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Genetic Dysregulation of Glutathione Synthesis Predicts Alteration of Plasma Thiol Redox Status in Schizophrenia

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Abstract

Genetic studies have shown an association between schizophrenia and a GAG trinucleotide repeat (TNR) polymorphism in the catalytic subunit (*GCLC*) of the glutamate cysteine ligase (GCL), the key enzyme for glutathione (GSH) synthesis. The present study was aimed at analyzing the influence of a GSH dysregulation of genetic origin on plasma thiols (total cysteine, homocysteine, and cysteine-glycine) and other free amino acid levels as well as fibroblast cultures GSH levels. Plasma thiols levels were also compared between patients and controls. As compared with patients with a low-risk *GCLC* GAG TNR genotype, patients with a high-risk genotype, having an impaired GSH synthesis, displayed a decrease of fibroblast GSH and plasma total cysteine levels, and an increase of the oxidized form of cysteine (cystine) content. Increased levels of plasma free serine, glutamine, citrulline, and arginine were also observed in the high-risk genotype. Taken together, the high-risk genotypes were associated with a subgroup of schizophrenia characterized by altered plasma thiols and free amino acid levels that reflect a dysregulation of redox control and an increased susceptibility to oxidative stress. This altered pattern potentially contributes to the development of a biomarker profile useful for early diagnosis and monitoring the effectiveness of novel drugs targeting redox dysregulation in schizophrenia. *Antioxid. Redox Signal.* 15, 2003–2010.

Introduction

Schizophrenia is a chronic and severe psychiatric complex disease that affects more than 30 million persons worldwide. It is caused by interplay of genetic, environmental, and developmental factors (2, 17, 43). Substantial evidence of oxidative damage has been observed in blood and other tissues of schizophrenia patients (8, 15, 20, 28, 35, 38, 44, 46, 68, 69). However, variability in these results could be due to the contribution of the diverse genotypes of the patients and the different tissues studied [review, see (16)]. It was unclear whether the responsible oxidative stress was due to environmental factors or was rather of genetic origin, preventing the affected brain areas from reacting adequately to oxidative stress. The genetic origin of a dysregulation of the redox system in schizophrenia was recently shown for the first time (25). We demonstrated an association between the disease and a trinucleotide repeat (TNR) polymorphism in the gene coding for the key glutathione (GSH)-synthesizing enzyme. GSH is the main intracellular nonprotein antioxidant and redox regulator, synthesized in two consecutive enzymatic reactions. The first reaction is catalyzed by the enzyme glutamate cysteine ligase (GCL), which consists of a catalytic (GCLC) and a modifier subunit (GCLM) (40) that conjugate glutamate and cysteine, while, in the second step, glycine is added after a reaction catalyzed by the enzyme glutathione synthase. A subgroup of patients with schizophrenia has a brain deficit in the GSH system that is of genetic origin: (a) GSH levels in the brain and cerebrospinal fluid of patients are decreased (19, 39, 66); (b) GCL activity and GSH synthesis are decreased in patients' fibroblasts under oxidative-stress conditions (25); and (c) allelic variants of the key GSH-synthesizing enzyme glutamate cysteine ligase (GCL)-modulatory subunit (*GCLM*) (58) and catalytic subunit (*GCLC*) (25) genes are associated with the disease. In particular, in two case–control studies (Swiss and Danish), a GAG trinucleotide with seven, eight, or nine repeat polymorphism in the 5′UTR of the *GCLC* gene showed a significant intergroup difference regarding the overall genotype distribution (25). The genotypes (8/7, 8/8, 8/9, and 9/9 GAG TNR), more abundant in patients as

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compared with control subjects, are termed "high-risk genotypes," whereas the genotypes (7/7 and 7/9 GAG TNR) more present in control subjects are called "low risk." This GAG TNR polymorphism has functional consequences: in fibroblast cultures under oxidative-stress conditions, the high-risk genotypes had lower GCL activity, GCLC protein expression, and GSH content than did those of subjects with low-risk genotypes. In the Swiss sample, the high-risk genotype is present in 36% of patients and is 3 times more frequent in patients than in control subjects. Interestingly, *GCLC* is located on chromosome 6p12, and several genetic studies have shown an association between schizophrenia and markers or genes on chromosome 6p, mostly between 6p23 and 6p21 (49). In addition, the GSH precursor *N*-acetyl cysteine, given to patients in a clinical trial, improves the negative symptoms (9), the auditory-evoked potentials mismatch negativity, (33) and the neuronal synchronization (12). Together with data on experimental models of GSH deficit that mimicked most morphologic, physiologic, and behavioral anomalies found in the disease (10, 11, 52–54) [for review, see (17, 18)], these data suggest that a developmental redox dysregulation constitutes one "hub" on which convergence takes place between genetic impairments of GSH synthesis and environmental vulnerability factors known to generate oxidative stress (17).

