

Association of gliadin antibodies, HLA alleles, and schizophrenia in Cuban population patients

Asociación de anticuerpos antigliadina, alelos HLA y esquizofrenia en pacientes cubanos

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ABSTRACT

Introduction: Several lines of evidence have suggested an interesting link between gluten ingestion and schizophrenia. For example, increased levels of gliadin and transglutaminase antibodies have been observed in patients with schizophrenia. **Methods:** To verify these observations we compared the prevalence of gliadin and transglutaminase antibodies, as well as the presence of the HLA alleles, HLA DQA1*0501-DQB1*02 (DQ2) and HLA-DQA1*0301-DQB1*0302 (DQ8), among patients with schizophrenia and healthy controls. A total of 108 patients with schizophrenia and 60 healthy controls were evaluated. Gliadin antibodies were determined by a visual semiquantitative assay and tissue transglutaminase antibodies were determined both by one-step immunochromatographic assay and ELISA. HLA typing was performed by PCR amplification using sequence-specific primers for each allele. **Results:** We found a strong association between the presence of gliadin antibodies and schizophrenia (OR 3.488; 95% CI, 1.43-8.44). However, tissue transglutaminase antibodies were not detected in either group neither by immunochromatographic or ELISA. No significant association was found for the DQ2 or DQ8 heterodimer and the disease, but a significant positive association between schizophrenia and HLA alleles DQA1*0301 and DQB1*02 was present (OR = 2.80; 95% CI, 1.27-6.17, and OR = 2.37, 95% CI, 1.24-4.53, respectively). **Conclusions:** The present study showed that the presence of gliadin antibodies was not correlated with the presence of HLA DQA1*0301 or DQB1*02 alleles within the group of patients with schizophrenia. Our study replicates the findings that anti-gliadin antibodies are associated with schizophrenia but also suggests that the presence of these antibodies and the HLA alleles DQB1*02 and DQA1*0301 are independently associated with susceptibility to schizophrenia.

Keywords: Celiac disease, gliadin antibodies, tissue transglutaminase antibodies, HLA, schizophrenia, Cuba.

RESUMEN

Introducción: Varias evidencias han sugerido un interesante enlace entre la ingestión de gluten y la esquizofrenia. Un aumento de los niveles de anticuerpos antigliadina y transglutaminasa se ha observado en pacientes con esquizofrenia. **Métodos:** Se comparó la prevalencia de anticuerpos antigliadina y transglutaminasa, así como la presencia de los alelos HLA, HLA DQA1 * 0501-DQB1 * 02 (DQ2) y HLA-DQA1 * 0301-DQB1 * 0302 (DQ8), entre pacientes con esquizofrenia y controles sanos. Se evaluaron un total de 108 pacientes con esquizofrenia y 60 controles sanos. Los anticuerpos antigliadina se determinaron mediante un ensayo de anticuerpos transglutaminasa tisular y semicuantitativos visuales fueron determinados por el ensayo inmunocromatográfico de un solo paso y ELISA. La tipificación de HLA se realizó mediante amplificación por PCR utilizando cebadores específicos de secuencia para cada alelo. **Resultados:** Se encontró una fuerte asociación entre la presencia de anticuerpos de la gliadina y la esquizofrenia (OR 3,488; IC del 95%, 1,43-8,44). Sin embargo, los anticuerpos transglutaminasa tisular no se detectaron en ninguno de los grupos ni por inmunocromatografía o ELISA. No se encontró asociación significativa para el DQ2 o DQ8 heterodímero y la enfermedad, sino una asociación positiva significativa entre la esquizofrenia y los alelos HLA DQA1 * 0301 y DQB1 * 02 estaba presente (OR = 2,80; IC del 95%, 01/27 a 06/17, y OR = CI 2,37, 95%, 1,24 a 4,53, respectivamente). **Conclusiones:** Se demostró que la presencia de anticuerpos de gliadina no se correlacionó con la presencia de HLA DQA1 * 0301 o DQB1 * 02 alelos dentro del grupo de pacientes con esquizofrenia. Nuestro estudio replica los hallazgos de que los anticuerpos anti gliadina están asociados con la esquizofrenia, pero también sugiere que la presencia de estos anticuerpos y la HLA alelos DQB1 * 02 y DQA1 * 0301 están asociados independientemente con la susceptibilidad a la esquizofrenia.

