotypes and susceptibility to T2DM or gestational diabetes (Van Den Berg et al., 2008; Alharbi et al., 2016; Wu et al., 2018). Nevertheless, the PON1 R allele, and not the Q allele, was a susceptible factor to develop T2DM in the South or East Asian population (Luo et al., 2018).

The second major finding of this study is that CMPAase PON1 activity is signficantly lowered in patients with DS and that effect is largely determined by the QQ genotype, which is strongly associated with DS. Recently, it was reported that PON1 activity is significantly lower in drug-naïve patients with first episode psychosis (FEP) (Noto et al., 2015) and additionally that lowered PON1 levels are inversely associated with increased cytokine levels, including IL-6, IL-4 and IL-10 (Brinholi et al., 2015). The latter authors suggested that PON1 activity in FEP may

play a role in the immune-inflammatory response that accompanies FEP through lowered anti-inflammatory effects. In patients with chronic schizophrenia, on the other hand, there was no significant decrease in PON1 activity (Boll et al., 2017), although the lack of any changes in PON1 activity could be explained by stimulatory effects of risperidone on PON1 activity (Noto et al., 2015). A recent study suggests a possible involvement of low CMPAase in the increased cardiovascular (CVD) risk in patients with SCZ (Pavăl et al., 2018). Gupta et al. (2011) found lower CMPAase PON1 activity in CVD patients as compared to controls, independent of age, sex, smoking, alcohol and HDL-C levels. Nevertheless, lowered PON1 activities are not specific for SCZ as lowered activities are also detected in affective disorders. Moreira et al. (2018; 2019) reported that lowered PON1 total and CMPAase activities may play a role in the pathophysiology of mood disorders through their impact on antioxidant defenses thereby increasing the risk towards lipid peroxidation, inflammation, bacterial proliferation, and neurotoxicity (by attenuating homocysteine thiolactone catabolism). As discussed above, lowered PON1 activity is associated with many conditions and diseases and this may be explained by the broad pattern of substrate specificities of PONs described as PONs are like “Jacks of all trades and masters of none” (Bar-Rogovsky et al., 2013).

The third major finding of this study is that a part of the variance in overall severity of SCZ (OSOS), negative symptoms, excitation, mannerism, PMR and FTD is explained by two different aspects of PON1 total activity, namely a) the combination of genetic load in an additive model and gene-associated CMPAase enzyme activities; and b) lowered AREase and CMPAase enzyme activities, which are largely independent from the Q192R gene. These findings indicate that SCZ phenomenology is in part explained by the combined effects of a gene-related decline in CMPAase PON1 activity and additionally that lowered activity levels of AREase and CMPAse PON1

activities not related to the PON1 gene may determine another part of the variance in OSOS. It is possible that the latter is associated with epigenetic changes in DNA methylation which frequently occur in SCZ (Kalayasiri et al., 2019) or that the PON1 activity might be partially inactivated in the presence of increased lipid peroxidation, which occurs in SCZ patients (Anderson and Maes, 2013). For example, lipid peroxidation may interact with PON's free sulphydryl group, causing inactivation of PON1 (Kucukalia et al., 2008). HDL-associated protein PON1 may be oxidatively modified and inactivated by myeloperoxidase (MPO) (Karlsson et al., 2015). During inflammatory conditions, key residues of the formed HDL-MPO-PON1 ternary complex may be targeted and oxidatively modified and inactivated (Huang et al., 2013). In addition, also dietary factors including high-fat diet and smoking may decrease PON1 activity and expression (Kucukalia et al., 2008), although in our study no significant effects of nicotine dependence were detected while Thai people do not consume high-fat-diets. All in all, our findings suggest that a primary deficit in CMPAase activity, in part associated with an increased frequency of the QQ genotype in DS may play a role in the immune and O&NS pathophysiology of DS, and that the latter may decrease AREase and CMPAAse PON1 activities, which may further aggravate the pathophysiology of DS.

