# Relationships between Complement Protein Concentration and Genotype in First-episode Psychosis: Evidence from C3 and C4 in Peripheral Blood

Yu Chen[1,2], Zhenguo Zhao[1,2], Fen Lin[1,2], Lifang Wang[1,2], Zheng Lin[3]* and Weihua Yue[1,2,4]*

[1] Institute of Mental Health, Peking University Sixth Hospital, Beijing, China, [2] Key Laboratory of Mental Health, National Clinical Research Center for Mental Disorders, Chinese Academy of Medical Sciences Research Unit (No. 2018RU006), Ministry of Health (Peking University), Beijing, China, [3] Second Hospital Zhejiang University School of Medicine (SAHZU), Hangzhou, China, [4] PKU-IDG/McGovern Institute for Brain Research, Peking University, Beijing, China

Schizophrenia is a common neuropsychiatric disorder with complex pathophysiology. Recent reports suggested that complement system alterations contributed to pathological synapse elimination that was associated with psychiatric symptoms in schizophrenia. Complement component 3 (C3) and complement component 4 (C4) play central roles in complement cascades. In this study, we compared peripheral C3 and C4 protein levels between first-episode psychosis (FEP) and healthy control (HC). Then we explored whether single nucleotide polymorphisms (SNPs) at C3 or C4 genes affect peripheral C3 or C4 protein levels. In total, 181 FEPs and 204 HCs were recruited after providing written informed consent. We measured serum C3 and C4 protein levels using turbidimetric inhibition immunoassay and genotyped C3 and C4 polymorphisms using the Sequenom MassArray genotyping. Our results showed that three SNPs were nominally associated with schizophrenia (rs11569562/C3: A > G, $p = 0.048$; rs2277983/C3: A > G, $p = 0.040$; rs149898426/C4: G > A, $p = 0.012$); one haplotype was nominally associated with schizophrenia, constructed by rs11569562–rs2277983–rs1389623 (GGG, $p = 0.048$); FEP had higher serum C3 and C4 (both $p < 0.001$) levels than HC; rs1389623 polymorphisms were associated with elevated C3 levels in our meta-analysis (standard mean difference, 0.50; 95% confidence interval, 0.30 to 0.71); the FEP with CG genotype of rs149898426 had higher C4 levels than that with GG genotypes ($p = 0.005$). Overall, these findings indicated that complement system altered in FEP and rs149898426 of C4 gene represented a genetic risk marker for schizophrenia likely through mediating complement system. Further studies with larger sample sizes needs to be validated.

Keywords: first-episode psychosis, complement protein concentration, single nucleotide polymorphism, Chinese Han population, case-control studies

## HIGHLIGHTS

- Peripheral C3 and C4 protein levels were increased in first-episode psychosis (FEP).
- The rs149898426 polymorphisms of C4 gene were associated with schizophrenia.
- The CG genotype of rs149898426 were associated with higher serum C4 level in FEP comparing with GG genotype.

## INTRODUCTION

Schizophrenia is a severe and complicated neuropsychiatric disorder characterized by hallucinations, delusions and cognitive dysfunction (Kahn and Keefe, 2013; Kahn et al., 2015; Owen et al., 2016). While many studies attributed the causes of schizophrenia to biological or environmental factors, the etiology of schizophrenia are still unclear. Several lines of evidence suggested that dysregulation of the immune system contributed to the development of schizophrenia. Epidemiological studies found that infection and autoimmune disorders were associated with schizophrenia (Brown and Derkits, 2010; Benros et al., 2011, 2014; Arias et al., 2012; Khandaker et al., 2013). Besides, many cross-sectional studies reported that proinflammatory cytokines and C-reactive protein (CRP) were increased in schizophrenia compared with healthy control (HC) (Miller et al., 2011; Fineberg and Ellman, 2013; Fernandes et al., 2016). Lastly, antipsychotics has been demonstrated to have immunomodulatory effects, for example, clozapine (Hinze-Selch et al., 1998; Roge et al., 2012). Thus, immune dysregulation could represent a vulnerability factor for schizophrenia.

Complement system, an important part of the innate immune system, play important roles in clearing microbes and damaged cells from an organism, triggering inflammation and destroying foreign invaders (Merle et al., 2015a,b). Recent evidence identified that complement system orchestrated the balance for neurodevelopmental processes (Stephan et al., 2012; Ricklin et al., 2016). For example, overactivation of complement system contributed to neurotoxicity and pathological synapse loss through microglia, leading to progressive dysfunction in Alzheimer disease (AD) (Shi et al., 2017; Heneka et al., 2018).

