Schizophrenia and Oxidative Stress: Glutamate Cysteine Ligase Modifier as a Susceptibility Gene


Oxidative stress could be involved in the pathophysiology of schizophrenia, a major psychiatric disorder. Glutathione (GSH), a redox regulator, is decreased in patients’ cerebrospinal fluid and prefrontal cortex. The gene of the key GSH-synthesizing enzyme, glutamate cysteine ligase modifier (GCLM) subunit, is strongly associated with schizophrenia in two case-control studies and in one family study. GCLM gene expression is decreased in patients’ fibroblasts. Thus, GSH metabolism dysfunction is proposed as one of the vulnerability factors for schizophrenia.

Schizophrenia (MIM 181500) is a major and frequent chronic psychiatric disorder with a strong genetic component. Converging evidence points to the involvement of oxidative stress and N-methyl D-aspartate (NMDA) receptor hypofunction in the pathophysiology of the disease. As a major cellular nonprotein antioxidant and redox regulator, glutathione (GSH) plays a major role in protecting nervous tissue against reactive oxygen species and in modulating redox-sensitive sites, including NMDA receptors (NMDA-R). It was shown elsewhere that the GSH levels were decreased in patients’ cerebrospinal fluid (−27%), in medial prefrontal cortex in vivo (−52%), and in striatum postmortem tissue. GSH-deficient models reveal morphological, electrophysiological, and behavioral anomalies similar to those observed in patients.

Here, we present strong evidence for an association between schizophrenia and the gene of the key GSH-synthesizing enzyme, glutamate cysteine ligase modifier (GCLM) subunit. The functional role of the GCLM gene variance in schizophrenia is supported by its low expression in patients’ fibroblasts and by the decreased stimulation of the enzyme activity when challenged by an oxidative stress.

To identify candidate gene(s) responsible for the low level of GSH observed in patients with schizophrenia, we studied steady-state levels of mRNA for 14 genes (data not shown) involved in GSH metabolism (fig. 1). Since GSH is ubiquitously present in cells, gene expression was studied in cultured skin fibroblasts. Two enzymes are responsible for GSH synthesis: glutamate cysteine ligase (GCL), also known as γ-glutamyl cysteine synthetase (Enzyme Commission number 6.3.2.2), and glutathione synthetase (GSS [Enzyme Commission number 6.3.2.3]). GCL, the first and rate-limiting enzyme, is composed of two subunits—GCL modifier (GCLM [light: 27.7 kDa]) and GCL catalytic subunit (GCLC [heavy: 72 kDa])—each encoded by separate genes. The specific mRNA steady-state levels were measured in fibroblasts obtained from 32 patients and 53 controls from a Swiss population (table 1). The subjects were recruited with fully informed written consent and guidelines for ethical treatment given by the University of Lausanne. All subjects were assessed using the Diagnostic Interview for Genetic Studies (DIGS) developed by the National Institute of Mental Health (NIMH). Additional measures of psychopathology of patients included the Positive and Negative Syndrome Scale (PANSS), which assessed the presence of symptoms within the same week as the blood collection and skin biopsy. Specific mRNA steady-state levels were measured in cultured skin fibroblasts grown for three passages, with the use of TaqMan chemistry and ABI Prism 7000 sequence detection system. cDNA corresponding to 10 ng of reverse-transcribed total RNA was amplified using TaqMan gene expression assays (Hs00155249 m1, Hs00157694 m1, and Hs00609286 m1) at the following amplification conditions: 1 cycle for 2 min at 50°C, 1 cycle for 10 min at 95°C, and 50 cycles for 15 s at 95°C, followed by 1 min at 60°C. Human glyceraldehyde-3-phosphate dehydrogenase (Applied Biosystem 4333764F) was used as endogenous control.

Case-control comparisons showed significant differences in GCLM (t = 1.989; P = .037) and GSS (t = 1.997; P = .030) mRNA levels between patients and control subjects, without effect from sex or age. For the GCLM mRNA,
Figure 1. GSH metabolic pathway. The genes for which expression was studied in fibroblast cultures from skin biopsies are highlighted. Black indicates no difference between controls and patients, and red indicates lower expression in patients compared with controls. These genes code for the enzymes highlighted in yellow. Substrates and products are highlighted in purple. Genes not directly involved in GSH metabolism but included in the expression study: Nrf1, Nrf2, and Nrf3 (NF-E2–related transcription factors 1, 2, and 3, respectively); xCT and 4F2 = genes encoding proteins involved in cystine/glutamate exchange; and MRP1 = multidrug resistance protein 1. ADP = adenosine diphosphate; GSSG = glutathione disulfide; RSH = reduced thiols; RS = disulfide; N-AC-CYS-X = N-acetyl-cysteine conjugate; Pi = inorganic phosphate.