Diagnosis in schizophrenia is currently based on clinical observation and thus can be uncertain in early phases, sometimes contributing to delay in adapted treatment (13, 50). No quantitative or empiric biologic tests are available to confirm this diagnosis. This causes difficulties not only in carrying out early treatment but also in the selection of patients for clinical trials of potentially novel therapeutics. In the search for schizophrenia biomarkers, altered free plasma amino acid levels were described. Chronic disease patients had increased plasma levels of amino acids such as glutamate, aspartate, glycine, serine, phenylalanine, or glutamine, although results were not always consistent (56, 57, 61).

The aim of the present study was to assess the influence of *GCLC* GAG TNR genotypes on systemic redox balance: on plasma thiols (total cysteine, homocysteine, and cysteine-glycine) and fibroblast GSH levels. To test the hypothesis that functional consequences of high-risk genotypes can be detected in the thiol redox status of peripheral tissues, we investigated the influence of the *GCLC* GAG TNR polymorphism, high-risk versus low-risk genotypes, on skin fibroblast GSH and plasma thiol levels of 54 schizophrenia patients. They were also assessed in 64 age-matched control subjects. Plasma levels of other free amino acids also were compared between high-risk and low-risk genotypes in an exploratory approach.

Materials and Methods

Subjects

All 54 patients (average age, 36.7 ± 11.1; 39 men, 15 women; duration of illness, $\geq$15 years) and 64 controls (average age, 35.7 ± 11.8; 35 men, 29 women) were recruited with fully informed written consent according to ethical guidelines of the Lausanne University. They were all of Caucasian origin and were assessed by using the Diagnostic Interview for Genetic Studies (DIGS), developed by the NIMH (42). Subjects with a major mood, psychotic, or substance-use disorder or a first-order relative with a psychotic disorder were excluded as controls. Patients, recruited from the in/outpatient schizophrenia units of the Psychiatry Department, Lausanne University Hospital, met the criteria for schizophrenia or schizoaffective disorder of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). Patients with a history of neurologic illness or head trauma, with mental retardation (IQ less than 60), or with a diagnosis of drug/alcohol dependence were excluded. Eight patients were untreated, and 46 were receiving antipsychotic medication (40 atypical, six typical) at therapeutic doses. To evaluate the effect of medication, it was computed to risperidone equivalents, as the majority of patients were treated with atypical antipsychotics (65).

Blood preparation

Blood was collected by venipuncture between 7 and 8:30 AM under restricted-activity conditions and fasting from the previous midnight. Then 20 ml of blood was allowed to drop in Vacutainer-tubes coated with Li-heparinate (Becton Dickinson), which were previously placed on ice. The blood was immediately centrifuged at 3,000 g, 5 min, 4°C; the pellet, corresponding to blood cells, was washed 2 times with 0.9% NaCl and frozen at −80°C until analysis. The supernatant, corresponding to the plasma, was recovered, sampled in aliquots, and kept at −80°C until analysis.

Amino acid analysis in plasma

The following free amino acids were quantified in plasma, as described in (51): taurine, hydroxyproline, threonine, serine, aspartate, glutamate, glutamine, proline, glycine, alanine, citrulline, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, ornithine, lysine, histidine, arginine. In brief, deproteinization was achieved by adding 50 μl 5-sulfosalicylic acid (160 g/L) containing the internal standards to 200 μl plasma. After mixing and standing (10 min), the sample was centrifuged 4 times at 14,600 g, 15 min, and the supernatant was kept at −80°C until analysis. Then 100 μl was injected into an amino acid analyzer (Biochrom, 30+ Model), and amino acids were detected by postcolumn reaction with nihydrine. Analytic performance was assessed for all amino acids by internal (IQC, home made) and external quality controls (ERNDIM; http://www.erndimqa.nl/). Typical coefficient of variation [CV (%), $N = 75$] were <3% for all amino acids except for Cit (<4%) and because of low concentrations in IQC sample for Asp (<18%; mean, 3 μM) and for Hyp (<22%; mean, 13 μM).