Complement component 3 (C3) and complement component 4 (C4) play dominant roles in complement cascades. The C3 gene is located on chromosome 19, consisting of 41 exons and the mRNA has 5101 bp. C3 is a convergent point for activation both classical and alternative complement pathways (Merle et al., 2015a,b). Genetic evidence suggests that the polymorphisms of C3 were associated with risk of schizophrenia (Rudduck et al., 1985; Fañanás et al., 1992; Ni et al., 2015; Zhang et al., 2018). And patients with schizophrenia had increased C3 protein levels in peripheral blood (Santos Sória et al., 2012; Ali et al., 2017) and serum C3 concentrations were positively correlated with PANSS scores (Li et al., 2016). The C4 gene has two isoforms, C4A and C4B, located within the major histocompatibility complex (MHC) region on chromosome 6. C4A was proved to increase the risk for schizophrenia through mediating synapse pruning during postnatal development (Sekar et al., 2016).

The elucidation of pathophysiology in the early stages of schizophrenia may help understand disease etiology. First-episode psychosis (FEP) refers to the first time a person outwardly experiences symptoms of psychosis. Although some symptoms were unspecific in FEP, the underlying biological processes has changed, especially upregulating inflammatory status. Given that C3 and C4 are major plasma proteins of the complement pathway and are widely measured parameters during clinical practice, we investigated whether C3 or C4 protein levels are different between FEP and HC from our clinical data. Considering previous reports that C3 or C4 gene has multiple single nucleotide polymorphisms (SNPs) that were at the genome-wide level (Yang et al., 2012), we selected several SNPs at C3 (rs11569562, rs2277983, rs1389623) and C4 (rs406658, rs2746414, rs149898426) genes to explore the associations between genotype and complement protein concentration in FEP.

## MATERIALS AND METHODS

### Subjects

A total of 181 FEPs (94 males, 87 females; mean age: $29.9 \pm 10.0$ years) and 204 HCs (96 males, 108 females; mean age: $30.0 \pm 10.2$ years) were recruited from Peking University Sixth Hospital. The diagnosis was made according to the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria using a Structured Clinical Interview and people with FEP did not take medication regularly. We excluded HC with a history of mental health or neurological diseases from communities through simple unstructured interviews conducted by psychiatrists. All participants were given written informed consent before the study began. The study was conducted under established ethical standards and was approved by the ethics committee of Peking University Sixth Hospital.

### SNP Selection and Genotyping

We collected 5 mL venous blood from each subject, separated out serum by centrifugation, and stored it at –80°C until laboratory analysis. The genomic DNA was extracted from using the QIAamp DNA Blood Mini Kit. We selected 3 haplotype-tagging single nucleotide polymorphisms (tagSNPs) by using the UCSC (GRCh37/hg19) genome browser[1] for C3 and C4, respectively (C3: rs11569562, rs2277983, rs1389623; C4: rs406658, rs2746414, rs149898426). Genotyping was conducted for 6 tagSNPs by using the platform of the Sequenom MassArray system (Sequenom, San Diego, CA, United States). Locus-specific polymerase chain reaction (PCR) and detection primers were designed with the MassARRAY Assay Design Version 3.0 software (Sequenom, San Diego, CA, United States). The DNA samples were amplified by multiplex PCR reactions and the obtained products were used for locus-specific single-base extension reaction. The final products were desalted and transferred to 384-element SpectroCHIP arrays. Allele typing was performed using MALDI-TOF MS spectroscopy. The mass spectrum was analyzed by

---

[1] http://genome.ucsc.edu/

the MassARRAY TYPER software (Sequenom, San Diego, CA, United States). All DNA samples were analyzed in technical duplicates. We genotyped each sample thrice to minimize genotyping errors and only consensus genotypes were processed for further analysis. Genotyping primer sequences are indicated in **Table 1**.

**TABLE 1 |** SNP information and PCR and extension primer sequences from Sequenom SNP genotyping.

| SNP | Position | Location | Second-PCRP | First-PCRP | UEP_SEQ |
|---|---|---|---|---|---|
| rs11569562 | 6678742 | Intron | ACGTTGGATGTACTATGCAATGAGAGTGAC | ACGTTGGATGTGACTGTAGTTTTCCGTGGC | CCATGAGGCTACAGTATT |
| rs2277983 | 6679552 | Intron | ACGTTGGATGGAAGATGAGAGGATAAGGGC | ACGTTGGATGCAGGTCTGAAGACTGAGAAC | CTCCCTCCAAAGACCA |
| rs1389623 | 6684186 | Intron | ACGTTGGATGATGATTGTGTACTTTCCTCC | ACGTTGGATGTGTGAGACCACAGCAATGAC | AGCAATGACCACGTAAG |
| rs406658 | 32028747 | Coding sequence | ACGTTGGATGTTGAAGGTCCTGAGTTTGGC | ACGTTGGATGGGACAGAAGCCAGTTAGATG | CAGTTTCTCAGGCGA |
| rs2746414 | 32029189 | Coding sequence | ACGTTGGATGGTTTTCTTCCAGGAAGCCTC | ACGTTGGATGAGCCCAGCACTTGCTTTCTC | TCCATCTCAAAGGCAA |
| rs149898426 | 32029352 | intron | ACGTTGGATGCAACAACCTCATGGCAATGG | ACGTTGGATGCACTCAGGGATCCTAAGGTC | GCTCACTGCCAGAGC |

