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Complement C4 associations with altered microbial biomarkers exemplify gene-by-environment interactions in schizophrenia

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Abstract

Schizophrenia is a complex brain disorder with genetic and environmental factors contributing to its etiology. Complement C4 genes are schizophrenia susceptibility loci and are activated in response to infections and gut microbiome imbalances. We hypothesize that C4 genetic susceptibility predisposes individuals to neuropathological effects from pathogen exposures or a microbiome in dysbiosis. In 214 individuals with schizophrenia and 123 non-psychiatric controls, we examined C4 gene copy number and haplotype groups for associations with schizophrenia and microbial plasma biomarkers. C4A copy number and haplotypes containing HERV-K insertions (C4A-long; C4AL-C4AL) conferred elevated odds ratios for schizophrenia diagnoses (OR 1.58–2.56, p<0.0001), while C4B-short (C4BS) haplogroups conferred decreased odds (OR 0.43, p<0.0001). Haplogroup-microbe combinations showed extensive associations with schizophrenia including C4AL with *Candida albicans* IgG (OR 2.16, p<0.0005), C4AL-C4BL with cytomegalovirus (CMV) IgG (OR 1.79, p<0.008), C4BS with lipopolysaccharide-binding protein (LBP) (OR 1.18, p<0.0001), and C4AL-C4AL with *Toxoplasma gondii* IgG (OR=17.67, p<0.0001). In controls, only one haplogroup-microbe combination was significant: C4BS with CMV IgG (OR 0.52, p<0.02). In schizophrenia only, LBP and CMV IgG levels were inversely correlated with C4A and C4S copy numbers, respectively (R^2=0.13–0.16, p<0.0001). C4 haplogroups were associated with altered scores of cognitive functioning in both cases and controls and with psychiatric symptom scores in schizophrenia. Our findings link complement C4 genes with a susceptibility to infections and a dysbiotic microbiome in schizophrenia. These

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Author Contributions

EGS conceived the idea for the paper and performed the data analyses. FL, AL and SY performed experiments and collected the data. EGS, FD and RHY wrote the paper.

Conflict of interest

Dr. Yolken is a member of the Stanley Medical Research Institute Board of Directors and Scientific Advisory Board. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies. None of the other authors report any potential conflicts of interest.

results support immune system mechanisms by which gene-environmental interactions may be operative in schizophrenia.

Keywords

complement system; microbiome; gut-brain axis; pathogens; gene-environment interactions; biomarkers

1. Introduction

Schizophrenia is a complex brain disorder with compelling evidence pointing to immune system dysfunctions as a place where genetic and environmental factors might converge and become neuropathological. Genetic studies show consistent associations of schizophrenia with polymorphisms in 6p21–6p22, a chromosomal region that contains the major histocompatibility complex (MHC) and that encodes hundreds of proteins integral to the immune system (Harrison, 2015). Environmental factors that interact with the immune system at elevated rates in schizophrenia include those involving specific pathogens such as bacteria, viruses, and parasites, as well as increased risks conferred by the infectious disease process (Benros et al., 2011; Kirch, 1993; Kohler et al., 2017; Miller et al., 2013; Severance and Yolken, 2020a; Torrey et al., 2012; Torrey and Peterson, 1976; Yolken and Torrey, 2008). In recent years, microbial associations with schizophrenia have taken a new direction, as research across many medical fields including psychiatry focuses on the gut microbiome as a potential source of novel disease mechanisms and treatments.

Residing in the MHC genomic region is complement C4, a gene that has long shown genetic and other biological associations with schizophrenia (Laskaris et al., 2019; Mayilyan et al., 2008b; Mondelli et al., 2020; Pouget, 2018; Prasad et al., 2018; Rey et al., 2020; Rudduck et al., 1985; Sekar et al., 2016; Woo et al., 2019). Complement C4 is an intriguing susceptibility gene candidate in schizophrenia because of its putative functional role in central nervous system synaptic pruning (Johnson and Stevens, 2018; Presumey et al., 2017; Sellgren et al., 2019). Prefrontal cortical and hippocampal dendritic spine deficiencies in schizophrenia may be a result of abnormally active synaptic pruning during adolescence (Bennett et al., 2013; Clarke et al., 2018). A C4 gene variant that is overexpressed or underexpressed during this sensitive time period would support a dysregulated pruning model mechanism for schizophrenia. Sekar et al (2016) demonstrated that increasing C4A copy number was associated with increased C4A expression in post-mortem brains of individuals with schizophrenia compared to controls. In that study, C4 protein localized to neuronal synapses, processes and cell bodies (Sekar et al., 2016).

