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Genetic polymorphism associated prefrontal glutathione and its coupling with brain glutamate and peripheral redox regulation in early psychosis

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Running title: Genetic polymorphism associated with brain GSH

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Abstract

Background

Oxidative stress and glutathione (GSH) metabolism dysregulation has been implicated in the pathophysiology of schizophrenia. GAG-trinucleotide repeat (TNR) polymorphisms in the glutamate-cysteine ligase catalytic gene (GCLC), the rate-limiting enzyme for GSH synthesis, are associated with schizophrenia. In addition, GSH may serve as a reserve pool for neuronal glutamate (Glu) through the γ-glutamyl cycle. The aim of this study is to investigate brain [GSH] and its association with GCLC polymorphism, peripheral redox indices and brain Glu.

Methods

Magnetic resonance spectroscopy was used to measure [GSH] and [Glu] in the medial prefrontal cortex (mPFC) of 25 early-psychosis patients and 33 controls. GCLC polymorphism was genotyped, glutathione peroxidases (GPx) and glutathione reductase (GR) activities were determined in blood cells.

Results

Significantly lower [GSH_{mPFC}] in GCLC high-risk genotype subjects were revealed as compared to low-risk genotype subjects independent of disease status. In male subjects, [GSH_{mPFC}] and blood GPx activities correlate positively in controls ($p = 0.021$), but negatively in patients ($p = 0.039$). In GCLC low-risk genotypes, [Glu_{mPFC}] are lower in patients, while it is not the case for high-risk genotypes.

Conclusions

GCLC high-risk genotypes are associated with low [GSH_{mPFC}], highlighting that GCLC polymorphisms should be considered in pathology studies of cerebral GSH. Low brain GSH levels are related to low peripheral oxidation status in controls but with high oxidation status in patients, pointing to a dysregulated GSH homeostasis in early psychosis patients. GCLC polymorphisms and disease associated correlations between brain GSH and Glu levels may allow patients stratification.

Keywords: schizophrenia, glutathione, oxidative stress, MRS, GCLC, glutamate, redox, glutathione peroxidase.

Introduction

Schizophrenia is a major psychiatric disorder which results from a complex interplay between genetic and environmental risk factors during neurodevelopment. There is increasing evidence suggesting that oxidative stress and dysregulation of the glutathione (GSH) metabolism play a role in the pathophysiology of the disease [1-4]. GSH serves as a major cellular redox regulator and antioxidant protecting cell from damages induced by reactive oxygen species. Decreased [GSH] have been reported in cerebro-spinal fluid (-27%) of patients with chronic schizophrenia[5]. A *postmortem* study also revealed lower caudate [GSH] in treated patients than in control subjects [6]. Furthermore, similar reduction of [GSH] was observed in blood plasma of treated and untreated patients [7-10].

Studies of cerebral [GSH] *in vivo* in schizophrenia are limited and exclusively achievable by using ^{1}H magnetic resonance spectroscopy (MRS). Do et al. reported a 52% reduction of [GSH] in medial prefrontal cortex of chronic schizophrenia patients at 1.5T (14 controls and 14 patients, in [5]; extended to 18 controls and 20 patients, unpublished results). However, two consecutive studies in schizophrenia patients did not reveal significant GSH alterations in the anterior cingulate cortex at 4T (9 controls and 11 patients) [11] or in posterior medial frontal cortex at 3T (16 controls and 20 patients), though low [GSH] in this later region were associated with more severe negative symptoms [12]. Reasons for such inconsistencies remain unclear. Differences in methodological aspects, such as magnetic field strengths, pulse sequences, number of subjects, and stage of disease may be relevant.

Whether alterations of cerebral [GSH] are already present in the early phase of psychosis (EP) remains an open question. Only one study has reported 22% higher [GSH] in medial temporal lobe of first episode patients using PRESS ^{1}H MRS sequence at 3T [13]. However, the mean Cramer-Rao lower-bound (CRLB) for the corresponding measurement of GSH was moderately high (~21%). Therefore, further investigations of cerebral [GSH] in EP patients including those in other brain regions are required.

One genetic factor has been reported to influence [GSH] in the periphery: a GAG trinucleotide repeat (TNR) polymorphism in the gene coding for the catalytic (GCLC) subunit of glutamate-cysteine ligase (GCL), the rate-limiting enzyme for GSH synthesis. Importantly, this polymorphism was associated with schizophrenia in two case-control studies [14]: the GCLC

high-risk genotypes (i.e. with 7/8, 8/8, 8/9 and 9/9 TNR) were more frequent in patients (30%) and were associated with lower GCLC protein expression, GCL activity and [GSH] in fibroblasts when challenged with oxidative stress conditions, as compared with GCLC low-risk genotypes (i.e. with 7/7 and 7/9 TNR). However, the peripheral GSH levels may not reflect GSH status in the central nervous system and the association between cerebral GSH levels and GCLC polymorphism has never been investigated. The understanding of their relation may shed light on current discrepancies concerning cerebral [GSH] in psychotic patients.

Studies on the various antioxidant systems in the peripheral tissue of schizophrenia patients showed large discrepancies [1, 4]. Altered levels of scavenging antioxidant enzymes were reported, however with mixed results [15]. Glutathione peroxidases (GPx) are important antioxidant enzymes that eliminate hydrogen and lipid peroxides by converting GSH into oxidized GSH (GSSG). Then GSSG is reduced back to GSH by glutathione reductase (GR) (Figure 1s). The precise quantification of blood GSH/GSSG balance in a psychiatric clinical setting is quite challenging and subjected to artefactual oxidations leading to unreliable results16. To capture the (dys)regulation of redox status, we assessed enzymatic activities of GPx and GR as well as their ratio (GPx/GR) in blood cells instead of GSH/GSSG ratio..

Besides its antioxidant properties, the tripeptide GSH has been proposed to constitute a reserve pool for neuronal glutamate (Glu) through the γ-glutamyl cycle [17]. γ-glutamyltranspeptidase (GGT) transfers γ-glutamyl moiety of extracellular GSH to an acceptor amino acid, generating γ-glutamyl amino acid and cysteinylglycine. On the one side, the cysteinylglycine can then provide substrates to synthesize GSH; on the other side, γ-glutamyl amino acid can be metabolized to amino acid and 5-oxoproline, which can be further catalyzed by the 5-oxoprolinase to form Glu, a precursor of GSH [18, 19]. The inhibition of 5-oxoprolinase reduces [Glu] in cortical and hippocampal neurons, with no changes in [GSH] [17]. This mechanism is of high interest in the context of the glutamatergic hypothesis in schizophrenia [20], and has never been evaluated *in vivo* in humans.

Therefore, the aims of this study were 1) to assess [GSH] in medial prefrontal cortex of early psychosis patients relative to control subjects 2) to test its association with GAG-TNR of GCLC genotypes and 3) with peripheral GSH redox indices (GPx, GR activities and their ratio GPx/GR) and finally, 4) to investigate the relationship between brain GSH and Glu.

Methods and Materials

Subjects

Twenty-five early psychosis patients (within the first 3 years of treatment for a psychotic disorder and having met psychosis threshold according to the Comprehensive Assessment of At Risk Mental States criteria) [21] were recruited from the Treatment and Early Intervention in Psychosis Program (TIPP, University Hospital of Lausanne, Lausanne, Switzerland) [22]. Medication prescribed to patients followed international recommendations for early psychosis treatment [23], was recorded in the medical file and adherence to treatment was rated by clinicians. CPZ equivalents were calculated on the basis of formulas using regression with power transformation suggested by Andreasen et al. [24] and of maximum daily doses [25] for the remaining medication (table 1).

Onset of illness was defined as the time when a definite diagnosis of psychosis was reached by the presence of clear evidence of delusions, hallucinations, first rank symptoms, catatonic symptoms for at least one week on the basis of elements gathered by treating psychiatrist and case managers through interviews with patients, their relatives and any mental health professional who would have been involved in the care of the patient at that time. Duration of illness was defined as the time that elapsed between onset of illness and the time when MRI was performed. Diagnosis for patients was based on DSM-IV criteria and is the result of an expert consensus procedure based on information gathered over the three years of treatment.

Thirty-three age, sex matched healthy controls were recruited and assessed by the Diagnostic Interview for Genetic Studies [26] with the following exclusion criteria: having major mood, psychotic, or substance-use disorder and a first-degree relative with a psychotic disorder. Neurological disorder and severe head trauma were exclusion criteria for all subjects. Demographic and clinical details of all participants are shown in table 1. All participants gave informed written consent prior to participate in this study which was approved by the ethics review board of the Lausanne University Hospital.

