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Joshua Inyangson Date

The Intersection Between Genetics and Infection in Maternal, Paternal and Offspring with Schizophrenia in an Ashkenazi Jewish Population

By

Joshua Inyangson
Master of Public Health

Epidemiology

_____

Brad Pearce
Committee Chair

# The Intersection Between Genetics and Infection in Maternal, Paternal and Offspring with Schizophrenia in an Ashkenazi Jewish Population

By

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Bachelor of Science
Carnegie Mellon University
2018

Thesis Committee Chair: Brad D. Pearce, Ph.D.

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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**Abstract**

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By Joshua Inyangson

Much of literature in the study of schizophrenia (SCZ) focuses on the interaction between its genetic and environmental components, but few studies have examined the intersection of genetics and infections in maternal, paternal, and the offspring with schizophrenia. In a sample of 539 subjects, we sought to examine the interaction of maternal cytomegalovirus (CMV) infection and a particular single nucleotide polymorphism carried by the proband on the risk for schizophrenia. We reasoned that maternal infection would be more likely to be a risk factor for schizophrenia in the offspring than paternal infection. We used regression analysis, chi-square, Kruskal-Wallis and Mann-Whitney analysis to assess the associations between infections (and other immune markers, both individually and collectively) and parental status. Age was found to be negative correlated with parental status, as mothers were more likely to be younger than fathers. Non-significant associations were found between infections (cytomegalovirus and toxoplasma) with parental status. Significant ($p<0.05$) associations were found between immune marker levels and parental status, as herpes virus type 6 (HHV6) levels were 19% higher in mothers than fathers, C-reactive protein (CRP) levels were 17% higher in mothers than fathers, and gliadin (GLIAD) levels were 14% higher in fathers than mothers. We were able to identify immune markers that differentiated mothers and fathers in SCZ triad samples. This data provides new insights into the immune dynamics of SCZ at the interface of genetics and infections relevant to neurodevelopment and gestational insults.

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**TABLE OF CONTENTS:**

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**Abstract**

Much of literature in the study of schizophrenia (SCZ) focuses on the interaction between its genetic and environmental components, but few studies have examined the intersection of genetics and infections in maternal, paternal, and the offspring with schizophrenia. In a sample of 539 subjects, we sought to examine the interaction of maternal cytomegalovirus (CMV) infection and a particular single nucleotide polymorphism carried by the proband on the risk for schizophrenia. We reasoned that maternal infection would be more likely to be a risk factor for schizophrenia in the offspring than paternal infection. We used regression analysis, chi-square, Kruskal-Wallis and Mann-Whitney analysis to assess the associations between infections (and other immune markers, both individually and collectively) and parental status. Age was found to be negative correlated with parental status, as mothers were more likely to be younger than fathers. Non-significant associations were found between infections (cytomegalovirus and toxoplasma) with parental status. Significant ($p<0.05$) associations were found between immune marker levels and parental status, as herpes virus type 6 (HHV6) levels were 19% higher in mothers than fathers, C-reactive protein (CRP) levels were 17% higher in mothers than fathers, and gliadin (GLIAD) levels were 14% higher in fathers than mothers. We were able to identify immune markers that differentiated mothers and fathers in SCZ triad samples. This data provides new insights into the immune dynamics of SCZ at the interface of genetics and infections relevant to neurodevelopment and gestational insults.

## Introduction

Schizophrenia (SCZ) is a chronic and debilitating group of brain disorders that has severe consequences for the individual and family members that are affected. Affecting about 0.5% to 1% of the population worldwide, it is characterized by delusions, hallucinations, disorganized thinking and speech, disorganized or abnormal motor behavior and negative symptoms (Hosak, 2013). Diagnosis most commonly occurs in late adolescence to early childhood, requiring lifelong treatment to keep symptoms under control and improve quality of life. There has been a large amount of genetic research into the etiology of schizophrenia. Twin concordance studies have been able to consistently conclude that heritability is high, about 81% (Sullivan et al, 2003). Family studies have been able to show that heritability for SCZ is very high, as having a first-degree relative with SCZ is associated with an odds ratio of almost ten (Sullivan, 2005). Although there are a number of additional risk factors for SCZ (season of birth, migrant status, biological sex), the size of the odds ratio for family history and high heritability in twin concordance studies suggests that there are underlying genetic and pathophysiological mechanisms that have not yet been discovered.