Technological advances in basic research and bioinformatic methods have made accessible the many encoded microbial genes and inferred metabolic pathways of the gut microbiome and as such represent unique opportunities to discover novel disease mechanisms and treatments in fields not traditionally considered to have an infectious basis. In psychiatry, for example, there are accumulating reports of alterations in diversities and abundances of presumably commensal gut microbial taxa which distinguish the schizophrenia microbiome from that of controls (Castro-Nallar et al., 2015; Dickerson et al., 2017; Li et al., 2020;

Nguyen et al., 2019; Shen et al., 2018; Xu et al., 2019; Yolken et al., 2015; Zheng et al., 2019). These findings have contributed a resurgence of interest in understanding the role of the gut brain-axis in psychiatric disorders and overall idea that properly functioning peripheral systems have important ramifications for brain health. Experiments in germ-free mice continue to shed light on the neurobiological mechanisms by which gut microbes enter the brain and control behaviors (Collins et al., 2012; Diaz Heijtz et al., 2011; Erny et al., 2015; Foster and McVey Neufeld, 2013; Hsiao et al., 2013; Luczynski et al., 2016; Sampson and Mazmanian, 2015; Stilling et al., 2014). As relevant for studies of gene-environmental interactions involving the immune system, the gut microbiome is also critical for immune system development and maturation (Chistiakov et al., 2014; Dinan and Cryan, 2015; Sandhya et al., 2016; Severance et al., 2018; Wekerle, 2017).

Evaluating C4 genotypes and functional changes in the microbiome have previously been experimentally cumbersome to manage. Complement C4A and C4B are extensively polymorphic loci with significant variation in copy number and structure, making it difficult to accurately genotype individuals. C4A and C4B genes are paralogous and each can contain a human endogenous retroviral sequence (HERV-K) that confers a long (L) or short (S) form of the gene (Figure 1). C4A and C4B can also vary in the number of copies with a typical diploid genome containing two to eight copies. The availability of algorithms to impute C4 genotypes from GWAS data has accelerated the progress of C4 gene analyses in schizophrenia (Sekar et al., 2016). Dysfunction in the microbiome can be assessed by direct nucleic acid sequencing of bacterial taxa in relevant mucosal biospecimens, or via indirect measures of host physiological changes that occur during gut dysbioses. For the latter, plasma biomarkers of gastrointestinal inflammation and microbial translocation are considered valuable surrogate measures of an unhealthy gut microbiome (Severance and Yolken, 2020b). Markers that specifically target the bacterial endotoxin, lipopolysaccharide (LPS), are important validators that systemic inflammation has a microbial source such as a disturbed microbiome. In this paper, we test the hypothesis that complement C4 associations with microbes contribute to gene-environmental interactions in schizophrenia by examining C4A and C4B gene associations with plasma biomarkers of pathogen exposures and a dysregulated gut microbiome. Plasma biomarkers examined here included C-Reactive Protein (CRP), LPS-binding Protein (LBP), soluble CD14 (sCD14), *Candida albicans* IgG, *Saccharomyces cerevisiae* IgG, Cytomegalovirus IgG, and *Toxoplasma gondii* IgG.

2. Material and methods

2.1 Study population

A total of 337 individuals were recruited from Sheppard Pratt located in Baltimore, MD, U.S.A.: 123 were control individuals with no history of psychiatric disorder; 214 individuals were diagnosed with schizophrenia. Diagnoses were made in accordance with DSM-IV-TR (APA, 2000) and have been previously described (Dickerson et al., 2015; Dickerson et al., 2013). For the schizophrenia group, individuals received a DSM-IV-TR diagnosis of schizophrenia, schizophreniform disorder, or schizoaffective disorder and were between the ages of 18 and 65. Individuals without a history of psychiatric disorder were interviewed to rule out current or past psychiatric disorders with the Structured Clinical

Interview for DSM-IV Axis I Disorders Non-Patient Edition (First, 1998). Controls were between the ages of 20 and 60, inclusive. Exclusion criteria for both groups included: mental retardation; clinically significant medical disorder that would affect cognitive performance; any history of intravenous substance abuse or a primary diagnosis of substance abuse or substance dependence. For controls, any active substance misuse was considered an exclusion criterion. Cognitive function was evaluated with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) Form A (Randolph, 1998) and psychiatric symptoms rated according to the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Mean total RBANS scores ± standard deviation (SD) were 85.10+11.71 for controls and 64.30±11.64 for schizophrenia. In individuals with schizophrenia, mean total PANSS scores ± SD were 77.74±13.82; mean PANSS positive symptom scores were 19.70±5.56; and mean PANSS negative symptom scores were 21.07±4.57. Basic demographic and other data (age, sex, race, body mass index (BMI)) for this study population are shown in Table 1. Age, sex, race and BMI were significantly different between diagnostic groups.