Thiols analysis in plasma

The following thiol containing amino acids and peptide were quantified in plasma as described before (62): total (protein-bound forms, free reduced and free oxidized form) cysteine, homocysteine, and cysteinyl-glycine. In brief, the thiols were reduced and/or decoupled from proteins by reaction with *tris*(2-carboxyethyl)phosphine. After deproteinization with perchloric acid, the thiols were derivatized with 7-fluorobenzofurazane-4-sulfonic acid (SBD-F). The SBD-F derivatives were analyzed by HPLC followed by fluorometric detection.

Analytic performance was assessed for total homocysteine by internal (IQC, home made) and external quality controls

(ERNDIM; http://www.erndimqa.nl/). Typical coefficient of variation [CV (%)] were <5.2% (at 5.5 μM; $N = 120$) and <3.3% (at 16.4 μM; $N = 143$). Total cysteine and total cysteine-glycine have higher CV (%), usually <10%. The levels of the oxidized form of cysteine, the free cystine levels in plasma were analyzed as described in the earlier section, "amino acid analysis."

Cell cultures

Human fibroblast cultures were established from skin biopsies, as described (58). Cells were grown with DMEM medium [0.5 l medium with 10 ml Ultroser-G plasma, 5 ml penicillin/streptomycin, 5 ml sodium pyruvate (100 mM)] at 37°C in a humidified atmosphere containing 5% CO_2/95% air.

GSH content in fibroblast

Cultures of fibroblast (four petri dishes of 10 cm diameter, confluent) were collected after three passages. They were removed from flasks with trypsin, washed, resuspended in 4 ml phosphate buffer (0.1 M, pH 7.4) and sonicated. GSH contents were measured by a colorimetric GSH assay kit (Calbiochem, San Diego, CA). This method is based on a colorimetric assay of a chromophoric thione formed specifically between the reagent and GSH. The protein levels of fibroblasts were determined by using the Biorad Kit with the Advanced Protein Assay reagent.

Genotyping

DNA was purified from blood samples by using NucleonBACC3 system (Amersham Pharmacia Biotech). *GCLC* GAG TNR polymorphism was assessed as originally described (63) with the following modifications: PCR amplification was performed in reaction mixtures of 25 μl containing 40 ng of each primer, 200 μM of each NTP and 0.3 U EuroTaq DNA polymerase with its buffer (Euroclone, Pero, Italy). Temperature cycling of the PCR was as originally described. PCR products were separated on an 8% polyacrylamide gel (6×8 cm) for 5 h with 40 V and stained by using SilverXpress Silver Staining Kit (Invitrogen, Carlsbad, CA).

Statistical analysis

Statistical analysis was performed by using the SAS statistical package version 9 (SAS Institute, Inc., Cary, NC). Multivariate analysis of variance (MANOVA) was assessed by using the amino acid levels as dependent variables and sex or *GCLC* GAG TNR genotype as fixed factors, and age and medication as covariables (GLM procedure, SAS/STAT User's Guide, version 6, 1989). A similar analysis was made between controls and patients groups by using age as covariable. Differences between genotype distributions were tested by using χ^2 or Fisher two-tailed test (FREQ procedure). Correlations between protein levels were studied by using the CORR procedure. A type I error, $p < 0.05$, was used to reject the null hypothesis.

Results

Thiols and amino acid levels in high- and low-risk GCLC *GAG TNR patients and in control subjects*

In the present sample, the *GCLC* GAG TNR polymorphism was differently distributed ($p = 0.038$) between 64 controls and 54 patients (genotypes 7/7, 7/9, 7/8, 8/9, 8/8 & 9/9: in controls, 33, 26, 2, 2, 0, and 1 vs. in patients 18, 19, 8, 4, 2, and 3). In patients, the polymorphism allowed us to separate 37 low-risk (genotypes 7/7 and 7/9) and 17 high-risk patients (genotypes 7/8, 8/8, 8/9, and 9/9). The two groups did not differ by age and sex. Low-risk patients had a mean age of 35.4 ± 10.1 years and a male-to-female ratio of 2.7, whereas high-risk patients had a mean age of 35.5 ± 11.6 years and a male-to-female ratio of 2.4.