Palabras clave: Enfermedad celíaca, anticuerpos anti gliadina, anticuerpos transglutaminasa tisular, HLA, esquizofrenia, Cuba.

Introduction

Celiac disease (CD) is one of the most common severe food intolerances in the Western world affecting, almost 1% of the population. The disease is an autoimmune reaction triggered by gluten ingestion in genetically susceptible children and adults. The conclusive diagnosis of CD is carried out by intestinal biopsy,

which provides evidence of the histological changes characteristic of this disease. However, serological screening methods, such as those detecting gliadin antibodies (AGA) and anti-tissue transglutaminase antibodies (tTGA), have gained attention because they are cheaper and less invasive.¹

CD and Gluten Sensitivity, a reaction of the innate immune system to wheat peptides, have been associa-

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ted with neurologic and psychiatric disorders including cerebellar ataxia, peripheral neuropathy, epilepsy, dementia, depression and autism.² Given that the prevalence of schizophrenia is almost 1%, the concurrence between schizophrenia and celiac disease would be expected to be rare; however a possible association between gluten and schizophrenia has been suggested in reports of sporadic cases, and comparisons between samples of persons with schizophrenia and healthy comparison groups.^{3,4} But another previous study based on the screening for specific CD antibodies among patients with schizophrenia suggested that there is no direct association between celiac disease and schizophrenia.⁵ In addition, some studies reported that psychotic symptoms occur in adult celiac disease, and schizophrenic symptoms can improve after gluten-free diet.⁶

A recent study hypothesized that gene-associated susceptibility and environment interaction could importantly contribute to schizophrenia. According to this hypothesis, gut disorders such as CD can lead to poor nutritional conditions and alteration in gut permeability; in this scenario, the gut could lose its capacity to block exogenous psychosis-causing substances that may enter the body promoting the development of schizophrenia and other mental conditions.⁷ In fact, CD and schizophrenia are complex disorders that have been reported to share genetic relationships. Susceptibility to CD is genetically determined by possession of specific HLA-DQ2 (DQA1*0501, DQB1*0201) and DQ8 (DQA1*0301, DQB1*0302) alleles located on chromosome 6, in concert with one or more non-HLA linked genes.⁸ The DQB1 locus has been associated with both celiac disease and schizophrenia.⁹

Genetic linkage studies for non-HLA loci in CD and schizophrenia suggest several areas of overlap. Recent studies have also shown susceptibility regions for CD in chromosome 11q23 that overlap with a potential schizophrenia susceptibility region.¹⁰ Another study has suggested that gene MYO9B on chromosome 19, which encodes a myosin molecule involved in actin remodeling of epithelial enterocytes, might be associated with an increased risk of developing celiac disease and schizophrenia.¹¹ However, a more recent study found no evidence for association between schizophrenia and Non-HLA CD genes, and suggests that these two conditions do not share any functional overlap.¹²

The objective of the present study was to compare the prevalence of gliadin antibodies, antitransglutaminase antibodies, and HLA DQA1*0501-DQB1*02 (DQ2) and HLA-DQA1*0301-DQB1*0302 alleles (DQ8) between Cuban patients with schizophrenia and healthy controls.

Methods

Subjects: The study was carried out to determine the presence of AGA and a tTGA in 108 outpatients with paranoid subtype schizophrenia, recruited at the Neuroscience Center in Havana, Cuba. Patients were evaluated by trained psychiatrists using the Spanish version of the Schedules for Clinical Assessment in Neuropsychiatry, and were diagnosed with paranoid schizophrenia according to the fourth edition of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders criteria (DSM-IV).^{13,14} There were 72 males and 36 females with average age of 35.44 years. The control group comprised 60 mentally normal volunteers, 31 females and 29 males, 37.61 years-old in average, who had never taken psychotropic drugs or presented any psychiatric disorder as determined by their reported history during the clinical interview and by structured diagnostic criteria categorized according to DSM-IV criteria. No individual reported to have any first-degree relatives with schizophrenia or psychosis. Individuals from both groups completed a questionnaire exploring sign and symptoms of celiac disease, and registering sociodemographic, nutritional and health habits items. This research was approved by the Ethics Research Com-

mittees of all institutions involved. Patients and controls had given written informed consent for participation in the study. All subjects included in the study agreed with the terms of the informed consent before the sample was selected.

