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Dear editor and reviewers:

Thank you for considering the revised version of our manuscript **ABCB9 polymorphism (rs61955196) is associated with schizophrenia in Chinese Han population** (Manuscript NO: 75378). We are thankful to the referees and the Editor for their suggestions and important modifications in the report. And we have thoughtfully taken into account these comments. The explanations for each comment and what we have changed in response to the reviewers' concerns are given point by point in the following pages.

To Reviewers:

Answering to 1st peer reviewer's major comments

1. In the methods section I would suggest the authors to mention more clearly about what are the inclusion and exclusion criteria that the authors used to select the study participants.

Our response: Thank you for the suggestion. We have modified the part introducing the study subjects in MATERIALS AND METHODS to make the criteria more clear and sound. Our selection and exclusion criteria for patients were summarized as follows: (1)The patients were Han Chinese coming from Jilin Province, China. (2)The patients were diagnosed according to the Tenth Revision of International Classification of Diseases (ICD-10) for SCZ, and the diagnoses were confirmed by at least two experienced psychiatrists. (3)Patients should have no other neurological disorders, severe organic lesions or drug dependence. And our selection criteria for healthy controls were as follows: (1)The subjects in the healthy control group were Han Chinese coming from Jilin Province, China. (2) The healthy controls were recruited matching the patients by gender and age, so that the propotion of individuals with prenatal famine exposure and the ratio of gender can be comparable between the two groups. (3) The healthy controls were required to have no history of mental illness, and should be in good health without any

known acute/chronic diseases at the time of recruitment and blood sample collection. Besides, all of the participants in both groups have provided written informed consent. The corresponding sentences are highlighted in yellow in the revised manuscript as follows:

According to the desired sample size and the inclusion and exclusion criteria, a total of 954 Han Chinese from Northeast China were finally recruited between 2010 and 2012, including 443 SCZ patients and 511 healthy people. The patients were recruited from the Siping Psychiatric Hospital and Sixth Hospital of Changchun City (Jilin, China). Each patients was diagnosed according to the Tenth Revision of International Classification of Diseases (ICD-10) for SCZ and confirmed by at least two experienced psychiatrists. Those with neurological disorders, severe organic lesions, and drug dependence were excluded. Subjects in the healthy control group matching the patients by gender and age were recruited from the Changchun Municipal Centre for Disease Control and Prevention, in order to get a comparable propotion of famine-exposed individuals between two groups and a similar ratio of gender. The healthy subjects were required to have no history of mental illness and were in good health without any known disease at the time of recruitment.

2. I would suggest the authors to work on spelling mistakes.

Our response: Thank you for the suggestion. We have carefully checked and revised the manuscript, and then passed it to professional English language editing company for further correction and got a new language certificate.

Answering to 2nd peer reviewer's major comments

1. Please correct the "2239681" to "rs2239681" in the Abstract if the authors are discussing the SNPs. Also, please go through the article to format the gene nomenclature correctly.

Our response: Thank you for the suggestion. We are sorry for the mistake, and we have corrected the "2239681" to "rs2239681" in the Abstract which is highlighted in yellow. We also checked for other possible mistakes regarding the gene nomenclature throughout the manuscript, and formatted them according to guildlines.

2. The sample size calculation should be mentioned in the Method section.

Our response: Thank you for the suggestion. We did calculation of sample size before the recruitment of study subjects. And we used the software Quanto and set parameters for a

desired statistical power of 0.8, which includes a unmatched case-control rate of 1.2, a population risk of 1% for the disease, a log-additive model gene with allele frequency of 0.1 and genetic effect of 1.5 and a type I error rate of 0.05 by 2-sided test. And the desired sample size for cases is 417 according to our calculation result. And we tried to enroll a little more participants to further improve the statistical power. We have added some details to the revised manuscript and the sentences are highlighted in yellow in the MATERIALS AND METHODS section as follows:

A desired sample size of 417 was calculated by the software Quanto with a proper power before the recruitment of participants, with a unmatched case-control rate of 1.2, an estimated population risk of 1% for SCZ, a log-additive model gene with allele frequency of 0.1 and genetic effect of 1.5 and a type I error rate of 0.05 by 2-sided test. According to the desired sample size and the inclusion and exclusion criteria, a total of 954 Han Chinese from Northeast China were finally recruited between 2010 and 2012, including 443 SCZ patients and 511 healthy people.