^{1}H Magnetic Resonance Spectroscopy

All MRS measurements were carried out on a 3T MR scanner (Magnetom TimTrio, Siemens Healthcare, Erlangen, Germany) with a transverse electromagnetic (TEM 3000) head coil (MR Instruments, Inc., Minneapolis, MN, USA). The magnetic field homogeneity was optimized by adjusting first- and second-order shims using FAST(EST)MAP [27, 28]. *In vivo* ^{1}H MR spectra were acquired from a volume of interest (VOI = 20x20x25mm^3) positioned in the medial prefrontal cortex (mPFC; Fig. 1A) using a short-TE spin-echo full-intensity acquired localized single voxel spectroscopy technique (SPECIAL) [29, 30]. The following scan parameters were used: TE/TR = 6/4000 ms, acquisition bandwidth = 2 kHz, number of averages = 148, vector size = 2048. Outer volume suppression (OVS) [31] and water suppression with variable-pulse power and optimized relaxation delays (VAPOR) [32] were applied prior to the SPECIAL localization sequence.

In addition, to evaluate tissue composition inside the VOI, three-dimensional T_1-weighted anatomical images were obtained using a magnetization-prepared rapid gradient-echo (MPRAGE) sequence [33] with a 32-channel head array coil, TE/TR = 2.98/2300 ms, TI = 900 ms, flip angle = 9 degree, FOV = 240×256 mm^2, matrix = 240×256, slice thickness = 1.2 mm.

Spectral quantification

[GSH_{mPFC}] and [Glu_{mPFC}] were obtained by analyzing water suppressed *in vivo* ^{1}H MR spectra using LCModel (Stephen Provencher, Inc., Oakville, ON, Canada) [34] with a basis-set consisting of 20 simulated individual metabolite spectra: alanine(Ala), aspartate (Asp), phosphocreatine (PCr), creatine (Cr), γ-aminobutyric acid (GABA), glutamine (Gln), Glu, phosphorylcholine (PCho), glycerophosphorylcholine (GPC), GSH, glucose (Glc), lactate (Lac), glycine (Gly), myo-inositol (mIns), N-acetylaspartylglutamate (NAA), N-acetylaspartylglutamate (NAAG), ascorbate (Asc), phosphorylethanolamine (PE), scyllo-inositol (Scyllo), taurine (Tau), and a experimentally measured macromolecule baseline [35]. The spectral fit obtained from LCModel is a linear combination of model spectral provided in the basis-set and the estimation of fitting error is provided by CRLB. Unsuppressed water ^{1}H MR spectra were used as an internal reference for the quantification of metabolite concentrations. The spectral range for analysis was set to 0.2 - 4.2 ppm. Tissue composition inside the VOI was calculated based on the segmentation of 3D T_1-weighted anatomical images using an in-house software [36]. Water concentrations, used in LCModel analysis, were calculated based on the volume fractions of white matter (WM), grey matter (GM) and cerebrospinal fluid (CSF) assuming water concentrations of WM, GM and CSF are 35880, 43300 and 55556 mM, respectively. Metabolite

concentrations were then divided by the fraction of WM and GM to correct for CSF inside the VOI, since metabolites are mainly present in WM and GM [37]. The signal-to-noise ratio (SNR) was obtained using NAA peak height at 2.01 ppm divided by the standard deviation of the noise.

Genotyping of GCLC tri-nucleotide polymorphism

DNA was extracted from whole blood. PCR was performed according to Walsh et al. [38] and modified as in Gysin et al. [14]: in a 20 μL reaction mix with GoTaq polymerase, 1.5 mM MgCl2, 0.25 μM of each primer (forward: TTCTGCGGGCGGCTGAGTGTCC; reverse: ATGGCGCTTGGTTTCCTCCC), 0.2mM of dNTP and 100 ng of genomic DNA. Amplification temperature were: 95° for 2 min followed by 32 cycles of 95° for 30 s, 62° for 30 s, 72° for 1 min and 1 step at 72° for 5 min . PCR products were separated on 10% polyacrylamide gels and were visualized under UV after 15min staining in GelRed solution. Classification into GCLC high- or GCLC low-risk genotype is based on the number of GAG repeats as defined in Gysin et al. (7/8, 8/8, 8/9, 9/9 and 7/7, 7/9 respectively) [14].

[GSH_B], GPx and GR enzymatic activities in blood

Blood was collected by venipuncture in Vacutainer-tubes coated with Li-heparinate (Becton Dickinson), between 7 and 8:30 AM under restricted activity conditions and fasting from the previous midnight. An aliquot of whole blood is sampled and frozen at -80°C until analysis of [GSH_B]. [GSH_B] was measured in 45 μL of whole blood using a diagnostic kit and according to manufacturer instructions (Calbiochem); [GSH_B] are normalized to blood volume. Blood cells were prepared as in [14]. All manipulations were performed rapidly with cooling to avoid artefactual oxidation of thiol compounds. Essentially, GR activity [39] was assessed in 8 μL of hemolyzed blood incubated in a phosphate buffer solution (100 mM, pH7.5) with EDTA (0.6 mM), oxidized glutathione (2.5 mM), NADPH (0.25 mM). GPx activity [40] was assessed in 8 μL of hemolyzed blood incubated in phosphate buffer solution (100 mM, pH7.5) with EDTA(0.6 mM), glutathione (2.5 mM), NADPH (0.25 mM), GR (0.84 U/ml; Fluka) and Tert-butyl hydroperoxide (0.8 mM, Fluka). Enzymatic activities were determined in triplicates as a function of the decrease in NADPH measured at 340 nm and normalized to hemoglobin content.

Statistical analysis

Differences between patients and controls in sex, smoking and handedness were assessed using Chi-square test or fisher's exact test accordingly. Differences in age, education of parents,

fractions of WM, GM and CSF, spectral quality (SNR and linewidth) between patients and controls, [GSH_{mPFC}] between smokers and non-smokers were evaluated by unpaired t-test (two-tailed). The effects of GCLC GAG-TNR polymorphism (low-risk *versus* high-risk), disease (controls *versus* patients), sex (male *versus* female) and age on [GSH_{mPFC}], [GSH_B], blood GPx and GR activities were investigated using generalized linear model in Matlab (R2013b,The MathWorks, Inc. USA). Two-tailed Mann-Whitney test was used to investigate the alterations in [Glu_{mPFC}]. All correlation tests were performed using the Spearman rank correlation (two-tailed) in GraphPad Prism (Version 5.04, GraphPad Software, Inc., USA). The difference between two independent correlation coefficients was tested online (Preacher, K. J. (2002, May). Calculation for the test of the difference between two independent correlation coefficients [Computer software]. Available from http://quantpsy.org.).

Results

There were no statistical differences in age, sex, handedness and education of parents between patients and controls indicating a good matching for these criteria (Table 1). All individuals were Caucasian. Since the high prevalence of smoking in schizophrenia patients [41], we also observed more smokers in patients.

A representative short-TE ^{1}H MR spectrum obtained from mPFC, LCModel spectral fit and individual metabolites fits are shown in Fig 1B. [GSH_{mPFC}] were measured by short-TE ^{1}H MRS at 3T and quantified using LCModel yielding CRLB of 10 ± 3% (mean ± s.d.). In addition to GSH, other 12 metabolites were quantified from LCModel fitting including Asp, Cr, PCr, Gln, Glu, Ins, NAA, total Choline (GPC+PCho) with mean CRLBs < 10% and GABA, Lac, PE, Tau with mean CRLBs < 20%. Spectral SNR and linewidth (LCModel output) were 104 ± 16, 0.031 ± 0.005ppm for controls and 97 ± 12, 0.033 ± 0.006ppm for patients, respectively. No significant differences ($p > 0.05$) in spectral quality (i.e. SNR and linewidth) between groups were observed. Tissue compositions inside the MRS voxel were measured and there is no differences of WM, GM and CSF fractions between patients and controls (Table 1s).

GSH levels in mPFC

To discern [GSH_{mPFC}] changes in early psychosis and its association with GCLC polymorphism, GCLC genotypes were included as a covariant together with age and sex to test

whether [GSH_{mPFC}] were different between 25 EP patients and 33 controls. There were significantly lower [GSH_{mPFC}] in subjects with the GCLC high-risk genotypes (1.15 ± 0.17 μmol/g) as compared to those with low-risk genotypes (1.34 ± 0.25 μmol/g; $p = 0.006$; Fig. 2), independent of the disease status ($p > 0.05$), age ($p > 0.05$) and sex ($p > 0.05$). To study the potential effect of medication, illness duration and smoking, we first correlated [GSH_{mPFC}] with CPZ equivalents and disease duration. No significant correlations were found between them ($p > 0.05$). [GSH_{mPFC}] were further compared between smokers and non-smokers, no significant difference was revealed ($p > 0.05$). Including smoking as one covariant showed no effect on the result and [GSH_{mPFC}] association with GCLC polymorphism still remained significant ($p = 0.006$).

