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Effect of oxidative stress on white matter integrity in early psychosis

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Abstract

Biomarkers are still missing for schizophrenia. An objective measure of disease extend or severity would greatly improve the stratification of patients which is an important step for the research, development and administration of stage specific treatments. During the past years, the development of brain imaging has allowed to highlight that abnormalities of white matter diffusion properties can be detected early in the time course of the disease and are rapidly widespread, involving all cerebral lobes. In parallel, a growing number of animal studies suggested that redox dysregulation and more specifically a deficiency in brain glutathione (GSH), could lead to oligodendrocyte impairment, alterations of white matter integrity and eventually to the development of schizophrenia.

Here, we propose that peripheral markers of oxidative stress can predict the level of cerebral white matter anomalies in early psychosis patients. Precisely, we first suggest that blood levels of the main cerebral anti-oxidant, namely GSH, but also levels of its related enzymes (i.e glutathione peroxidase (GPx) and glutathione reductase (GR)) and precursors (i.e. cysteine and cysteine) will be reduced in early psychosis patients when compared to healthy controls. Secondly, we anticipate that GSH, GPx, GR, cysteine and cysteine blood levels will be well correlated with generalized fractional anisotropy (gFA) - a proxy for cerebral white matter integrity - computed from magnetic resonance imaging (MRI) diffusion brain scans of early psychosis patients.

We selected a subgroup of 49 psychotic patients for which we had blood and MRI data from the full sample of the TIPP (Treatment and early Intervention in Psychosis Program) and chronic schizophrenia cohort. 64 age and sex matched healthy controls were also recruited for a total of 113 participants aged between 18 and 35 years old.

Independent t-tests were performed to compare average gFA, cysteine, cystine, GPx, Gr and GSH levels between patients and healthy control groups. We also tested the correlations between average gFA values and blood marker values (i.e., cysteine, cystine, GPx, Gr, and GSH) in the two groups independently.

We first observed that cerebral white matter integrity estimated using average gFA was reduced in early psychosis patients when compared to healthy controls. Second, the activity levels of GPx, the enzyme responsible for the elimination of peroxides were lower in patients than in controls. In contrast, GSH and its precursors cystine and cysteine were higher in the group of patients. Third, we reported that GSH levels were positively correlated with gFA in healthy controls and that cysteine levels could predict the level of cerebral white matter anomalies in early psychosis patients.

Although further exploration is needed to better understand the precise relationships between peripheral blood antioxidants and alterations of brain anatomy, peripheral cysteine levels could represent a quick and economic way to stratify patients and assess the extent of their cerebral alterations.

Key words: Schizophrenia, generalized fractional anisotropy, redox dysregulation, glutathione, cysteine

Introduction

I. Schizophrenia

According to the fifth revision of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V)(1), schizophrenia is a mental disorder characterized by symptoms such as delusions, hallucinations, disorganized speech, disorganized or catatonic behaviour and negative symptoms (see table 1 for full diagnostic criteria)(1). Despite a relatively low incidence of 15.2 per 100'000 persons per year(2), this mental disorder affects 4 per 1000 persons worldwide (3). This moderate prevalence despite a relatively low incidence is probably due to chronicity or multiple relapses in the majority of patients and its common beginning early in life, during adolescence or young adulthood. Schizophrenia represents the 9th cause of global disability in 1990 (4) but the causes of this illness are still unknown and no cure exists. Biomarkers that could facilitate early detection of patients and etiological treatments are still missing. Similar to other psychiatric conditions, a combination of genetic and environmental factors may play a role in the pathophysiology of the disorder.

1) Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated). At least one of these must be (1),(2),(3).
   1. Delusions.
   2. Hallucinations.
   3. Disorganized speech (e.g., frequent derailment or incoherence).
   4. Grossly disorganized or catatonic behavior.
   5. Negative symptoms (i.e., diminished emotional expression or avolition).
2) For a significant portion of the time since the onset of the disturbance, level of functioning in one or more major areas, such as work, interpersonal relations, or self-care, is markedly below the level achieved prior to the onset (or when the onsets is in childhood or adolescence, there is failure to achieve expected level of interpersonal, academic, or occupational functioning).
3) Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of disturbance may be manifested by only negative symptoms or by two or more symptoms listed in Criterion A present in attenuated form (e.g., odd beliefs, unusual perceptual experiences).
4) Schizoaffective disorder and depressive or bipolar disorder with psychotic features have been ruled out because either 1) no major depressive or manic episodes have occurred concurrently with the active-phase symptoms, or 2) if mood episodes have occurred during active-phase symptoms, they have been present for a minority of the total duration of the active and residual periods of the illness.
5) The disturbance is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition.
6) If there is a history of autism spectrum disorder or a communication disorder of childhood onset, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations, in addition to the other required symptoms of schizophrenia, are also present for at least 1 month (or less if successfully treated).

Table 1 Diagnostic criteria of schizophrenia according to DSM-V(1).

II. Treatment and early intervention in psychosis program (TIPP-Lausanne)(5)

A staging model approach provides a better understanding of illnesses with complex development and allows to plan specific interventions. A good example of that is cancer therapy in which the TNM (Tumour, Nodule, Metastasis) classification directs to either a chirurgical approach, chemotherapy or to radiotherapy depending on disease extension(6). In the same way, the development of schizophrenia could be described in three stages: the prodrome, the first psychotic episode, and the chronic phase(7). This 3-stage model was initially developped by McGorry and colleagues(8). It is supported by neuroimaging findings suggesting a progression of the disease, at the level of brain anatomy, from the prodromal state to the first episode(9). Several clinical programmes focusing specifically on early interventions during the course of psychosis have been developed worldwide (10)(11)(12)(13)(14)(15).

In Lausanne, the treatment and early intervention in psychosis program (TIPP) was launched in 2004 with the aim to improve engagement and quality of treatment for early psychosis patients. 8 years later, more than 350 patients have been included in the program and 90 of them have participated in the neurobiological research, providing blood samples, skin biopsies and magnetic resonance imaging (MRI) scans. The delay between program entry and harvesting of these data varies between 2 and 24 months. Patients included in the TIPP program must satisfy selection criteria similar to those in other first-episode psychosis samples. They are between 18 and 35 years old. They never had any previous treatment with antipsychotics for more than 24 weeks. Finally, they have crossed the psychosis threshold according to Comprehensive Assessment of At-Risk Mental States (CAARMS) criteria that consist in a semi-structured interview in which the severity, the duration, the frequency, the relation with drug abuse and the date of apparition and ending of 28 items are listed (Table 2)(16). During the 3-year follow-up, patients can benefit of different kind of support. The outpatient's team, composed of nurses and social workers that collaborate with psychiatrists, help the patients by promoting engagement, psychoeducation, integration of the psychotic experience, relapse prevention and recovery. If a patient needs a closer monitoring, an intensive mobile team, called ACT (assertive community treatment) team, support him. It could be an alternative to hospital admission too. When it is unavoidable, patients are hospitalised while maintaining homogeneity in their ages and interests. Team members trained in early intervention principles follow them and contacts are kept with the outpatient's team.