3. How the healthy controls were included? What are the inclusion and exclusion criteria for both groups?

Our response: Thank you for the question. We are sorry to realize that this part was not explicit enough and we should describe the criteria more clear and sound. In summary, our criteria for cases were set as follows: (1)The patients were Han Chinese from Jilin Province, China. (2)The patients were diagnosed according to the Tenth Revision of International Classification of Diseases (ICD-10) for SCZ, and the diagnoses were confirmed by at least two experienced psychiatrists. (3)The patients should have no other neurological disorders, severe organic lesions or drug dependence. And for the healthy controls, the following criteria should be satisfied: (1)The subjects in the healthy control group were Han Chinese coming from Jilin Province, China. (2)They were recruited matching the patients by gender and age, so that the propotion of individuals with prenatal famine exposure and the ratio of gender can be comparable between the two groups. (3)The healthy controls should have no history of mental illness and no known diseases at the time of recruitment. We have revised and reorganized the corresponding contents in Study subjects in MATERIALS AND METHODS section, which are highlighted in yellow for quick inspection and shown here as follows:

According to the desired sample size and the inclusion and exclusion criteria, a total of 954 Han Chinese from Northeast China were finally recruited between 2010 and 2012, including 443 SCZ patients and 511 healthy people. The patients were recruited from the Siping Psychiatric Hospital and Sixth Hospital of Changchun City (Jilin, China). Each

patients was diagnosed according to the Tenth Revision of International Classification of Diseases (ICD-10) for SCZ and confirmed by at least two experienced psychiatrists. Those with neurological disorders, severe organic lesions, and drug dependence were excluded. Subjects in the healthy control group matching the patients by gender and age were recruited from the Changchun Municipal Centre for Disease Control and Prevention, in order to get a comparable propotion of famine-exposed individuals between two groups and a similar ratio of gender. The healthy subjects were required to have no history of mental illness and were in good health without any known disease at the time of recruitment.

4. There are thousands of SNPs that are reported to be associated with schizophrenia. How did the authors decide to choose the four SNPs in this study? Or just took the potluck randomly? The rationale of locus selection should be discussed clearly.

Our response: Thank you for the comment. We basically selected the SNPs from previous population-based studies which were somehow related with schizophrenia and prenatal famine, and we would like to explain the details here.

First, rs11917047 in *PTPRG* was tested by a genome-wide association study (GWAS) of mQTLs with a considerably significant *trans*-acting association with CpG of gene *ID2*[1]. And *ID2* gene is responsible for negative regulation of activating *bHLH* proteins[2] which was reported to be associated with development of the nervous system[3]. Thus, we think it is possible that rs11917047 could be a meQTL SNP related to SCZ.

Second, rs61955196 in *ABCB9* was reported in a study mapping DNA methylation and identifying CpGs that differed between SCZ patients and controls, in which rs61955196 was determined to be a risk SNP for SCZ in the Psychiatric Genomics Consortium GWAS and also a meQTL in the cortex DNA methylation. Thus, we assume that rs61955196 should be associated with both DNA methylation and SCZ and worth digging in our study.

Third, the rest of the two SNPs, rs2239681 in *IGF2* and rs3842756 in *INSIGF*, were selected from a study on prenatal famine and genetic variation in a Dutch famine population[4]. Both of the SNPs were assessed to be closely associated with DNA methylation and had no linkage disequilibrium. Also, it was reported that both the *INSIGF* methylation and *IGF2 DMR0* methylation were significantly lower in the famine-exposed individuals as compared to their unexposed siblings. These findings suggest that these two SNPs have a great chance in acting as genetic tools for the investigation of DNA methylation and prenatal famine. Meanwhile, there have been

studies on the relationship between *IGF2* and neurological disorders including SCZ[5].

Based on the above facts, we suppose these four SNPs are quite convincing for the investigation of DNA methylation associated with prenatal famine and SCZ, and thus we selected them into this study to verify their effect and associations. Also, we have revised this part and added some of the references (No. 21,24,25,26) to the revised manuscript as we haven't been able to describe it clearly before. The related sentences are highlighted in yellow in the section Genomic DNA Extraction and Genotyping of MATERIALS AND METHODS as follows:

Combining the feasibility of the detection method and the previous publications, we selected four SNPs (rs11917047 in *PTPRG*、rs2239681 in *IGF2*、rs3842756 in *INSIGF* and rs61955196 in *ABCB9*) which have been confirmed as meQTLs, and the SNPs themselves or the genes they belong to were assessed to be associated with SCZ[21, 24-26].

5. As the authors mentioned in the limitation, "other possible confounding factors, such as medication use in the sample, any alcohol and drug use, evidence of folic acid intake in the form of prescription or over the counter" are not considered. Also, the duration of illness, severity of psychopathology, and other illness-related parameters are not mentioned. All these confounding factors are strongly contributed to the results of this study and failure to control them will lead to misinterpretation of current findings.

Our response: Thank you for the comment. We are sorry that our previous expression was not clear and might have caused a misunderstanding, and we would like to give a further explanation. Our original intention was to say that it was a pity that we were not able to investigate the interference of medication for SCZ, as we were not able to collect only first-episode antipsychotic drug-free SCZ patients to fully rull out therapeutic impact. Meanwhile, we excluded the patients who had taken any medication in the past three months before recruitment, and those who had any abused of alcohol, as described in the MATERIALS AND METHODS part. Although we could not conduct comparison analyses discussing these confoundings in this study, they were under consideration and control, which would not interfere our results.