These studies were approved by the Institutional Review Boards (IRB) of Sheppard Pratt and the Johns Hopkins Medical Institution following established guidelines. All participants provided written informed consent after study procedures were explained. This research was performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

2.2 Laboratory tests

Blood was drawn at the time of interview using Becton-Dickinson's Cell Preparation Tubes containing sodium citrate. Plasma was separated and stored at −80°C. Peripheral blood mononuclear cells (PBMCs) were isolated and stored at −80oC. DNA was extracted from PBMCs using Qiagen's DNAeasy kit and stored at −80oC.

2.3 Biomarkers of pathogen exposures and gut dysbioses

The following biomarkers were measured in plasma using commercially-available enzyme-linked immunosorbent assays (ELISAs): CRP, LBP, sCD14, *Candida albicans* IgG, *Saccharomyces cerevisiae* IgG, Cytomegalovirus IgG, and *Toxoplasma gondii* IgG. Methods and analyses reporting psychiatric case and control levels of these biomarkers were previously described (Dickerson et al., 2007a; Dickerson et al., 2007b; Leweke et al., 2004; Severance et al., 2012; Severance et al., 2016a; Severance et al., 2013).

2.4 Data analyses

Complement C4A, C4B, C4S and C4L haplotypes and copy numbers were imputed from GWAS SNPs data using previously described methods (Sekar et al 2016; https://github.com/freeseek/imputec4). T-tests were used to detect bivariate associations between continuous variables. Chi-square analyses were used to detect bivariate associations between categorical variables. Multiple linear regression models were used to examine correlations among continuous variables. Multivariate logistic regression models were used to assign odds ratios for copy numbers and haplotype group associations with diagnoses, plasma biomarker levels, RBANS scores, and PANSS scores. We focused on the most common haplogroups

found in our population and as characterized by Sekar et al (2016). Haplogroup associations in multivariate models were further analyzed in relation to homozygous and heterozygous status compared to individuals in whom the haplogroup was absent. Multivariate regressions included the covariates: age, gender, race, BMI; an assay plate covariate was included with the ELISA data analyses to correct for plate-to-plate variation. These regression models applied robust standard error corrections to accommodate multiple samples per individual. We did not formally account for multiple comparisons in order to prevent the possibility that a too stringent threshold would lead to type B errors. Therefore, a p-value of less than 0.05 was considered significant. However, it is of note that for most of the analyses, our p-value fell well below 0.01.

3. Results

We found that the haplogroup containing two copies of C4A-long (C4AL-C4AL) was associated with an elevated odds of schizophrenia compared to controls (Table 2; OR=2.56, 95th%CI 1.05–6.27, p<0.0001). The haplogroup containing one copy of C4B-short (C4BS) was associated with a significantly decreased odds of a schizophrenia diagnosis (OR=0.43, 95th%CI 0.20–0.94, p<0.0001). None of the other haplogroups shown in Table 2 were associated with a significantly altered odds for schizophrenia. In comparisons of mean C4 copy number differences between cases and controls, we found significantly greater copy numbers of C4A in schizophrenia and greater numbers of C4B in controls (controls vs schizophrenia, mean levels ± standard error: C4A 1.94±0.04 vs 2.08±0.04, t=−2.53, p<0.01; C4B 1.91±0.03 vs 1.76±0.03, t=3.19, p<0.002). In multivariate logistic models, C4A copy numbers conferred increased odds of a schizophrenia diagnosis compared to controls (Table 2; OR=1.58, 95%CI 1.00–2.53, p<0.0001). There were no detectable differences in mean number of copies for C4B, C4S or C4L between diagnostic groups. C4A copy numbers were significantly inversely correlated with C4B copy numbers in controls and schizophrenia (controls: R^2=0.16, coefficient=−0.44, 95th% CI=−0.62−−0.27, p<0.0001; schizophrenia: R^2=0.46, coefficient=−0.85, 95th% CI=−0.97−−0.73, p<0.0001). Similarly, C4S copy numbers were significantly inversely correlated with C4L copy numbers in controls and schizophrenia (controls: R^2=0.69, coefficient=−0.78, 95th% CI=−0.87−−0.69, p<0.0001; schizophrenia: R^2=0.77, coefficient=−0.73, 95th% CI=−0.79−−0.67, p<0.0001).

