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No correlation between HLA-DQ 2.5, DQ 8.1 and DQ 6.2 and circulating levels of antibodies against gliadins in schizophrenia

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ABSTRACT

It has been suggested that gluten consumption is linked to schizophrenia, with this link strengthened through the presence of circulating anti-native gliadin antibodies (AGAs). The human leukocyte antigen (HLA) system is crucial for antigen presentation and antibody secretion but no study has examined the relationship between HLA-II variants and circulating antibodies against gliadin peptides. In this study, HLA-II variants were genotyped in patients with schizophrenia and the relationship between these variants and plasma AGA levels was examined. Although there was no association found, HLA-AGA associations could potentially serve as a marker of gluten sensitivity in patients with schizophrenia.

1. Introduction

A link between the development of schizophrenia and gluten consumption has long been suggested, with studies, including double-blind case studies, demonstrating the recurrence of symptoms under gluten challenge and their alleviation with gluten-free diets (Eaton et al., 2015; Lionetti et al., 2015; Vlissides et al., 1986). The finding that increased levels of circulating anti-native gliadin antibody (AGA) levels are associated with schizophrenia suggests that this link may be immunological and is concurrent with other findings, implying an involvement of the immune system in the pathophysiology of schizophrenia, including genetic associations for the human leukocyte antigen (HLA) system (Reichelt and Landmark, 1995; Samaroo et al., 2010; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). The mechanistic role of AGAs in schizophrenia is unclear although they have been linked to peripheral inflammatory markers and increased levels of kynurenine (Kelly et al., 2018; Okusaga et al., 2016).

Both HLA-DQ2 and HLA-DQ8 variants are strongly associated with celiac disease, while altered AGAs in schizophrenia were not associated with these two variants (Dickerson et al., 2010). Previously, we demonstrated significant alterations of plasma levels of antibodies against linear peptide antigens derived from a component of wheat gluten, called gliadin, in schizophrenia (McLean et al., 2017). When compared with control subjects, the levels of plasma IgG against the γ-gliadin-derived fragment designated AAQ6C were significantly increased in patients with schizophrenia, while the levels of plasma IgG against the α-gliadin-derived fragment AL2G1 were decreased. As HLA-II molecules play a crucial role in presenting peptide-derived antigens to the adaptive immune system, the present study was undertaken to examine HLA-II variants with levels of plasma IgG against indigestible gliadin-derived fragments and native gliadin in schizophrenia.

2. Materials and methods

2.1. Patient samples

A total of 169 matched plasma and DNA samples from patients with schizophrenia were used in this study (132 males and 37 females, aged 42.0 ± 13.3 years). These samples were collected through the University of Aberdeen between 2003 and 2008, and had been stored long term at −80°C until they were aliquoted for antibody testing as detailed elsewhere (International Schizophrenia Consortium et al., 2009). All patients were diagnosed as having schizophrenia based on the DSM-IV criteria and were classified as British Caucasian individuals. Informed written consent to donate blood samples for schizophrenia research was received from every patient. This study was approved by a local ethics committee and conformed to the provisions of the Declaration of Helsinki and its amendments. Antipsychotic drugs used by schizophrenia patients at the time of sampling were listed in our previous study (McLean et al., 2017). Of these 169 patients, 128 were taking a single antipsychotic drug, 14 taking more than one drug and 27 without medication details, although medication status did not predict

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circulating AGA levels (McLean et al., 2017).

2.2. Selection of gliadin-derived antigens

Based on our initial findings that schizophrenia patients had an increase in anti-AAQ6C IgG levels and a decrease in anti-AL2G1 IgG levels (McLean et al., 2017), previously collected data on plasma levels of these antibodies in patients with schizophrenia were used to perform regression analysis to determine the associations between these antibody levels and HLA-II variants. Additionally, plasma levels of AGA IgG were also analysed as reported in our previous study (McLean et al., 2017).

2.3. Genotyping of HLA-II variants

HLA-II tagging SNPs were selected according to the SNP map constructed by de Bakker et al. (2006) to genotype HLA-II variants, including rs2187668 tagging to the DRB1*0301-DQA1*0501 and DQB1*0201 haplotypes that encode HLA-DQ2.5, rs7454108 tagging to the DRB1*04-DQA1*0301 and DQB1*0302 haplotypes that encode HLA-DQ8.1 and rs3135388 tagging to the DRB1*1501-DQA1*01-DQB1*0602 haplotypes that encode HLA-DQ6.2. Genotyping was performed using TaqMan-based PCR protocol as described previously (Halley et al., 2013). Briefly, PCR amplification took place in a 10 µl volume containing 2 X master mix (Applied Biosystems), 20 X assay mix (primer probe set) and 5–20 ng DNA. The PCR was performed under the following conditions: 50°C for 2 min, 95°C for 10 min, 50 cycles of 95°C for 15 s and 60°C for 1 min with OneStep real-time PCR system (Applied Biosystems). Genotype calls were made manually based on the distribution of the three clusters resulting from plotting the amplification of allele 1 versus allele 2.