Anti-gliadin antibodies detection: AGA were measured by AuBioDOT™ gliadin (HerberBiotec S.A, La Habana, Cuba) as described in detail elsewhere.¹⁵ Briefly, AuBioDOT™ gliadin is a semiquantitative visual immunoassay based on 8 well opaque-white polystyrene strips coated with wheat gliadin (Sigma, USA). After incubation with a serum or plasma sample, AGA was detected with a protein A-gold conjugate probe. Silver ion solution is used to enhance the reaction, and PBS-Tween washings are included after each step. The intensity of the color is proportional to the quantity of AGA in the samples. A highly positive control serum and a cutoff standard are used to categorize samples as negatives or positives.

Anti-transglutaminase antibodies detection: The tTGA were detected in blood by using a fast one-step immunochromatographic assay (HeberFast Line® anti-transglutaminase, Heber Biotec S.A, Havana, Cuba) that has been shown to have high sensitivity and specificity for diagnosis of the disease. Details of this assay have been described elsewhere.¹⁶ HeberFast Line® anti-transglutaminase is a qualitative assay and consists of a nitrocellulose strip inserted in a plastic cassette, which is filled with blood obtained from a finger puncture. After 20 minutes, a positive result consists in signals in both the reactive zone and the control zone. A signal only in the control zone represents a negative result. The HeberFast Line® anti-transglutaminase assay detects both IgA and IgG antibodies responses, but cannot distinguish between them. Additionally, IgA anti-transglutaminase antibodies were determined in serum by ELISA (Celikey Pharmacia & Upjohn, Freiburg, Germany) using cut-offs values as recommended by the manufacturer.

DNA Isolation and HLA Typing: DNA was isolated from whole blood using a Wizard Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's instructions. Selected HLA alleles DQ2 (HLA-DQA1*0501-DQB1*02) and DQ8 (HLA-DQA1*3-DQB1*0302) genotyping, was performed by PCR amplification, using sequence-specific primers (PCR-SSP), as previously described.¹⁷ Primers were obtained from the Department of Oligonucleotide Synthesis at the Center of Genetic Engineering and Biotechnology (Havana, Cuba) and used at 0.25 µmol/L. Amplified products were analyzed in 3% agarose gels and visualized under a UV transilluminator after ethidium bromide staining.

Definition of Celiac Disease: CD was defined by a combination of tTGA and HLA DQ2 or DQ8 positives, as first-line diagnostic criteria, considering the high negative predictive value of the tTGA and HLA markers for celiac disease. We also considered the presence of CD-associated symptoms and AGA to complement the first-line diagnostic criteria.

Statistical Analysis: The Fisher exact test was used to compare HLA allele frequencies between patients and controls. A precise correction method for multiple comparisons was applied using the formula $\alpha=1-(0.951/N)$, where N is the number of comparisons one or more of which shows a significant result.¹⁸ The χ^2 test with Yates' correction was used to compare the positivity for gliadin antibodies in patients and controls in 2 x 2 tables. Odds ratios with 95% confidence intervals were also estimated. Statistical significance was considered for p values <0.05 for gliadin antibodies comparisons, and p values <0.017 for HLA alleles associations. All the analysis was made using the SPSS software version 15. In order to determine the correlation between the presence of gliadin antibodies and specific HLA DQ alleles in the group of patients with schizophrenia, the correlation coefficient Phi (ϕ) was calculated using the Pearson correlation with Yates' correction for continuity implemented in SPSS software version 15. For multiple tests we applied Bonferroni corrections.

Results

Celiac Disease Symptoms: No symptoms of celiac disease were detected on the basis of the criteria established in the questionnaire. There were also no symptoms found for other disorders associated with so-called "silent" celiac disease.

Serological Test: Gliadin antibodies were detected in 34 (31.48%) patients with schizophrenia compared with only in 7 (11.66%) individuals in the control group ($p < 0.05$). We found a strong association between high levels of gliadin antibodies and schizophrenia, $OR = 3.478$ (95% CI, 1.433- 8.443) (Table 1), suggesting that the presence of these antibodies could increase the probability of having schizophrenia more than three times (Table 1). However, no serum sample from patients or controls was positive for tTGA, as determined both by enzyme-linked immunosorbent assay and by immunochromatographic.