The fourth major finding of this study is that there is a significant association between the recessive Q192R model and translocation of Gram-negative bacteria whereby carriers of the Q allele have increased IgA responses to the LPS of *Pseudomonas aeruginosa, Morganella morganii,* and *Pseudomonas putida* as compared with RR carriers. PONs not only display anti-oxidant and anti-inflammatory effects (see above), but also quorum quenching properties, namely PONs may hydrolyze N-(3-oxo-dodecanoyl)-homoserine lactone, which is a quorum-sensing molecule that modulates the virulence and biofilm formation properties of many Gram-negative bacteria (Aybey and Demirkan, 2016; Koul and Kalia, 2017).

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It is interesting to note that mammalian PONs may be related to bacterial, PON-like lactonases with quorum quenching properties (Elias and Tawfik, 2012). However, the mammalian ancestor, unlike bacterial PON, hydrolyzes lactones other than homoserine lactones and as such preceded the detoxifying functions that diverged later (Draganov et al., 2005). The recruitment of homoserine lactonase activity obtained from endosymbiotic pathogens or bacteria became part of the innate immunity due to the quorum sensing properties of PON (Bar-Rogovsky et al., 2013). For example, the PON enzyme family has activity against biofilm formation by *P. aeruginosa* which utilize N-acyl-homoserine lactonase to regulate bacterial virulence and promote biofilm formation (Marsillach et al., 2008; Veesenmeyer et al., 2009). As such, PON1 activity is part of the innate immune system offering a first line protection against Gram-negative bacteria by attenuating quorum sensing. These mechanisms may explain that SCZ patients with the Q allele or QQ genotype and with lowered PON1 activities may be at risk to develop increased bacterial load and that genetically-determined deficits in CMPAase activity may increase risk towards DS through attenuated innate immune defenses including quorum quenching. Moreover, DS is accompanied by breakdown of the gut tight and adherens junctions with increased bacterial translocation whereby increased load of Gram-negative bacteria is associated with breakdown of the blood-brain barrier (BBB) and increased neurocognitive deficits (Maes et al., 2019b; 2019d). As such, lowered CMPAase PON1 activities in DS may lead to increased LPS load and its consequences.

As described in the Introduction, DS is the outcome of enhanced neurotoxic pathways including CCL11, TRYCATs, IgM levels to NO-cysteinyl and bacterial LPS and lowered anti-oxidant, anti-inflammatory and antibacterial protection through lowered natural IgM levels against OSEs, including azelaic acid and malondialdehyde, which is a key part of the innate immune

system (Maes et al., 2019b; 2019c). By inference, lowered PON1 activities and lowered natural IgM are two key impairments in innate immunity leading to greater impact of inflammatory, oxidative and nitrosative and Gram-negative microbiota.

Conclusions.

DS is significantly associated with the Q allele and the QQ genotype and with lowered CMPAase PON1 activities, while the overall severity of SCZ is associated with the Q192R gene as well as CMPAase and AREase PON1 activity. Lowered PON1 activities are additionally associated with negative symptoms, formal thought disorders, psychomotor retardation and excitation. The Q allele is associated with increased signs of bacterial translocation namely increased IgA responses to *Pseudomonas aeruginosa, Morganella morganii,* and *Pseudomonas putida.* It is suggested that lowered PON1 activities in DS constitute a deficit in the innate immune system which together with lowered natural IgM responses to OSE may predispose towards greater neurotoxic effects of immune-inflammatory, oxidative and nitrosative pathways and Gram-negative microbiota.

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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. All authors contributed to interpretation of the data and writing of the manuscript. All authors approved the final version of the manuscript.