**TABLE 2 |** Comparison of genotype and allele distribution of 6 SNPs of C3 and C4 gene between FEP and HC.

| Makers/gene | Genotype N (Freq.) | | | Chi-square (df = 2) | p-Value | HWE P | Allele N (freq.) | | Chi-square (df = 1) | p-Value | OR (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| rs11569562/C3 | A/A | A/G | G/G | NA | | | A | G | | | |
| FEP | 54 (0.298) | 89 (0.492) | 38 (0.210) | | 4.325 | 0.114 | 0.905 | 197 (0.544) | 165 (0.456) | 3.886 | 0.048[a] | 0.751 (0.566–0.999) |
| HC | 43 (0.211) | 107 (0.525) | 54 (0.265) | | | | 0.457 | 193 (0.473) | 215 (0.527) | | | |
| rs2277983/C3 | A/A | A/G | G/G | | | | | A | G | | | |
| FEP | 54 (0.298) | 90 (0.497) | 37 (0.204) | | 4.484 | 0.106 | 0.964 | 198 (0.547) | 164 (0.453) | 4.199 | 0.040[a] | 0.743 (0.559–0.987) |
| HC | 43 (0.212) | 106 (0.522) | 54 (0.266) | 1 | | | 0.499 | 192 (0.473) | 214 (0.527) | | | |
| rs1389623/C3 | A/A | A/G | G/G | | | | | A | G | | | |
| FEP | 3 (0.017) | 41 (0.227) | 137 (0.757) | | 0.178 | 0.915 | 0.973 | 47 (0.130) | 315 (0.870) | 0.040 | 0.841 | 1.044 (0.683–1.596) |
| HC | 4 (0.020) | 43 (0.211) | 157 (0.767) | | | | 0.603 | 51 (0.125) | 357 (0.875) | | | |
| rs406658/C4 | A/A | A/C | C/C | | | | | A | C | | | |
| FEP | 1 (0.006) | 33 (0.189) | 141 (0.806) | 6 | 1.939 | 0.379 | 0.529 | 35 (0.100) | 315 (0.900) | 1.480 | 0.224 | 0.736 (0.468–1.158) |
| HC | 3 (0.015) | 47 (0.233) | 152 (0.752) | 2 | | | 0.769 | 53 (0.131) | 351 (0.869) | | | |
| rs2746414/C4 | A/A | A/G | G/G | | | | | A | G | | | |
| FEP | 0 (0) | 29 (0.160) | 152 (0.840) | | 1.977 | 0.372 | 0.241 | 29 (0.080) | 333 (0.920) | 0.479 | 0.489 | 0.811 (0.490–1.341) |
| HC | 2 (0.001) | 35 (0.174) | 164 (0.816) | 3 | | | 0.931 | 39 (0.097) | 363 (0.903) | | | |
| rs149898426/C4 | C/C | C/G | G/G | | | | | C | G | | | |
| FEP | 0 (0) | 28 (0.156) | 152 (0.844) | 1 | 7.775 | 0.021[a] | 0.258 | 28 (0.078) | 332 (0.922) | 6.344 | 0.012[b] | 0.527 (0.325–0.853) |
| HC | 3 (0.016) | 47 (0.245) | 142 (0.740) | 12 | | | 0.690 | 53 (0.138) | 331 (0.862) | | | |

*FEP, first-episode psychosis; HC, healthy control; Freq., frequencies; HWE P, The p-value for Hardy-Weinberg equilibrium tests; OR, odds ratio; CI, confidence interval; NA indicates a missing value.*
[a]*Denotes a significant association (P < 0.05).*
[b]*Denotes a significant association after Bonferroni adjustment (P < 0.05/3 = 0.017).*

## Measurement of Complement Protein

Serum C3 and C4 levels were measured using the turbidimetric inhibition immunoassay in an automatic chemistry analyzer, ROCHE (Roche Molecular Systems, Inc., Basel, Switzerland) module Cobas 8000 (C702). The assays were performed strictly by the manufacturer's recommended protocol. All samples were analyzed in duplicates and the mean of the two measurements was used in the analysis.

## Statistical Analyses

Pairwise linkage disequilibrium (LD), haplotype construction, and genetic association analysis were performed by using Haploview 4.2 software[2]. Deviation of the genotype and allele frequency from the Hardy–Weinberg equilibrium were analyzed using a chi-square goodness-of-fit test. We conducted $t$-tests and a univariate analysis of covariance (ANCOVA) to examine the difference of serum complement protein levels (C3 and C4) between FEP and HC, considering that age and sex may affect serum complement protein levels. A two-way analysis of variance (ANOVA) was used to explore the interaction effects of group and genotype. *Post hoc* analyses were performed using a Tukey test. When the result from $F$-test was marginal significant ($0.05 < p < 0.1$), we would conduct a meta-analysis of the SNP for case group and control group to increase the statistical power. Statistical analyses were performed using R statistical software[3]. Data were expressed as the mean $\pm$ standard deviation (SD) or as number (proportion). Results were considered nominally significant at $p < 0.05$ (two-tailed). Multiple testing adjustment will be controlled via Bonferroni adjustment for comparison of genotype and allele distribution for C3 and C4 gene. $P < 0.05/3$ was considered as significant for the reason that the total number of independence tests (excluding LD) was 3 for all 6 SNPs across two genes.