To decipher whether blood GSH reflects cerebral GSH, we studied the relationship between them. No correlation was found between [GSH_B] and [GSH_{mPFC}] (Supplementary Fig. 1s) and [GSH_B] were not affected by disease, age, sex and GCLC polymorphism (Supplementary Table 2s).

Relationship between [GSH_{mPFC}] and [Glu_{mPFC}]

We revealed that [GSH_{mPFC}] were affected by GCLC polymorphism and additionally GSH may serve as a reservoir for neuronal Glu [17], which is involved in the NMDA hypofunction hypothesis of schizophrenia. Therefore, we evaluated [Glu_{mPFC}] (CRLB of 2.0 ± 0.5%) between patients and controls in GCLC low-risk and high-risk group respectively and revealed a 8 % reduction of [Glu_{mPFC}] in GCLC low-risk patients relative to low-risk controls ($p = 0.01$), while no difference was found between high-risk genotypes in controls and patients (Fig. 3A). In GCLC low-risk controls, [Glu_{mPFC}] positively correlated with [GSH_{mPFC}] ($r = 0.5133$, $p = 0.010$; Fig. 3B); however this correlation was absent in low-risk genotypes in patients. Interestingly, correlations were observed neither in controls nor in patients with high-risk genotypes (Fig. 3C).

Correlation between brain GSH and peripheral redox enzymatic activities

To test the association between cerebral GSH and peripheral redox indices, we investigated enzymatic activities of GPx and GR in blood cells, and their correlations with [GSH_{mPFC}]. No significant differences were observed for GPx and GR activities between patients and controls,

nor between GCLC high-risk and low-risk genotype (Supplementary Table 2s). Female subjects showed higher GR activities than male subjects (p = 0.015, Table 2s), which is consistent with [42], therefore, following analyses were performed separately for each sex group.

[GSH_{mPFC}] were positively correlated with GPx activities in male controls (Fig. 4A; r = 0.5543, p = 0.021). In contrast, in male patients an inverse correlation was obtained between [GSH_{mPFC}] and GPx activities (Fig. 4D; r = -0.4899, p = 0.039). There is a trend of a positive correlation between GR activities and [GSH_{mPFC}] in male controls (Fig. 4B; r = 0.3973, p = 0.114) but not in male patients (Fig. 4E; r = 0.0111, p = 0.966). The ratio of GPx/GR activity, which reflects the regulation of redox status, tends to correlate negatively with [GSH_{mPFC}] in male patients (Fig. 4F; r=-0.4760, p=0.0534), whereas such correlation was absent in male controls (Fig. 4C). We further tested the significance of the difference between patients and controls for the correlation coefficients of [GSH_{mPFC}] with GPx activities, and with GPx/GR ratio. Patients and controls demonstrated significant differences in correlation coefficients of [GSH_{mPFC}] with GPx activities (z=2.07, p=0.002) and with GPx/GR ratio (z=2.18, p=0.029). For female controls or patients, we did not observe any significant correlations (Supplementary Fig. 2s).

Discussion

In this study, we assessed [GSH_{mPFC}] of 25 early psychosis patients and 33 control subjects. We showed that GAG-TNR polymorphisms in GCLC, which have been previously linked to schizophrenia, are associated with [GSH_{mPFC}] irrespectively of disease status. Additional analyses also revealed that correlation between cortical GSH levels and peripheral redox marker, i.e. blood cells GPx enzymatic activities, differed in patients and controls: their correlation is positive in controls and negative in patients. Low [GSH_{mPFC}] are associated with low peripheral oxidation status in controls but with high oxidation status in patients, suggesting a dysregulation of GSH homeostasis under oxidative conditions. Furthermore, in GCLC low-risk genotypes, [Glu_{mPFC}] are lower in patients as compared to controls, while it is not the case for GCLC high-risk genotypes. This suggests a predominant pathogenic role of glutamatergic system impairments in GCLC low-risk genotypes.

In vivo measurement of cerebral GSH

The *in vivo* measurement of GSH concentration in human brain using (short-TE) ^{1}H MRS is challenging because of its low concentration and strong spectral overlap mainly with resonances

e.g. originated from glutamate and glutamine. Therefore, spectral editing schemes [43-45] combined with localization sequences have been developed for the detection of GSH. Although an editing approach provides a resolved measurement of GSH, several downside aspects remain, such as signal reduction induced by editing efficiency [44], sensitive editing performance to the frequency drift of the narrow band editing pulse [46], T_2 relaxation assumption for quantification and restricted number of measureable metabolites [44]. As an alternative approach, short-TE MRS has been employed to detect GSH at 3T and above [11, 29, 47, 48]. The main benefit of short-TE MRS, apart from GSH measurement, is the possibility to simultaneously assess a large number of metabolites. Nevertheless, due to spectral overlap, GSH quantification relies on deconvolution methods such as LCModel [34]. In the current study, we performed GSH measurement by short-TE ^{1}H MRS to obtain other metabolic information, e.g. Glu, in parallel. A previous validation study of Terpstra et al. compared GSH measurements using both, MEGA-PRESS and short-TE STEAM methods, at 4T and demonstrated a good agreement between these two methods [11]. Recently, Wijtenburg et al. showed an excellent reproducibility of GSH measurement using short-TE MRS at 3T [48]. We further evaluated the accuracy and precision of GSH measurement by the short-TE MRS method at 3T showing that comparable spectral quality in terms of SNR and linewidth would allow the accurate measurement of GSH changes between groups [49]. Therefore, the matched spectral quality is a prerequisite for the detection of GSH alterations. This requirement was well satisfied in this work as judged from equivalently high spectral quality of both patient and control groups.

Association of [GSH_{mPFC}] with GCLC polymorphism

The current study provided the original finding that GCLC high-risk genotypes are associated with low [GSH_{mPFC}]. The functional impact of short GAG repeats on GCLC regulation is still being debated: at the molecular level, a recent study suggested that the length of the TNR modulates mRNA translation [50], but it is controversial whether longer repeats increase or decrease GCLC and subsequently GSH levels. Interestingly, Nichenametla et al. suggest that the effect might be cell and tissue specific. Indeed, *in vitro* [50], in tumor cell lines [51], and in mononuclear blood cells [50], the 7/7 genotype (i.e. GCLC low-risk) is associated with low GSH and low GCL activity as compared to 9/9 genotype (i.e. GCLC high-risk). But in other systems, such as red blood cells or fibroblasts, submitted to oxidative stress the regulation is opposite: 7/7 genotype display more GSH and higher GCL activity than 9/9 genotype [14, 50, 52]. Similarly, we now show that GCLC low-risk genotypes (7/7 and 7/9) are associated with higher [GSH_{mPFC}]

than high-risk genotypes (9/9, 8/8, 8/9, 7/8). Therefore, our study highlights which type of regulation is linked with GAG repeats in cerebral cortex. Since GCLC high-risk genotypes were more frequent in patients [14], this result may thus clarify the potential implication of GCLC high-risk genotypes in psychiatric diseases.

In addition, this polymorphism in GCLC could be a plausible reason for not revealing [GSH_{mPFC}] changes in the patient cohort of the current study, i.e. group differences depend on the proportion of the various GAG-TNR genotypes. Indeed, in contrast to the larger cohort of patients with chronic schizophrenia previously studied, in which the GCLC high-risk genotypes were more frequently observed in the disease group [14], this is not the case for the present small sample in which the percentage of high-risk controls subjects (27%) was similar to that of EP patients (31%). Importantly, this could thus be an explanation for inconsistent results observed for brain GSH levels in schizophrenia patients, i.e. essentially different GAG-TNR genotypes distribution in the very small cohorts of previous studies leading to discrepancies. In this regard, it is important to note that allele frequencies vary strongly with the ethnic background and therefore not controlling for this factor may induce bias [52, 53]. In the context of a redox dysregulation hypothesis in schizophrenia pathophysiology [2, 3], the presence of GCLC high-risk genotypes in the control population suggests the possibility of a protective factor in healthy subjects carrying these variants.

Moreover, stratification of patients based on this genetic marker may help identifying distinct pathogenic mechanisms in GCLC low-risk *vs* high-risk schizophrenia patients. Indeed, in GCLC low-risk individuals [Glu_{mPFC}] are lower in patients as compared to controls, while it is not the case in GCLC high-risk genotypes (Fig. 3A). Furthermore, a positive correlation was found between [Glu_{mPFC}] and [GSH_{mPFC}] in GCLC low-risk controls, but not in low-risk patients (Fig. 3B). This contrasts with GCLC high-risk genotypes, in which the tight correlation between [GSH_{mPFC}] and [Glu_{mPFC}] is absent both in controls and patients (Fig. 3C). However the latter result was based on a small number of high-risk subjects; a relationship between brain GSH and Glu levels may emerge with a larger number of subjects. Together, these data suggest that aberrant functions of enzymes in the γ-glutamyl cycle or other glutamatergic system impairments may be critical, at least in GCLC low-risk patients. Note that Glu and GSH were both quantified using water as an internal reference, therefore, water can be a potential driving factor in the correlation between GSH and Glu. However, such effect should be general for all

groups, yet we did not see a correlation e.g. in low-risk patients, suggesting that such an effect is likely of limited importance.

