Gene Reports 4 (2016) 249–252

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

Association of polymorphism in glutamate-cysteine ligase catalytic subunit gene with schizophrenia: A case-control study in a Tunisian population

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ARTICLE INFO

Article history:
Received 9 May 2016
Accepted 12 July 2016
Available online 16 July 2016

Keywords:
Glutathione
GCLC
Oxidative stress
Schizophrenia

ABSTRACT

Glutathione (GSH) is a key regulator of oxidative balance in all mammals, especially in the central nervous system. The first step of glutathione synthesis is catalysed by glutamate cysteine ligase (GCL), which is composed of catalytic and modifier subunits (*GCLC* and *GCLM*, respectively). The purpose of this study was to test the hypothesis that the -129C/T polymorphism in the promoter region of the glutamate-cysteine ligase catalytic subunit *(GCLC)* gene may be associated with the schizophrenia disease and if this polymorphism could affect plasma GSH concentrations. The *GCLC* -129C/T genotypes were determined in 138 schizophrenic patients and 123 healthy individuals using a PCR-based restriction fragment length polymorphism (RFLP) method. Glutathione levels: total glutathione (GSHt), reduced glutathione (GSHr) and oxidized glutathione (GSSG) were determined by spectrophotometry. The subjects with -129C/T and T/T genotypes are highly associated with schizophrenia. No association was found between glutathione levels and the -129C/C genotype, C/T and T/T genotypes in schizophrenic patients. The present finding indicated that the *GCLC* polymorphism seems to be associated with schizophrenia disease in at least a Tunisian population.

1. Introduction

Schizophrenia is a devastating psychiatric disease with a complex genetic etiology. Accumulating evidence suggests that oxidative stress associated with impaired metabolism of the antioxidant glutathione (GSH) plays a key role in the pathogenesis of schizophrenia (Do et al., 2000; Yao et al., 2006; Raffa et al., 2009). The cellular glutathione synthesis occurs in two steps. In the first step, the formation of γ-glutamyl cysteine is catalysed by glutamate cysteine ligase (GCL). In the next step, formation of glutathione is catalysed by glutathione synthase (Lu, 2013). These synthetic steps can occur in neurons and glial cells in the brain (Dringen and Hirrlinger, 2003). In glutathione biosynthesis, GCL is considered the rate-limiting enzyme because the product of GCL, γ-glutamyl cysteine, is present at a low concentration when glutathione synthase is active (Dalton et al., 2004). GCL is composed of catalytic and modifier subunits named *GCLC* and *GCLM*, respectively (Lu, 2013). Previous studies have shown that expression levels of these two GCL subunit genes are regulated by trans-activation of antioxidant-response element (Jaiswal, 2004; Lu, 2013). Glutathione suppresses oxidative stress-induced cellular damage by acting alone or reacting with glutathione peroxidase to reduce superoxide radicals, hydroxyl radicals and peroxynitrites (Smeyne and Smeyne, 2014). Because of their potential effect on GSH production, *GCLC* gene polymorphisms have been the concern of many studies. Among them, the -129C/T polymorphism (rs17883901) within the 5′ regulatory region of the gene is of particular interest. This substitution was shown to correlate with an impaired gene expression and has been associated with cardiovascular diseases in two independent studies (Zuo et al., 2007; Koide et al., 2003). Glutathione suppresses oxidative stress-induced cellular damage by acting alone or reacting with glutathione peroxidase to reduce superoxide radicals, hydroxyl radicals and peroxynitrites (Smeyne and Smeyne, 2014). In addition to detoxification, many other cellular functions that involve the intracellular thiol-redox state have

Abbreviations: CI, confidence interval; DNA, deoxyribonucleic acid; dNTP, deoxynucleotide-tri phosphate; DSM-IV-TR, diagnostic and statistical manual of mental disorders; EDTA, ethylenediaminetetraacetic acid; GCL, glutamate cysteine ligase; *GCLC*, glutamate cysteine ligase catalytic; *GCLM*, glutamate cysteine ligase modifier; GSH, glutathione; GSHt, total glutathione; GSHr, reduced glutathione; GSSG oxidized, glutathione; ml, milliliter; MgCl2, magnesium chloride; NS, no significant; OR, odds ratio; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SD, standard deviation; TNR, trinucleotide repeat.

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http://dx.doi.org/10.1016/j.genrep.2016.07.007
2452-0144/

been ascribed to GSH, including involvement in cell proliferation (Shi et al., 2000), signal transduction, gene expression, apoptosis, and posttranslational protein modification (Butticaz et al., 2011). Because of this large number of functions, GSH metabolism is critical for maintaining optimal cellular homeostasis, and decreased GSH levels have been observed in a wide range of human disorders such as schizophrenia (Steullet et al., 2006; Gysin et al., 2007), Parkinson disease (Bharath et al., 2002), cardiovascular diseases (Campolo et al., 2007) and diabetes (Bekris et al., 2007). The aim of the present study was to investigate the relationship between the *GCLC* -129C/T polymorphism and schizophrenia in a Tunisian population. We have further correlated the -129C/T genotypes with the glutathione levels in the all studied subjects.