## RESULTS

### Single Polymorphism Analysis

We observed no association of rs11569562, rs1389623, s406658, and rs2746414 polymorphism with schizophrenia for genotypes and alleles. There were two nominally significant associations between rs11569562 ($p = 0.048$) and rs2277983 ($p = 0.040$) alleles distributions and schizophrenia but no association between genotypes and schizophrenia. The genotypes ($p = 0.0205$) and alleles ($p = 0.012 < 0.05/3$) of rs149898426 were significantly different between FEP and HC. The genotypes and alleles frequencies of each polymorphism were presented in **Table 2**.

### Haplotype-Based Association Analysis

The LD map and block structures of C3 and C4 polymorphisms were shown in **Figure 1**. A haplotype block of C3 was constructed by rs11569562, rs2277983, and rs1389623: GGG haplotype was significantly associated with schizophrenia ($\chi^2 = 3.922$, $p = 0.048$); while after 10,000 times of permutation tests, the
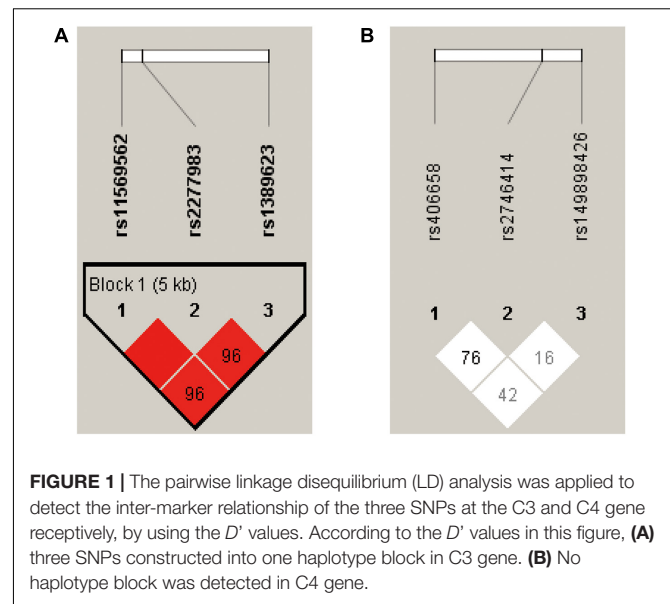
**FIGURE 1 |** The pairwise linkage disequilibrium (LD) analysis was applied to detect the inter-marker relationship of the three SNPs at the C3 and C4 gene receptively, by using the $D'$ values. According to the $D'$ values in this figure, **(A)** three SNPs constructed into one haplotype block in C3 gene. **(B)** No haplotype block was detected in C4 gene.

difference of haplotype GGG frequencies between FEP and HC were not significant (empirical $p$-value was 0.104) (see details in **Table 3** and **Figure 1A**). No haplotype blocks were found in C4 gene (**Figure 1B**).

## Complement Protein Levels

One sample testing C3 protein levels was excluded for being three SDs above the mean. The serum C3 protein levels (mean $\pm$ SD) in cases and controls were $1.18 \pm 0.22$ and $1.07 \pm 0.20$ g/L; the serum C4 protein levels (mean $\pm$ SD) in cases and controls were $0.28 \pm 0.08$ and $0.25 \pm 0.08$ g/L, respectively. $T$-tests showed that FEP had higher serum C3 ($t = 5.11$, $p < 0.001$, Cohen's $d = 0.52$) and C4 ($t = 4.21$, $p < 0.001$, Cohen's $d = 0.44$) protein levels than HC (**Figures 2A,B**). Although the differences of serum C3 and C4 protein levels were not significant considering age and sex in cases and controls, respectively (see details in **Table 4**), age- and sex-adjusted comparisons were performed with ANCOVA: the differences of serum C3 ($F = 26.55$, $p < 0.001$, $\eta^2 = 0.065$) and C4 ($F = 18.27$, $p < 0.001$, $\eta^2 = 0.046$) protein levels remained significant.