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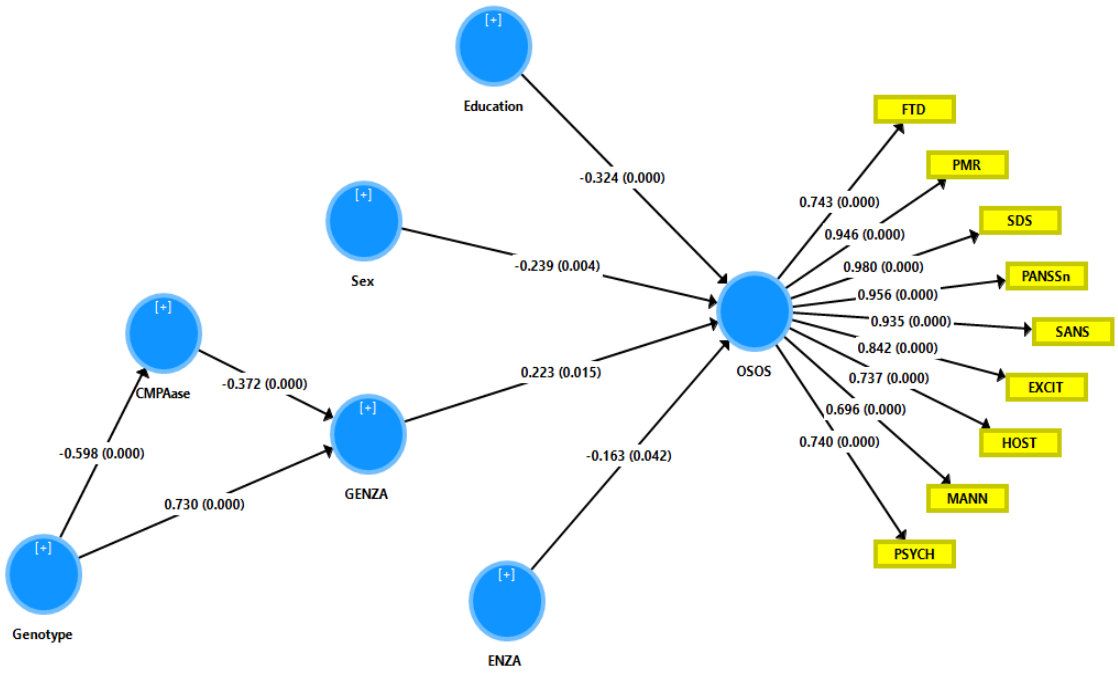
Figure 1. Results of consistent Partial least Squares analysis (5000 bootstraps) with overall severity of schizophrenia (OSOS) as output variable and a combination of the additive model of PON1 Q192R genotype and CMPAase activity (GENZA), PON1 enzymatic activity (ENZA), education and sex as input variables.



Shown are the loadings (with p values) for the outer model and path coefficients (with p values) for the inner model.

CMPAase: 4-(chloromethyl)phenyl acetate hydrolysis

SANS: total score on the Scale for the Assessments of Negative Symptoms, PANSSn: total score on the Negative Syndrome Scale of the PANSS; SDS: total score on the Schedule of the Deficit Syndrome; FTD: formal thought disorders, PMR: psychomotor retardation; EXCIT: excitation; HOST: hostility; MANN: mannerism: PSYCH: psychosis.

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Table 1: Demographic and clinical data of all participants divided according to their PON1 Q192R genotype.

Variables	QQ [A] (n=16)	QR [B] (n=48)	RR [C] (n=56)	F/X²	df	p
Age (years)	39.4(10.8)	41.1(11.6)	39.0(12.2)	0.44	2/112	0.644
Sex (Male/Female)	9/7	17/31	27/29	2.81	2	0.243
BMI (kg/m^2)	25.4(5.8)	24.4(4.8)	23.9(4.7)	0.59	2/112	0.550
TUD (No/Yes)	16/0	45/3	52/4	0.10	-	0.554
Employment (No/Yes)	10/6	22/26	13/38	5.29	2	0.071
Education (years)	10.7(4.0)	12.8(4.8)	13.8(4.1)	3.02	2/117	0.053
Total SANS score	41.1(30.5) [B,C]	23.1(25.1) [A]	18.7(22.0) [A]	5.23	2/116	0.007
PANSS negative subscale score	22.1(11.5) [B,C]	15.2(10.0) [A]	13.1(9.3) [A]	5.14	2/116	0.007
Psychosis (z scores)	0.46(1.11)	-0.07(0.88)	-0.08(1.06)	2.03	2/116	0.136
Hostility (z scores)	0.26(1.14)	0.04(1.10)	-0.11(0.86)	0.93	2/116	0.396
Mannerism (z scores)	0.39(1.51)	-0.05(1.44)	-0.07(1.66)	0.58	2/116	0.563
Formal thought disorders (z scores)	0.42(1.04)	-0.10(0.94)	-0.04(1.03)	1.65	2/116	0.196
Psychomotor retardation (z scores)	0.87(1.11) [B,C]	0.00(0.96) [A]	-0.25(0.87) [A]	8.74	2/116	<0.001
PON1 CMPAase (U/mL)	22.2(9.3) [B,C]	37.0 (9.5) [A,C]	44.7(9.8) [A,B]	35.21	2/117	<0.001
PON1 AREase (U/mL)	212.2(95.8)	223.9(59.4) [C]	180.1(44.8) [B]	7.31	2/117	0.001