Despite substantial evidence that genetic factors have an impact in the risk of SCZ, much of its genetic variance remains undiscovered. Genetic association studies (GWAS) have been used over the past decade to identify genetic risk factors for common diseases like schizophrenia, bipolar disorder and other polygenic traits. Ultimately, GWAS uses genetic factors to make predictions about who is at risk and identify biological underpinnings of disease susceptibility for developing new prevention and treatment strategies (Bush et al, 2012). Early GWAS, although large at the time, were underpowered to detect associations when appropriately correcting for multiple tests, given the effect size of risk genes was generally below 1.2, much lower than expected (Bergen et al, 2012). Fortunately, GWAS studies have been able to incrementally identify more risk loci. Recently, the Psychiatric Genomics Consortium-Schizophrenia Workgroup (PGC-SCZ) published a GWAS study establishing a list of greater than 100 genetic loci, illustrating the risk of SCZ at accepted standards of statistical significance. From their study, Lencz et al were able to identify several examples of 'low-hanging fruit' – genes that were plausibly connected to SCZ pathophysiology, likely to be influenced by the SNPs identified by the PGC-SCZ GWAS (Lencz et al., 2015). Multiple genes that are already well known like DRD2, GRM3 and other lesser known genes were implicated in their study, adding to prior evidence that SCZ is a polygenic disorder. Additionally, another GWAS study done in 2014, involving over 150,000 cases and controls across multiple ancestry lines was able to find 108 physically distinct loci, 83 of which had not been previously implicated in SCZ (Nature, 2014). GWAS studies such as these not only shed light on the polygenicity of SCZ but also provide biological insight into its etiology.

Environmental factors also undoubtedly play a role in risk for SCZ. Recent epidemiological studies have implicated different infectious agents in the pathology of SCZ. Several studies have suggested that SCZ patients have a higher proportion of antibodies against CMV and that the reactivity of their sera, as measured by enzyme immunoassay in optical density, was significantly stronger than that of control individuals (Torrey et al, 2006). Toxoplasma gondii (TOXO), responsible for causing severe central nervous system symptoms in immunocompromised individuals, was found to be elevated in 15 different studies of SCZ (Torrey et al, 2012). Viruses in the Herpes Simplex Family, like Herpes Simplex Virus 1 (HSV1), have been consistently implicated as an independent predictor of cognitive impairment in individuals with SCZ (Dickerson et al, 2003; Shirts et al, 2008; Prasad et al, 2012). Human

Herpes Virus Type 6 (HHV6), another virus of prime interest in SCZ research, have shown a significant association with SCZ in select populations (Niebuhr et al, 2007). However, findings from previous studies have been inconsistent in their findings (Aria et al, 2011; Gutiérrez-Fernández et al, 2015). With such a diverse list of infectious agents and factors that have been implicated in SCZ, these statistical associations could be as a result of the response to infection and immune activation rather than the specific infectious agents (Avramopoulos et al, 2015). In fact, research in the etiology of SCZ has expanded to immune responses to gliadin (GLIAD) with a few studies showing an association with SCZ although most study results have been inconsistent (Dickerson et al, 2010). Antibodies to this protein are indicative of autoimmunity, and several studies have suggested an increase predilection to autoimmune disease among people with SCZ. C-Reactive Protein (CRP), useful in the diagnosis and monitoring of many acute and chronic inflammatory conditions, has a growing body of literature supporting an association with schizophrenia (Miller et al, 2014). Longitudinal associations between adolescents CRP levels and adult SCZ diagnosis have been able to potentially indicate the important role of inflammation in the pathogenesis of SCZ (Metcalf et al, 2016).