Table 1. Association of gliadin antibodies (AGA) in patients with schizophrenia and controls

AGA	Patients N=108	Controls N=60	Odds Ratio (95% CI)	χ^2 test P value
positive	34 (31.48%)	7 (11.66%)	3.479 (1.433- 8.443)	0.0041*
negative	74 (68.52%)	53 (88.34%)		

* P value < 0.05

HLA Typing: In this study, DQ2 or DQ8 heterodimers were not associated with schizophrenia. Nonetheless, single alleles DQA1*0301 and DQB1*020 were positively associated with schizophrenia, indicating that their presence confers disease susceptibility ($OR = 2.803$, 95% CI: 1.273-6.172; and $OR = 2.368$, 95% CI: 1.237-4.534, respectively). In contrast, association of the HLA allele DQB1*0301 with schizophrenia ($p < 0.05$; $OR = 2.357$, 95% CI: 1.064-5.221) was not significant after Bonferroni correction. It is important to emphasize that although the correction for multiple independent comparisons is particularly recommended when more than five significant associations are found, we preferred to be more conservative and applied it in this case even for three statistically significant associations (Table 2).

Correlation analysis of anti-gliadin antibodies with HLA DQ chains: In order to determine if the observed association between schizophrenia and HLA alleles was not the result of a confounding bias, due to the relationship of these HLA alleles and celiac disease (or associated markers), we test the correlation between gliadin antibodies and HLA DQ alleles within the group

of patients with schizophrenia. In this study sample, the presence of gliadin antibodies was not correlated with any of the HLA DQ alleles as shown in Table 1II; phi (ϕ) values are close to zero and none of the X^2 tests were statistically significant after correction for multiple tests. Only the HLA DQB1*0301 allele showed weak to modest correlation ($\phi = 0.24$, $p < 0.05$) (Table 3).

Table 3. Analysis of correlation between gliadin antibodies and the HLA DQ chains in schizophrenic patients, phi (ϕ) correlation coefficient for the 2x2 table of DQ2a, DQ2b, DQ8a and DQ8b alleles with gliadin antibodies applying the Pearson correlation coefficient with Yates correction

HLA allele	Phi (ϕ)	Pearson Chi square	P value
DQA1*0501	+0.1	0.59	0.442419
DQB1*02	-0.03	0.99	1
DQA1*0301	-0.06	0.2	0.654721
DQB1*0302	0.24	4.97	0.025791*

* P value < 0.05

Discussion

Schizophrenia and celiac disease each affect almost 1% of general population worldwide. A similar epidemiological characteristic have been reported for the Cuban population.¹⁹ A high prevalence of comorbidity between the two conditions would be unexpected by chance. However, some epidemiological studies⁴ and sporadic case reports³ suggest a possible association. Some studies reported that psychotic symptoms often occur in adult celiac disease and schizophrenic symptoms can improve after gluten-free diet.⁶

In our study we did not find celiac disease in patients with schizophrenia. But we found a strong association between high AGA level and schizophrenia in patients as compared with controls (Table 1). The higher prevalence of AGA in patients with schizophrenia suggests that Gluten Sensitivity confers susceptibility to schizophrenia. It is important to note that in this setting, gliadin antibody prevalence among individuals of the control group was similar to that reported for larger population studies: 2–12%.²⁰

Antibodies to tTG and the HLA markers for CD have high negative predictive value for CD. Since antibodies to tTG were not found in both groups, and, as well, the fact that there were no significant differences in the presence of susceptibility genes for celiac disease in schizophrenia and control subjects, our study suggests that these patients do not have CD. Therefore, these data suggest a potential connection between gluten and schizophrenia. This is in agreement with others' results, particularly with a

Table 2. Association of HLA alleles DQA1*0501, DQB1*02 and DQA1*0301, DQB1*0302 and the DQ2 and DQ8 heterodimers in patients with schizophrenia and controls

HLA	Patients N=104	Controls N=60	Odds Ratios (95% CI)	Fisher exact P value
DQA1*0501	42 (40.38%)	31 (51.66%)	0.634 (0.334-1.202)	0.108
DQB1*02	67 (64.42%)	26 (43.33%)	2.368 (1.237-4.534)	0.007*, **
DQA1*0301	37 (35.57%)	10 (16.66%)	2.803 (1.273-6.172)	0.006*, **
DQB1*0302	33 (31.73%)	10 (16.66%)	2.357 (1.064-5.221)	0.0230*
DQ2+	19 (18.26%)	13 (21.66%)	0.808 (0.367-1.781)	0.369
DQ8+	13 (12.5%)	10 (16.66%)	0.714 (0.292-1.746)	0.302