Plasma biomarkers of pathogen exposure and gut dysbiosis were extensively associated with C4 haplogroups in schizophrenia but minimally in controls. In multiple linear regression models, cytomegalovirus IgG was inversely correlated with C4S copy numbers in schizophrenia but not controls (R^2=0.16, regression coefficient=−0.30, 95th%CI=−0.56−−0.05, p<0.0001). Also, only in schizophrenia, LBP was inversely correlated with C4A copy numbers (R^2=0.13, regression coefficient=−2.41, 95th%CI=−4.03− −0.79, p<0.0001). As shown in Table 3, *C. albicans* IgG, cytomegalovirus IgG, LBP and *T. gondii* IgG all showed significantly elevated odds ratios for associations with specific C4 haplogroups and significantly reduced odds ratios for other C4 haplogroups in schizophrenia. These associations varied according to homozygous and heterozygous states. For haplogroup-biomarker combinations associated with significantly elevated odds ratios, we further depicted these relationships for exploratory purposes by charting several representative biomarker levels according to homozygous and heterozygous status of the haplogroup, as

shown in Figure 2. In controls, only one haplogroup-microbe combination was significant: C4BS with CMV IgG (OR 0.52, 95th%CI=0.29–0.95,−p<0.02). Overall, no significant associations with C4 were observed for *S. cerevisiae* IgG, CRP or sCD14.

In analyses of cognitive functioning, decreased RBANS scores were significantly associated with schizophrenia in individuals who were homozygous for the haplogroups C4AL and C4BS compared to individuals with schizophrenia who did not have these haplogroups (Table 4; C4AL: OR 0.68, 95th%CI 0.55–0.83, p<0.0001; C4BS: OR 0.82, 95th%CI 0.76–0.90, p<0.0001. Controls who were homozygous for the haplogroup C4AL-C4BS showed decreased RBANS scores compared to those who did not have this haplotype (OR 0.94, 95th%CI 0.89–1.00, p<0.03). Control individuals who were homozygous for the haplotype C4AL-C4BL showed elevated scores on RBANS compared to those who did not have this haplotype (OR 1.07, 95th%CI 1.00–1.14, p<0.02).

In multivariate models, homozygosity of the C4BS haplogroup and heterozygosity of the C4AL-AL haplogroup were associated with less severe positive symptoms (C4BS: OR 0.54, 95th%CI 0.33–0.88, p<0.0001; C4AL-AL: OR 0.92, 95th%CI 0.85–1.00, p<0.0001). Homozygotes and heterozygotes of the C4AL haplogroup showed increased severity of negative psychiatric symptoms compared to those who did not have this haplogroup (Heterozygotes: OR 1.24, 95th%CI 1.09–1.40, p<0.0001; Homozygote: OR 8.62, 95th%CI 1.48–50.33 p<0.0001).

4. Discussion

Complement C4 is an interesting susceptibility gene candidate for studies of gene-environmental interactions in schizophrenia because of its multifactorial neurobiological ties to the immune system. Not only is it located in the MHC, an immune gene region long associated with schizophrenia, but it is activated following environmental immune challenges such as infections that are known risk factors for schizophrenia. It also likely has a functional role in central nervous system synaptic pruning, a neurobiological mechanism relevant to schizophrenia (Nimgaonkar et al., 2017; Presumey et al., 2017; Sekar et al., 2016; Stevens et al., 2007). Here we demonstrated that in people with schizophrenia, complement C4A and C4B polymorphisms conferred a diversity of phenotypes related to microbial environmental variables such as pathogen exposures and gut dysbioses. These findings are especially relevant as examples of how gene and environmental variables may be interactive in schizophrenia via the immune system. We also observed that C4A and C4B copy numbers were inversely correlated with each other, likely reflecting the nonrandom linkage of C4A and C4B loci. Our study further detected significant associations of C4A and C4B haplotypes not only with a diagnosis of schizophrenia and environmental factors, but with psychiatric symptoms and cognitive functioning. These phenotypes in schizophrenia appeared most extreme when haplogroups were in the homozygous state.