All results are shown as mean (SD). BMI: Body Mass Index, TUD: Tobacco Use Disorder, SANS: Scale for the Assessments of Negative Symptoms, PANSS: Positive and Negative Syndrome Scale, CMPAase: 4-(chloromethyl)phenyl acetate hydrolysis, PON1: Paraoxonase; AREase: arylesterase

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Table 2: Associations between PON1 Q192R genotypes (full data and different models) and deficit (DS) and non-deficit (NDS) schizophrenia (SCZ) versus healthy controls (HC).

Models	Classes	Total	HC	NDS	DS	X^2/ψ	df	p
1	QQ	16	1 (-81.2%)	4 (-2.5%)	11 (+106.3%)	0.31	-	0.018
	QR	48	17 (+6.3%)	16 (0%)	15 (-6.2%)			
	RR	56	22 (+17.9%)	20 (+7.1%)	14 (-25%)			
2	Q allele	80	19 (-28.7%)	24 (-10%)	37 (+38.7%)	9.71	2	0.008
	R allele	160	61 (+14.4%)	56 (+5%)	43 (-19.4%)			
3	Recessive allele	104	39 (+12.5%)	36 (+3.8%)	29 (-16.3%)	0.31	-	0.003
	QQ	16	1 (-81.2%)	4 (-25%)	11 (+106.3%)			
4	Dominant allele	64	18 (-15.6%)	20 (-6.2%)	26 (+21.9%)	3.48	2	0.175
	RR	56	22 (+17.9%)	20 (+7.1%)	14 (-25%)			
5	Homozygotes	72	23 (-4.2%)	24 (0%)	25 (+4.2%)	0.21	2	0.901
	QR	48	17 (+6.3%)	16 (0%)	15 (-6.2%)			

Shown are the effects of different genotypic models, i.e. (1): full data, no model; (2): allelic model; (3) dominant model; (4): recessive model; and (5) over-dominant model.

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Table 3: Results of GLM analysis with total PON1 status as dependent variable and diagnosis (deficit and non-deficit schizophrenia and controls) as explanatory variable while adjusting for background variables.

Type	Dependent variable	Explanatory variable	F	df	p	Partial Eta Squared
Multivariate	CMPAase, AREase, GENZA PC, ENZA PC	Diagnosis	3.00	6/212	0.008	0.078
		Sex	1.37	3/106	0.255	0.037
		TUD	0.44	3/106	0.722	0.012
		Age	0.53	3/106	0.661	0.015
		BMI	0.78	3/106	0.507	0.022
Tests for between-subject effects	PON1 CMPAase	Diagnosis	6.30	2/108	0.003	0.105
	PON1 AREase	Diagnosis	0.99	2/108	0.374	0.018
	GENZA PC	Diagnosis	6.15	2/108	0.003	0.102
	ENZA PC	Diagnosis	1.05	2/108	0.353	0.019

Diagnosis: Deficit and non-deficit schizophrenia, BMI: Body Mass Index, TUD: Tobacco Use Disorder, CMPAase: 4-(chloromethyl)phenyl acetate hydrolysis, PON1: Paraoxonase 1, GENZA PC: first PC reflects the Q allele and associated lowered CMPAase activity, ENZA PC: second PC reflects PON1 plus CMPAAse activities relatively independent from the genotypes.

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Table 4. Model-generated estimated marginal mean values in z scores obtained by multivariate GLM analysis shown in Table 3.