However, to our knowledge, the study of the intersection between genetic and infectious risk factors has not been well studied, especially among family triads. Family triads are used in research when there are interrelationships within the family. From prior literature, we already know that there is a familial component in the prognosis of schizophrenia. Exploring the link between prenatal insults and the risk of schizophrenia, there is accumulating evidence from epidemiological studies that have implicated maternal infection in the disease's etiology (Brown et al, 2010). Results for prenatal maternal infections differ between studies but there is general agreement that prenatal exposure to a range of infections and inflammatory responses have been associated with the risk of schizophrenia in offspring. (Khandaker et al, 2012). Finding a link between genetics and infection, Borglum et al found that maternal infection with CMV had a fivefold increased likelihood of SCZ developing in the offspring, but only if the child carried a specific host genetic variant (SNP rs7902091). Pursuing research in the intersection of genetics and infection in familial triads would be able to provide clearer evidence about parental genetic interaction and infection in offspring with schizophrenia. In addition, familial triads better control for environmental factors and other covariates seen in the traditional family. Therefore, in this current study, we examine the association of SCZ with infection status and immune marker level in familial triads. We used an existing cohort of family triads (mother, father and child) from an Ashkenazi Jewish population to try to replicate the association found by Borglum et al. If Borglum's relationship is correct in our sample, we expect to see a saturation of seropositive CMV mother and their recessive allele proband pairings compared to a seronegative CMV mother and their recessive allele proband pairing. We also expect to see no difference regardless of seropositivity between the father and their recessive allele proband. We then tested the association between parental status and infection, the association between parental status and cumulative infection and the association between parental status and immune marker level.

## Methods

*Subjects*

The institutional review board at Johns Hopkins University approved the recruitment methods, protocols and informed consent documents used in this study. Subjects provided written informed consent forms to be able to participate in this study. If the subject was a minor, written consent needed to be obtained from both the subject and their parent/guardian or written

informed consent was obtained from the parent/guardian and an assent procedure was performed with the subject. If the subject was not capable of consenting, the parent/guardian provided written informed consent (Avramopoulos et al, 2015). Research participants were genetically homogenous and of Ashkenazi Jewish descent. The original research cohort consisted of participants diagnosed with schizophrenia (including schizoaffective disorder) or bipolar disorder. The population sample was recruited over a 15-year period between 1996 and 2011. Recruitment methods mainly consisted of advertisements, talks, letters to the leaders' service providers of the Jewish population and a study website (Avramopoulos et al, 2015). All cases were ascertained reporting four grandparents of known AJ descent. Subjects that were diagnosed with schizophrenia were interviewed in person and diagnosed through a consensus procedure found in other literature. Examiners were blinded to the subject's diagnosis. Most of the subjects were seen in their homes and parents were examined by a doctoral level psychologist (Avramopoulos et al, 2015). It was possible for parents to be positively diagnosed as cases however the likelihood of this was less than 10% and likely had little impact on overall statistical differences. The blood for DNA and plasma samples were collected and frozen at -80$^{oC}$.

For this current study, we had serology data from 2660 subjects and genotype data from 2179 subjects. No control samples were available in this study therefore father and mothers were used as the two main comparison groups for this case study. Only schizophrenic and schizoaffective family triads of a father, mother and child (proband) were created utilizing the family ids and case ids obtained from the dataset. Organizing the subjects into triads allowed for a proxy control group (fathers) while controlling for factors within each family. 86 subjects with substantial missing serology were excluded from the final count of subjects. After data cleaning, the final study size was 539 schizophrenia/schizoaffective subjects (185 family triads) (Table 1).

*Immunoassay Measurements and Data Cleaning*

Plasma Igb class antibodies for anti-HSV1, anti-HHV6, anti-CMV, anti-TOXO and anti-GLD were measured using immunoassay methods described in previous literature (Avramopoulos et al, 2015). The reagents and assay kits for anti-HSV1 were obtained from Focus Laboratories, Cypress CA. The reagents for ant-HHV6 were obtained from Advanced Biotechnologies Incorporated, Columbia, MD. The reagents that were used for anti-CMV, anti-TOXO and for measurement of CRP were obtained from IBL Laboratories, Hamburg, Germany. Reagents for anti-GLD were obtained from Iowa Diagnostics, San Diego, California (Avramopoulos et al, 2015). Avramopoulos et al. in their previous study had been able to show near-normal density plot distributions for anti-GLD, anti-HHV6 and CRP while their anti-HSV1, anti-CMV and anti-TOXO showed clearly bimodal density plot distributions. A cut point was determined for the near-normal distributions and converted to dichotomous variables. For the clearly bimodal distributions, a threshold was chosen based on the lowest points between the two density plot peaks and then converted to binary variables (Avramopoulos et al, 2015).