Pathogen exposures have been studied as risk factors for the development of schizophrenia for a long time (Severance and Yolken, 2020a). More recently, interest in the microbiome and gut-brain axis has expanded the scope of the focus on infectious agents to the trillions of commensal microbes that inhabit the gastrointestinal tract and other mucosal surfaces.

Alterations in the microbiome have been associated with gut dysbiosis, a condition that is increased in individuals with schizophrenia and other serious psychiatric disorders. This gut dysbiosis generally reflects an inflammatory state in the GI tract which leads to microbial translocation and a toxic cycle of systemic inflammation and leaky endothelial barriers at the blood-gut and blood-brain barriers (Dickerson et al., 2017; Severance et al., 2012; Severance et al., 2014; Severance et al., 2015; Severance et al., 2016c). Exposures to pathogens and a gut in dysbiosis represent immune-related environmental variables that are potentially impacted by a dysregulated complement system. In our study, only one of the measured biomarkers was associated with C4 haplogroups in controls, whereas in schizophrenia, there were widespread associations of these environmental immune factors with C4 haplogroups. For example, case-control differences in peripheral biomarkers in conjunction with C4 haplotypes were detected for IgG class antibodies to *C. albicans*, cytomegalovirus, and *T. gondii*.

The IgG-based markers can be generally categorized as biomarkers of exposure to pathogens; however, there are additional implications of these markers for GI-specific pathologies. *C. albicans* IgG, for example, has been shown in our previous studies to be associated with GI conditions in schizophrenia (Severance et al., 2016a; Severance et al., 2017; Severance et al., 2015; Severance et al., 2016b; Severance et al., 2016c). CMV is a virus that can infect the GI tract and has been implicated as a source of inflammation in inflammatory bowel diseases such as ulcerative colitis (Jentzer et al., 2020; Sager et al., 2015). Furthermore, studies of animal models implicate *T. gondii* in gut-related inflammation and dysbiosis, as this parasite primarily infects its host by the GI tract (Severance et al., 2016a; Severance et al., 2016b; Severance et al., 2016c). Here, the C4AL-AL haplogroup showed the strongest association with a schizophrenia diagnosis and with *T. gondii* IgG antibodies. *T. gondii* is historically one of the best-replicated pathogens that is associated with an increased risk of schizophrenia (Torrey et al., 2012; Yolken et al., 2009). The non-IgG based measure, LBP, on the other hand, specifically reflects the response to microbial translocation and the circulation of bacterial LPS, as would be apparent if a gut microbiome were in dysbiosis (Severance et al., 2013). In individuals with schizophrenia but not controls, LBP was inversely correlated with C4A copy numbers, a finding of interest since C4A copy numbers showed the strongest association with a schizophrenia diagnosis. While these markers are useful surrogate indices of microbial exposures, it will be important to compare marker patterns with direct measures of the microbiome in future studies.

Most of the common C4 haplogroups studied were associated with altered severity of psychiatric symptoms and measures of cognitive functioning, especially C4 haplogroups in a homozygous state. For example, the C4BS haplogroup was associated with decreased severity of positive psychiatric symptoms (OR 0.54, $p<0.0001$) and the C4AL haplogroup was associated with increased severity of negative psychiatric symptoms (OR 8.62, $p<0.0001$). C4 haplogroups were associated with scores of cognitive functioning in people with schizophrenia and in controls suggesting an interaction between C4 and cognition irrespective of its association with schizophrenia. Interestingly, in controls the C4AL-BS haplogroup was associated with worse performance on the RBANS while C4AL-BL conferred a beneficial effect (i.e high scores). The effects of C4 variants on cognition in schizophrenia and controls have previously been examined in various forms. For example,

C4A RNA expression predicted from C4A structural variation was associated with poorer memory recall in individuals with schizophrenia compared to healthy controls. Healthy controls with higher predicted C4A expression, however, had reduced cortical activity during a visual processing task (Donohoe et al., 2018). Likewise, a strong association of complement pathway genes on cognitive function was observed independently of C4A structural variation and independently of an association with schizophrenia (Holland et al., 2019).