**TABLE 3 |** Comparison of haplotype frequencies at the C3 gene between FEP and HC.

| Haplotypes | Freq. case | Freq. control | Chi-square | $p$-Value | Empirical $p$-Value |
|---|---|---|---|---|---|
| Block (rs11569562–rs2277983–rs1389623) | | | | | |
| GGG | 0.524 | 0.452 | 3.922 | 0.048 | 0.104 |
| AAG | 0.351 | 0.415 | 3.320 | 0.069 | 0.158 |
| AAA | 0.125 | 0.129 | 0.092 | 0.761 | 1.000 |

*FEP, first-episode psychosis; HC, healthy control; Freq., frequency. Haplotypes with frequency < 0.01 in FEP and HC were dropped. Empirical p-value: the p-value of the permutation tests (number of iterations, 10,000).*
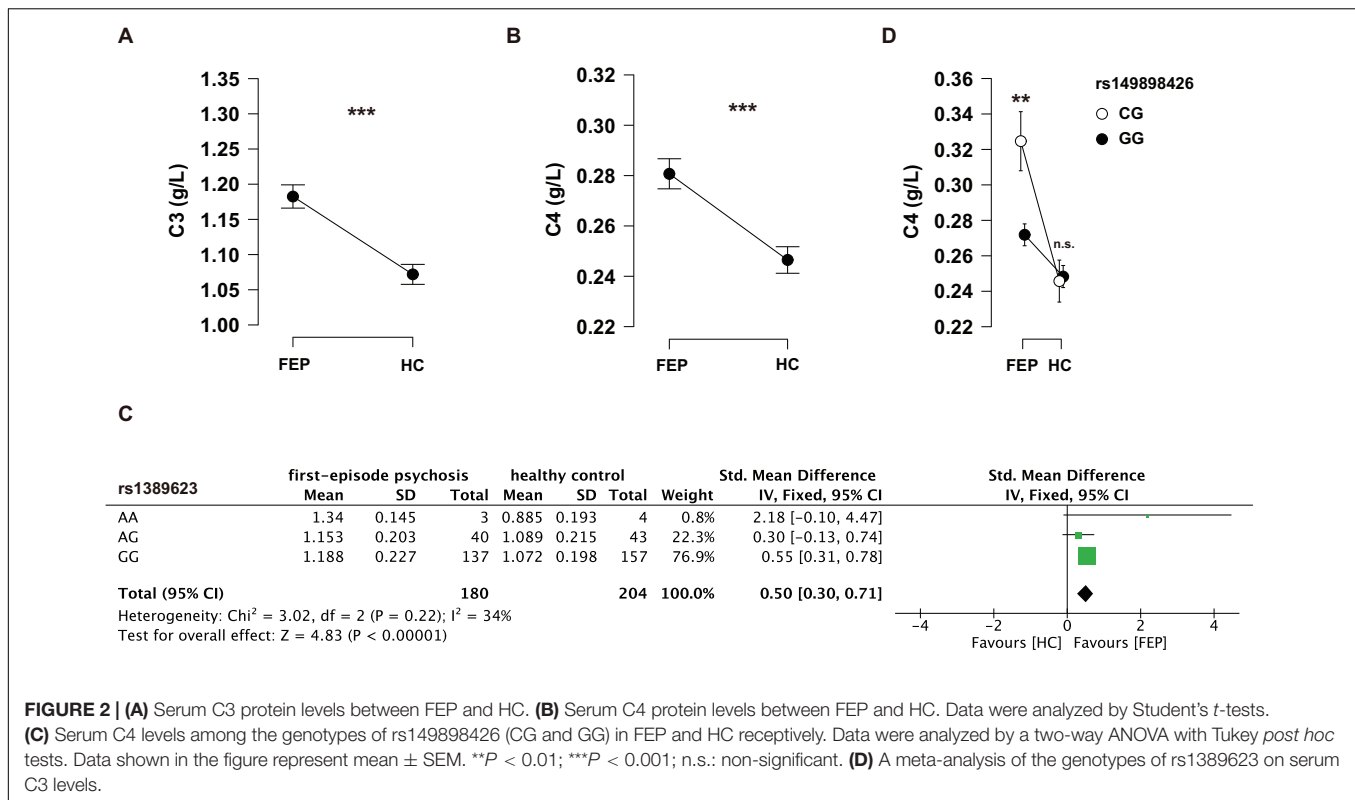
FIGURE 2 | (A) Serum C3 protein levels between FEP and HC. (B) Serum C4 protein levels between FEP and HC. Data were analyzed by Student's *t*-tests.
(C) Serum C4 levels among the genotypes of rs149898426 (CG and GG) in FEP and HC receptively. Data were analyzed by a two-way ANOVA with Tukey *post hoc*
tests. Data shown in the figure represent mean ± SEM. **$P$ < 0.01; ***$P$ < 0.001; n.s.: non-significant. (D) A meta-analysis of the genotypes of rs1389623 on serum
C3 levels.