*Genotyping and Cleaning*

The Gentra Puregene Kit or the QIAGEN DNeasy Blood and Tissue Kit was used to extract DNA from the blood samples. Previous literature described the genotyping methods performed using the Affymetrix Human Genome-Wide SNP Array 6.0 at Emory University (Mulle et al, 2010). Genotypes were called using the corrected robust linear mixture model (CRLMM), an algorithm for preprocessing and genotype calling of Affymetrix SNP array data (Scharpfel et al, 2011). The software PLINK was used to perform the genotype data cleaning

(Purcell, 2007). The cleaning steps that were used are outlined by the Psychiatric GWAS consortium (Ripke et al, 2013) and used in previous literature (Avramopoulos et al, 2015).

*Statistical Analysis*

GWAS analyses were performed using PLINK. Data cleaning was conducted using SAS version 9.4. All statistical analyses were performed using the software SPSS unless otherwise stated. All figures were created in GraphPad PRISM. Univariate associations between individual infection and parental status were calculated using chi-square analysis. An additional variable was created to illustrate dichotomous positive and negative infection levels of CMV, HSV1 and TOXO. The positive and negative binary variables used in analysis were created from the log base two transformed CMV, HSV1 and TOXO density plot residual scores, which were adjusted for the plate and storage years recorded. Stratified by parental status, positive and negative infection status can be found in Table 3 and Figure 2. Associations between cumulative infection and parental status were calculated using an independent samples Kruskal-Wallis test. A cumulative infection index variable (0-3) was created, which indicated how many infections of interest were seropositive in fathers and mothers, seen in Table 4 and Figure 3. Associations between immune markers levels and parental status were calculated using a series of Mann-Whitney tests. Mean and standard deviation values were calculated for each immune marker using the log base two transformed density plot residual scores measured. These values were then stratified by parental status and recorded accordingly (Table 5, Figure 4). Regression analysis was used to account for maternal and paternal age in individual infection, cumulative infection and immune marker level. For statistical significance, 95% confidence interval used and a p-value below 0.05 was deemed sufficient.

## Results

*Demographic information*

Demographic information is provided in Table 1. 234 females consisted of 43.41% of the sample population. There were 186 (34.51%) diagnosed cases of schizophrenia/schizoaffective disorder. There were 354 parents in this population composed of 177 fathers and 177 mothers. The average age at plasma for fathers in this population was 68.06 years old (St.D = 8.96). The average age at plasma for mothers was 64.28 years old (St.D = 8.46) and the average age at plasma for proband was 41.18 (St.D = 10.02).

**Table 1** Sample Demographic Information

| N = 539 | N (%) or Mean (St.D) |
|---|---|
| | |
| **Sex (Female)** | 234 (43.41%) |
| **Sz/Sza*** | 186 (34.51%) |
| **Parents** | 354 (65.68%) |
| **Age at plasma** | |
| **Fathers** | 68.06 (8.96) |
| **Mothers** | 64.28 (8.46) |
| **Proband** | 41.18 (10.02) |

*Schizophrenia and schizoaffective disorder were classified together for this study