UHR status
Group 1: Attenuated psychosis group
Subthreshold intensity: • Severity scale score of 3–5 on disorders of thought content subscale, 3–4 on perceptual abnormalities subscale and/or 4–5 on disorganized speech subscale of the CAARMS. • Frequency scale score of 3–6 on disorders of thought content, perceptual abnormalities and/or disorganized speech subscale of the CAARMS for at least 1week. OR • Frequency scale score of 2 on disorders of thought content, perceptual abnormalities and disorganized speech subscale of the CAARMS on more than two occasions. *Subthreshold frequency:* • Severity scale score of 6 on disorders of thought content subscale, 5–6 on perceptual abnormalities subscale and/or 6 on disorganized speech subscale of the CAARMS. • Frequency scale score of 3 on disorders of thought content, perceptual abnormalities and/or disorganized speech subscale of the CAARMS. (for both categories) • Symptoms present in past year and for not longer than 5 years.
Group 2: BLIPS group
• Severity scale score of 6 on disorders of thought content subscale, 5 or 6 on perceptual abnormalities subscale and/or 6 on disorganized speech subscale of the CAARMS. • Frequency scale score of 4–6 on disorders of thought content, perceptual abnormalities and/or disorganized speech subscale. • Each episode of symptoms is present for less than 1 week and symptoms spontaneously remit on every occasion. • Symptoms occurred during last year and for not longer than 5 years.
Group 3: Vulnerability
• Family history of psychosis in first degree relative OR schizotypal personality disorder in identified patient. • 30% drop in GAF score from premorbid level, sustained for 1 month. • Change in functioning occurred within last year and maintained at least 1 month.
Psychotic disorder threshold
• Severity scale score of 6 on disorders of thought content subscale, 5 or 6 on perceptual abnormalities subscale and/or 6 on disorganized speech subscale of the CAARMS. • Frequency scale score of greater than or equal to 4 on disorders of thought content, perceptual abnormalities and/or disorganized speech subscale. • Psychotic symptoms present for longer than 1 week.

Table 2 CAARMS-defined ultra high risk and psychotic disorder threshold criteria. (BLIPS = Brief Limited Intermittent Psychotic Symptoms; GAF = Global Assessment of Functioning; UHR = ultra high risk)(16)

III. Biomarkers

A biomarker is a measured or evaluated variable used as indicator of a normal biological process, a physiopathology process or a pharmacological response to a therapeutic intervention(17). To be useful for clinical practice, a biomarker must be sensitive, specific and needs to have an acceptable predictive value(18). In the case of cancer treatment, the use of biomarkers is already individualized and allows the development of novel drugs that target specific molecular-signalling pathways related to genetic mutations(19). Unfortunately, the nosology of biomarkers is still inaccurate

in neuropsychiatric diseases in which the path from genes to behaviors is influenced by a lot of complex interactive links that remain unknown(18). Moreover, there is no gold standards for psychiatric diagnoses that are only based on combinations of symptoms(18). Davis and al(20), propose to classify biomarkers into 6 distinct categories: i. biomarkers of risk, ii. diagnostic or trait biomarkers, iii. state or acuity biomarkers, iv. stage biomarkers, v. treatment response biomarkers and vi. prognostic biomarkers (table 3). In the context of schizophrenia, our poor understanding of the disease doesn't allow us to have biomarkers in each category. Several biomarkers have been suggested for risk (e.g. general cognitive ability (21)) and state (e.g. brain-derived neurotrophic factor (22)), but diagnosis biomarkers allowing to diagnose schizophrenia do not exist yet (23). A biological signature of schizophrenia can be identified in the blood serum of patients through 34 analytes (such as ICAM 1, GST, betacellulin), but some of these are present in other pathologies such as major depressive disorder or bipolar disorder(24) and they lack specificity. Abi-Dargham & Horga(18) highlight the potential of neuroimaging techniques to be used as diagnostic and predictive biomarkers. However, such techniques still need to demonstrate sufficient precision and reliability, as well as their capacity to predict a clinical diagnosis or outcome. Moreover, the development of such precise and reliable biomarkers would require the understanding of underlying mechanisms of disease dimensions, symptoms and their progression.

Biomarkers of risk	A measurable characteristic that predicts the risk of an individual developing a neuropsychiatric disorder to allow for identification of at-risk individuals
Biomarkers of diagnosis/trait	A measurable characteristic that reflects the presence of a disease state and allows for definitive diagnosis of disease, ideally with no overlap between disorders or disruption by confounders
Biomarkers of state/acuity	A measurable characteristic that reflects the severity of a particular disease process, which delineates the present acuity and severity of an individual disease episode
Biomarkers of stage	A measurable characteristic reflecting extant classifications of staging, which indicates an individual's stage of illness
Biomarkers of treatment response	A measurable characteristic capable of indexing the probability of response to a given treatment to potentially assist clinicians in selecting therapeutic options for an individual patient
Biomarkers of prognosis	A measurable characteristic that could predict the likely course and outcome of an illness

Table 3 overview of the proposed classification theme with biomarker types and their definition according to Davis et al. (20)

IV. Introduction to diffusion magnetic resonance imaging (MRI)

Introduced in the mid-1980s, diffusion MRI allows us to reveal the microarchitecture of the tissues measuring the random motion of water molecules(25). This method is useful for brain imaging due to the fibrillar structure of neuronal tissue(26). Indeed, the alignment of axons surrounded by glial cells and mainly organised in bundles result in micrometric movements of water molecules parallel to the axons(26). Such diffusion is called anisotropic because the molecules move in a preferential direction (opposed to isotropic diffusion where they move in all directions)(figure 1). For example, we know that a bundle is anisotropic, if we need to know the direction of their fibres, a diffusion tensor, usually represented by an ellipsoid or an orientation distribution function, allows us to characterize diffusion in 3D space (figure 1)(26). It is possible to use fractional anisotropy (FA) to convert the diffusion tensor into a scalar value. This consists in a complex equation that compares each eigenvalue with the mean of all eigenvalues(26). Although FA is sensitive to a number of tissue properties (axonal ordering, axonal density, degree of myelinisation, water in extracellular space), it isn't specific to any of them(27). Moreover, this measure depends significantly on the way the axons are arranged in the voxel(27). Thus, differences in FA between two groups are useful only if data is proven to be robust, reliable and reproducible. Since the first use of diffusor tensing imaging (DTI) in schizophrenia in 1998, more than 200 studies have been published about the disease(28). Early studies have rapidly brought evidence for white matter impairments in patients(28). Moreover, several recent studies that have applied new methods and measures to address the issue of FA and suggest a major role of myelin in the diffusion abnormality of schizophrenia(28).

