CACNA1C Polymorphism (rs2283291) Is Associated with Schizophrenia in Chinese Males: A Case-Control Study

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1. Introduction

Schizophrenia (SCZ) is a serious mental illness with a lifetime prevalence of approximately 1%, with symptoms of affective disorder, cognitive disorder, and volitional behavior disorder [1], and its etiology is complex and involves genetic and environmental factors. DNA methylation plays a vital role in SCZ and can directly act as a pathogenesis or biomarker of SCZ [2]. Wilkinson [3] used genome-wide analysis in white blood cells, and global methylation results showed lower levels of DNA methylation in SCZ patients; thus, DNA methylation with the ability to regulate gene expression has shown its relationship with SCZ [4].

China experienced a serious three-year natural disaster from 1959 to 1961 (also known as the "famine" period), during which food was scarce and people were severely malnourished. Exposure to famine during pregnancy has a serious impact on the development of the foetus, and maternal protein deficiency especially methionine and folic acid deficiency can cause DNA methylation changes [5, 6]; folic acid is a key component of DNA methylation [7], and insufficient dietary supplementation of methionine, folic acid, vitamin B6, etc., can alter DNA methylation, thereby changing an offspring’s phenotype. Retrospective studies have revealed that a poor utero environment caused by famine during pregnancy can lead to differences in DNA methylation in offspring [8]. Thus, people born during famine years may have altered DNA methylation due to nutrient deficiency.

Xu et al. [5] explained that exposure to famine during pregnancy has been identified as a risk factor for SCZ. Evidence from Dutch winter hunger from 1944-1945 and the 1959-1961 famine period in China shows that people exposed to famine during the foetal period especially in the first three months had a twofold increased risk of SCZ in adult life [9].
Harmful environmental events (like famine and nutritional deficits)—possibly through epigenetic mechanisms—may lead to SCZ [10]. DNA methylation is an inheritable epigenetic modification that can alter gene expression [11]; as a result, people born in the famine years may have an increased risk of SCZ due to altered DNA methylation of genes. This study explores the association between DNA methylation-related sites (rs2283291/rs4648635) and the incidence of SCZ as well as the effect of famine exposure at the genetic level, thus providing clues to reveal the pathogenesis of SCZ.

2. Materials and Methods

2.1. Subjects. Between 2010 and 2012, 960 SCZ patients and healthy people born in the famine years (1960-1962) and nonfamine years (1963-1965) in Jilin Province in the northeast of China were recruited. Exposure to famine during the prenatal period was defined based on birthdate, and the subjects were then divided into four groups: famine and SCZ group, famine and healthy control group, nonfamine and healthy control group, and nonfamine and SCZ group. A total of 445 SCZ patients were recruited from the Sixth Hospital of Changchun City and Siping Psychiatric Hospital. At least two independent experienced psychiatrists diagnosed the patients according to the tenth edition of International Classification of Diseases diagnostic criteria (ICD-10) [12]. Birthdate- and gender-matched control subjects were recruited from the Changchun Municipal Center for Disease Control and Prevention, and 515 healthy controls were free from mental illness. This study was approved by the Ethics Committee of the School of Public Health in Jilin University (approval number: 2014-05-01), and written informed consent was obtained from all subjects.

2.2. Genomic DNA Extraction and SNP Genotyping. Genomic DNA was extracted using Column Blood Clot DNA out (Win Honor Bioscience) and identified using a microplate nucleic acid protein analyzer (BioTek, USA), according to OD260/OD280 values to determine DNA content and purity.

SNPs were selected from an article “Mapping DNA methylation across development, genotype and schizophrenia in the human frontal cortex” [13] published in Nature Neuroscience based on MAF > 0.1. Then, according to the feasibility and applicability of the detection method, we selected only part of SNPs that can be detected. Furthermore, it has been found that CACNA1C is often considered a susceptibility gene for SCZ, while PLCH2 has been reported to be associated with mental retardation. Therefore, two SNPs including rs2283291 (in intron 12 of CACNA1C) and rs4648635 (in intron 1 of PLCH2) were selected for detection.