The present study did not reveal altered [GSH_{mPFC}] in early psychosis patients and therefore contrasts with findings in patients with chronic schizophrenia[5]. Apart from the genetic factor mentioned above, other hypothesis can be mentioned 1) GSH measurement was performed under resting conditions and alterations that can be reliably measured may only appear under particular conditions, such as psychological stress, which leads to oxidative stress at cellular levels [54]. Indeed, lower [GSH] in fibroblasts of GCLC high-risk subjects as compared to low-risk genotypes were only observed under oxidative stress conditions, suggesting a deficit in the induction of regulatory mechanisms or adaptive responses. 2) GSH concentration determination by MRS does not allow the discrimination of GSH in the neuronal, glial or extracellular pools [55]. As [GSH] is about ten times higher in astrocytes than in neurons, a differential neuronal regulation might be masked. 3) Early psychosis patients might have an intermediary phenotype between control subjects and patients with chronic schizophrenia. There could be a stage specificity for oxidative stress in the brain: alterations of cerebral [GSH] might be more prominent in chronic phase of the disease, while soluble superoxide dismutase-1 defects have recently been suggested to be an early phase marker [56]. Importantly, our data indicates that medication and smoking are probably not biases, as we did not find a correlation between [GSH_{mPFC}] and CPZ equivalents, and as a covariant smoking has no effects on [GSH_{mPFC}] and the current results. There is a possibility that antipsychotics may have antioxidant effect and raise GSH to levels comparable to healthy controls. However no difference in [GSHmPFC] between medicated (n=20) and few non-medicated patients (n=5) was observed (p=xxx). The medication effect on cerebral GSH levels has never been documented, thus requiring further investigation.

Dysregulated GSH redox activity in patients

Glutathione is critically involved in redox regulation: A balanced activity of GPx to eliminate peroxides by metabolizing GSH and of GR to recycle GSH is crucial to maintain GSH homeostasis (see fig. 1s). Disturbed GSH redox coupling with the antioxidant defense system was suggested in the *postmortem* caudate, supported by the lack of correlations between GSH and GPx or GR in patients [6]. Our study indicates similar defects of redox regulation *in vivo* in early psychosis (Fig. 4). In male subjects, blood GPx activities and brain GSH levels correlate

negatively in patients (Fig. 4D), but positively in controls (Fig. 4A) with a significant difference between the two correlation coefficients. Similarly, we observed a trend of inverse correlation between blood GPx/GR enzymatic ratio and brain GSH levels in patients (Fig. 4F) but not in controls (Fig. 4C), with a significant difference between the two correlation coefficients. On the other hand, blood GR activities and brain GSH levels were not correlated in both patients and controls. These correlation analyses of brain GSH levels with blood GPx and GPx/GR activities imply a proper regulation of the redox balance in controls and a disrupted redox homeostasis in patients. Specifically, low brain GSH levels are associated with low peripheral oxidation status in controls but with high oxidation status in patients, as indicated by their high blood GPx activities, pointing to a dysregulated GSH homeostasis under oxidative conditions in early psychosis patients.

Lack of link between peripheral and central GSH levels

The lack of relationship between [GSH_B] and [GSH_{mPFC}] is consistent with the reported lack of significant transport system for GSH across the blood brain barrier [57]. Moreover peripheral GSH levels measurements showed large discrepancies in the literature due to not only differences in analytical methodologies but also in testing materials (whole blood vs. serum vs. blood cells vs. plasma), exposure to medication (drug naïve vs. drug withdrawal vs. medicated), stages of the disease (early psychosis vs. chronic or active vs. remission phase), lifestyle or dietary pattern [1]. It is also important to note that blood GSH levels are susceptible to artefactual oxidation during preparation and longtime storage of samples, unless special care is taken to prevent it immediately after blood collection (cold conditions, thiol blocking reagents…), measures difficult to guarantee in psychiatric clinical settings. To our knowledge, the present study is the first allowing a comparison between [GSH_B] and [GSH_{mPFC}] revealing that they are not correlated. This suggests that peripheral GSH levels unlikely reflects brain levels, in keeping with Wu et al[58] and the reported predominant liver origin of blood GSH[59, 60]. Raffa et al. reported decreased plasma GSH levels in drug naïve first episode patients [9, 10]. On the other hand, in the current study in which maximal care was taken to minimize artefactual factors during blood collection and processing, no difference was found in total blood GSH levels (blood cells + plasma) of medicated patients in the early phase of psychosis versus matched controls. Indeed, GSH levels quantification in total blood was chosen instead of plasma levels as the latter are susceptible to more variability due to the interference of high intracellular GSH due to hemolysis[61]. In regards to relationship to GCLC genotypes, we did not observe

differences in [GSH_B] between long and short repeats, which may be ascribed to the fact that we assessed the free GSH instead of both free and protein bound GSH as measured in [50]. This result is consistent with our previous study in chronic SZ patients[61] showing no link between blood GSH levels and GCLC genotypes (unpublished data).

Limitations

This study has some limitations. Firstly, given their later age of onset, the number of female patients is relatively small in the current study, thus affecting the generality of the result of regulation of GSH redox activity. Therefore future study with more female patients is required for further investigation of GSH redox regulation in female cohort. Secondly, GCLC high-risk subjects represent about 30% of the patient population[14]. As we do not know the genotype at the time of recruitment, it is challenging to recruit high number of GCLC high-risk subjects. Lastly, the potential medication effect is a general confounding factor for most schizophrenia patients studies, although we did not observe differences in GSH levels between medicated and unmedicated patients,

Conclusions

We show for the first time that GAG-TNR of GCLC gene affects [GSH_{mPFC}], with high-risk genotypes associated with low [GSH_{mPFC}]. These results extend to the central nervous system previous results in fibroblasts [14] and in red blood cells [50], therefore highlight that GCLC genotypes should be taken into account for the investigation of cerebral GSH levels in any pathology studies. Moreover, low [GSH_{mPFC}] are associated to low peripheral oxidation status in controls but with high oxidation status in patients, pointing to a dysregulated GSH homeostasis in early psychosis patients and paving the way for the search of central and peripheral markers of the disease. Lastly, GCLC polymorphisms and disease associated correlations between [Glu_{mPFC}] and [GSH_{mPFC}] may allow the stratification of patients and open new avenues to biomarker guided treatment strategies.

Acknowledgments

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Competing financial interests

The authors declare no competing financial interests.

Figure caption

Figure 1. (A) T_1-weighted MPRAGE images with the volume of interest (20x20x25mm^3) for MRS in the medial prefrontal cortex. (B) A representative ^{1}H MR spectrum acquired with the SPECIAL sequence at 3T (TE/TR = 6/4000 ms, number of averages = 148), the corresponding LCModel spectral fit, fit residual, macromolecules, baseline and individual metabolite fits including GSH.

Figure 2. [GSH_{mPFC}] are lower in GCLC high-risk genotypes (7/8, 8/8, 8/9 and 9/9; n = 17) than in low-risk genotypes (7/7 and 7/9; n = 41). From left to right: [GSH_{mPFC}] in low-risk genotype controls, low-risk genotype patients, high-risk genotype controls and high-risk genotype patients, respectively. Each horizontal bar indicates the mean value of the group. ** p=0.006.

Figure 3. (A) [Glu_{mPFC}] in GCLC low-risk controls (n=24), low-risk patients (n=17), high-risk controls (n=9) and high-risk patients (n=8). The bars represent mean values of each data group. * $p<0.05$. Different associations between [GSH_{mPFC}] and [Glu_{mPFC}] in GCLC low-risk (B) and high-risk (C) subjects. In GCLC low-risk controls, [GSH_{mPFC}] positively correlated with [Glu_{mPFC}]. The correlations between [GSH_{mPFC}] and [Glu_{mPFC}] were absent in GCLC low-risk patients, high-risk controls and high-risk patients.

Figure 4. Correlations between [GSH_{mPFC}] and blood redox enzymatic activities of GPx, GR and ratio GPx/GR in male controls (n=17) and in male patients (n=17). [GSH_{mPFC}] were positively correlated with GPx activities in controls (A). Conversely, in patients [GSH_{mPFC}] were negatively correlated with GPx activities (D). There is a trend of positive correlation between GR activities and [GSH_{mPFC}] in controls (B) but not in patients (E). The ratio of GPx/GR activity, reflecting the regulation of redox status, correlated negatively with [GSH_{mPFC}] in patients (F) not in controls (C).

Table 1 Demographic characteristics of controls and early psychosis patients.