TABLE 4 | Subgroups of serum C3 and C4 levels according to sex and
clinical information.

|    | Group | Sex | N | Mean ± SD (g/L) | P |
|----|-------|-----|---|-----------------|---|
| C3 | FEP | Male | 94 | 1.16 ± 0.24 | 0.44 |
|    |     | Female | 86 | 1.20 ± 0.20 | |
|    | HC | Male | 96 | 1.07 ± 0.22 | 0.77 |
|    |    | Female | 108 | 1.08 ± 0.19 | |
| C4 | FEP | Male | 94 | 0.28 ± 0.09 | 0.45 |
|    |     | Female | 87 | 0.27 ± 0.07 | |
|    | HC | Male | 96 | 0.24 ± 0.08 | 0.68 |
|    |    | Female | 108 | 0.24 ± 0.07 | |

*FEP, first-episode psychosis; HC, healthy control. One sample information (C3 for
Female in cases) was lost for technical errors.*

## SNP-Group Interactions in Complement Proteins

Serum C3 and C4 protein levels of individual genotypes
were evaluated between the case and control groups using
two-way ANOVAs. We found that the interaction effects of
group (FEP vs. HC) and rs1389623 were marginal significant
($F$ = 2.81, $p$ = 0.062, partial $\eta^2$ = 0.015) on serum C3 levels
(see **Supplementary Tables 1–2**). A meta-analysis showed that
rs1389623 polymorphism were associated with elevated serum
C3 levels ($Z$ = 4.83, $p$ < 0.001) (see details in **Figure 2C**). There
was no interaction effect between other genotypes and group
on serum C3 protein levels (see **Supplementary Tables 3–6**).
Besides, we excluded all CC genotypes in a two-way ANOVAs

(group: FEP, HC × rs149898426 polymorphisms: CG, GG) on
C4 protein levels, for the reason that there were three subjects
with CC genotypes in HC and no subject in FEP. The interaction
effects of group and rs149898426 were significant ($F$ = 7.33,
$p$ = 0.007, partial $\eta^2$ = 0.02) (see **Supplementary Tables 7–8**).
*Post hoc* tests found that FEP who carry the CG genotypes of
rs149898426 had higher serum C4 protein levels than the patients
who carry the GG genotypes ($t$ = 3.34, $p_{tukey}$ = 0.005) while HC
not ($t$ = −0.198, $p_{tukey}$ = 0.997) (**Figure 2D**). No other interaction
of genotypes and group was significant in serum C4 protein levels
(see **Supplementary Tables 9–12**).

## DISCUSSION

The present study confirmed that complement system
overactivated in FEP, which were correlated with genetic
factors. We observed that FEP had higher serum C3 and C4
protein levels than HC, suggesting that aberrant expression
of complement proteins may be potential biomarkers for
schizophrenia; C4 polymorphism affected serum C4 protein
levels in FEP but not in HC, suggesting that genotype may be
considered as an important risk factor for the development
of schizophrenia.

The first finding was that FEP had increased serum C3 and
C4 protein levels compared with HC. The results replicated
many previous findings in schizophrenia (Maes et al., 1997)
and extended to patients that were not FEP. But two other
studies had no similar findings in FEP (Kopczynska et al., 2019;

Laskaris et al., 2019). One possible reason is that the sample size may not be sufficient to reach the statistical differences; both studies had lower sample sizes than our study. Another potential cause for this divergence of outcomes is race or ethnicity. In our study, study participants are limited to the Chinese Han population, while various racial groups were involved in other studies. However, one study pointed that individuals at ultra-high risk (UHR) for psychosis had elevated C3 and C4 protein levels (Laskaris et al., 2019), suggesting that alteration of complement system accompanied the development of schizophrenia. These results should be considered exploratory and further studies with larger cohorts would be required to confirm results.

To our knowledge, this is the first survey that compares serum C3 and C4 levels with their SNP in FEP of the China Han population. Two recent papers investigated the roles of C3 polymorphism in susceptibility to schizophrenia in the China Han population (Ni et al., 2015; Zhang et al., 2018). Although SNP rs11569562 in C3 was not associated with schizophrenia in the Chinese Han population (Zhang et al., 2018) consisting of 1086 patients with schizophrenia and 1154 HCs, patients with all genotypes of rs11569562 have higher serum C3 levels than the controls similar to our study. Other studies using expression quantitative trait locus (eQTL) analysis revealed that rs2277984, which is adjacent to rs2277983 in our study, regulates C3 expression in the liver (Zhang et al., 2017), suggesting that SNPs in the C3 gene affected the expression of C3 genes to further impact the C3 protein translation. Similarly, one study found that in the region near C4 of chromosome 6, the more strongly an SNP associated with schizophrenia, the more strongly it correlated with predicted C4A expression (Sekar et al., 2016). In short, the genotype of the complement gene had effects on its expression, which concurs with our results.

The present study has a major limitation that the sample size was small and may underpower the whole study in terms of C3 and C4 genotype distribution. Therefore, our results should be interpreted with caution. Further studies with larger sample sizes will be required to achieve sufficient statistical power and elucidate a potential link between complement system and schizophrenia in China Han population.

## CONCLUSION

Our results showed that first-episode psychosis had higher serum C3 and C4 protein levels than healthy control. C4 gene polymorphisms affected C4 protein levels in peripheral blood: the FEP carrying CG genotypes of rs149898426 had higher serum C4 protein levels than that of GG genotypes. The available evidence that complement proteins were elevated in FEP and polymorphisms of complement genes played a contributing role in regulating peripheral complement proteins concentration.