A trend toward a decrease could be observed ($t = 2.000; P = .064$) in patients.

We tested, therefore, whether the reduced GCLM and GSS mRNA levels observed in patients with schizophrenia could be due to a primary defect. We studied eight SNPs in the GCLM gene and nine SNPs in the GSS gene for possible association with schizophrenia (fig. 2). Sixteen SNPs were chosen from a group of 60 SNPs selected from publicly available databases (SNP Consortium and dbSNP) on the basis of the estimated level of polymorphism in our population. One SNP (ss60197536) at the GCLM 5′ end, described by Nakamura et al., is being submitted to dbSNP. The dbSNP-annotated SNP numbers and their positions in each gene are shown in figure 2. Genotyping was performed with DNA extracted from peripheral blood by the use of either Sequenom technology or sequencing. The list of specific primers is shown in table 2.

A pilot association study was performed with a relatively small sample (40 patients and 31 controls from a Swiss population) (fig. 2). Most, although not all, of the subjects from the Swiss population were used in both gene expression and association studies. The details about subject groups are given in the tables: demographic data in table 1, genotype and allele frequencies for each SNP in table 3, and the allelic frequencies compared with those known for other populations in table 4. All SNPs were in Hardy-Weinberg equilibrium.

<table>
<thead>
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<th>Study and Population</th>
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<th>Age$^a$ (years)</th>
<th>Sex$^b$</th>
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$^a$ Expressed as the mean and range.

$^b$ Ratio of males to females.

$^c$ International Classification of Diseases, 10th Revision.
Figure 2. Map of GCLM and GSS genomic structures, with the positions of SNP markers and results of two case-control studies. Tables show P values for each SNP in case-control studies of subjects from two independent populations, from Switzerland and Denmark. Values in italics indicate a significant difference, and the value in bold italics indicates a significant difference after correction for multiple testing.

Table 2. Primers Used in Genotyping Studies

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

Table 3. Genotype and Allele Frequencies for Each SNP Studied in Two Populations

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.
is localized, like rs2301022, in intron 1. We analyzed different genotype pattern classes for these three markers (ss60197536, rs2301022, and rs718875) in a region of ~3,000 bp that includes GCLM exon 1. We identified 14 different genotype patterns (table 5), among which 2 were specifically present in affected individuals. Nine patterns of these three SNPs showed different frequencies between patients and unaffected controls, with \( \chi^2 = 30.39 \) and \( P = .004 \). Two particular combinations, TT/GG/TC and CC/GG/TT, had odds ratios (ORs) of 4.89 and 4.17, respectively.

At present, we cannot completely exclude the possibility of a second functionally associated region in the vicinity of SNP rs3170633. The association test for this SNP showed low \( P \) values in both populations (\( P = .009 \)) (fig. 2). However, these values are not significant after correction for multiple testing.

Genotype frequency distribution in the Danish population suggests a dominant mode of transmission for the two SNPs of interest (rs2301022 and rs3170633), with the G allele dominant in each of them (fig. 3). For rs2301022, genotypes AA, AG, and GG show respective ORs of 0.37, 1.22, and 1.12. Thus, we pooled AG and GG genotypes, since both are associated with disease, to approximately the same degree. The corresponding OR for disease then was 2.72 (\( P = .0005 \)). For rs3170633, which showed weaker association, OR = 1.77 (\( P = .002 \)).

Thus, case-control studies of two independent populations provided strong evidence of an association of the GCLM gene and schizophrenia. These data were supported with an additional linkage study of the families from the NIMH cohort, shown in table 6. Genotyping of 275 individuals from 72 families for seven SNPs in the GCLM gene showed supportive (although by no means significant) evidence of linkage between schizophrenia and two GCLM markers. The highest LOD score, 1.382 (corresponding to \( P = .012 \)), was obtained under assumed dominant inheritance for rs2064764, which is located within 2 kb (~0.002 cM) of rs3170633.

To test whether there is association in the presence of linkage, we ran a family-based association statistic test (FBAT v. 1.7.2) in NIMH families (table 7). The results showed that there is significant evidence (\( Z = 3.247 \); \( P = .0012 \)) to accept the alternative hypothesis of association in the presence of linkage between SNP marker rs2301022 and the schizophrenia phenotype: allele G at the marker appeared as significantly overtransmitted to the affected offspring. Moreover, the \( P \) value remains significant, experiment-wise, after correction for multiple testing (table 7).