2. Methods

2.1. Subjects

One hundred thirty-eight (120 males and 18 females) patients with schizophrenia and 123 (99males and 24 females) healthy controls were involved in this case–control study. The socio-demographic characteristics of the study subjects are summarized in Table 1. Patients were recruited from consecutive admissions at the psychiatric department of the Teaching Hospital of Monastir (Tunisia). They had DSM-IV-TR criteria for schizophrenia based on the structured Clinical interview for DSM-IV-TR (American Psychiatric Association, 2000). Inclusion criteria of patients were aged between 18 and 60 years, and having chronic schizophrenia. These patients had no other psychiatric disorders; including major depression, schizoaffective disorder, substance abuse (except tobacco consumption), or mental retardation. The healthy controls were recruited from the blood centre of the Teaching Hospital of Monastir; they had no personal or family history of major psychiatric disorders including schizophrenia, bipolar disorder or major depression. Their current mental status and personal or family history of mental disorder were assessed by unstructured interviews. This study was approved by the local ethical committee. All subjects signed informed consent after a full explanation of the study.

2.2. DNA preparation

For genotyping, 4 ml blood were drawn into an EDTA tube and stored at −20 °C until DNA extraction was carried out. Genomic DNA was isolated from whole peripheral blood using the standard salt precipitation method (Miller et al., 1988).

Table 1
The socio-demographic characteristics of the patients and control groups.

Variables	Schizophrenic patients (n = 138)	Controls (n = 123)	Results of comparisons
Mean age ± SD (years)	32.67 ± 7.44	31.28 ± 5.28	NS
Gender: n (%)			
Male	120 (86.9%)	99 (80.5%)	NS
Female	18 (13.0)	24 (19.5%)	
Smoking status: n (%)			
Smokers	83 (60.1%)	61 (49.6%)	NS
Non smokers	55 (39.8%)	62 (50.4%)	
Educational level: n (%)			
Primary school	66 (47.8%)	22 (17.9%)	$\chi^2 = 27.11$ ($df = 2$)
Secondary school	55 (39.8%)	70 (56.9%)	$p < 0.001$
University	17 (12.3%)	31 (25.2%)	
Marital status: n (%)			
Single	88 (63.8%)	66 (53.6%)	$\chi^2 = 15.31$ ($df = 2$)
Married	40 (29.0%)	57 (46.3%)	$p = 0.0004$
Divorced	10 (7.2%)	0 (0%)	

SD: standard deviation, df: degrees of freedom.

2.3. Genotyping

We identified the -129C/T polymorphism. The genotypes of this polymorphism were determined by the PCR-based restriction fragment length polymorphism (RFLP) method. The -129C/T polymorphism creates another novel site for the *Tsp*45I restriction enzyme in the presence of the T allele. A set of primers was designed to amplify a 613-base pair (pb) fragment of the *GCLC* promoter by PCR (forward: 5-TCGTCCCAAGTCTCACAGTC-3; reverse: 5-CGCCCTCCCCGCTGCTCCTC-3-, encompassing the -129C/T polymorphic site and an additional site for *Tsp*45I as a control. Subjects with the CC genotype were identified by the presence of 500- and 113-pb bands; those with the TT genotype were identified by the presence of 302, 198, and 113-pb bands; and those with the CT genotype were identified by the presence of all four bands, as shown in Fig. 1.

2.4. Biochemical procedures

5 ml of blood were drawn from control subjects and patients by simple venipuncture between 7.00 and 9.00 a.m., after overnight fasting and tobacco abstinence for >12 h. Each evaluated parameter was assayed in duplicate for all samples. Throughout the investigations, the biochemical assays were conducted ignoring the available clinical information. The glutathione levels (GSHt, GSHr, and GSSG), were measured spectrophotometrically in deproteinized blood samples by the method of Akerboom and Sies (1981), using 5,5 dithiobis (2-nitrobenzoic acid). Absorbance values were compared with standard curves generated from known amounts of GSH standards.

2.5. Statistical analysis

Genotype frequencies were compared by Chi-squared test ($\chi 2$). Odds ratios and their 95% confidence interval (CI) were calculated. Quantitative variables were presented as mean ± SD and comparisons were performed using the Student's *t*-test. Qualitative variable comparisons were performed using the $\chi 2$ test. $p < 0.05$ was considered statistically significant. The statistical analyses were carried out with the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL) release 11.0 for Windows.

3. Results

3.1. Case control analysis

Two hundred sixty-one subjects were enrolled in the study: 138 were schizophrenic patients (mean age 32.67 ± 7.44 years) and 123 were healthy controls (mean age 31.28 ± 5.28 years). There was no significant difference between patients and controls in terms of age, gender, and smoking status (Table 1). The -129TT, CT, and CC genotypes were present in 2 (1.44%), 66 (47.82%), and 70 (50.72%) of the 138 patients with schizophrenia, respectively, and they were present in 2

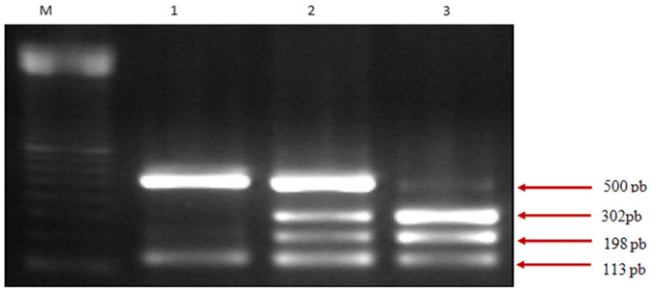

Fig. 1. Genotyping of GCLC by RFLP PCR. M: 100 bp DNA marker. Lane 1: CC genotype (500 pb and 112 pb). Lane 2: CT genotype (500 pb, 302 pb, 198 pb and 113 pb). Lane 3: TT genotype (302 pb, 198 pb, 113 pb).