Skin fibroblasts of high-risk patients had decreased GSH levels, as compared with fibroblasts from low-risk patients (Table 1). Considering age and sex as covariables, MANOVA analysis revealed that the thiols and amino acid pattern was different in high risk, as compared with low-risk patients ($p = 0.029$). Univariate analysis showed that high-risk patients had significantly lower levels of total cysteine (oxidized + reduced form + protein bound; minus 20.4%; $p = 0.004$), but higher levels of free cystine (+43.3%, p = 0.005), than low-risk patients (Table 1). Moreover threonine, glutamine, citrulline, and arginine levels were also significantly higher in high-risk genotype patients (Table 2).

The thiols levels were also compared between all schizophrenia patients and control subjects. Free cystine (+20.7%; $p = 0.033$) were higher in patients (+20.7%; $p = 0.033$) as compared with control subjects. Interestingly, as compared with control subjects (36.3 μM), plasma free cystine levels were similar in low-risk genotype patients (36.9 μM), whereas they were 46% higher in high-risk genotypes (52.9 μM). Likewise, plasma cysteine levels, compared with control subjects, were higher in high-risk but not different in low-risk genotypes (Tables 1 and 3). Furthermore, fibroblast GSH levels were lower in high-risk but not different in low-risk genotypes. In the control-subjects group, the cysteine and cystine levels did not differ between the 59 low-risk and five high-risk genotypes. For cysteine, they amounted, in micromoles per liter, to, respectively, 191.7 ± 46.2 and 188.0 ± 20.4,

Table 1. Levels of Plasma Thiols and GSH Fibroblasts in High- and Low-risk *GCLC* GAG TNR Genotypes in Schizophrenia Patients

Amino acids	*Patients with low-risk genotypes*	*Patients with high-risk genotypes*	*ANOVA* (p)
Cystine	36.9 ± 19.2	52.9 ± 21.3	0.005
Cysteine	195.9 ± 43.6	156.0 ± 46.4	0.004
Cysteine-glycine	44.1 ± 8.3	42.7 ± 7.8	n.s.
Homocysteine	9.7 ± 2.8	8.6 ± 2.0	n.s.
GSH (fibroblast)	33.6 ± 7.2	29.0 ± 7.4	0.013

Plasma thiols levels were assessed in 17 high-risk and 37 low-risk *GCLC* GAG TNR schizophrenia patients and are presented in micromoles per liter as mean ± SD. The levels of total (protein-bound forms, free reduced and free oxidized form) of cysteine, cysteine-glycine and homocysteine were quantified while the levels of free cystine in plasma were measured. Multivariate analysis of variance (MANOVA) was assessed by using the four amino acid levels as dependent variables, *GCLC* GAG TNR Genotype (Wilks' Lambda $F = 3.15$, $p = 0.02$) and Sex ($p = 0.02$) as fixed factors, and Age ($p = 0.04$) as covariables. The p values are univariate type I errors. n.s., not significant. Fibroblast GSH levels were assessed in 14 high-risk and 25 low-risk *GCLC* GAG TNR schizophrenia patients and are presented in micromoles per liter as mean ± SD. The p value (0.013) comes from the univariate analysis of variance (ANOVA).

Table 2. Plasma Amino Acids Levels in High-risk and Low-risk *GCLC* GAG TNR Genotypes of Schizophrenia Patients