	Controls (n=33)	Patients (n=25)	p value
Age, years (mean ± s.d.)	25.4 ± 4.5	24.8 ± 6.1	n.s[a]
Sex, Male/Female	18/15	18/7	n.s[b]
Ethnicity	Caucasian	Caucasian	-
Education of parents, years (mean ± s.d.)	14.4 ± 3.6	15.1 ± 4.6	n.s[a]
Cigarettes, smokers/non-smokers	4/29	13/12	0.001[b]
Handedness, Right/Left/Ambidextrous	28/5/0	22/2/1	n.s[c]
Illness duration, days (mean ± s.d.)	-	933 ± 867	-
CPZ equivalents, mg (mean ± s.d.)	-	325 ± 317[d]	-
Antipsychotic medications			
Amisulpride		3	
Aripiprazole		5	
Clozapine		1	
Olanzapine		2	
Paliperidone		1	
Quetiapine		5	
Risperidone		3	
None		5	

[a]Unpaired two tailed Student *t* test; [b]chi-square; [c]fisher's exact test; [d]five patients without antipsychotic medication; CPZ equivalents, chlorpromazine equivalents; n.s., not significant ($p > 0.05$), s.d., standard deviation.

References

1. Do KQ, Bovet P, Cabungcal JH, Conus P, Gysin R, Lavoie S, Steullet P, Cuenod M. Redox Dysregulation in Schizophrenia: Genetic Susceptibility and Pathophysiological Mechanisms. *Handb Neurochem Mol Neurobiol*. 3rd ed: Springer; 2009.

2. Do KQ, Cuenod M, Hensch TK. Targeting Oxidative Stress and Aberrant Critical Period Plasticity in the Developmental Trajectory to Schizophrenia. *Schizophrenia bulletin* Jul 2015;41(4):835-846.

3. Steullet P, Cabungcal JH, Monin A, Dwir D, O'Donnell P, Cuenod M, Do KQ. Redox dysregulation, neuroinflammation, and NMDA receptor hypofunction: A "central hub" in schizophrenia pathophysiology? *Schizophr Res* Jul 4 2014.

4. Yao JK, Keshavan MS. Antioxidants, redox signaling, and pathophysiology in schizophrenia: an integrative view. *Antioxid Redox Signal* Oct 1 2011;15(7):2011-2035.

5. Do KQ, Trabesinger AH, Kirsten-Kruger M, et al. Schizophrenia: glutathione deficit in cerebrospinal fluid and prefrontal cortex in vivo. *Eur J Neurosci* Oct 2000;12(10):3721-3728.

6. Yao JK, Leonard S, Reddy R. Altered glutathione redox state in schizophrenia. *Disease markers* 2006;22(1-2):83-93.

7. Altuntas I, Aksoy H, Coskun I, Caykoylu A, Akcay F. Erythrocyte superoxide dismutase and glutathione peroxidase activities, and malondialdehyde and reduced glutathione levels in schizophrenic patients. *Clinical chemistry and laboratory medicine : CCLM / FESCC* Dec 2000;38(12):1277-1281.

8. Mico JA, Rojas-Corrales MO, Gibert-Rahola J, et al. Reduced antioxidant defense in early onset first-episode psychosis: a case-control study. *BMC psychiatry* 2011;11:26.

9. Raffa M, Atig F, Mhalla A, Kerkeni A, Mechri A. Decreased glutathione levels and impaired antioxidant enzyme activities in drug-naive first-episode schizophrenic patients. *BMC psychiatry* 2011;11:124.

10. Raffa M, Mechri A, Othman LB, Fendri C, Gaha L, Kerkeni A. Decreased glutathione levels and antioxidant enzyme activities in untreated and treated schizophrenic patients. *Prog Neuropsychopharmacol Biol Psychiatry* Oct 1 2009;33(7):1178-1183.

11. Terpstra M, Vaughan TJ, Ugurbil K, Lim KO, Schulz SC, Gruetter R. Validation of glutathione quantitation from STEAM spectra against edited 1H NMR spectroscopy at 4T: application to schizophrenia. *Magma* Nov 2005;18(5):276-282.

12. Matsuzawa D, Obata T, Shirayama Y, et al. Negative correlation between brain glutathione level and negative symptoms in schizophrenia: a 3T 1H-MRS study. *PloS one* 2008;3(4):e1944.

13. Wood SJ, Berger GE, Wellard RM, Proffitt TM, McConchie M, Berk M, McGorry PD, Pantelis C. Medial temporal lobe glutathione concentration in first episode psychosis: a 1H-MRS investigation. *Neurobiol Dis* Mar 2009;33(3):354-357.

14. Gysin R, Kraftsik R, Sandell J, et al. Impaired glutathione synthesis in schizophrenia: convergent genetic and functional evidence. *Proc Natl Acad Sci U S A* Oct 16 2007;104(42):16621-16626.

15. Flatow J, Buckley P, Miller BJ. Meta-Analysis of Oxidative Stress in Schizophrenia. *Biological psychiatry* Sep 15 2013;74(6):400-409.

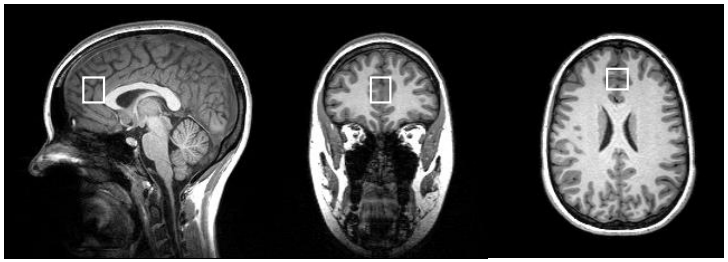
16. Rossi R, Milzani A, Dalle-Donne I, Giustarini D, Lusini L, Colombo R, Di Simplicio P. Blood glutathione disulfide: in vivo factor or in vitro artifact? *Clin Chem* May 2002;48(5):742-753.

17. Koga M, Serritella AV, Messmer MM, Hayashi-Takagi A, Hester LD, Snyder SH, Sawa A, Sedlak TW. Glutathione is a physiologic reservoir of neuronal glutamate. *Biochem Biophys Res Commun* Jun 17 2011;409(4):596-602.
18. Hanigan MH, Pitot HC. Gamma-glutamyl transpeptidase--its role in hepatocarcinogenesis. *Carcinogenesis* Feb 1985;6(2):165-172.
19. Meister A, Larsson A. Glutathione synthetase deficiency and other disorders of the γ-glutamyl cycle. *The Online Metabolic and Molecular Bases of Inherited Disease*: The McGraw-Hill Companies, Inc.; 1995.
20. Egerton A, Stone JM. The glutamate hypothesis of schizophrenia: neuroimaging and drug development. *Current pharmaceutical biotechnology* Jun 2012;13(8):1500-1512.
21. Yung AR, Yuen HP, McGorry PD, et al. Mapping the onset of psychosis: the Comprehensive Assessment of At-Risk Mental States. *Aust N Z J Psychiatry* Nov-Dec 2005;39(11-12):964-971.
22. Baumann PS, Crespi S, Marion-Veyron R, et al. Treatment and early intervention in psychosis program (TIPP-Lausanne): implementation of an early intervention programme for psychosis in Switzerland. *Early Interv Psychiatry* Aug 2013;7(3):322-328.
23. Lambert M, Conus P, Lambert T, McGorry PD. Pharmacotherapy of first-episode psychosis. *Expert opinion on pharmacotherapy* May 2003;4(5):717-750.
24. Andreasen NC, Pressler M, Nopoulos P, Miller D, Ho BC. Antipsychotic dose equivalents and dose-years: a standardized method for comparing exposure to different drugs. *Biological psychiatry* Feb 1 2010;67(3):255-262.
25. Gardner DM, Murphy AL, O'Donnell H, Centorrino F, Baldessarini RJ. International consensus study of antipsychotic dosing. *Am J Psychiatry* Jun 2010;167(6):686-693.
26. Nurnberger JI, Jr., Blehar MC, Kaufmann CA, et al. Diagnostic interview for genetic studies. Rationale, unique features, and training. NIMH Genetics Initiative. *Arch Gen Psychiatry* Nov 1994;51(11):849-859.
27. Gruetter R. Automatic, localized in vivo adjustment of all first- and second-order shim coils. *Magn Reson Med* Jun 1993;29(6):804-811.
28. Gruetter R, Tkac I. Field mapping without reference scan using asymmetric echo-planar techniques. *Magn Reson Med* Feb 2000;43(2):319-323.
29. Mekle R, Mlynarik V, Gambarota G, Hergt M, Krueger G, Gruetter R. MR spectroscopy of the human brain with enhanced signal intensity at ultrashort echo times on a clinical platform at 3T and 7T. *Magn Reson Med* Jun 2009;61(6):1279-1285.
30. Mlynarik V, Gambarota G, Frenkel H, Gruetter R. Localized short-echo-time proton MR spectroscopy with full signal-intensity acquisition. *Magn Reson Med* Nov 2006;56(5):965-970.
31. Tkac I, Andersen P, Adriany G, Merkle H, Ugurbil K, Gruetter R. In vivo 1H NMR spectroscopy of the human brain at 7 T. *Magn Reson Med* Sep 2001;46(3):451-456.
32. Tkac I, Starcuk Z, Choi IY, Gruetter R. In vivo 1H NMR spectroscopy of rat brain at 1 ms echo time. *Magn Reson Med* Apr 1999;41(4):649-656.
33. Mugler JP, 3rd, Brookeman JR. Three-dimensional magnetization-prepared rapid gradient-echo imaging (3D MP RAGE). *Magn Reson Med* Jul 1990;15(1):152-157.
34. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med* Dec 1993;30(6):672-679.
35. Schaller B, Xin L, Cudalbu C, Gruetter R. Quantification of the neurochemical profile using simulated macromolecule resonances at 3 T. *NMR Biomed* May 2013;26(5):593-599.