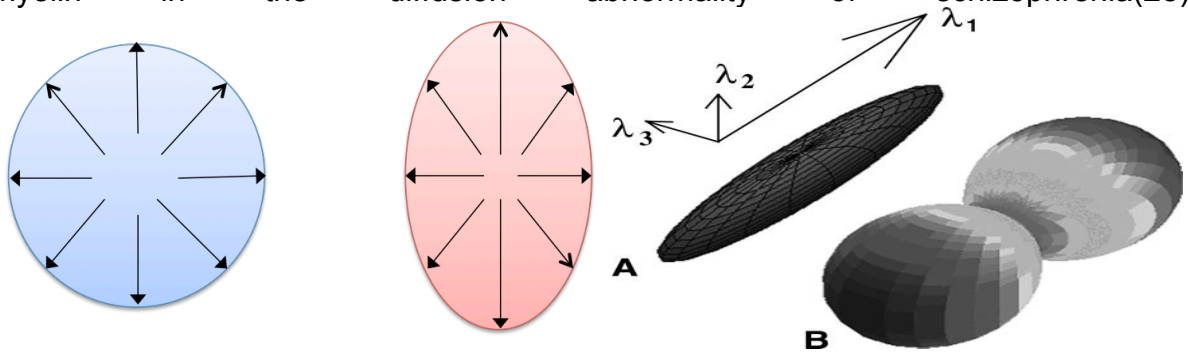

Figure 1 To left, the figure shows the difference between an isotropic diffusion (blue circle) and an anisotropic diffusion. To right, diagrams of diffusor tensors are represented as an ellipsoid (A) and as an orientation distribution function (B). Adapted from reference (26).

V. White matter impairments

Functional MRI (fMRI) findings converge to say that schizophrenia is associated with connectivity reductions, mainly in the frontal lobe(29). Despite the low number of investigations in early psychosis groups, this seems to apply across all stages of the disorder(29). As mentioned before, DTI is commonly used to study the disease and perhaps detect white matter impairments through myelin abnormalities(28). As in fMRI, these anomalies are also noticeable at all stages of the disease and concern the whole brain(29), with a preponderance in the white matter of the frontal and temporal lobe(30)(31)(32). A recent DTI study using one of the largest samples of patients with chronic schizophrenia demonstrated that more than 40% of the cerebral white matter is impaired and that 52% of the connections in the human connectome

are disrupted(33). In this research, the connectivity between central hubs seem to be more extensively disturbed than the rest of the brain(33). Whitford and al. suggested coupling DTI measures with event related potential (ERP) measures. They realized that the origin of these impairments were linked to defected axonal integrity and conduction velocity(34).

Many studies suggest that oligodendrocytes and myelinisation are impaired in schizophrenia and could participate in these alterations of brain morphology(35). The use of light and electron microscopy in post-mortem studies has demonstrated the presence of ultrastructural abnormalities of myelin in both, the prefrontal cortex (PFC) and caudate nucleus (35). With such methods, dystrophic and destructive changes in the oligodendrocytes were found in these regions(36) and a morphometric study has demonstrated the presence of damaged myelinated fibres in both grey and white matter(36). A decrease in the amount of oligodendroglial cells has also been mentioned, specifically in different layers of the PFC(37)(38)(39) and in the anterior thalamic nucleus(40).

Micro-array analyses have indicated that some cell-cycle pathways are disrupted in the anterior cingulate gyrus (ACG), these disruptions could lead to the oligodendroglial deficit observed in schizophrenia(41). Other studies using the same method, have shown a down-regulation of certain genes, such as the myelin-associated glycoprotein (MAG), implicated in the formation and maintenance of myelin sheaths and in the oligendodrocyte function(42)(43)(44).

Concerning animal studies, rodents help to explore the formation of mature oligodendrocytes and myelin sheaths(45). Moreover, genetic findings mentioned prior have allowed the development of animal models in order to study cerebral dysfunctions. MAG-knockout mice, missing one of the myelin-related genes, have altered layer III pyramid cells in the PFC(46). Although the mutant mice have normal spatial learning and memory abilities, anomalies in their behaviour are observed, such as the incapacity to maintain balance on a rotating cylinder compared to wild-type mice(46).

These different studies suggest a genetic origin and a neurodevelopmental component in white matter anomalies associated to schizophrenia.

VI. The redox dysregulation

Redox signalling has a key role in many cellular functions such as cell proliferation, regulation of cellular apoptosis or the maintenance of thiol redox potential in cells keeping sulfhydryl groups of proteins in the reduced form(47). Moreover, the glutathione (GSH) system (illustrated in figure 2) is very important for the cellular defence against reactive oxygen species (ROS)(47). When this system is overbooked by the ROS, macromolecular damage appears(48). This process is called “oxidative stress”. In this way, a redox dysregulation can affect cellular cycle, energy metabolism and neurotransmission(49).

An increasing number of studies propose that redox dysregulation is central in the pathophysiology of schizophrenia(50). Post-mortem cerebral tissues of patients have shown reduced levels of GSH and glutathione peroxidase (GPx), the enzyme that allows GSH to supply an electron to ROS (illustrated in figure 2), in the PFC(51). This finding has been confirmed in vivo with magnetic resonance spectroscopy (MRS); a brain imaging technology that can measure bioactive substances such as GSH(52)(53). This method also allowed to determine a negative correlation between

GSH levels and negative symptoms(52). A significant decreased level of total GSH in the cerebrospinal fluid as well as in the PFC has been observed in drug free patients(53).