The association between C4A and an increased risk of schizophrenia has been previously demonstrated in several studies. However, the driving force behind the inverse relationship between C4B and schizophrenia risk may be more tenuous (Mayilyan et al., 2008a; Rudduck et al., 1985; Sekar et al., 2016; Woo et al., 2019). It is not clear if the decreased C4B copy number confers a protection against risk for the disorder or if the decrease reflects an associated pathological protein deficit. The finding of an inverse correlation between C4A and C4B and between C4L and C4S copy numbers more likely reflects that these loci may be in linkage disequilibrium with risk (C4A and C4L) vs protection (C4B and C4S) at either end of the spectrum. Based on studies of chemical-binding preferences, C4A has an affinity for amino groups and C4B for hydroxyl groups, suggesting that C4A may function more to bind immune complexes and antigenic proteins and C4B in binding to antigens with carbohydrate-rich domains such as microbes (Presumey et al., 2017). Because a multitude of studies demonstrate exposure to pathogens as a risk factor for schizophrenia (Severance and Yolken, 2020a), a deficiency of C4B protein in schizophrenia may reflect compromised microbe-binding ability. Conversely, it is also conceivable that low C4B copy number confers a degree of protection with respect to preserving the body's microbiome from C4B-mediated over-harvesting of beneficial microbes. In a study of pediatric inflammatory bowel disease, a low C4B copy number was associated with less inflammation and greater diversity of gut microbes than individuals with higher C4B gene copy numbers (Nissila et al., 2017). As reports of the gut microbiome in schizophrenia begin to populate the literature, it will be important to examine C4 genotypes in conjunction with microbiome profiling. It will also be necessary to further interrogate these haplotype associations to rule out that variation in nearby HLA genes which segregate with specific C4 alleles via linkage disequilibrium are not driving the observed immune traits (Kamitaki et al., 2020).

The understanding of complement C4 genotypes and C4A/C4B dynamics is just beginning to be elucidated as improved genomic techniques illuminate previously inaccessible regions of the genome in larger and more diverse study populations. Our finding of several deleterious haplotype combinations and associations with pathogens, inflammatory gut processes, psychiatric symptom severity and cognitive functioning suggests that C4 gene screening might aid the identification of subsets of individuals with a potentially more severe disease course. Complement-based therapies with the aim of decreasing C4A activity and/or increasing C4B activity in different genetic backgrounds will require examination in a research setting.

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Abbreviations:

BMI	Body mass index
C4AL	C4A-long
C4AS	C4A-short
C4BL	C4B-long
C4BS	C4B-short
CMV	Cytomegalovirus
CRP	C-reactive protein
ELISA	Enzyme-linked immunosorbent assay
HERV	Human endogenous retrovirus
HMZ	Homozygous
HTZ	Heterozygous
IgG	Immunoglobulin G
LBP	Lipopolysaccharide binding protein
MHC	Major histocompatibility complex
OR	Odds ratio
PBMCs	Peripheral blood mononuclear cells
sCD14	Soluble CD14

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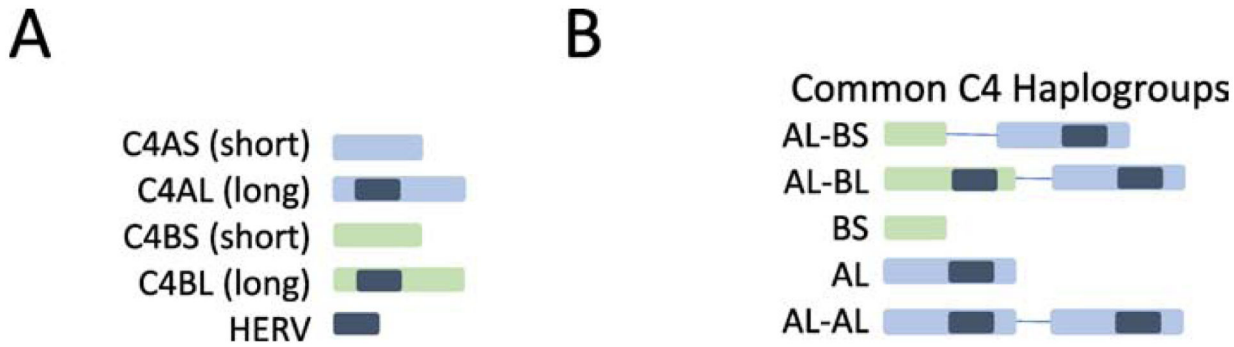

Figure 1. Structural complexity of the complement C4 gene

Panel A: C4 genes exist in four forms (1) C4A gene short (C4AS - no HERV insertion); (2) C4A gene long (C4AL – contains HERV insertion); (3) C4B gene short (C4BS no HERV insertion); (4) C4B gene long (C4BL – contains HERV insertion). Panel B: Five common C4 haplogroups that reflect copy number variation and presence/absence of the HERV insertion. This diagram is based on material from Sekar et al (2016)(Sekar et al., 2016).