Amino acids	*Patients with low-risk genotypes*	*Patients with high-risk genotypes*	*ANOVA* (p)
Alanine	368.3 ± 98.9	365.7 ± 106.7	n.s.
Arginine	76.6 ± 17.6	92.9 ± 21.9	0.004
Asparagine	44.5 ± 10.2	52.1 ± 16.5	(0.051)
Aspartate	4.6 ± 1.7	5.0 ± 1.4	n.s.
Citrulline	26.8 ± 5.5	34.9 ± 10.6	<0.001
Glutamine	521.6 ± 94.9	594.7 ± 122.7	0.007
Glutamate	57.5 ± 43.6	51.5 ± 41.4	n.s.
Glycine	215.3 ± 64.0	249.3 ± 71.8	n.s.
Histidine	76.3 ± 15.0	83.7 ± 22.3	n.s.
Hydroxyproline	9.4 ± 5.6	9.7 ± 4.0	n.s.
Isoleucine	63.2 ± 18.5	68.1 ± 20.5	n.s.
Leucine	122.6 ± 29.3	133.7 ± 39.2	n.s.
Lysine	162.9 ± 41.4	183.9 ± 50.9	n.s.
Methionine	22.1 ± 4.3	24.6 ± 7.1	n.s.
Ornithine	51.3 ± 20.3	58.8 ± 19.4	n.s
Phenylalanine	50.4 ± 12.8	52.5 ± 19.1	n.s.
Proline	214.8 ± 64.9	203.0 ± 62.9	n.s.
Serine	92.0 ± 22.5	102.8 ± 25.9	n.s.
Taurine	50.7 ± 16.0	48.3 ± 15.9	n.s.
Threonine	112.8 ± 40.0	139.9 ± 48.5	0.035
Tyrosine	60.7 ± 16.6	63.3 ± 19.6	n.s.
Valine	230.7 ± 54.9	244.6 ± 70.1	n.s.

Plasma amino acid levels were assessed in 17 high-risk and 37 low-risk *GCLC* GAG TNR schizophrenia patients and are presented in micromoles per liter as mean ± SD. Multivariate analysis of variance (MANOVA) procedure was assessed by using the amino acid levels as dependent variables and *GCLC* GAG TNR Genotype (n.s.) and Sex (n.s.) as fixed factors and Age (Wilks' Lambda $F = 2.02$; $p = 0.04$) as covariable. The p values come from the univariate analysis of variance (ANOVA) done as part of the MANOVA procedure. n.s., not significant.

whereas for cystine, 35.6 ± 16.7 and 36.9 ± 13.5. Therefore, it is justified to pool them for comparison with the patients.

Influence of antipsychotic medication

Clinical studies have shown that antipsychotic medication can influence amino acid levels. To evaluate the effect of antipsychotics treatment on amino acid levels, the medication doses were computed into risperidone equivalents (65). Negative correlations were observed between citrulline levels ($R = -0.315$ $p = 0.031$ respectively) and leucine levels ($R = 0.300$; $p = 0.041$) with the medication doses. No dose effect of treatment was observed on all other amino acid and thiols levels. Moreover, no difference of antipsychotic dose was observed between high- and low-risk genotype patients ($p = 0.11$, ANOVA). Among the 40 patients who received atypical antipsychotics, 27 were with low-risk and 13 with high-risk genotypes. For the six patients receiving typical antipsychotics, five were with low risk and one with high-risk genotypes. No difference (Fisher test, $p = 0.65$) was observed in exposure of the patients to typical vs. atypical antipsychotic drugs in the high- vs. low-risk patient genotype groups. It is of note that there were eight untreated patients, five with low-risk genotype (13.5% of all low risk), and three with high-risk genotype (17.6% of all high risk). The distribution of these untreated patients between the two genotypes was not significantly different ($p = 0.7$; Fisher test). Interestingly, citrulline levels were increased in high-risk patients, as compared with low-risk patients. Comparing citrulline levels between high-risk patients (34.9 μM) and controls (29.1 μM) revealed a significant difference ($p = 0.010$) difference in exposure.

Table 3. Plasma Thiols and Fibroblast GSH Levels in Schizophrenia Patients and Control Subjects

Amino acids	*Control subjects*	*Schizophrenia patients*	*ANOVA* (p)
Cystine	36.3 ± 16.2	43.8 ± 22.0	0.033
Cysteine	188.8 ± 44.1	179.2 ± 49.8	n.s.
Cysteine-glycine	45.9 ± 8.6	43.3 ± 8.0	n.s.
Homocysteine	10.9 ± 11.5	9.3 ± 2.6	n.s.
GSH (fibroblast)	34.3 ± 8.7	31.8 ± 7.1	n.s.

Plasma thiols levels were assessed in 64 control subjects and 54 schizophrenia patients and are presented in micromoles per liter as mean ± SD. The levels of total (protein-bound forms, free reduced and free oxidized form) of cysteine, cysteine-glycine, and homocysteine were quantified while the levels of free cystine in plasma were measured. The p values were derived from univariate analysis of variance (ANOVA). n.s., not significant.