Cysteine, a semi-essential amino acid, is the limiting precursor of GSH (illustrated in figure 2)(54)(55). The oxidized form, cystine, is stored in the serum and used to maintain the intracellular rate of GSH in case of oxidative stress (56). Consequently, a disturbance in cysteine or cystine may lead to the dysfunction of the antioxidant GSH system(57)(58). A case-control study effected by Yang and al. has observed an increased rate of cystin in the urine and a decreased rate in the serum of patients with schizophrenia compare to healthy controls(59). Recently, Wang and al. have found a correlation between the serum levels of cysteine and cognitive function in patients with schizophrenia(60). In the same study, the cysteine rates in the serum were higher in patients than in healthy controls(60). These discrepancies might be due to differences in analytical methodologies, testing materials (blood cells vs. plasma or serum), exposure to medication (naïve vs. drug withdrawal vs. medicated), stage of disease (acute vs. chronic or active vs. remission phase), lifestyle or dietary pattern, and origin of patients populations.

Glutamate cysteine ligase modifier (Gclm) and glutamate cysteine ligase catalase (Gclc) are the two genes for the key GSH-synthesizing enzyme: the glutamate cysteine ligase (GCL)(illustrated in figure 2). Studies about cultured skin fibroblasts from schizophrenia patients have discovered a strong association between schizophrenia and polymorphisms in these genes (61)(62). Gclc high risk genes are present in 30% of patients and are linked with lower Gclc protein expression, GLC activity and GSH concentration in the PFC(62)(63). Gclc and Gclm polymorphisms are also associated with modifications in the blood anti-oxidant system(64). Genotyping of different polymorphisms of the glutathione S-transferase, an enzyme used to detoxify products generated by the oxidative damages, in peripheral blood samples revealed an other predisposition for schizophrenia (65). Proteomics studies in the brain and peripheral tissues of patients have shown that mitochondrial function may be altered in the illness. This can lead to many perturbations among which an increased production of ROS(66).

Animal models such as Gclm-KO mice could allow us to study the behavioural and cognitive consequences of redox dysregulation in the context of psychiatric diseases(67). Indeed, in Gclm-KO mice, brain GSH rates are decreased(68) with some brain regions showing signs of oxidative stress(69). Moreover, early-life insults induce retarded maturation of parvalbumine interneurons in this model(69). Other mice models have demonstrated the role of some genes that are indirectly implicated in the redox dysregulation process such as DISC1(70). Finally, a study about transgenic mice has revealed the potential of the GSH precursor N-acetyl cysteine as a treatment of cognitive deficits(71).

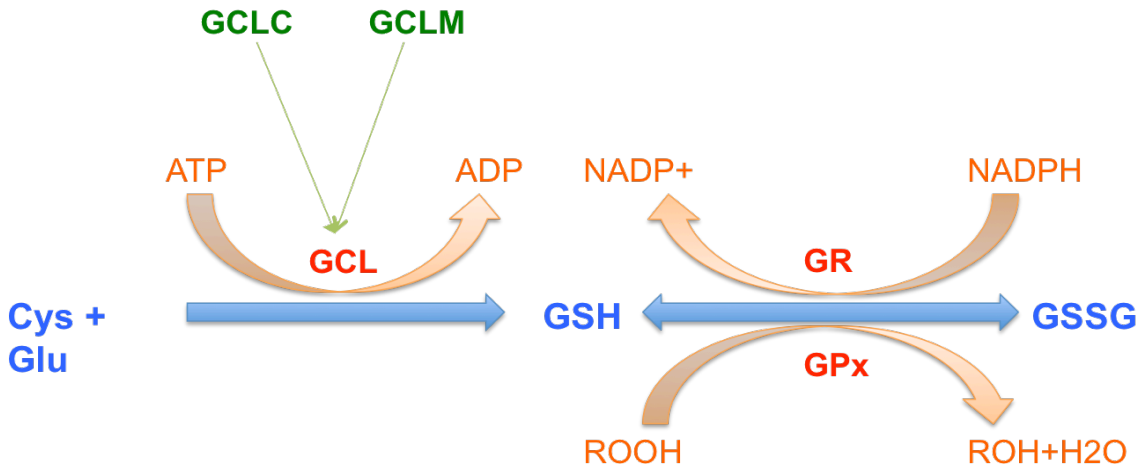

Figure 2 Reduced glutathione (GSH) supplies an electron to reactive oxygen species (ROS) and becomes an oxidized glutathione (GSSG). This transfer is facilitated by glutathione peroxidase (GPx). After that, GSSG is reduced in GSH. The glutathione reductase (GR) catalyses this reduction using NADPH as a cofactor(72). Glutamate cysteine ligase modifier (GCLM) and glutamate cysteine ligase catalase (GCLC) are two genes and subunits for the glutamate cysteine ligase (GCL) that is the key GSH-synthesizing enzyme using cysteine or cystine and glutamate. ROOH, organic peroxide; ROH, organic hydroxide.

VII. Effect of oxidative stress on white matter integrity

Using MRS and diffusion spectrum imaging (DSI) in early psychosis patients, a relationship between medial PFC GSH levels and white matter integrity has been observed in the cingulum bundle. This demonstrates that there may be a potential effect of oxidative stress on white matter integrity during cerebral development(73).

To understand the effect of oxidative stress on white matter integrity, many studies used cell cultures from rat brains. These researches allowed us to create a pathophysiologic model of oligodendrocyte differentiation. Indeed, oligodendrocytes are sensitive to oxidative stress due to their intrinsic properties and functions(74). They have a high metabolism and high iron rates, especially during the myelinisation process.(75)(45)(76). On the other hand, these cells contain surprisingly low antioxidant levels(77)(78). In stage-specific rat oligodendrocyte cultures, mature oligodendrocytes seem to have higher rates of antioxidant enzymes than their progenitors and therefore better resistance to oxidative stress(79). Moreover, glutathione levels were higher in differentiated oligodendrocytes, which were less susceptible to glutathione concentration decreases caused by oxidative stress than their progenitors(80).

The intracellular redox state affects oligodendrocyte differentiation and proliferation(81)(82). Oxidizing agents disrupt oligodendrocyte differentiation by two mechanisms(83). Firstly, the expression of key genes promoting this differentiation are decreased while genes known to inhibit differentiation are overexpressed(83). Secondly, global histone acetylation persists under oxidative stress conditions(83). At a molecular level of genetic expression, oxidizing agents disrupt a pathway of tyrosine kinase receptor signalling required for cell division, the platelet-derived growth factor receptor (PDGFR) pathway(81)(84). These pro-oxidative changes activate Fyn kinase, leading to a secondary activation of c-CBL ubiquitin ligase which inhibits the PDGFR pathway(81). In a post-mortem study effected in the PFC(85), an

increase in Fyn kinase rates has been found in the fibroblasts of early psychosis patients with high-risk Gclc genotypes(73).