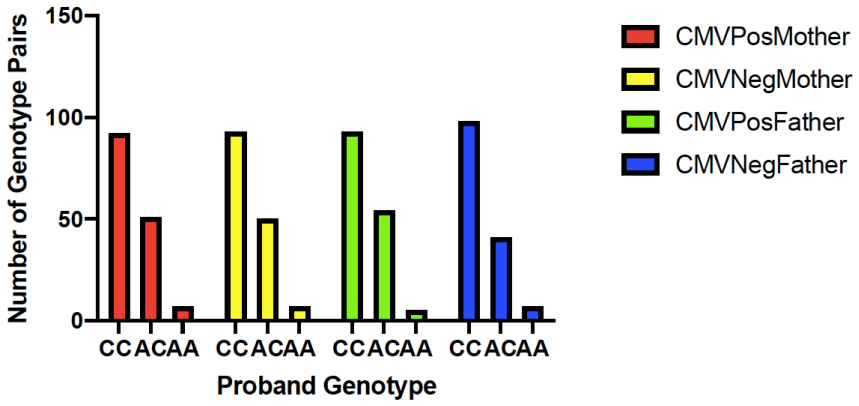
*Replicating Borglum's relationship*

To replicate Borglum's relationship seen in his Danish population, we compared the relationship between seropositive CMV mothers and their recessive allele proband to seronegative mothers and their recessive allele proband (Borglum et al, 2013). According to Borglum's relationship, the pairing of a seropositive CMV mother and their recessive allele proband should be the most common in this sample. When looking at the pairing of a seronegative CMV mother and their recessive allele proband, there should be no difference in this relationship among the three alleles. Similarly, regardless of the seropositivity of the father, there should be no difference in this relationship among the three alleles. In this sample (Table 2, Figure 1), there were a total of seven pairings of seropositive CMV mothers and their recessive allele proband and seven pairings of a seronegative CMV mother and their recessive allele proband. There was a total of five pairings of a seropositive CMV father and their recessive allele proband and there were seven pairings of a seronegative CMV father and their recessive allele proband. Regardless of seropositivity, the dominant allele was consistently enriched in this population with 92 CMV positive mother and CC proband pairings, 93 CMV negative mother and CC proband pairings, 93 CMV positive father and CC proband pairings and 98 CMV negative father and CC proband pairings (Table 2, Figure 1).

**Table 2** Replicating Borglum's Aim Comparing CMV Seropositivity Proband Genotype Pairings [*N*] by Parent

| Variable | Mother | | Father | |
|---|---|---|---|---|
| | *CMV Positive* | *CMV Negative* | *CMV Positive* | *CMV Negative* |
| **CC Proband** | *92* | *93* | *93* | *98* |
| **AC Proband** | *51* | *50* | *54* | *41* |
| **AA Proband** | *7* | *7* | *5* | *7* |

**Figure 1**




*Individual Infection between Father and Mother*

Because all the probands had schizophrenia, and maternal (gestational) infection is a risk factor for the child to develop schizophrenia later in life, it was hypothesized that in this cohort infections with the potential to affect the developing fetus would be more common in mothers than fathers. Demographic information is provided in Table 3 and Figure 2. A series of chi-

square tests were performed to test this prediction. The differences in CMV infection status were not significant between fathers and mothers, $X^2(1, 354) = 2.551$, $p = 0.110$. Differences between fathers and mothers in TOXO infection status were also not significant, $X^2(1, 354) = 0.773$, $p = 0.379$. Similarly, differences between fathers and mothers in HSV1 infection status were not significant, $X^2(1, 354) = 2.582$, $p = 0.108$. No differences in infection status between fathers and mothers were found in this sample population. Bivariate logistic regression was used to tests the association between individual infection and parental status (0=father, 1=mother) as the outcome variable. As shown in Table 4 below, individual infection was not significantly different between mother and fathers in models adjusted for age. No differences in individual infection were seen between fathers and mothers. Individual infection is not a predictor of parental status, with this model explaining 6.6% of the variance (Nagelkerke R Squared).

**Table 3** Comparing Individual Infection Status to Parental Status

| Variable (N = 177) | CMV | | TOXO | | HSV1 | |
|---|---|---|---|---|---|---|
| | *Mother* | *Father* | *Mother* | *Father* | *Mother* | *Father* |
| **Positive Serology, (*N*) (%)** | *86 (48.6)* | *101 (57.1)* | *70 (39.5)* | *62 (35.0)* | *92 (52.0)* | *70 (39.5)* |
| **Negative Serology, (*N*) (%)** | *91 (51.4)* | *76 (42.9)* | *107 (60.5)* | *115 (65.0)* | *85 (48.0)* | *107 (60.5)* |