## REFERENCES

Ali, F. T., Abd El-Azeem, E. M., Hamed, M. A., Ali, M. A. M., Abd Al-Kader, N. M., and Hassan, E. A. (2017). Redox dysregulation, immuno-inflammatory alterations and genetic variants of BDNF and MMP-9 in schizophrenia:

## DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the Peking University Open Research Data repository, accession number 10635 (http://opendata.pku.edu.cn/api/access/datafile/10635).

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking University Sixth Hospital. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

WY provided the funds and designed the study. YC analyzed the data and wrote the draft of the manuscript. ZZ recruited schizophrenia patients and collected peripheral blood samples. Primers were designed by FL and LW. ZL supervised this study. All authors contributed to the article and approved the submitted version.

## FUNDING

## ACKNOWLEDGMENTS

We thank the participants who were involved in the study.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2021.647246/full#supplementary-material

pathophysiological and phenotypic implications. *Schizophr. Res.* 188, 98–109. doi: 10.1016/j.schres.2017.01.016

Arias, I., Sorlozano, A., Villegas, E., de Dios Luna, J., McKenney, K., Cervilla, J., et al. (2012). Infectious agents associated with schizophrenia: a meta-analysis. *Schizophr. Res.* 136, 128–136. doi: 10.1016/j.schres.2011.10.026

Benros, M. E., Nielsen, P. R., Nordentoft, M., Eaton, W. W., Dalton, S. O., and Mortensen, P. B. (2011). Autoimmune diseases and severe infections as risk factors for Schizophrenia: a 30-year population-based register study. *Am. J. Psychiatry* 168, 1303–1310. doi: 10.1176/appi.ajp.2011.11030516

Benros, M. E., Pedersen, M. G., Rasmussen, H., Eaton, W. W., Nordentoft, M., and Mortensen, P. B. (2014). A Nationwide Study on the Risk of Autoimmune Diseases in Individuals With a Personal or a Family History of Schizophrenia and Related Psychosis. *Am. J. Psychiatry* 171, 218–226. doi: 10.1176/appi.ajp. 2013.13010086

Brown, A. S., and Derkits, E. J. (2010). Prenatal infection and Schizophrenia: a review of epidemiologic and translational studies. *Am. J. Psychiatry* 167, 261–280. doi: 10.1176/appi.ajp.2009.09030361

Fañanás, L., Moral, P., Panadero, M. A., and Bertranpetit, J. (1992). Complement genetic markers in schizophrenia: C3, BF and C6 polymorphisms. *Hum. Hered.* 42, 162–167. doi: 10.1159/000154060

Fernandes, B. S., Steiner, J., Bernstein, H. G., Dodd, S., Pasco, J. A., Dean, O. M., et al. (2016). C-reactive protein is increased in schizophrenia but is not altered by antipsychotics: meta-analysis and implications. *Mol. Psychiatry* 21, 554–564. doi: 10.1038/mp.2015.87

Fineberg, A. M., and Ellman, L. M. (2013). Inflammatory cytokines and neurological and neurocognitive alterations in the course of Schizophrenia. *Biol. Psychiatry* 73, 951–966. doi: 10.1016/j.biopsych.2013.01.001

Heneka, M. T., McManus, R. M., and Latz, E. (2018). Inflammasome signalling in brain function and neurodegenerative disease. *Nat. Rev. Neurosci.* 19, 610–621.

Hinze-Selch, D., Becker, E. W., Stein, G. M., Berg, P. A., Mullington, J., Holsboer, F., et al. (1998). Effects of clozapine on in vitro immune parameters: a longitudinal study in clozapine-treated schizophrenic patients. *Neuropsychopharmacology* 19, 114–122.

Kahn, R. S., and Keefe, R. S. E. (2013). Schizophrenia is a cognitive illness time for a change in focus. *JAMA Psychiatry* 70, 1107–1112. doi: 10.1001/jamapsychiatry. 2013.155

Kahn, R. S., Sommer, I. E., Murray, R. M., Meyer-Lindenberg, A., Weinberger, D. R., Cannon, T. D., et al. (2015). Schizophrenia. *Nat. Rev. Dis. Primers* 1:15067. doi: 10.1038/nrdp.2015.67

Khandaker, G. M., Zimbron, J., Lewis, G., and Jones, P. B. (2013). Prenatal maternal infection, neurodevelopment and adult schizophrenia: a systematic review of population-based studies. *Psychol. Med.* 43, 239–257. doi: 10.1017/s0033291712000736

Kopczynska, M., Zelek, W., Touchard, S., Gaughran, F., Di Forti, M., Mondelli, V., et al. (2019). Complement system biomarkers in first episode psychosis. *Schizophr. Res.* 204, 16–22. doi: 10.1016/j.schres.2017.12.012