SNP genotyping was carried out via the iMDRTM multiple SNP typing technology (Shanghai Tian Hao Biological Technology Co. Ltd. Genetic Analysis Center). Primer sequences of each SNP were as follows: rs2283291-F: GTGT TTGGCCCTCGAGATGTGTT and rs2283291-R: GTTGCC AACCTCAGGCTTGGA and rs4648635-F: CCTAGAGGCC CAGAACCAGG and rs4648635-R: GCAAAGGATGC CCTCTTAAG. The main steps of genotyping were as follows. (1) The region where the SNPs are located was firstly amplified by a multiplex PCR reaction in one system. (2) The amplified products were subjected to exonuclease and phosphokinase (ExoI/SAP) purification for subsequent ligase reaction. (3) In a ligation reaction, each site contains two 5′ end allele-specific probes (the 3′ ends are two allele-specific bases or sequences, respectively, for insertion of a deletion polymorphism) as well as a fluorescently labelled specific probe next to a 3′ end site. The ligation product was differentiated by capillary electrophoresis of ABI 3730XL, and the original data file was analyzed using GeneMapper 4.1 software.

2.3. Statistics. Statistical analysis was performed using SPSS 24.0 and GMDR 0.9 software. Continuous variables were expressed as mean ± SD. Categorical variables were expressed as N (%). The chi-squared test was carried out to establish whether the genotype frequency distribution of SNPs was consistent with Hardy-Weinberg equilibrium (HWE), the genotype distributions and allele frequency distributions were compared using the chi-squared test or Fisher’s exact test, and Bonferroni-adjusted \( P = 0.05/2 = 0.025 \) was used as the critical value. SNP Stats online genetic analysis software [14] was used for genetic model analysis, with the smallest value of the Akaike Information Criterion (AIC) as the optimal genetic model. The gene-environment interactions were analyzed by logistic regression analysis while the gene-by-gene interactions were analyzed using the GMDR 0.9 software [15].

3. Results

3.1. Descriptive Analysis. Table 1 shows the baseline demographic characteristics between the four groups. The average age was 46.70-49.77 years, while the patients consisted of more males than females and more rural area people than urban area people. Maternal smoking, maternal drinking, maternal illness, and education level were similar among the four groups.

3.2. The Hardy-Weinberg Equilibrium Analysis. The genotype distributions of the two SNPs in both SCZ and healthy control groups satisfied the Hardy-Weinberg equilibrium (all \( P > 0.05 \)) except for rs2283291 in the SCZ group (\( P = 0.036 \)). About 10% of the samples randomly selected from the SCZ group were examined, and the accuracy of genotyping was more than 99%.

3.3. The Allele and Genotype Analysis. There was a significant association between rs2283291 genotype and SCZ in male patients (\( \chi^2 = 8.151, P = 0.017 \)), and difference still existed after correction using the Bonferroni method. There was no association in females (\( P > 0.05 \)), while rs4648635 and SCZ had no significant association for both males and females (all \( P > 0.05 \)). Among the male subjects, the rs2283291 GA genotype of SCZ patients was significantly higher, while the frequencies of the GG and AA genotype were relatively lower, and there was no such difference among the female subjects. There was no significant difference in genotype distributions and allele frequencies of the rs2283291/rs4648635...
between SCZ patients and healthy control groups or between famine and nonfamine groups (all \( P > 0.05 \)). The associations between two SNPs and SCZ by famine exposure and gender stratification were further analyzed, and the results are presented in Tables 2 and 3.