Variables (z scores)	HC [A]	NDS [B]	DS [C]
PON1 CMPAase	0.338 (0.303) [C]	0.282 (0.278) [C]	-0.393 (0.276) [A,B]
PON1 AREase	0.601 (0.309)	0.351 (0.283)	0.146 (0.282)
GENZA PC	-0.288 (0.302) [C]	-0.203 (0.277) [C]	0.427 (0.275) [A,B]
ENZA PC	0.493 (0.309)	0.321 (0.283)	0.060 (0.281)

The categories are: deficit (DS) and non-deficit (NDS) schizophrenia versus healthy controls (HC). CMPAase: 4-(chloromethyl)phenyl acetate hydrolysis, PON1: Paraoxonase 1, GENZA PC: first PC reflecting the Q allele and associated lowered CMPAase activity, ENZA PC: second PC reflecting PON1 plus CMPAAse activities independently from the genotypes

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Table 5: Results of multiple regression analysis with schizophrenia symptom domains as dependent variables.

Dependent variable	Explanatory variables	β	t	p	F_{model}	df	p	R^2
SANS	**Model**				11.06	3/115	<0.001	0.224
	Education	-0.300	-3.57	0.001				
	Sex	-0.221	-2.68	0.009				
	GENZA PC	0.218	2.58	0.011				
PANSS negative	**Model**				10.26	3/115	<0.001	0.211
	Education	-0.266	-3.20	0.002				
	Sex	-0.184	-2.21	0.029				
	CMPAase	-0.285	-3.42	0.001				
Excitement	**Model**				9.04	3/115	<0.001	0.189
	Education	-0.275	-3.27	0.001				
	Sex	-0202	-2.40	0.018				
	CMPAase	-0.223	-2.66	0.009				
Mannerism	**Model**				9.87	3/115	<0.001	0.205
	Education	-0.293	-3.46	0.001				
	Sex	-0.301	-3.61	<0.001				
	ENZA PC	-0.178	-2.11	0.037				
FTD	**Model**				8.97	3/115	<0.001	0.190
	Education	-0.295	-3.45	0.001				
	ENZA PC	-0.249	-2.92	0.004				
	Sex	-0.233	-2.77	0.007				
PMR	**Model**				10.04	3/115	<0.001	0.208
	Education	-0.255	-2.95	0.004				

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	GENZA PC	0.320	3.78	<0.001				
	AREase	-0.177	-2.09	0.039				
OSOS	**Model**				12.59	3/115	<0.001	0.247
	Education	-0.315	-3.87	<0.001				
	Sex	-0.247	-3.04	0.003				
	CMPAase	-0.238	-2.93	0.004				

SANS: Scale for the Assessments of Negative Symptoms, PANSS: Positive and Negative Syndrome Scale, FTD: formal thought disorders, PMR: psychomotor retardation, OSOS: overall severity of schizophrenia, CMPAase: 4-(chloromethyl)phenyl acetate hydrolysis, PON1: Paraoxonase 1, GENZA PC: first PC reflecting the Q allele and associated lowered CMPAase activity, ENZA PC: second PC reflecting PON1 plus CMPAAse activities relatively independent from the genotypes.

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Table 6: Associations of PON1 Q192R genotype (recessive model) with IgA levels directed to Gram-negative bacteria (in z scores).

IgA responses directed to	Dominant allele (n=62)	RR (n=56)	F	df	p
Hafnia alvei	+0.16 (1.15)	-0.18 (0.78)	3.40	1/116	0.068
Pseudomonas aeruginosa	+0.23 (0.98)	-0.26 (0.97)	7.35	1/116	0.008
Morganella morganii	+0.22 (1.10)	-0.25 (0.82)	6.78	1/116	0.010
Pseudomonas putida	+0.21 (1.02)	-0.23 (0.93)	5.96	1/116	0.016
Klebsiella pneumoniae	+0.13 (1.14)	-0.15 (0.80)	2.40	1/116	0.124
Sum 5 IgA LPS	+0.24 (1.07)	-0.25 (0.85)	7.82	1/116	0.006

All results are shown as mean (SE) and in z scores. All results of GLM analyses.

Sum 5 IgA LPS: z unit-weighted composite score based on the 5 IgA responses to Gram-negative bacteria