Discussion

The *GCLC* GAG TNR polymorphism is a good candidate as schizophrenia-susceptibility gene in two Caucasian samples (17, 25). In the present study, patients ($n = 54$) had also a significant different distribution of the *GCLC* GAG TNR genotypes, as compared with controls ($n = 64$). With the aim to assess the effect of this risk genotype for an impaired GSH synthesis on the plasma redox thiol status, we compared the plasma thiols levels including free cystine and total homocysteine, cysteine, and cysteine-glycine between the high- and low-risk genotypes schizophrenia patients. Plasma levels of other amino acids, skin fibroblast GSH levels, and *GCLC* GAG TNR genotypes of 54 schizophrenia patients and 64 controls subjects were also determined. Patients had a significantly different distribution of the *GCLC* GAG TNR genotypes, as compared with controls. High-risk, as compared with low-risk genotype patients, had decreased fibroblast GSH levels. More important, they also displayed lower levels of total cysteine and higher levels of its free oxidized form, cystine. High risk, as compared with low-risk genotype patients, are thus characterized by a fibroblast/plasma thiols pattern reflecting an impaired redox status and oxidative stress. Comparing the effects of genotypes within the patient group allows neutralizing some potential confounding factors, likely to be common to all patients.

Plasma redox thiol status: methodologic considerations

Cysteine, its disulfide cystine, GSH and glutathione disulfide constitute the major low-molecular-weight thiol/disulfide redox control systems in mammals (30). However, in plasma, other aminothiols such as cysteine-glycine and homocysteine, also contribute to thiol redox status, although in much smaller proportion (37, 59). In plasma, cysteine is the most abundant aminothiol (total concentration about 200 μM); about 65% is protein bound, 30% is free oxidized

(free cystine), and 3–4% is reduced. The plasma concentration of cysteine-glycine is less (total, $\sim 40\,\mu M$); 60% is protein bound, 30% is free oxidized, and 10% is reduced. At physiologic conditions, total homocysteine levels amount to $\sim 10\,\mu M$, which encompass the predominant protein-bound, the free oxidized fraction, and the small amounts of free reduced form (60). Most ($\sim 65\%$) plasma glutathione (total concentration, $\sim 6\,\mu M$) is in the reduced form (3, 4, 37, 59), but its high intracellular concentration (40) makes the determination unreliable in the presence of hemolysis (1% hemolysis can result in an increase of $\sim 7\,\mu M$ GSH in plasma). Therefore, GSH levels in plasma were not measured in the present study. Moreover, rapid oxidation or disulfide exchange reactions and redistribution of thiol species or both may also take place in freshly prepared plasma. In general, these factors were not taken into consideration (15): little information was given concerning free versus total thiols measurements and what precautions were taken to avoid artifacts. In the present study, although maximal care was taken to minimize these artificial factors during blood collection and processing (lowering temperature and pH), we thus favored, in a relatively difficult psychiatric clinical setting, the quantification of total thiols (cysteine, cysteine-glycine, and homocysteine) to their free reduced forms. Although precise redox states (E_h) of the cysteine/cystine, as imputed in experimental studies (31), could not be calculated, it still allows a robust and reliable overall picture of the redox thiol status in plasma.

GCLC *genotypes related imbalance of plasma thiols*

Schizophrenia is a complex dynamic disease with variations of symptoms over time (34). Plasma amino acid concentrations usually do not directly correlate with brain amino acid concentrations, and they are influenced by many parameters, including age, sex, medication, duration of disease, and lifestyle (21, 23, 57). In the present study, blood collection was done under fasting conditions and parameters like age, sex, and medication were taken into consideration. Moreover, all the patients were in stable and chronic situation with at least 15 years of illness. Alterations observed among patients only, according the *GCLC* GAG TNR high-risk versus low-risk genotypes, are probably less biased by confounding factors such as medication, illness duration, and lifestyle, and were analyzed by using age and sex as covariates.