In animal models such as Gclm-KO mice, the redox dysregulation generated seems to contribute to the impaired white matter observed during neurodevelopment(86). Indeed, the GSH deficit observed affects the ventricular size and the integrity of the fornix-fimbriae and anterior commissure(86). Interestingly, these mice have an increase in Fyn mRNA and protein expression in the developing ACC(73).

These observations strongly suggest that redox dysregulation plays a central role in developmental anomalies of oligodendrocytes and myelin disruptions which can be observed in schizophrenia(87)(74).

VIII. Aim and hypotheses

We propose that peripheral markers of oxidative stress can predict the level of cerebral white matter anomalies in early psychosis patients.

Precisely, we first suggest that blood levels of the main cerebral anti-oxidant, namely GSH, but also levels of its related enzymes (i.e GPx and glutathione reductase (GR)) and precursors (i.e. cysteine and cysteine) will be higher in healthy controls than in early psychosis patients.

Secondly, we anticipate that GSH, GPx, GR, cysteine and cysteine blood levels will be well correlated with fractional anisotropy values computed from MRI diffusion brain scans of early psychosis patients.

Methods

I. Participants

We selected a subgroup of 49 psychotic patients for which we had blood and MRI data from the full sample of the TIPP and chronic schizophrenia cohort. These young patients had crossed the psychosis threshold within the last 24 months and have never had any previous treatment with antipsychotics for more than 24 weeks. 64 age and sex matched healthy controls were also recruited for a total of 113 participants aged between 18 and 35 years old. (Table 4).

II. Lifestyle factors and medication

We used the body-mass index (BMI), the smoking level (i.e., number of cigarettes consumed per day) and the chlorpromazine equivalent (CPZ) to investigate the possible effects of lifestyle factors and medication on peripheral antioxidants levels, the. CPZ were calculated according to the defined daily dose (DDD) recommended by the WHO Collaborating Centre for Drug Statistics Methodology(88) and this for each medicated patient.

III. Images and white matter values acquisition

As previously described in the section *"Treatment and early intervention in psychosis program (TIPP-Lausanne)"*, the delay between program entry and MRI scanning varied between 2 and 24 months. All participants were scanned with the same MRI machine and acquisition sequences (Siemens Trio at CHUV). The diffusion spectrum

imaging (DSI) approach was used to estimate water diffusion properties in cerebral white matter. The diffusion tensors were converted into scalar values using generalized fractional anisotropy (gFA). Each gFA map was normalised to MNI standard space using non-linear registration procedures (FSL 5.0.7, http://fsl.fmrib.ox.ac.uk/fsl/ fslwiki/) and smoothed with a Gaussian kernel of SD = 1 mm. A white matter mask was created by thresholding FMRIB58_FA_1mm template from FSL at 2500. Average gFA within the white matter mask was then extracted for each participant using fslmeants command in FSL.

IV. Blood samples acquisition

The blood samples were collected at the same time-point as MRI brain scans. As in Gysin & al(64) and Baumann and al(89) studies, they were collected by venipuncture between 7 and 8:30 AM under restricted activity conditions and fasting from the previous midnight. 20 ml of blood were collected in Vacutainer-tubes coated with Li-heparinate (previously stored in ice) to be immediately centrifuged at 3'000 g and 4°C during 5 min. The blood cells retrieved were washed twice with 0,9% NaCl and the plasma was recovered and distributed in aliquots. Both samples were stocked at -80°C until analysis. The GSH rates, measured in 45 µl of whole blood, were quantified by a colorimetric approach using a diagnostic kit: the Glutathione Assay kit (San Diego, CA, USA)(64). The GR activity was measured by incubating 8 µl of haemolysed blood in a phosphate buffer solution (100 mM, pH 7.5) with EDTA (0.6 mM), oxidized GSH (3 mM), NADPH (0.25 mM), and Tert-butyl hydroperoxide (0.8 mM; Sigma-Aldrich). The GPx activity was determined by incubating 8 µl of haemolysed blood in a phosphate buffer solution (100 mM, pH 7.5) with EDTA (0.6 mM), oxidized GSH (3 mM), NADPH (0.25 mM), Gr (0.84 U ml−1; Sigma-Aldrich, St. Louis, MO, USA) and Tert-butyl hydroperoxide (0.8 mM; Sigma-Aldrich). Both of enzymatic activities were assessed by using a function of the decrease of NADPH measured at 340 nm and normalized to haemoglobin content(63)(90)(89). Cysteine was measured in the blood using high performance chromatography on plasma samples. Precisely, the thiol group was reduced from the protein by reaction with tris(2-carboxyethyl)phosphine. After that, a deproteinization with perchloric acid was effected to allow a derivatization of the thiol group with 7-fluorobenzofurazane-4-sulfonic acid (SBD-F). Finally, the SBD-F resulting from these reactions was analysed by HPLC and fluorometric detection(91)(64). Another technique was used to measure the cystine level in blood. This latter was deproteinized by adding 50 ml 5-sulfosalicylic acid (160 g=L) containing the internal standards to 200 ml plasma. After 10 min, a centrifugation of the solution was repeated 4 times at 14'600 g during 15 min. The result was stocked at -80°C until the next phase which consisted of injecting the supernatant into an amino acid analyser (Biochrom, 30+Model) to detect amino acids by postcolumn reaction with nihydrine.

V. Statistical analyses

Statistical analyses were performed using the 23th version of Statistical Package for the Social Sciences (SPSS). The means of age and BMI for the early psychosis patient group (PAT) and the healthy control group (HC) were compared using an independent t-test. The proportions of gender and current smoking status (i.e., smoker vs non-smoker) across the two groups were compared using a chi-square test. Independent t-tests were performed to compare gFA means, cysteine, cystine, GPx, Gr and GSH levels between PAT and HC groups. Distribution of variables for

each statistically significant difference was represented using box-plots. We used partial correlations to test for possible correlations between lifestyle factors (i.e., BMI and smoking level) or medication (i.e. CPZ) and blood biomarkers or gFA. We also tested the correlations between mean gFA values and blood marker values (i.e., cysteine, cystine, GPx, Gr, and GSH) in the PAT and HC groups independently. These correlations have been controlled for age and gender in HC group and for age, gender and CPZ in PAT group.

Results

Demographics

There were no significant differences between the PAT and HC regarding mean age (p-value = 0.956) and gender (p-value = 0.634) (Table 4 and Figure 3). We found that there was a higher number of smokers in PAT than in HC (p-value < 0.001). Similarily, average BMI was higher in PAT than in HC (p-value < 0.005) (Table 4).