**Figure 2**

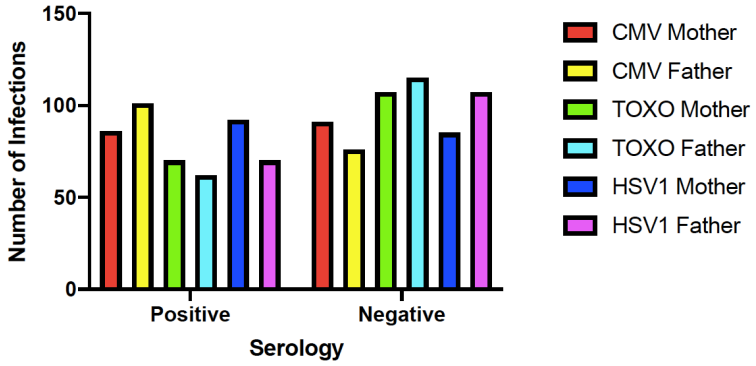



**Table 4** Variables in the Individual Infection Equation

| | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for Exp(B) Lower | Upper |
|---|---|---|---|---|---|---|---|---|
| Age at plasma | -.038 | .012 | 10.003 | 1 | .002 | .963 | .940 | .986 |
| CMV Infection | .199 | .224 | .793 | 1 | .373 | 1.220 | .787 | 1.892 |
| TOXO Infection | -.326 | .230 | 2.013 | 1 | .156 | .721 | .460 | 1.133 |
| HSV1 Infection | .280 | .226 | 1.539 | 1 | .215 | 1.323 | .850 | 2.058 |
| Constant | 2.486 | .858 | 8.384 | 1 | .004 | 12.009 | | |

a. Variable(s) entered on step 1: age_at_plasma years, RecodeCMV (dichotomized 0=negative, 1=positive), RecodeTOXO ((dichotomized 0=negative, 1=positive),, RecodeHSV (dichotomized 0=negative, 1=positive).

*Cumulative Infection between Father and Mother*

It was hypothesized that there would be a difference between mothers and fathers in the number of infections carried. Demographic information is provided in Table 5 and Figure 3. An independent samples Kruskal-Wallis test was performed to determine if there was a statistically significant difference in cumulative infection between the father and mother. The mean rank score for fathers was 171.04, and the mean rank score for mothers was 183.96. No statistical differences in cumulative infection between fathers and mothers were found, (Kruskal-Wallis H = 21.181, $df = 1$, $p = 0.214$). No differences in cumulative infection between fathers and mothers were found in this sample population. Bivariate logistic regression was used to tests the association between cumulative infection and parental status (0=father, 1=mother) as the outcome variable. As shown in Table 6 below, cumulative infection was not significantly different between mother and fathers in models adjusted for age. No differences in cumulative infection were seen between fathers and mothers. Cumulative infection is not a predictor of parental status, with this model explaining 5.2% of the variance (Nagelkerke R Squared).

**Table 5** Comparing Cumulative Infection to Parental Status

| Variable (N=354) | Zero Infections | One Infection | Two Infections | Three Infections |
|---|---|---|---|---|
| **Mother** *(N, %)* | *37 (10.5)* | *60 (16.9)* | *52 (14.7)* | *28 (7.9)* |
| **Father** *(N, %)* | *23 (6.5)* | *65 (18.4)* | *62 (17.5)* | *27 (7.4)* |