High-risk *GCLC* GAG TNR patients represent around one third of all patients (17, 25). While the levels of total cysteine and of its free oxidized form, cystine, were similar between low-risk genotype patients and control subjects, high-risk genotype patients had 20% lower total cysteine and 43% higher free cystine levels as compared with low-risk genotypes. No alteration was observed with the other plasma thiols such as cysteine-glycine and homocysteine. Conditions causing oxidative stress have been reported to consume extracellular antioxidants, including vitamin C, protein thiols, bilirubin, urate, and α-tocopherol (26). However, reduced cysteine, the most abundant low-molecular-mass thiol component in plasma, represents an important extracellular antioxidant defense system. The decreased levels of total cysteine and increased levels of its oxidized form in high-risk genotype patients reflected enhanced oxidative stress processes or impaired redox regulation or both, as a consequence of the genetically impaired GSH synthesis. Combined with the decreased GSH levels in fibroblasts of high-risk genotype patients, these results show that compromised synthesis of the ubiquitous GSH system in patients with high-risk *GCLC* GAG TNR polymorphisms affected the thiols status in peripheral tissue, such as blood and fibroblasts. It should be noted that, in control subjects, both cysteine and cystine levels are not different between the two GAG TNR genotypes. Thus, in this respect, the patients carrying high-risk genotype are different from the high-risk controls. This is in line with the existence of additional risk factor(s) not yet identified in patients.

Other plasma amino acid changes

Plasma levels of other amino acids were also compared between high-risk and low-risk genotypes. High-risk patients had 16% higher glycine and 12% higher serine levels, as compared with low-risk patients. Glycine and serine have drawn particular attention in schizophrenia research. Some studies observed elevated plasma levels of glycine and serine in schizophrenia patients (5, 64), although others could not confirm these alterations (22, 47). These contradictory results may be explained by the difference of high-risk, low-risk genotypes distribution in these various schizophrenia patients' samples.

Compared with low-risk *GCLC* GAG TNR patients, high-risk patients had also increased levels of glutamine, citrulline, and arginine. Interestingly, increased glutamine levels have been observed in chronic schizophrenia patients (1), and increased levels of citrulline in drug-naïve patients (47). Moreover, as arginine and citrulline are, respectively, precursor and product in nitric oxide (NO) synthesis, their increased levels in high-risk patients might reflect higher NO metabolism. Higher NO levels or NO synthase activity were reported in red blood cells (27), platelets (14), and in postmortem brain (67) of schizophrenia patients. As excess of NO levels can lead to generation of the free radical peroxynitrite (6); this result further supports the involvement of nitrosative stress pathology in high-risk patients (35, 45). The negative correlation between citrulline levels and antipsychotic medication is consistent with the report that treatments with chlorpromazine, haloperidol, or clozapine normalize NO synthase activity (14).

Comorbidities

Apart from psychiatric symptoms, schizophrenia is known to have major somatic concerns like an increased risk for cardiovascular diseases (24), or for diabetes mellitus (29, 55), also in drug-naïve patients (48). Interestingly, all these three diseases were found to be associated with polymorphisms of *GCLC* and *GCLM*. The *GCLC* GAG TNR polymorphism was shown to be associated with schizophrenia and with diabetes (7, 25). The $-129\,C/T$ of *GCLC* and the $-588\,C/T$ of *GCLM* have been associated with an increased risk for cardiovascular events and myocardial infarction (32, 41).

In conclusion, based on clinical and preclinical studies, the *GCLC* GAG TNR polymorphism was proposed as a schizophrenia susceptibility factor. The present results show that the high-risk *GCLC* GAG TNR genotypes lead to alterations of plasma thiols levels that reflect a dysregulation of redox control, and an increased susceptibility to oxidative stress. This indicates that the high-risk *GCLC* genotypes are associated with a subgroup of schizophrenia characterized by

redox imbalance. The plasma/fibroblast thiol levels could therefore potentially contribute to a biomarker profile useful for early diagnosis and monitoring the effectiveness of novel drugs targeting redox dysregulation in schizophrenia.

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Author Disclosure Statement

The authors state that no competing financial interests exist.

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Abbreviations Used

ANOVA = univariate analysis of variance
GCL = glutamate cysteine ligase
GCLC = glutamate cysteine ligase catalytic subunit
GCLM = glutamate cysteine ligase modulatory subunit
GSH = glutathione
MANOVA = multivariate analysis of variance
NO = nitric oxide
TNR = trinucleotide repeat