Laskaris, L., Zalesky, A., Weickert, C. S., Di Biase, M. A., Chana, G., Baune, B. T., et al. (2019). Investigation of peripheral complement factors across stages of psychosis. *Schizophr. Res.* 204, 30–37. doi: 10.1016/j.schres.2018.11.035

Li, H., Zhang, Q., Li, N., Wang, F., Xiang, H., Zhang, Z., et al. (2016). Plasma levels of Th17-related cytokines and complement C3 correlated with aggressive behavior in patients with schizophrenia. *Psychiatry Res.* 246, 700–706. doi: 10.1016/j.psychres.2016.10.061

Maes, M., Delange, J., Ranjan, R., Meltzer, H. Y., Desnyder, R., Cooremans, W., et al. (1997). Acute phase proteins in schizophrenia, mania and major depression: modulation by psychotropic drugs. *Psychiatry Res.* 66, 1–11. doi: 10.1016/s0165-1781(96)02915-0

Merle, N. S., Church, S. E., Fremeaux-Bacchi, V., and Roumenina, L. T. (2015a). Complement system part I - molecular mechanisms of activation and regulation. *Front. Immunol.* 6:262. doi: 10.3389/fimmu.2015.00262

Merle, N. S., Noe, R., Halbwachs-Mecarelli, L., Fremeaux-Bacchi, V., and Roumenina, L. T. (2015b). Complement system part II: role in immunity. *Front. Immunol.* 6:257. doi: 10.3389/fimmu.2015.00257

Miller, B. J., Buckley, P., Seabolt, W., Mellor, A., and Kirkpatrick, B. (2011). Meta-analysis of cytokine alterations in Schizophrenia: clinical status and antipsychotic effects. *Biol. Psychiatry* 70, 663–671. doi: 10.1016/j.biopsych.2011. 04.013

Ni, J., Hu, S., Zhang, J., Tang, W., Lu, W., and Zhang, C. (2015). A preliminary genetic analysis of complement 3 gene and Schizophrenia. *PLoS One* 10:e0136372. doi: 10.1371/journal.pone.0136372

Owen, M. J., Sawa, A., and Mortensen, P. B. (2016). Schizophrenia. *Lancet* 388, 86–97. doi: 10.1016/s0140-6736(15)01121-6

Ricklin, D., Reis, E. S., and Lambris, J. D. (2016). Complement in disease: a defence system turning offensive. *Nat. Rev. Nephrol.* 12, 383–401. doi: 10.1038/nrneph. 2016.70

Roge, R., Moller, B. K., Andersen, C. R., Correll, C. U., and Nielsen, J. (2012). Immunomodulatory effects of clozapine and their clinical implications: What have we learned so far? *Schizophr. Res.* 140, 204–213. doi: 10.1016/j.schres.2012. 06.020

Rudduck, C., Beckman, L., Franzén, G., and Lindström, L. (1985). C3 and C6 complement types in schizophrenia. *Hum. Hered.* 35, 255–258. doi: 10.1159/000153555

Santos Sória, L. D., Moura Gubert, C. D., Ceresér, K. M., Gama, C. S., and Kapczinski, F. (2012). Increased serum levels of C3 and C4 in patients with schizophrenia compared to Eutymic patients with bipolar disorder and healthy. *Rev. Bras. Psiquiatr.* 34, 119–120.

Sekar, A., Bialas, A. R., de Rivera, H., Davis, A., Hammond, T. R., Kamitaki, N., et al. (2016). Schizophrenia risk from complex variation of complement component 4. *Nature* 530, 177–183. doi: 10.1038/nature16549

Shi, Q., Chowdhury, S., Ma, R., Le, K. X., Hong, S., Caldarone, B. J., et al. (2017). Complement C3 deficiency protects against neurodegeneration in aged plaque-rich APP/PS1 mice. *Sci. Transl. Med.* 9:eaaf6295. doi: 10.1126/scitranslmed. aaf6295

Stephan, A. H., Barres, B. A., and Stevens, B. (2012). The complement system: an unexpected role in synaptic pruning during development and disease. *Annu. Rev. Neurosci.* 35, 369–389.

Yang, X., Sun, J., Gao, Y., Tan, A., Zhang, H., Hu, Y., et al. (2012). Genome-wide association study for serum complement C3 and C4 levels in healthy Chinese subjects. *PLoS Genet.* 8:e1002916. doi: 10.1371/journal.pgen.1002916

Zhang, C., Zhang, Y., Cai, J., Chen, M., and Song, L. (2017). Complement 3 and metabolic syndrome induced by clozapine: a cross-sectional study and retrospective cohort analysis. *Pharmacogenomics J.* 17, 92–97. doi: 10.1038/tpj. 2015.68

Zhang, S., Zhou, N., Liu, R., Rao, W., Yang, M., Cao, B., et al. (2018). Association between polymorphisms of the complement 3 gene and Schizophrenia in a Han Chinese population. *Cell. Physiol. Biochem.* 46, 2480–2486. doi: 10.1159/000489654