To define possible functional variants associated with the disease, we estimated pairwise linkage disequilibrium (LD) for all 17 SNPs of GCLM and GSS genes in 348 unaffected subjects from Denmark. The resulting LD map showed very strong association among all markers within each of the two genes (fig. 4). Thus, any association with this region might suggest the presence of a functional variant associated with schizophrenia.

On the basis of the strong association of two SNPs (rs2301022 and rs718875) in the 5′ region of the GCLM gene and schizophrenia, we examined the relationship of these variants and the GCLM expression level in the Swiss
population. The mRNA steady-state level in cultured fibroblasts showed a significant correlation with these two SNPs ($P = .040$). This result confirms the functional effect of the $GCLM$ variants on the schizophrenia phenotype. However, it is still unknown in which way these variants affect the $GCLM$ gene expression, since their localization is not directly related to any of the currently known regulatory sequences. It is worth noting that the $GCLM$ gene is localized on chromosome 1p21, the region shown by previous linkage studies to be one of the several regions critical for schizophrenia.$^{30,31}$

Several genes involved in GSH metabolism have already been considered as potential candidates for schizophrenia. Association of the glutathione-S-transferase M 1 gene was shown in a subgroup of patients with schizophrenia in Japanese$^{32}$ and Korean$^{33}$ populations. Case-control studies of glutathione-S-transferase P1 and glutathione peroxidase 1 showed no association.$^{34,35}$

Although GCLM is not essential for survival, its interaction with the GCLC subunit increases, by four- to five-fold, the catalytic efficiency of the holoenzyme.$^{36}$ The GCLM knockout (KO) mice exhibit an increased sensitivity of GCL activity, apparently resulting in brain GSH levels 52% below normal reported for patients with schizophrenia.$^{37}$ This is strikingly similar to the 40% of normal.$^{36,37}$ This is strikingly similar to the levels 52% below normal reported for patients with schizophrenia.$^{36,37}$ Thus, GCLM KO mice can be used as a model for further studies of GSH deficit in schizophrenia.

GCLM KO mice showed an increased feedback inhibition of GCL activity, apparently resulting in brain GSH levels ~40% of normal.$^{36,37}$ This is strikingly similar to the levels 52% below normal reported for patients with schizophrenia.$^{36,37}$ Thus, GCLM KO mice can be used as a model for further studies of GSH deficit in schizophrenia. A GCL dysregulation could lead to cellular alterations in the surroundings of dopaminergic terminals, affecting the synaptic contacts on dendritic spines of prefrontal cortical neurons that are particularly rich in dopamine innervations. Indeed, the metabolism of dopamine generates reactive oxygen species (e.g., hydrogen peroxide and quinones), which, in GSH-deficit conditions, are not adequately neutralized and, thus, induce cellular damage.$^{11}$ Interestingly, in rat models, GSH deficit and excess dopamine during development mimic structural and functional anomalies observed in patients: they exhibited a decrease in spine density of pyramidal neurons (F. Gheorghita, unpublished data) and, selectively, in GABA-parvalbumin immunoreactivity of prefrontal cortex,21 similar to patients.$^{38,39}$ The same rat model presented an impairment in object recognition$^{18,19}$ and in integration of olfactory information,$^{40}$ reproducing some cognitive deficits of schizophrenia.

Furthermore, NMDA-R hypofunction is implicated in schizophrenia, since the NMDA-R antagonist phencyclidine induces a psychotic syndrome.$^{41}$ In the case of GSH deficit, NMDA-R activity could be depressed through interaction at their redox sites.$^{12,13}$ Similarly, in rat hippocampal slices, GSH depletion impaired NMDA-dependent synaptic plasticity.$^{20}$

In conclusion, these studies provide converging evidence of a link between schizophrenia and $GCLM$ genetic variations, which affect the function of the encoded protein in its ability to promote GSH synthesis when challenged by an oxidative stress. They support the new concept that a dysregulation of GSH metabolism is one of the vulnerability factors contributing to the development of the disease.

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**Table 6. Family Linkage Analysis of the $GCLM$ Gene**

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*. 

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**Table 7. Family-Based Association Test for SNP Markers Linked to $GCLM$**

<table>
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<tr>
<th>Marker</th>
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*Note.*—Cells highlighted in bold show significantly different values.
Web Resources
The URLs for data presented herein are as follows:

International Classification of Diseases (ICD-10), http://www3.who.int/icd/currentversion/fr-icd.htm
Online Mendelian Inheritance in Man (OMIM) http://www.ncbi.nlm.nih.gov/Omim/ (for schizophrenia)

References


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