* P value < 0.05

** P value corrected after bonferroni $P < 0.017$ ($\alpha = 1 - 0.95^{1/N}$)

recently published study that also found that most patients with schizophrenia and elevated AGA titers do not have tTGA and do not appear to have celiac disease.^{5,21} Besides, these authors argue that sample size in their study was not large enough to exclude the possibility of celiac-associated-markers (HLA-DQ2/DQ8 genes, tTGA, anti-deamidated gliadin antibodies), and thereby celiac disease, being more prevalent in patients with schizophrenia than in normal healthy individuals. However, our study has more than six times the sample size of the previously mentioned one but we found the same results. In another study Cascella et al. reported an increased prevalence of celiac disease in patients with schizophrenia: 5.4% versus 0.80% in the control group, based on the presence of tTGA. But curiously they found no differences in the presence of anti-endomysial antibody between two groups in the same study.⁵ This last finding seems contradictory since antibodies to endomysial tissue are highly sensitive and specific; many studies have reported values in the range of 95-100% for both of these parameters.²²

We found an association between schizophrenia and the presence of alleles DQB1*02 and DQA1*0301, which is in line with previous reports.²³ Wei and Hemmings⁷ hypothesized that combinations of some DQB1 alleles and defects of the gene CLDN5, which forms permeability barriers in the gut and in the blood-brain barrier may confer a susceptibility to schizophrenia. Our data do not support this hypothesis at least in the context of a similar relationship between anti-gliadin immune response and the proposed gene-defect in gut barrier function, in the framework of coexistence of particular HLA DQ alleles. In our data the presence of high levels of AGA in the serum of patients was independent of the presence of the alleles DQB1*02 and DQA1*0301. Although high levels of anti-gliadin antibodies were correlated with the allele DQB1*0301, this allele is not associated with schizophrenia. These data reinforce our view that the relationship of schizophrenia with high levels of AGA, and these specific DQ alleles associations with schizophrenia are independent.

Gluten is linked to neurological harm in patients, both with and without evidence of celiac disease.^{3,12} The neurotoxic potential of AGA is a contentious issue. Some authors report an increase of AGA levels in neurological diseases. Pellecchia et al.²⁴ reported the occurrence of high AGA levels in 18.7% of multiple system atrophy affected-patients and Bushara et al.²⁵ reported 37% and 44% AGA prevalence in patients with hereditary ataxia and Huntington's disease respectively.

The neurotoxicity of Gliadin antibodies could be conditioned by their capacity to gain access to the central nervous system.⁶ As a matter of fact, AGA has been found in the cerebrospinal fluid in patients with gluten sensitivity and neurological dysfunction. Furthermore, in patients with gluten ataxia, the occurrence of lymphocytic infiltration of the perivascular space and the neutrophil by CD4 and CD8 T cells has been observed, suggesting the presence of a cell-mediated response, which in turn may compromise blood-brain barrier and allow the access of AGA into the central nervous system.⁶ On the other hand, AGA levels may be only a reflection of increased intestinal permeability. If this function of the gut is altered, some of the environmental factors, for example, potentially pathogenic components from ingested foods could easily enter the blood stream through the damaged permeability barrier. Consequently, a disease affecting the brain could be triggered.²⁶ In this context, it could be of interest to evaluate gluten peptide presence in cerebrospinal fluid of patients with schizophrenia.

Some authors suggest that there is an increased permeability of the gut irrespective of the genetic predisposition to autoimmunity. The passage of gliadin through the gut barrier could be mediated by receptors. In a recent study, Lammers et al. identified the chemokine receptor CXCR3 as a receptor for gliadin. Their

data suggest that gliadin binds CXCR3 on epithelial cells and triggers the increase in intestinal permeability through MyD88-dependent release of zonulin. This protein enables paracellular passage of gliadin (and possibly other nonself antigens) from the intestinal lumen to the gut mucosa.²⁷

Conclusions

Our results reveal an association between AGA and schizophrenia. Further studies are needed to elucidate the clinical consequences of this finding, to clarify mechanisms underlying potential pathophysiological relationships among gliadin, AGA and schizophrenia, and to investigate the potential of gluten-free diet as an environmental modifier of the disease.

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