White matter integrity

We found that mean gFA in cerebral white matter was significantly decreased in PAT when compared to HC (p-value < 0.005) (Table 5 and Figure 3).

Peripheral antioxidants

GPx activity in blood was significantly decreased in PAT when compared to HC (p-value < 0.005). In contrast, PAT had higher blood levels of cystine (p-value < 0.005), cysteine (p-value < 0.05) and GSH (p-value < 0.05) than HC. We observed no difference regarding Gr activity between PAT and HC (Table 5 and Figure 3).

Correlations between medication or lifestyle factors and peripheral antioxidants

We found a positive correlation between CPZ and blood cysteine levels (p-value < 0.05) in PAT (Table 9). We found no correlation between smoking level or BMI and peripheral antioxidants in both PAT or HC (Table 6-9).

Correlations between white matter integrity and peripheral antioxidants

In HC, we found a positive correlation between peripheral blood GSH levels and mean gFA in cerebral white matter. (p-value < 0.05) (Table 10 and Figure 4).

In PAT, peripheral blood cysteine was negatively correlated with mean gFA in cerebral white matter (p-value < 0.05) (Table 11 and Figure 5).

	Healthy controls	Early psychosis patients	Test stat.	P-value
Age, mean (SD)	28.6 (8.4)	28.5 (9.2)	T=-0.055	0.956
Male, % (No.)	60.9% (39)	65.3% (32)	Chi sq.=0.227	0.634
Smoking, % (No.) *	5.4% (3/55)	53.3% (24/45)	Chi sq.=29.321	0.000
Body-mass index, mean (SD) **	23.1 (2.4)	25.8 (5.4)	T=-3.064	0.003
Antipsychotic medication (CPZ equivalent), mean (SD)	0 (0)	414.5 (338.1)	T=8.581	0.000

Table 4: Demographics. No statistically significant differences regarding age and gender between early psychosis patients and healthy controls. Statistically significant differences are present for the number of smokers and body-mass index between the two groups. *, data were missing for 13 participants (9 controls and 4 patients)., data were missing for 11 participants (4 controls and 7 patients).**

	Healthy controls	Early psychosis patients	Test stat.	p-value
gFA, mean (SD)	0.234 (0.009)	0.229 (0.01)	T=-3.022	0.003
Cysteine, mean (SD)	239.65 (32.62)	252.9 (34.45)	T=2.087	0.039
Cystine, mean (SD)	41.25 (10.93)	51.36 (20.13)	T=3.409	0.001
GPx, mean (SD)	25.95 (2.2)	20.08 (2.21)	T=-3.077	0.003
Gr, mean (SD)	3.482 (1.408)	3.965 (01.95)	T=-1.526	0.130
GSH, mean (SD)	1.578 (0.635)	1.859 (0.716)	T=2.168	0.033

Table 5: Comparison of mean values between early psychosis patients and healthy controls. gFA, generalized fractional anisotropy; cysteine (µmol/l); cystine (µmol/l); GPx, glutathione peroxidase (µmol/min/g of Hb); Gr, glutathione reductase (µmol/min/g of Hb); GSH, glutathione (µmol/ml)

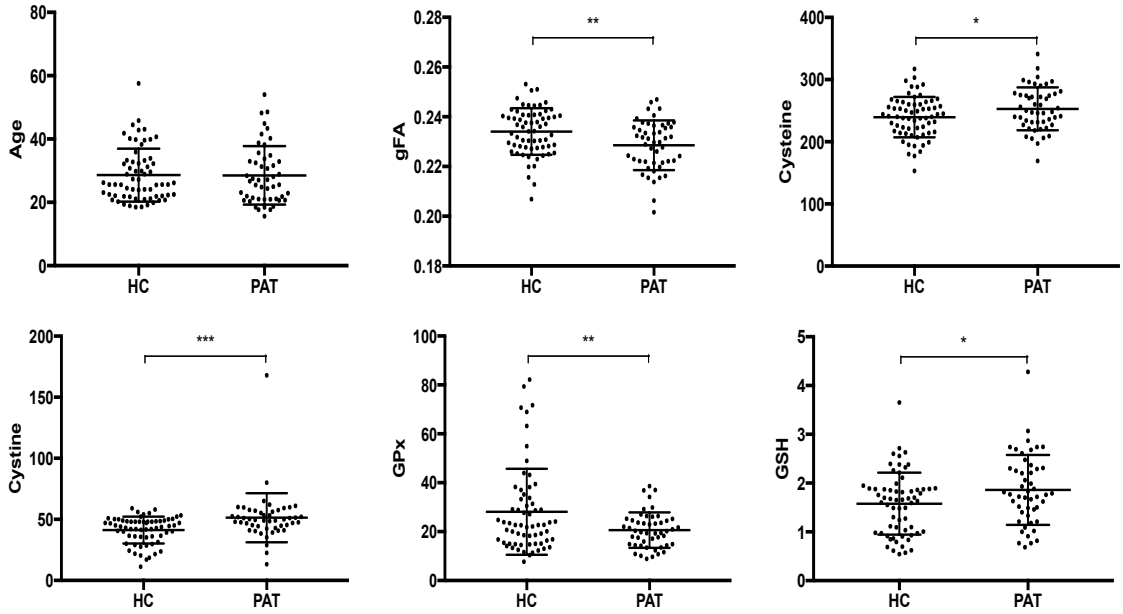

Figure 3: From left to right, from top to bottom: dot plot for age, cerebral gFA and peripheral cysteine (µmol/l), cystine (µmol/l), GPx (µmol/min/g of Hb) and GSH (µmol/ml). HC, healthy control; PAT, psychosis; gFA, generalized fractional anisotropy; GPx, glutathione peroxidase; GSH, glutathione; *, p < 0,05; **, p < 0,005; ***, p < 0,0005

HC	**BMI**	
Controlled by age and gender	**Correlation**	**p-value**
Cysteine	0.172	0.197
Cystine	0.105	0.435
GPx	-0.088	0.509
Gr	-0.000	0.997
GSH	0.118	0.379
gFA	0.153	0.250

Table 6: Partial correlations controlled by age and gender in HC group. There was no statistically significant correlation between BMI and blood markers or gFA. HC, healthy controls; gFA, generalized fractional anisotropy; BMI, body-mass index; cysteine (µmol/l); cystine (µmol/l); GPx, glutathione peroxidase (µmol/min/g of Hb); Gr, glutathione reductase (µmol/min/g of Hb); GSH, glutathione (µmol/ml)