On the other hand, it is true that we did not collect information about parameters including the duration of illness or severity of psychopathology. We agree that they might have impact on DNA methylation in SCZ patients. However, our primary target was to verify the associations between the four SNPs and SCZ along with prenatal famine, and the genetic variants generally remain constant since we were born and thus be free of these confounders. As a result, we suppose this would not disturb the

interpretation of our present results either.

We have revised the corresponding sentences highlighted in yellow in the last paragraph of DISCUSSION to clarify the limitations more clearly. Despite these explanations, we truly appreciate the comment and believe that it is preferable to conduct a subgroup analysis for SCZ patients regarding these factors to gain more valuable results, and we will try to enrich our design in future research. The revised part is shown here as follows:

Meanwhile, this study had several limitations. First, we only adjusted for gender as we mainly focused on the genetic variants, and we were not able to explore some underlying confounders such as medication as we have directly excluded those who had any medical treatment in the past three months before enrollment. Second, as we did not collect sufficient information from the patients regarding illness-related parameters such as the severity or duration of disease, we could not rull out the possibility that the SNPs could be associated with SCZ under some specific conditions although we got negative results.

6. What is the significance of this study? As already known, the ABCB9 polymorphism is associated with many disorders, including schizophrenia. The novelty of this study is the role of prenatal famine exposure in schizophrenia, postulating the ABCB9 gene could be a marker or a modulator for the later psychosis. However, the negative finding was found in this study. The novelty of this study is questionable.

Our response: Thank you for the comments. We have discussed and reconsidered the novelty of this study, and we would like to summarize it here, and make complement in the revised manuscript.

Firstly, we think the primary idea is one major novelty in our study design, as we intend to link prenatal famine exposure with the risk of schizophrenia through DNA methylation related sites. We hypothesized that prenatal famine might induce alteration of DNA methylation in individuals and thus increase their risk of having SCZ as DNA methylation plays an important role in the etiology of SCZ. And our study is among the first to investigate the relationship between prenatal famine and SCZ through SNPs associated with DNA methylation. Although we did not get many positive results, we believe that this field is promising and the design is feasible.

Secondly, regarding this topic, we did literature searching and found that basically there are only two sources that may serve for recruiting a remarkable number of study subjects for prenatal famine which are the Dutch and Chinese Famines[6]. Here we purposely recruited 443 SCZ patients from local hospitals with confirmed diagnosis

along with 511 healthy controls in Jilin Province, China. And 492 of them were exposed to prenatal famine. Using this study population, there have already been works of our group successfully published[7, 8]. We regard this study population to be a valuable and representative sample with a considerable size for research on SCZ and famine.

Thirdly, it was for the first time that the four SNPs we selected were detected and analyzed among Chinese subjects, and the positive association between rs61955196 and SCZ susceptibility in the Chinese Han population was initially been determined in this study. Although we got many other negative findings, we believe our results on the Chinese population should be interpreted cautiously for other regions or races, and the idea is still interesting and promising. We are glad to continue our work on this topic, hoping that we may accomplish more positive results in the future.

We have also revised and added some of these thoughts to our manuscript in the last paragraphs of Introduction and Discussion which are highlighted in yellow, as well as in the newly-written research highlight part. Some of the revised sentences are shown here as follows:

Introduction part: As DNA methylation tends to be sensitive to environmental factors, DNA methylation quantitative trait locus (meQTLs) seems more promising. They can be derived by GWAS mapping levels of DNA methylation in genotyped individuals and defined loci at which DNA methylation is influenced by genetic variation[20], with a superiority of higher consistency throughout one's life than DNA methylation itself. There have already been reports revealing the role of meQTLs in SCZ risk, which promote the feasibility of them serving as a useful tool for SCZ-related research[21, 22]. However, the results from GWAS studies are often not repeatable due to the enormous number for detection and heterogeneity of genetic information regarding people from different races and regions[23]. Given the high SCZ prevalence in China and current lack of available genetic data covering native patients, we find it necessary to conduct reseach collecting genetic data among Chinese individuals.

We recruited SCZ patients and healthy controls with comparable age including individuals born between 1959 and 1961 with prenatal famine exposure, and collected their peripheral blood samples for genotyping. We selected four SNPs which were previously reported as meQTLs, and determined their associations between SCZ and prenatal famine along with their interactions.

Research motivation part:

The lifetime prevalence of SCZ is approximately 1% around the world, and study has reported a highest age-standardized prevalence of SCZ for China. Meanwhile, the

Chinese famine of 1959-1961 is a proper source of study subjects to investigate the effect of prenatal famine on SCZ with little available genetic data. As a result, we intend to conduct analyses for SCZ and prenatal famine using native subjects with collected genetic information, which may provide insights specifically for Chinese researchers and patients.