3.4. The Inheritance Model Analysis. As shown in Table 4, the differences between SCZ and rs2283291 in males were statistically significant. According to the AIC values, the best genetic model was overdominant inheritance for an rs2283291 locus in the male subjects. In the overdominant model (AA+GG vs. GA genotype), it was found that the high risk rs2283291 locus in the male subjects. In the overdominant genetic model was overdominant inheritance for an rs2283291 was associated with SCZ in Chinese males. The results indicated that methylation may be potentially associated with SCZ in a Chinese population. Furthermore, the results showed that the GA genotype of the methylation site rs2283291 increased the risk of SCZ in the overdominant model. Overdominance, or an advantage of a heterozygote over both homozygotes, is usually adapted to the new optimal phenotype. The phenotype of the heterozygote is more prominent in the context of overdominance [17]. In addition, the rs2283291 was found to be a methylation-related site; thus, the heterozygous GA of rs2283291 reflects the methylation level which is the most dominant. Some reports indicate that heterozygotes can cause changes in the methylation level of SCZ; for example, Zong et al. [18] reported a significant association between the genotypes of SNP and the promoter DNA methylation (5mC) levels, and the heterozygous genotype (CT) of rs3811997 was correlated with the decreased 5mC levels in SCZ patients.

SCZ is a complicated hereditary disease, and its etiology is still unknown [19, 20]. Epigenetics is considered to account for the gaps in SCZ etiology research. This is because epigenetic modifications influence the development of organisms, especially in the embryonic and postnatal neural development and brain function. Increasing evidence shows that epigenetic changes are involved in the pathophysiology of SCZ. DNA methylation is one of the most important epigenetic modifications which regulate gene expression. It participates in neural development, and hence it may be a vital factor in the pathogenesis of brain diseases [4, 11, 20]. Through their studies using mouse models, Zhang et al. [21] concluded that the CACNA1C gene was related to SCZ and is a risk gene for many mental disorders such as SCZ and depression. CACNA1C encodes L-type voltage-dependent calcium channel Cav1.2 alpha-1c which modulates the permeability of the cell membrane to calcium ion, leading to intracellular signal transduction, gene transcription, and synaptic plasticity change, and this plays a vital role in the adjustment of the brains’ major complex functions such as cognition, emotion,
The current study confirmed that there was a significant association between rs2283291 in the CACNA1C gene and SCZ. A significant difference was found in genotype frequencies of the CACNA1C rs2283291 among male SCZ patients, but female patients did not exhibit a significant correlation. Strohmaier et al. [22] suggested that CACNA1C has a distinct sex-specific effect and that it was involved in the internal phenotypic genetic structure of affective disorders and SCZ. In males, the A allele of rs1006737 within CACNA1C was associated with lower resilience and higher emotional lability, but the A allele was associated with stronger resilience and lower emotional lability in females. Although it is not the same SNP, their results showed a link to the gender difference in CACNA1C; it is therefore believed that CACNA1C may be different in male and female phenotypes. CACNA1C rs2283291 was examined in a case-control analysis, and it was found that the GA genotypes increase the risk of SCZ in males (OR: 1.62, 95% CI: 1.13-2.33, \( P = 0.0086 \)). In addition to genetic factors, some reports suggest that gender differences in SCZ may be due to hormonal differences, the disease itself, and differences in other behavioral patterns. It is considered that the sex hormone oestradiol is used to induce a stabilizer-like effect on psychotic symptoms and has a neuroprotective effect on females [23, 24]. The most important reason is the difference in social baseline levels, with higher incidence of SCZ in males than in females. Moreover, before suffering from SCZ, females have fewer adverse social behaviors than males because of their late-onset age, higher education, work experience, and social ability, which facilitate their self-regulation and higher treatment compliance after the onset of psychiatric symptoms, and it is also a fact that females are able to cope better with the disease [25, 26].

Similar results from the Netherlands and Chinese famine investigation have proven that famine-induced maternal folic acid deficiency is a risk factor for SCZ [7]. Folic acid is