36. Van Leemput K, Maes, F., Vandermeulen D., Suetens P. Automated Model-Based Bias Field Correction of MR Images of the Brain. Paper presented at: IEEE Transactions on Medical Imaging, 1999.
37. Provencher S. LCModel & LCMgui User's Manual. Available at: http://s-provencher.com/pages/lcm-manual.shtml.
38. Walsh AC, Li W, Rosen DR, Lawrence DA. Genetic mapping of GLCLC, the human gene encoding the catalytic subunit of gamma-glutamylcysteine synthetase, to chromosome band 6p12 and characterization of a polymorphic trinucleotide repeat within its 5' untranslated region. *Cytogenet Cell Genet* 1996;75(1):14-16.
39. Long WK, Carson PE. Increased Erythrocyte Glutathione Reductase Activity in Diabetes Mellitus. *Biochem Bioph Res Co* 1961;5(5):394-399.
40. Gunzler WA, Kremers H, Flohe L. Improved Coupled Test Procedure for Glutathione Peroxidase (Ec 1.11.1.9.) in Blood. *Zeitschrift fur klinische Chemie und klinische Biochemie* 1974;12(10):444-448.
41. Lohr JB, Flynn K. Smoking and schizophrenia. *Schizophr Res* Dec 1992;8(2):93-102.
42. Habif S, Mutaf I, Turgan N, Onur E, Duman C, Ozmen D, Bayindir O. Age and gender dependent alterations in the activities of glutathione related enzymes in healthy subjects. *Clinical biochemistry* Nov 2001;34(8):667-671.
43. Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous in vivo spectral editing and water suppression. *NMR Biomed* Oct 1998;11(6):266-272.
44. Terpstra M, Marjanska M, Henry PG, Tkac I, Gruetter R. Detection of an antioxidant profile in the human brain in vivo via double editing with MEGA-PRESS. *Magn Reson Med* Dec 2006;56(6):1192-1199.
45. Trabesinger AH, Weber OM, Duc CO, Boesiger P. Detection of glutathione in the human brain in vivo by means of double quantum coherence filtering. *Magn Reson Med* Aug 1999;42(2):283-289.
46. Harris AD, Glaubitz B, Near J, et al. Impact of frequency drift on gamma-aminobutyric acid-edited MR spectroscopy. *Magn Reson Med* Nov 11 2013;72(4):941-948.
47. Tkac I, Oz G, Adriany G, Ugurbil K, Gruetter R. In vivo 1H NMR spectroscopy of the human brain at high magnetic fields: metabolite quantification at 4T vs. 7T. *Magn Reson Med* Oct 2009;62(4):868-879.
48. Wijtenburg SA, Gaston FE, Spieker EA, Korenic SA, Kochunov P, Hong LE, Rowland LM. Reproducibility of phase rotation STEAM at 3T: focus on glutathione. *Magn Reson Med* Sep 2014;72(3):603-609.
49. Xin L, Gruetter R. Glutathione measurement using short-TE 1H MRS at 3T: accuracy and precision assessment. Paper presented at: Intl. Soc. Mag. Reson. Med. 23, 2015; Toronto, Ontario, Canada.
50. Nichenametla SN, Lazarus P, Richie JP, Jr. A GAG trinucleotide-repeat polymorphism in the gene for glutathione biosynthetic enzyme, GCLC, affects gene expression through translation. *FASEB J* Jul 2011;25(7):2180-2187.
51. Walsh AC, Feulner JA, Reilly A. Evidence for functionally significant polymorphism of human glutamate cysteine ligase catalytic subunit: association with glutathione levels and drug resistance in the National Cancer Institute tumor cell line panel. *Toxicological sciences : an official journal of the Society of Toxicology* Jun 2001;61(2):218-223.
52. Nichenametla SN, Ellison I, Calcagnotto A, Lazarus P, Muscat JE, Richie JP, Jr. Functional significance of the GAG trinucleotide-repeat polymorphism in the gene for the catalytic subunit of gamma-glutamylcysteine ligase. *Free radical biology & medicine* Sep 1 2008;45(5):645-650.

53. Willis AS, Freeman ML, Summar SR, Barr FE, Williams SM, Dawson E, Summar ML. Ethnic diversity in a critical gene responsible for glutathione synthesis. *Free radical biology & medicine* Jan 1 2003;34(1):72-76.
54. Do KQ, Cabungcal JH, Frank A, Steullet P, Cuenod M. Redox dysregulation, neurodevelopment, and schizophrenia. *Curr Opin Neurobiol* Apr 2009;19(2):220-230.
55. Dringen R, Hirrlinger J. Glutathione pathways in the brain. *Biol Chem* Apr 2003;384(4):505-516.
56. Coughlin JM, Ishizuka K, Kano SI, et al. Marked reduction of soluble superoxide dismutase-1 (SOD1) in cerebrospinal fluid of patients with recent-onset schizophrenia. *Mol Psychiatry* Jan 2013;18(1):10-11.
57. Dringen R. Metabolism and functions of glutathione in brain. *Prog Neurobiol* Dec 2000;62(6):649-671.
58. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. *J Nutr* Mar 2004;134(3):489-492.
59. Bartoli GM, Sies H. Reduced and oxidized glutathione efflux from liver. *FEBS Lett* Feb 1 1978;86(1):89-91.
60. Ookhtens M, Kaplowitz N. Role of the liver in interorgan homeostasis of glutathione and cyst(e)ine. *Seminars in liver disease* 1998;18(4):313-329.
61. Gysin R, Kraftsik R, Boulat O, et al. Genetic dysregulation of glutathione synthesis predicts alteration of plasma thiol redox status in schizophrenia. *Antioxid Redox Signal* Oct 1 2011;15(7):2003-2010.