To Editors:

Answering to comments from Science Editor:

1. This invited manuscript focused on the association between ABCB9 polymorphism (rs61955196) and schizophrenia in Chinese Han Chinese, which is an interesting topic for clinical work. However, there are some defects in the authors' experimental design, which should be further improved.

Our response: Thank you for the comments. We truly appreciate the approbation of the editor on our topic. Combining the relevant comments from previous reviews, we suppose the 'defects' here refer to our little consideration of certain confounding factors. As we mentioned in the previous answer, we admit that it is a pity that we did not document illness-related parameters for further analyses, while it is really difficult to collect this information for a supplement as the subjects were recruited several years ago. Also, as we controlled conditions on medication and alcohol use in SCZ patients by directly excluding all cases with these issues, we did not discuss these factors either. We feel sorry for the lack of this information, but we see them as underlying limitations rather than defects needing improvement as our primary target was to investigate SCZ and prenatal famine using genetic variants, and the SNPs are supposed to be free of these confounders. Furthermore, we have added more detailed explanations to the revised manuscript which are highlighted in yellow regarding the confounders in both the inclusion and exclusion criteria of subjects and discussion of limitations, and some of the sentences are shown as follows:

Each patients was diagnosed according to the Tenth Revision of International Classification of Diseases (ICD-10) for SCZ and confirmed by at least two experienced psychiatrists. Those with neurological disorders, severe organic lesions, and drug dependence were excluded. Subjects in the healthy control group matching the patients by gender and age were recruited from the Changchun Municipal Centre for Disease Control and Prevention, in order to get a comparable propotion of famine-exposed individuals between two groups and a similar ratio of gender. The healthy subjects were

required to have no history of mental illness and were in good health without any known disease at the time of recruitment.

Meanwhile, this study had several limitations. First, we only adjusted for gender as we mainly focused on the genetic variants, and we were not able to explore some underlying confounders such as medication as we have directly excluded those who had any medical treatment in the past three months before enrollment. Second, as we did not collect sufficient information from the patients regarding illness-related parameters such as the severity or duration of disease, we could not rull out the possibility that the SNPs could be associated with SCZ under some specific conditions although we got negative results.

2. The authors also need to supplement the details of the research method so that readers can better understand it.

Our response: Thank you for the comments. We have added some content regarding the previous comments including the inclusion and exclusion criteria of subjects and selection of the four SNPs. And we have revised other parts in MATERIALS AND METHODS with a few modifications. Some main revised sentences are shown as follows:

A desired sample size of 417 was calculated by the software Quanto with a proper power before the recruitment of participants, with a unmatched case-control rate of 1.2, an estimated population risk of 1% for SCZ, a log-additive model gene with allele frequency of 0.1 and genetic effect of 1.5 and a type I error rate of 0.05 by 2-sided test. According to the desired sample size and the inclusion and exclusion criteria, a total of 954 Han Chinese from Northeast China were finally recruited between 2010 and 2012, including 443 SCZ patients and 511 healthy people. The patients were recruited from the Siping Psychiatric Hospital and Sixth Hospital of Changchun City (Jilin, China). Each patients was diagnosed according to the Tenth Revision of International Classification of Diseases (ICD-10) for SCZ and confirmed by at least two experienced psychiatrists. Those with neurological disorders, severe organic lesions, and drug dependence were excluded. Subjects in the healthy control group matching the patients by gender and age were recruited from the Changchun Municipal Centre for Disease Control and Prevention, in order to get a comparable propotion of famine-exposed individuals between two groups and a similar ratio of gender. The healthy subjects were required to have no history of mental illness and were in good health without any known disease at the time of recruitment.

3. Furthermore, the number of total references is few and a bit outdated, maybe a little

more related references could also be cited.

Our response: Thank you for the comments. We have tried to replace as many references as possible with more recent ones regarding the same topics, and we added some new references through the process of manuscript revising. The number of references has increased from 26 to 40 now.

Answering to comments from Company Editor-in-chief:

Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is 'original', the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2022.

Our response: We sincerely appreciate your reminder regarding the formatting of figures and tables. We have separately prepared a single PPT containing our figure and a word document containing our tables, and formatted them according to your requirements and the guidelines from your website.

We truly regard the comments to be highly constructive and helpful for us to restructure the manuscript. We hope that all these changes fulfil the requirements, and we are looking forward that the new version of the manuscript can be accepted for publication. All authors have read and approved the revised manuscript, and there is no conflict of interest among authors. Neither the entire paper nor any part of its content has been published or accepted for publication elsewhere.

Sincerely yours,

Qiong Yu, Ph.D.

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