| Genotype/allele | SCZ \(( n = 225)\) | Famine | Nonfamine | \( \chi^2 \) | \( P \) | SCZ \(( n = 220)\) | HC \(( n = 267)\) | \( \chi^2 \) | \( P \) |
|----------------|-----------------|---------|-----------|---------|--------|-----------------|----------|---------|--------|
| rs2283291      |                 |         |           |         |        |                 |          |         |        |
| GG             | 106 (47.1)      | 117 (47.2) | 2.386     | 0.303   | 94 (42.7) | 129 (48.3) | 3.464   | 0.177   |
| GA             | 104 (46.2)      | 105 (42.3) | 107 (48.6) | 11 (1.2) | 108 (40.4) | 30 (11.2)  |         |         |
| AA             | 15 (6.7)        | 26 (10.5) | 19 (8.6)  |         | 30 (11.2) |            |         |         |
| G              | 316 (70.2)      | 339 (68.3) | 0.390     | 0.532   | 295 (67.0) | 366 (68.5) | 0.247   | 0.619   |
| A              | 134 (29.8)      | 157 (31.7) | 145 (33.0) |         | 168 (31.5) |            |         |         |
| rs4648635      |                 |         |           |         |        |                 |          |         |        |
| CC             | 176 (78.2)      | 198 (79.8) | —         | 0.915*  | 185 (84.1) | 216 (80.9) | —       | 0.617*  |
| CT             | 46 (20.4)       | 47 (19.0) | 33 (15.0) |         | 49 (18.4) |            |         |         |
| TT             | 3 (1.3)         | 3 (1.2)  | 2 (0.9)   |         | 2 (0.7)   |            |         |         |
| C              | 398 (88.4)      | 443 (89.3) | 0.181     | 0.671   | 403 (91.6) | 481 (90.1) | 0.661   | 0.416   |
| T              | 52 (11.6)       | 53 (10.7) | 37 (8.4)  |         | 53 (9.9)  |            |         |         |

SCZ: schizophrenic; HC: healthy control. *Fisher’s exact test.
essential for normal DNA methylation. Since humans cannot synthesize it, they usually obtain it from diet. Its deficiency impedes the generation of methyl donors and DNA methylation, which affects adjustment of gene expression associated with neurodevelopmental processes; low levels of maternal folic acid may influence the risk of offspring SCZ [27, 28]. The results in this study did not find any association between prenatal famine exposure and DNA methylation sites (rs2283291/rs4648635). Tobi et al. [29] suggested that prenatal famine exposure may cause changes in DNA methylation. There is a need to further explore the relationship between famine exposure and DNA methylation.

Table 4: Association analysis of SNP genotype distributions in SCZ patients and the healthy control group as stratified by the gender.

| SNP      | Model | Genotype | SCZ vs. HC (male) | SCZ vs. HC (female) |
|----------|-------|----------|-------------------|---------------------|
|          |       |          | OR (95% CI)       | P       | OR (95% CI) | P       |
| rs2283291|       | GG       | 1.00 (ref)        | 0.017   | 1.00 (ref) | 0.74    |
|          |       | GA       | 1.52 (1.04-2.22)  | 0.93    | 0.63-1.39 |
|          |       | AA       | 0.70 (0.37-1.31)  | 0.76    | 0.38-1.55 |
|          | Dominant | GG       | 1.00 (ref)        | 0.13    | 1.00 (ref) | 0.59    |
|          |       | GA+AA    | 1.32 (0.92-1.89)  | 0.90    | 0.62-1.32 |
|          |       | GG+GA    | 1.00 (ref)        | 0.064   | 673.1      | 0.49 |
|          | Recessive | AA       | 0.57 (0.31-1.04)  | 0.79    | 0.40-1.56 |
|          | Overdominant | GG+AA    | 1.00 (ref)        | 0.0086  | 669.7      | 0.89    |
|          |       | GA       | 1.62 (1.13-2.33)  | 0.97    | 0.66-1.42 |
| rs4648635|       | CC       | 1.00 (ref)        | 0.07    | 673.2      | 0.57    |
|          |       | CT       | 0.82 (0.52-1.29)  | 1.05    | 0.64-1.73 |
|          |       | TT       | —                 | 0.36    | 0.04-3.09 |
|          | Dominant | CC       | 1.00 (ref)        | 0.6     | 676.3      | 0.97    |
|          |       | CT+TT    | 0.89 (0.57-1.39)  | 0.99    | 0.61-1.61 |
|          | Recessive | CC+CT    | 1.00 (ref)        | —       | —         |
|          |       | TT       | —                 | 0.35    | 0.04-3.06 |
|          | Overdominant | CC+TT    | 1.00 (ref)        | 0.35    | 675.7      | 0.80    |
|          |       | CT       | 0.81 (0.51-1.27)  | 1.07    | 0.65-1.75 |

SCZ: schizophrenic; HC: healthy control.