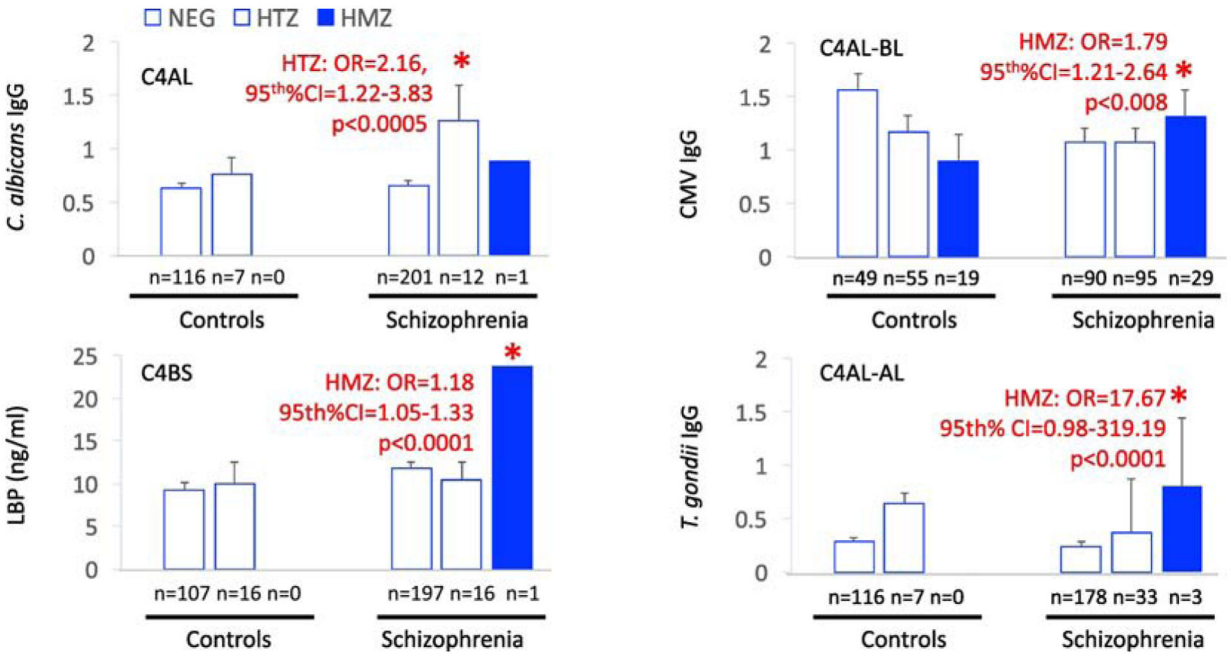

Figure 2. C4 haplogroup associations with microbial biomarkers in schizophrenia

NEG refers to individuals not having the listed haplogroup; HTZ refers to heterozygous for the listed haplogroup; HMZ refers to homozygous. Plotted are mean levels of plasma biomarkers. Error bars indicate standard error of the mean. Asterisk designates significance at $p<0.05$ of multivariate models that included age, race, sex, BMI and assay plate as covariates.

Table 1.

Demographics and other variables of the study populations.

Population	n Individuals	Age Mean years+SD[1]	Sex n (% Female)	Race n (% Caucasian)	BMI Mean score+SD
Controls	123	32.34+11.20[2]	79 (64.22)[3]	80 (65.04)[4]	26.76+6.33[5]
Schizophrenia	214	37.40+12.43	73 (34.11)	105 (49.06)	30.67+8.07

[1] SD refers to standard deviation;

[2] T =−4.66, two-tailed p<0.001;

[3] Chi-square = 28.61, p<0.001;

[4] Chi-square = 8.05, p<0.005;

[5] T =−4.65, two-tailed p<0.0001

Table 2.