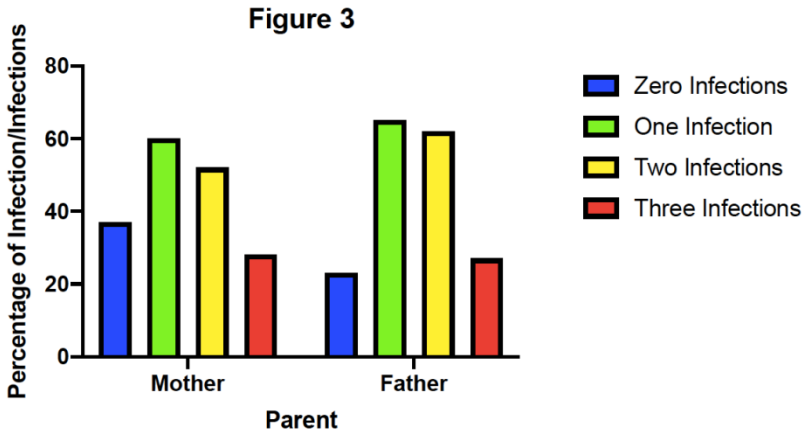



**Table 6** Variables in the Cumulative Infection Equation

| | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for Exp(B) Lower | Upper |
|---|---|---|---|---|---|---|---|---|
| Age at plasma | -.040 | .012 | 10.806 | 1 | .001 | .961 | .939 | .984 |
| Infection | -.052 | .118 | .198 | 1 | .657 | .949 | .753 | 1.195 |
| Constant | 2.688 | .783 | 11.789 | 1 | .001 | 14.709 | | |

a. Variable(s) entered on step 1: age_at_plasma, years, infection-ordinal variable 0, 1, 2, 3

*Levels of Immune Markers between Father and Mother*

While HHV6 is an infection, we previously found that most people were likely exposed and hence had a range of titers against this infection. Immune marker level information is provided in Table 7. A series of Mann-Whitney tests were performed to determine if there was an association between HHV6, GLIAD and CRP levels and parental status. Consistent with the previous hypothesis, a statistically significant difference in HHV6 between fathers and mother were found, (Mann-Whitney U = 12969, $p = 0.005$). The mean rank for fathers was 162.27 and the mean rank for mothers 192.73, illustrating that HHV6 was higher for mothers than fathers in this sample. In addition, statistically significant differences in CRP between fathers and mothers were found, (Mann-Whitney U = 13266, $p = 0.013$). The mean rank for fathers was 163.95 and the mean rank for mothers was 191.05, illustrating that CRP was higher in mothers than fathers in this sample. Similarly, statistically significant differences in gliadin between fathers and mothers were found, (Mann-Whitney U = 13615, $p = 0.033$). The mean rank for fathers was 189.08 and the mean rank for mothers was 165.92, illustrating that gliadin was higher in fathers than in mothers. In this sample population, mothers are more likely to have higher levels of HHV6 and CRP in their blood while fathers are more likely to have higher levels of gliadin in their blood. Bivariate logistic regression was used to tests the association of between the remaining immune parameters (entered as log2 transformed continuous variables) and parental status (0=father, 1=mother) as the outcome variable. As shown in Table 8 below, the level of immune markers (including sero-intensity for HHV6) were significantly different between mother and fathers in models adjusted for age. Mothers had higher levels of HHV6 and CRP, while fathers had higher levels of GLIAD. Thus, each of these markers were independent predictors of parental status, with the model explaining 11.2% of the variance (Nagelkerke R Squared).

**Table 7** Average Immune Marker Level by Parental Status (Values are Log2 Transformed)

| Variable | Gliadin | HHV6 | CRP |
|---|---|---|---|
| **Mother *(N, StD)*** | *-0.165 (1.355)* | *0.042 (1.458)* | *0.241 (1.979)* |
| **Father *(N, StD)*** | *0.121 (1.254)* | *-0.524 (1.947)* | *-0.135 (1.648)* |

**Table 8** Variables in the Immune Marker Equation

| | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for Exp(B) Lower | Upper |
|---|---|---|---|---|---|---|---|---|
| Age at Plasma | -.037 | .012 | 9.795 | 1 | .002 | .963 | .941 | .986 |
| HHV6 level | .193 | .072 | 7.290 | 1 | .007 | 1.213 | 1.054 | 1.396 |
| CRP level | .154 | .062 | 6.201 | 1 | .013 | 1.167 | 1.033 | 1.318 |
| Gliadin level | -.175 | .089 | 3.851 | 1 | .050 | .839 | .705 | 1.000 |
| Constant | 2.493 | .795 | 9.840 | 1 | .002 | 12.098 | | |

a. Variable(s) entered on step 1: age_at_plasma, years, hhv6 zr1 mainvar (log2 transformed HHV6 serointensity), crp zr1 mainvar (Log2 transformed CRP level), gliadin zr1 mainvar (Log2 transformed gliaden level)