Table 5: Crossover analysis of the interactions between rs2283291/rs4648635 and famine factor with SCZ.

| Interaction term | SCZ | HC | OR (95% CI) | P    |
|------------------|-----|----|-------------|------|
| rs2283291 Famine | +   | +  | 1.247 (0.867, 1.793) | 0.235 |
|                  | +   | -  | 1.253 (0.875, 1.794) | 0.218 |
|                  | -   | +  | 1.243 (0.856, 1.807) | 0.253 |
|                  | -   | -  | 1 (ref)         |      |
| rs4648635 Famine | +   | +  | 1.144 (0.737, 1.777) | 0.548 |
|                  | +   | -  | 0.801 (0.499, 1.286) | 0.358 |
|                  | -   | +  | 1.038 (0.782, 1.377) | 0.797 |
|                  | -   | -  | 1 (ref)         |      |

SCZ: schizophrenic; HC: healthy control; rs2283291 (+): mutant (GA+AA); rs4648635 (+): mutant (CT+TT); rs4648635 (-): wild-type (CC).

Figure 1: GMDR 2D interaction model in rs2283291 and rs4648635. The left bar represents the positive score and the right bar represents the negative score, and high risks are represented by dark shading and low risks by light ones. 0, 1, and 2 in the figure are the GG, GA, AA genotypes of rs2283291 and the CC, CT, TT genotypes of rs4648635, respectively.

This study had some limitations. One was to reflect the level of methylation through methylation-related loci. Since the results in this work were preliminary, further research is...
needed to study the exact methylation levels among the population born in the famine years to fully validate this hypothesis. In addition, there is a need to further investigate more possible confounding factors of SCZ, such as smoking and drinking.

5. Conclusions
This study is the first to report that the methylation site rs2283291 on CACNA1C was associated with SCZ in Chinese males and the GA genotype of rs2283291 increased the risk of SCZ among Chinese males in the overdominant model.

Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that there is no conflict of interest.