a



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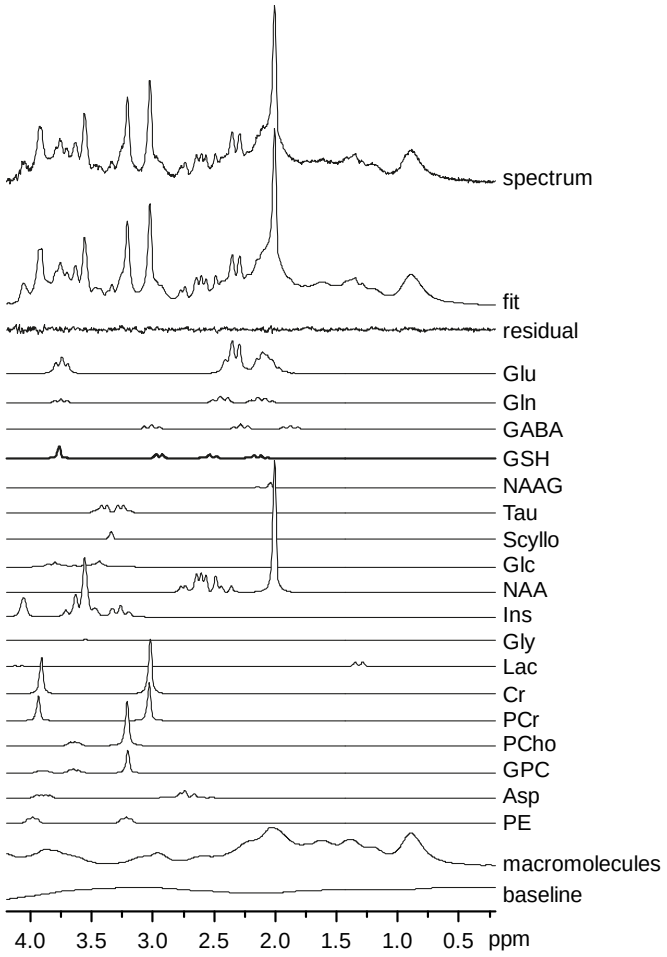
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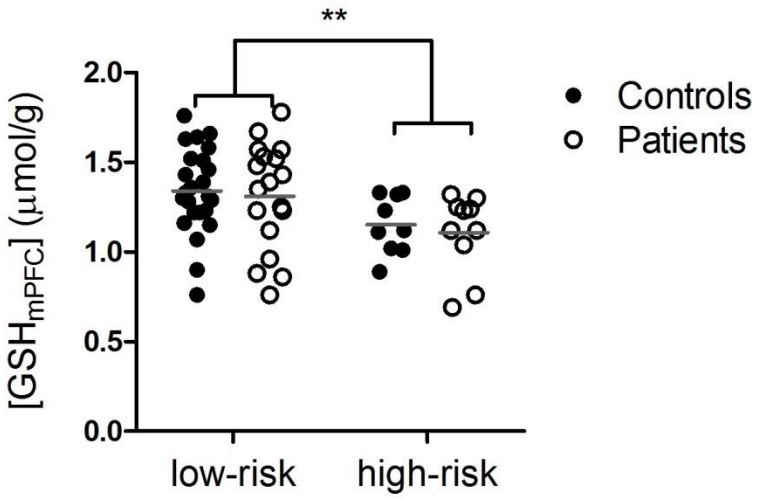
**
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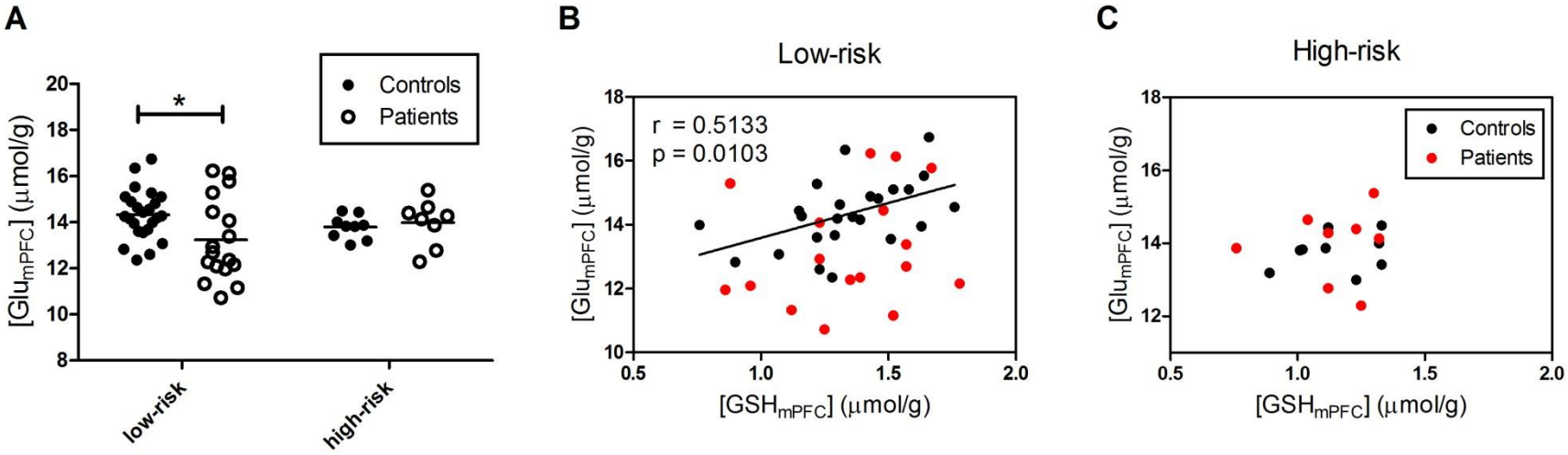
1.0
0.5
0.0
[GSH$_{mPFC}$] (µmol/g)
low-risk
high-risk
Controls
Patients


A
B
C
Controls
Patients
*
[Glu$_{mPFC}$] (µmol/g)
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high-risk
Low-risk
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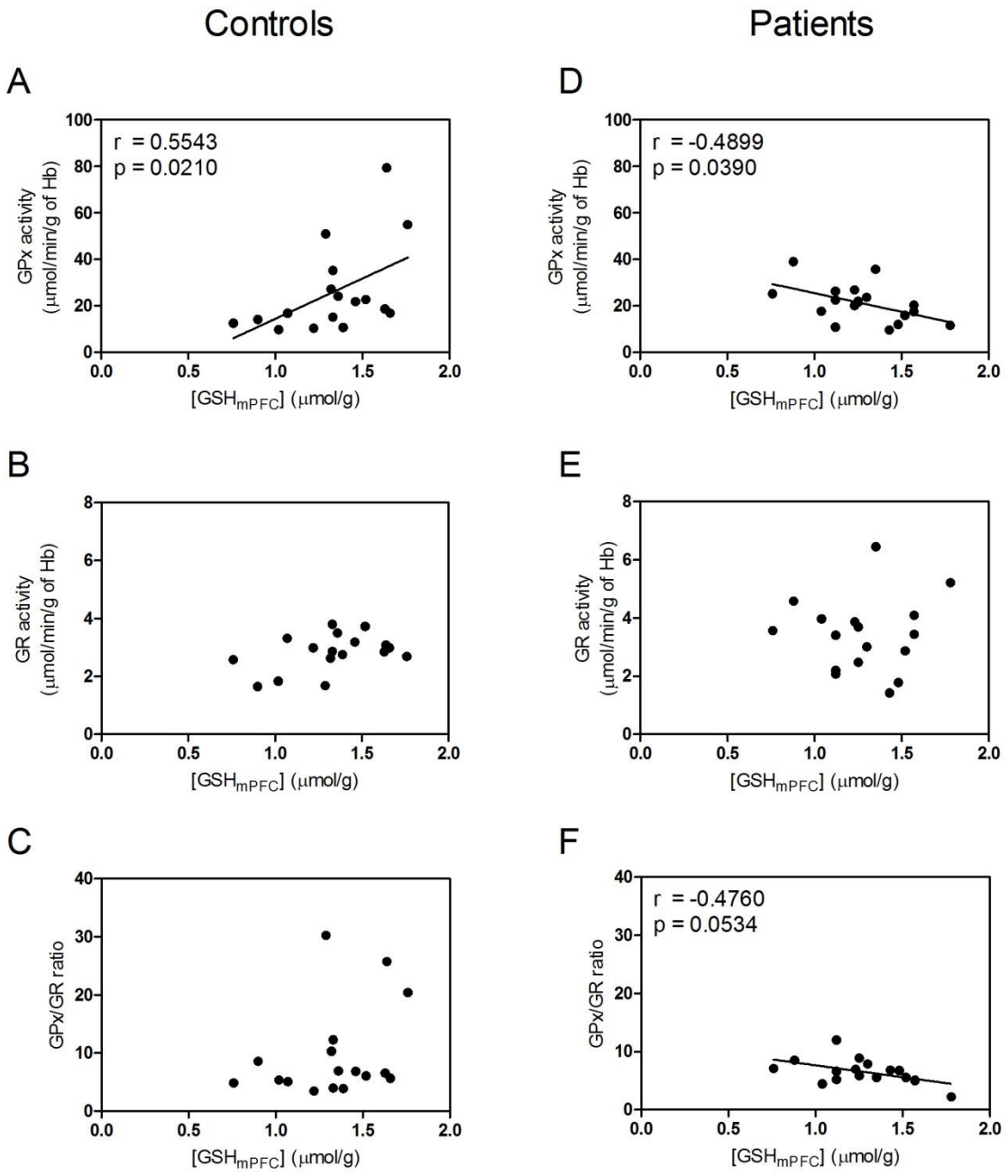
p = 0.0103
[GSH$_{mPFC}$] (µmol/g)
High-risk


Controls
Patients
A
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p = 0.0210
GPx activity (µmol/min/g of Hb)
[GSH$_{mPFC}$] (µmol/g)
D
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p = 0.0390
GPx activity (µmol/min/g of Hb)
[GSH$_{mPFC}$] (µmol/g)
B
GR activity (µmol/min/g of Hb)
[GSH$_{mPFC}$] (µmol/g)
E
GR activity (µmol/min/g of Hb)
[GSH$_{mPFC}$] (µmol/g)
C
GPx/GR ratio
[GSH$_{mPFC}$] (µmol/g)
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GPx/GR ratio
[GSH$_{mPFC}$] (µmol/g)