PAT	BMI	
Controlled by age, gender and CPZ	Correlation	p-value
Cysteine	0.144	0.382
Cystine	-0.111	0.501
GPx	-0.116	0.313
Gr	-0.024	0.885
GSH	-0.088	0.596
gFA	0.041	0.803

Table 7: Partial correlations controlled by age, gender and CPZ in PAT group. There was no statistically significant correlation between BMI and blood markers or gFA. CPZ, chlorpromazine; PAT, psychotic patients; BMI, body-mass index; gFA, generalized fractional anisotropy; cysteine (µmol/l); cystine (µmol/l); GPx, glutathione peroxidase (µmol/min/g of Hb); Gr, glutathione reductase (µmol/min/g of Hb); GSH, glutathione (µmol/ml)

PAT	Smoking	
Controlled by age, gender and CPZ	Correlation	p-value
Cysteine	0.201	0.201
Cystine	0.037	0.815
GPx	-0.156	0.323
Gr	0.022	0.891
GSH	-0.006	0.971
gFA	0.158	0.318

Table 8: Partial correlations controlled by age, gender and CPZ in PAT group. There was no statistically significant correlation between smoking levels and blood markers or gFA. CPZ, chlorpromazine; PAT, psychotic patients; smoking, cigarette consumption (per day); gFA, generalized fractional anisotropy; cysteine (µmol/l); cystine (µmol/l); GPx, glutathione peroxidase (µmol/min/g of Hb); Gr, glutathione reductase (µmol/min/g of Hb); GSH, glutathione (µmol/ml)

PAT	CPZ	
Controlled by age and gender	Correlation	p-value
Cysteine	0.334	0.022
Cystine	-0.075	0.615
GPx	-0.071	0.637
Gr	-0.153	0.311
GSH	0.115	0.443
gFA	-0.099	0.507

Table 9: Partial correlations controlled by age and gender in PAT group. There was a statistically significant positive correlation between cysteine levels and CPZ. PAT, psychotic patients; CPZ, chlorpromazine; gFA, generalized fractional anisotropy; cysteine (µmol/l); cystine (µmol/l); GPx, glutathione peroxidase (µmol/min/g of Hb); Gr, glutathione reductase (µmol/min/g of Hb); GSH, glutathione (µmol/ml).

HC	Mean gFA mask	
Controlled by age and gender	Correlation	p-value
Cysteine	-0.089	0.492
Cystine	0.000	0.997
GPx (blood)	-0.043	0.742
Gr (blood)	-0.032	0.805
GSH	0.275	0.032

Table 10: Partial correlations controlled by age and gender in HC group. There was a statistically significant positive correlation between GSH and mean gFA. HC, healthy controls; gFA, generalized fractional anisotropy; cysteine (µmol/l); cystine (µmol/l);

GPx, glutathione peroxidase (µmol/min/g of Hb); Gr, glutathione reductase (µmol/min/g of Hb); GSH, glutathione (µmol/ml)

PAT	Mean gFA mask	
Controlled by age, gender and CPZ	Correlation	p-value
Cysteine	-0.296	0.046
Cystine	-0.042	0.781
GPx (blood)	0.176	0.243
Gr (blood)	0.106	0.490
GSH	-0.080	0.599

Table 11: Partial correlations controlled by age, gender and CPZ in PAT group. Negative correlation statistically significant between cysteine and mean gFA. CPZ, chlorpromazine; PAT, psychosis; gFA, generalized fractional anisotropy; cysteine (µmol/l); cystine (µmol/l); GPx, glutathione peroxidase (µmol/min/g of Hb); Gr, glutathione reductase (µmol/min/g of Hb); GSH, glutathione (µmol/ml)

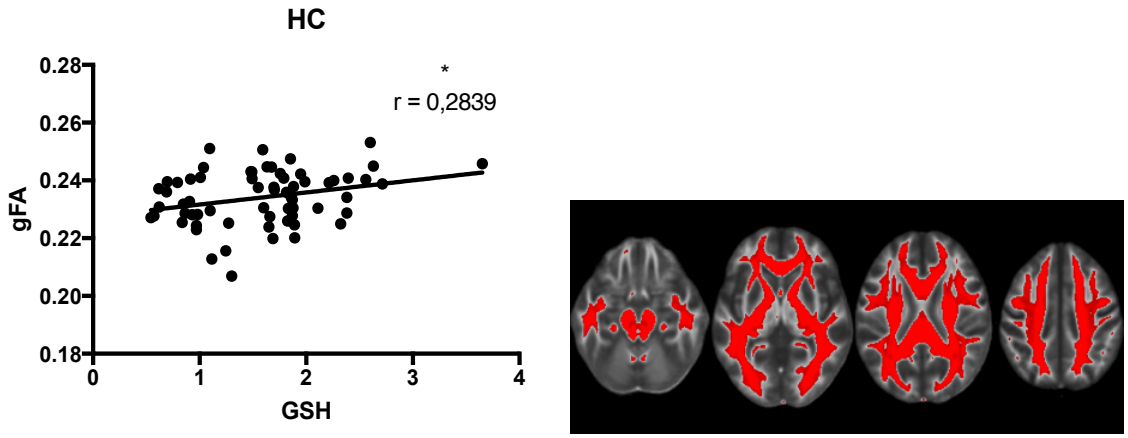

Figure 4: to the left, generalized fractional anisotropy associated with a significant increase in the control group correlated positively with blood glutathione in controls. This correlation is not controlled by age and gender. To the right, the mask of the white matter is illustrated. gFA, generalized fractional anisotropy; GSH, glutathione (µmol/ml); HC, controls; Pearson r is given; *, $p < 0.05$