C4 haplogroup & copy number odds ratios for a schizophrenia diagnosis as compared to controls

	OR	95th%CI	p-value
Haplogroup			
AL-BS	1.07	0.76–1.51	NS
AL-BL	0.94	0.66–1.34	NS
BS	0.43	0.20–0.94	0.0001
AL	0.73	0.28–1.90	NS
AL-AL	2.56	1.05–6.27	0.0001
Copy Number			
C4A	1.58	1.00–2.53	0.0001
C4B	0.55	0.29–1.06	NS
C4S	0.85	0.61–1.18	NS
C4L	1.20	0.89–1.60	NS

Controls n=123, Schizophrenia n=214.

OR – odds ratio; CI – confidence interval; NS - not statistically significant at $p<0.05$.

Table 3.

Odds ratios for C4 haplogroup associations with biomarkers of pathogen exposures and gut dysbioses

	***C. albicans* IgG**		***CMV*IgG**		**LBP**		***T. gondii* IgG**	
	OR (95th%CI) p		**OR (95th%CI) p**		**OR (95th%CI) p**		**OR (95th%CI) p**	
	HTZ	**HMZ**	**HTZ**	**HMZ**	**HTZ**	**HMZ**	**HTZ**	**HMZ**
Control								
AL-BS	NS	NS	NS	NS	NS	NS	NS	NS
AL-BL	NS	NS	NS	NS	NS	NS	NS	NS
BS	NS	NA	0.52 (0.29–0.95) 0.02	NA	NS	NA	NS	NA
AL	NS	NA	NS	NA	NS	NA	NS	NA
AL-AL	NS	NA	NS	NA	NS	NA	NS	NA
Schizophrenia								
AL-BS	NS	NS	0.62 (0.44–0.87) 0.001	0.62 (0.43–0.90) 0.001	NS	NS	NS	NS
AL-BL	NS	NS	NS	1.79 (1.21–2.64) 0.008	0.97 (0.94–1.00) 0.002	NS	NS	NS
BS	0.20 (0.05–0.82) 0.0001	NS	1.55 (1.01–2.38) 0.0001	0.0003 (1E-6–0.10) 0.0001	NS	1.18 (1.05–1.33) 0.0001	NS	0.43 (0.26–0.70) 0.001
AL	2.16 (1.22–3.83) 0.0005	NS	1.56 (1.09–2.24) 0.0001	0.20 (0.06–0.62) 0.0001	NS	1.09 (1.02–1.16) 0.0001	NS	0.33 (0.18–0.60) 0.0001
AL-AL	NS	NS	NS	NS	0.96 (0.92–0.99) 0.0001	NS	NS	17.67 (0.98–319.19) 0.0001

OR – odds ratio; CI – confidence interval; p – p-value; HTZ – heterozygote; HMZ – homozygote; NS - not statistically significant at $p<0.05$; NA - not applicable (due to absence of homozygotes in the control group); CMV - cytomegalovirus

Comparison is HTZ and HMZ relative to absence of haplotype.

Table 4.

Odds ratios for C4 haplogroup associations with PANSS and RBANS scores

	PANSS positive		PANSS negative		RBANS	
	OR (95th%CI) p		OR (95th%CI) p		OR (95th%CI) p	
	HTZ	HMZ	HTZ	HMZ	HTZ	HMZ
Control						
AL-BS	NA	NA	NA	NA	NS	0.94 (0.89–1.00) 0.03
AL-BL	NA	NA	NA	NA	NS	1.07 (1.00–1.14) 0.02
BS	NA	NA	NA	NA	NS	NA
AL	NA	NA	NA	NA	NS	NA
AL-AL	NA	NA	NA	NA	NS	NA
Schizophrenia						
AL-BS	NS	NS	NS	NS	NS	NS
AL-BL	NS	NS	NS	NS	NS	NS
BS	NS	0.54 (0.33–0.88) 0.0001	NS	NS	NS	0.82 0.76–0.90 0.0001
AL	NS	NS	1.24 (1.09–1.40) 0.0001	8.62 (1.48–50.34) 0.0001	NS	0.68 (0.55–0.83) 0.0001
AL-AL	0.92 (0.85–1.00) 0.0001	NS	NS	NS	NS	NS

OR – odds ratio; CI – confidence interval; p – p-value; HTZ – heterozygote; HMZ – homozygote; NS - not statistically significant at $p<0.05$; NA - not applicable (due to absence of homozygotes in the control group); PANSS positive refers to positive psychiatric symptoms; PANSS negative refers to negative psychiatric symptoms.

Comparison is HTZ and HMZ relative to absence of haplotype.