**Discussion**

The present study looked at the different associations between SCZ and infection status and SCZ and immune marker levels in parents in an Ashkenazi Jewish population. Borglum's relationship seen in his Danish population was not able to be replicated in this study. No significant associations between individual infection and parental status were found. In addition, no significant associations were found between cumulative infection and parental status were found. Possible reasons for this could be the smaller sample size used in this population and the genetic differences found in the Ashkenazi Jewish population that was sampled from. The relationship between maternal infection and rs7902091 may be specific to the Danish population however further analysis needs to be done to support this conclusion. However, immune marker level was found to be a predictor parental status, as parents with higher levels of HHV6 and CRP were more likely to be mothers and parents who had higher levels of gliadin were more likely to be fathers. Several studies have been able to prove that HHV6 has a higher seroprevalence in females than males although inconsistencies do exist (Braun et al, 1997). Similar to CMV, HHV6 infection may be reactivated during pregnancy in those effected, alluding to the possibility that there may be perinatal transmission of the virus during pregnancy. Another study was able to show through Polymerase Chain Reactions (PCR) that HHV6 DNA was detected more frequently in the cervices of pregnant women than nonpregnant women (Okuno et al, 1995). These findings could provide some insight into why HHV6 might be higher in SCZ mothers than father. SCZ patients are already predisposed for increased CRP levels, as there is evidence for abnormal levels of acute phase proteins, plasma proteins synthesized by the liver in response to inflammation, including CRP (Miller et al, 2014). In addition, maternal CRP levels have been found to be significantly associated with schizophrenia in offspring, with findings remaining significant even after adjusting for confounders (Canetta et al, 2014). Therefore, findings in this study are reflective of other studies that have been able to show sex-related differences in CRP. However, the mechanics of such differences are unknown. Most likely multifactorial, the sex-related differences in CRP observed in this study could be as a result of hormonal environment, menopausal status, estrogen replacement or increased adiposity in women compared to men (Ford et al, 2004; Khera et al, 2005; Khera et al, 2009). However, inconsistencies in this relationship between CRP and sex still abound therefore further analysis needs to be done to find more consistent findings. Recent work has been able to find that the gluten link to SCZ symptoms is gliadin, such that native anti-gliadin antibodies are significantly elevated in SCZ patients. The links between SCZ, celiac disease and anti-gliadin have been studied in several studies dating back to the 1950s. Anti-gliadin antibodies seroprevalence has not been found to sex-dependent in literature however drawing from the links between celiac disease and gliadin in literature, it would be expected that mothers would showcase higher levels of anti-gliadin in this dataset, as celiac disease tends to be more prevalent in women (Green et al, 2007; Bai et al, 2005; Ciacci et al, 1995). Other literature specifies that clinically diagnosed celiac disease is more common in women (two to three times more) but screening-detected celiac disease is equally prevalent in men and women (Rubio-Tapia et al, 2009; Fasano et al, 2003). Therefore, further research needs to be done to properly understand the links between SCZ, celiac disease, gliadin and sex.

There were a number of limitations in our study. We did not have a large sample size, as the use of several stratifications reduced the number of participants in the overall dataset. The effects of medications were not considered, which could have had a confounding effect not accounted for in this study. In addition, other confounders such as socio-economic status, which

has been found to correlate with infections like CMV (Staras et al, 2006) were not accounted for in this study. In addition, as this was a case study done with an Ashkenazi Jewish population, this data cannot necessarily be generalized to a wider population. However, this study was able to account for temporal trends, as the data collected for this study was done over a 15-year period. In addition, this study was able to evaluate these trends in an underrepresented ethnic minority with a rare mental disorder and provide a larger picture of what SCZ looks like in a different ethnic population.

This study provides insight into the dynamics of infection among SCZ parents. We report on the differences in the immune marker levels of HHV6, CRP and gliadin among SCZ parents in an Ashkenazi Jewish population. Additional studies should be performed to be done to help define the relationships between SCZ and infection status and SCZ and immune markers. More evidence about these relationships could lead to a better understanding of the dynamics of SCZ.

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