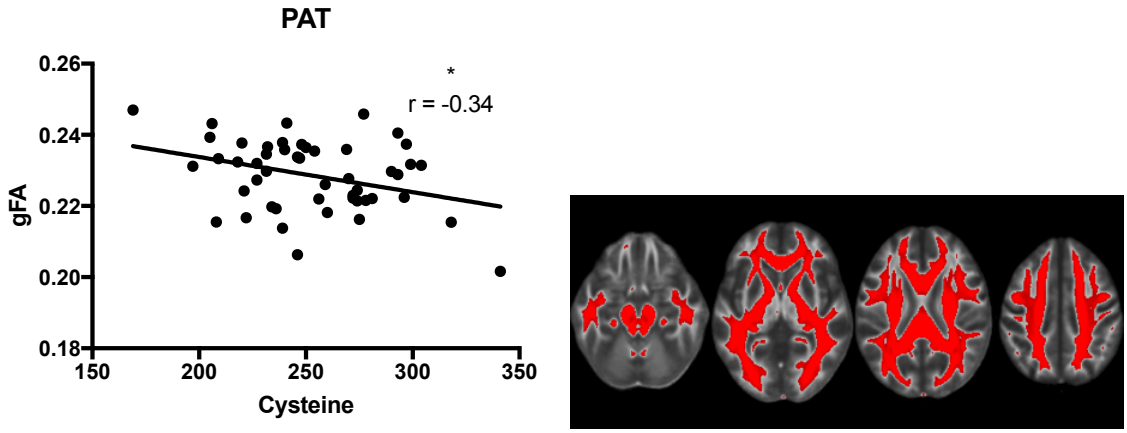

Figure 5: to the left, generalized fractional anisotropy associated with a significant reduction in the patient group correlated negatively with blood cysteine in patients. This correlation is not controlled by age, gender and CPZ. To the right, the mask of the white matter is illustrated. gFA, generalized fractional anisotropy; cysteine (µmol/l); PAT, psychosis; Pearson r is given; *, p < 0.05

Discussion

Biomarkers are still missing for schizophrenia. An objective measure of disease extend or severity would greatly improve the stratification of patients which is an important step for the research, development and administration of stage specific treatments. During the past years, the development of brain imaging has allowed to highlight that abnormalities of white matter diffusion properties can be detected early in the time course of the disease and are rapidly widespread, involving all cerebral lobes. In parallel, a growing number of animal studies suggested that redox dysregulation and more specifically a deficiency in brain GSH, could lead to oligodendrocyte impairment, alterations of white matter integrity and eventually to the development of schizophrenia.

Accordingly, we first observed that cerebral white matter integrity estimated using mean gFA was reduced in early psychosis patients when compared to healthy controls. Second, the activity levels of GPx, the enzyme responsible for the elimination of peroxides were lower in patients than in controls. In contrast, GSH and its precursors cystine and cysteine were higher in the group of patients. Third, we reported that GSH levels were positively correlated with gFA in healthy controls and that cysteine levels could predict the level of cerebral white matter anomalies in early psychosis patients.

Finding of a lower gFA in the group of patients than in the group of controls is consistent with the existing literature reporting widespread alterations of fractional anisotropy in the cerebral white matter of patients with schizophrenia(92)(33). Indeed, gFA reflects modifications of water diffusion properties and a decreased value could be considered as a “proxy” for alterations of white matter integrity in patients.

The results regarding the different components of the antioxidant defense system and their interpretation were more complex. Blood cells GPx activity was lower in PAT than in HC, but GSH blood levels were increased in PAT. These two observations could be explained by a decreased activity of GPx leading to an

accumulation of GSH that could not be metabolized to eliminate peroxides(63). This interpretation may thus be consistent with the redox dysregulation hypothesis. Another explanation could be that the GSH was measured in whole blood and not in the cerebrospinal fluid or cerebral tissue as in previously cited studies. It is notable that Xin & al.(63) have found a lack of relationship between GSH in blood and GSH in the medial PFC using MRI spectroscopy.

Similarly to GSH, free cystine and total cysteine levels were both increased in the plasma of patients. Increased free cystine levels may reflect elevated oxidative status in patients. This observation is consistent with similar results observed in chronic SZ patients (64). In this study, the difference of plasmatic total cysteine levels between PAT and HC could be due to the effect in PAT of the medication, which was positively correlated with the blood cysteine concentration. Interestingly, Wang & al.(60) reported an increased serum free cysteine levels in chronic SZ patients as compared to matched control subjects. According to them, two hypotheses could explain this increase; a) an augmentation of cysteine levels could be due to a defect of enzymatic system that converts this amino acid into GSH; b) elevated cysteine levels could be a compensatory response to the excess of ROS produced in schizophrenia patients. However the effect of medication was not investigated in Wang et al. In our study, plasmatic total cysteine levels were quantified [i.e. free cysteine (3-4%, measured in Wang et al) + free cystine (30%; also measured in our study) + protein bound forms (65%)]. It is possible that the increased total cysteine levels reported here could reflect not only the increased free cystine levels as observed but also the elevated cysteine protein bound forms. This hypothesis is currently under investigation in our lab.

Concerning the partial correlations, two correlations have been found between blood biomarkers and gFA values. Firstly, blood GSH levels were positively correlated with gFA values in HC but not in PAT. Interestingly, similar positive association was observed between prefrontal GSH levels and gFA along the cingulum bundle in early psychosis patients and matched control subjects (73). Taken together, it is tempting to speculate that in control subjects, i.e. in conditions of redox homeostasis, both peripheral and central GSH are well regulated and may contribute to brain white matter integrity. This is not the case in schizophrenia patients. Secondly, total cysteine plasma levels were negatively associated with gFA values in PAT but not in HC. In the alternative that increased total cysteine plasma levels would reflect elevated oxidation of the cysteine bound form of proteins, such negative association with white matter integrity in patients would be also consistent with the proposed critical implication of redox dysregulation in schizophrenia.

It should be noted that our study is limited by several factors, including the heterogeneity of our sample regarding the stages of the disease (acute vs. chronic or active vs. remission phase), the dietary pattern and the ethnicity of patients. The testing materials could also explain the difference with the literature. While free cystine levels were measured, total levels of cysteine were quantified. Indeed, in plasma, the total cysteine levels reflect the protein bound forms (65%), 30% are related to the free oxidized form (i.e., free cystine), and only 3–4% represent the free reduced form. Finally, a multiple comparison issue possibly limits the statistical significance of our findings. On the one hand, the choice of the variables that we wanted to test was strongly driven by the literature and our a priori hypotheses and this could have limited the risk of reporting false positive findings based on uncorrected alpha set at 0.05. However, the scientific rigor of this approach is

debated and it could be argued that we should have corrected the alpha level for multiple comparisons. Using a classical Bonferroni procedure would have set the alpha level to 0.008 (0.05/6) for the group comparison and to 0.01 (0.05/5) for the correlation analyses. In this last scenario, only the group difference for gFA, cystine and GPx would have survived the correction for multiple comparisons.

Although further exploration is needed to better understand the precise relationships between peripheral blood antioxidants and alterations of brain anatomy, peripheral cysteine levels could represent a quick and economic way to stratify patients and assess the extent of their cerebral alterations.

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