2.4. Data analysis

To examine the association between HLA-II genotypes and anti-gliadin IgG levels, genotypes were inputted into a spreadsheet in numeric format, i.e. 0 = homozygous for the major allele, 1 = heterozygous, 2 = homozygous for the minor allele. Regression analysis was then applied with antibody levels as the dependent variable and HLA-II genotype, age and gender as independent variables. Where the residuals were skewed, log transformation was applied to achieve a normal distribution of the residuals.

3. Results and discussion

Regression analysis demonstrated no significant associations between plasma anti-gliadin IgG levels and any of the HLA-II genotypes tested at a significance level of $p < 0.05$ (Table 1). The lack of a genetic association between AGA IgG levels and HLA-DQ 2.5 ($p = 0.391$), which is involved in the presentation of the immunodominant gliadin epitope in celiac disease, provides further evidence that anti-gliadin antibodies in patients with schizophrenia are distinct from those present in celiac disease and is consistent with the study from Dickerson et al. (2010) showing that AGAs in schizophrenia were not associated with HLA-DQ2/8 alleles (Samaroo et al., 2010).

Serological and genetic markers of the anti-gliadin immune response in celiac disease are well characterised but this is not the case in schizophrenia. Previously, we reported an increase in plasma anti-AAQ6C IgG levels in this cohort of schizophrenia patients despite lack of altered AGA IgG or IgA levels. It is worth noting that elevated AGA levels in schizophrenia have been sustained in the literature, especially in meta-analysis (Čiháková et al., 2018; Lachance and McKenzie, 2014; McLean et al., 2017). Since HLA-II molecules are crucial for the presentation of antigen to the adaptive immune system to induce the production of antibodies, the identification of HLA-associations with IgG against this γ-gliadin-derived peptide in patients with schizophrenia could strengthen the association between anti-AAQ6C IgG and schizophrenia. Furthermore, HLA-II associations with AGA levels could also provide more information regarding the anti-gliadin immune response in schizophrenia. Finally, the identification of HLA-variants associated with anti-gliadin IgG levels has the potential to serve as genetic tests for a gluten-sensitive subgroup in schizophrenia.

Although no predictive associations were found in this study, further investigation of examining the relationship between HLA-II genotypes and anti-gliadin antibodies should be undertaken for the reasons as mentioned above. The present study has focussed on HLA-II genotypes associated with other diseases, including diabetes, celiac disease and systemic lupus erythematosus, all of which have previously been reported to be associated with schizophrenia to varying degrees and all have potential autoantibody involvement. Based on the latest update of the human major histocompatibility database (https://www.ebi.ac.uk/ipd/imgt/hla/), there are more than 5000 HLA-II alleles identified among the general population; however, the work reported in this study represents only a few HLA-types. Future studies attempting to examine the genetic association of anti-gliadin immune responses with schizophrenia should focus on screening other HLA-II alleles. An alternative hypothesis proposed is that AGAs might reflect cross-reactivity of gliadin molecules to autoantibodies in the circulation due to sequence homology or polyreactive autoantibodies. Therefore, analysis of the associations between HLA-II variants and circulating autoantibodies in schizophrenia may also be warranted. A limitation of this study is that we did not have any dietary records available although serological measures from patients on gluten-free diets may provide useful evidence in support of this hypothesis.

Declaration of interests

All authors declared that they had no conflict of interest.

Table 1
Associations between anti-gliadin IgG levels and HLA-DQ variants.

Antibody	*R*	adj. r^2	df	*F*	*p*	HLA-II Variant	Coefficient β	Standard Error	Standardised Coefficient β	*p*
AL2G1 IgG	0.214	0.010	5	1.282	0.275	DQ 6.2	0.011	0.031	0.029	0.736
						DQ 2.5	0.073	0.043	0.146	0.092
						DQ 8.1	−0.008	0.053	−0.013	0.878
AAQ6C IgG	0.230	0.017	5	1.490	0.197	DQ 6.2	0.062	0.039	0.139	0.115
						DQ 2.5	0.051	0.053	0.083	0.336
						DQ 8.1	0.067	0.066	0.088	0.312
Gliadin IgG	0.194	0.002	5	1.051	0.391	DQ 6.2	−0.058	0.051	−0.099	0.260
						DQ 2.5	−0.061	0.070	−0.074	0.391
						DQ 8.1	−0.105	0.087	−0.104	0.233

Regression analysis examining the relationship between IgG against gliadin molecules and HLA-DQ variants in patients with schizophrenia. Levels of IgG against gliadin molecules were not dependent upon any HLA-DQ variant measured in this schizophrenia cohort ($p < 0.05$). Age and gender were covariates in this analysis, but were not significant predictors of levels for any IgG measured ($p < 0.05$). The presence of HLA-DQ2.5 was not predictive of levels of IgG against native gliadin in patients with schizophrenia.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2018.12.001.

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