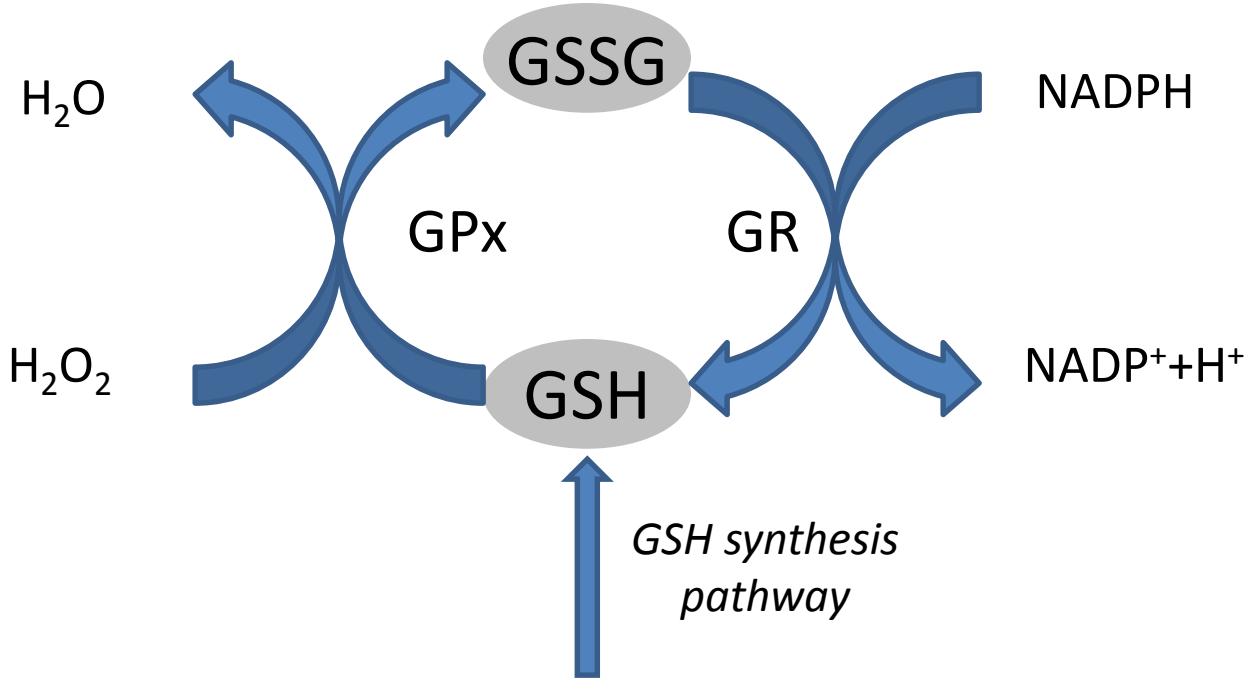

Figure 1s. Glutathione (GSH) redox cycle for eliminating reactive oxygen species(ROS). The ROS such as hydrogen peroxide can be reduced by GSH peroxidase (GPx) through converting GSH to oxidized GSH (GSSG). Then GSSG is reduced back to GSH by GSH reductase (GR).

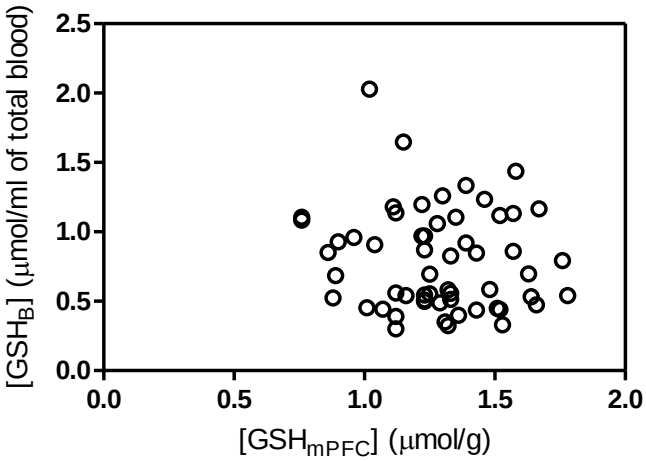

Figure 1s. No correlation between [GSH$_{mPFC}$] and [GSH$_B$] (n=57).

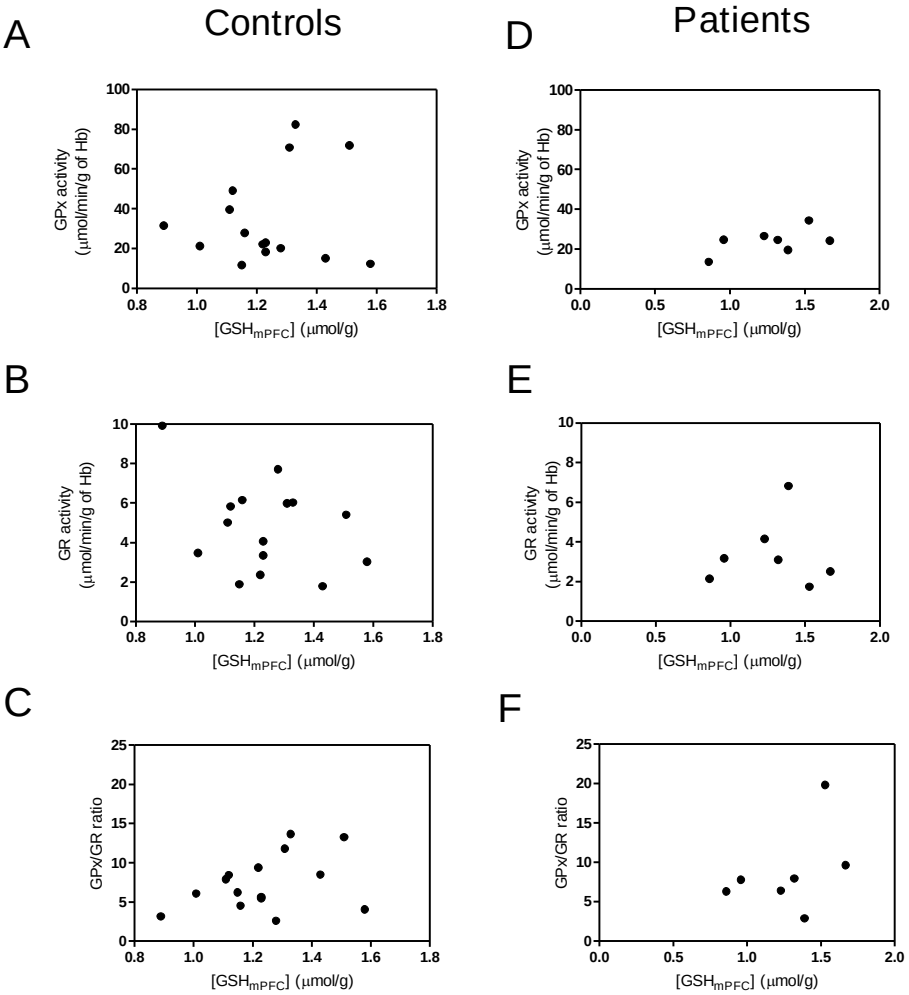

Figure 2s. No correlation between [GSH$_{mPFC}$] and blood enzymatic activities of GPx , GR and GPx/GR in female controls (n=15; A-C) and in female patients (n=7; D-F).

Table 1s. Tissue compositions of the MRS voxel.

Tissue fraction (%)	Controls (n=33)	Patients (n=25)	p value
White matter	23.5 ± 4.1	23.5 ± 4.4	n.s.
Grey matter	59.1 ± 3.5	57.5 ± 5.2	n.s.
Cerebrospinal fluid	17.3 ± 2.8	18.9 ± 4.6	n.s.

P values obtained from unpaired two tailed Student *t* test; n.s., not significant.

Table 2s. P values obtained from generalized linear model to test the effects of disease(controls versus patients), gender (male versus female), age and GCLC GAG-TNR polymorphism (low-risk versus high-risk) on blood GPx, GR activities, GPx/GR ratio and blood GSH. ***** p<0.05.

	GPx activity (μmol/min/g of Hb)	GR activity (μmol/min/g of Hb)	GPx/GR	$[GSH_B]$ (μmol/ml of total blood)
Disease	0.154	0.794	0.289	0.928
Gender	0.258	0.015*****	0.549	0.973
Age	0.077	0.076	0.154	0.246
GCLC polymorphism	0.583	0.867	0.444	0.592

Table 3s. mPFC Glu and GSH levels in different groups.

	Patients (n=25)	Controls (n=33)	Male (n=36)	Female (n=22)	High-risk (n=17)	Low-risk (n=41)	Treated patients (n=20)	Untreated patients (n=5)
GSH, μmol/g (mean ± s.d.)	1.28 ± 0.26	1.29 ± 0.23	1.30 ± 0.26	1.25 ± 0.22	1.15 ± 0.17	1.34 ± 0.25	1.31 ± 0.26	1.15 ± 0.24
Glu, μmol/g (mean ± s.d.)	13.47 ± 1.59	14.18 ± 0.98	13.87 ± 1.36	13.88 ± 1.28	13.87 ± 0.76	13.88 ± 1.49	13.74 ± 1.60	12.4 ± 1.13