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References
[1] J. Ryan and R. Saffery, “Crucial timing in schizophrenia: role of DNA methylation in early neurodevelopment,” Genome Biology, vol. 15, no. 10, p. 495, 2014.
[2] C. Montano, M. A. Taub, A. Jaffe et al., “Association of DNA methylation differences with schizophrenia in an epigenome-wide association study,” JAMA Psychiatry, vol. 73, no. 5, pp. 506–514, 2016.
[3] J. Wilkinson, “Blood cells show differences in DNA methylation in schizophrenia patients,” Epigenomics, vol. 4, no. 3, pp. 247–248, 2012.
[4] J. Liu, P. Siyahhan Julnes, J. Chen, S. Ehrlich, E. Walton, and V. D. Calhoun, “The association of DNA methylation and brain volume in healthy individuals and schizophrenia patients,” Schizophrenia Research, vol. 169, no. 1-3, pp. 447–452, 2015.
[5] J. Xu, G. He, J. Zhu et al., “Prenatal nutritional deficiency reprogrammed postnatal expression in mammal brains: implications for schizophrenia,” International Journal of Neuropsychopharmacology, vol. 18, no. 4, article pu054, 2015.
[6] E. Reynolds, “Vitamin B12, folic acid, and the nervous system,” The Lancet Neurology, vol. 5, no. 11, pp. 949–960, 2006.
[7] J. M. McCellan, E. Susser, and M. C. King, “Maternal famine, de novo mutations, and schizophrenia,” JAMA, vol. 296, no. 5, pp. 582–584, 2006.
[8] P. Dominguez-Salas, S. E. Cox, A. M. Prentice, B. J. Hennig, and S. E. Moore, “Maternal nutritional status, C, metabolism and offspring DNA methylation: a review of current evidence in human subjects,” Proceedings of the Nutrition Society, vol. 71, no. 1, pp. 154–165, 2012.
[9] M. Q. Xu, W. S. Sun, B. X. Liu et al., “Prenatal malnutrition and adult schizophrenia: further evidence from the 1959-1961 Chinese famine,” Schizophrenia Bulletin, vol. 35, no. 3, pp. 568–576, 2009.
[10] L. K. Pries, S. Guloksuz, and G. Kenis, “DNA methylation in schizophrenia,” Advances in Experimental Medicine and Biology, vol. 978, pp. 211–236, 2017.
[11] B. Rukova, R. Staneva, S. Hadjidekova, G. Stamenov, V. Milanova, and D. Toncheva, “Whole genome methylation analyses of schizophrenia patients before and after treatment,” Biotechnology & Biotechnological Equipment, vol. 28, no. 3, pp. 518–524, 2014.
[12] W. Rao, N. Zhou, H. Zhang et al., “A case-control study of the association between polymorphisms in the fibrinogen alpha chain gene and schizophrenia,” Disease Markers, vol. 2017, Article ID 3104180, 5 pages, 2017.
[13] A. E. Jaffe, Y. Gao, A. Deep-Soboslay et al., “Mapping DNA methylation across development, genotype and schizophrenia in the human frontal cortex,” Nature Neuroscience, vol. 19, no. 1, pp. 40–47, 2016.
[14] X. Sole, E. Guino, J. Valls, R. Iniesta, and V. Moreno, “SNPStats: a web tool for the analysis of association studies,” Bioinformatics, vol. 22, no. 15, pp. 1928–1929, 2006.
[15] X. Y. Lou, G. B. Chen, L. Yan et al., “A generalized combinatorial approach for detecting gene-by-gene and gene-by-environment interactions with application to nicotine dependence,” The American Journal of Human Genetics, vol. 80, no. 6, pp. 1125–1137, 2007.
[16] Schizophrenia Working Group of the Psychiatric Genomics Consortium, “Biological insights from 108 schizophrenia-associated genetic loci,” Nature, vol. 511, no. 7510, pp. 421–427, 2014.
[17] J. Draghi and M. C. Whitlock, “Overdominance interacts with linkage to determine the rate of adaptation to a new optimum,” Journal of Evolutionary Biology, vol. 28, no. 1, pp. 95–104, 2015.
[18] L. Zong, L. Zhou, Y. Hou et al., “Genetic and epigenetic regulation on the transcription of GABRB2: genotype-dependent hydroxymethylation and methylation alterations in schizophrenia,” Journal of Psychiatric Research, vol. 88, pp. 9–17, 2017.
[19] B. Rukova, R. Staneva, S. Hadjidekova, G. Stamenov, V. Milanova, and D. Toncheva, “Genome-wide methylation profiling of schizophrenia,” Balkan Journal of Medical Genetics, vol. 17, no. 2, pp. 15–23, 2014.
[20] M. Nishioka, M. Bundo, K. Kasai, and K. Iwamoto, “DNA methylation in schizophrenia: progress and challenges of epigenetic studies,” Genome Medicine, vol. 4, no. 12, p. 96, 2012.
[21] S. Y. Zhang, Q. Hu, T. Tang et al., “Role of CACNA1C gene polymorphisms and protein expressions in the pathogenesis of schizophrenia: a case-control study in a Chinese population,” Neurological Sciences, vol. 38, no. 8, pp. 1393–1403, 2017.
[22] J. Strohmaier, M. Amelang, L. A. Hothorn et al., “The psychiatric vulnerability gene CACNA1C and its sex-specific relationship with personality traits, resilience factors and depressive symptoms in the general population,” Molecular Psychiatry, vol. 18, no. 5, pp. 607–613, 2013.
[23] A. Riecher-Rossler and H. Hafner, “Gender aspects in schizophrenia: bridging the border between social and biological
psychiatry,” *Acta Psychiatrica Scandinavica*, vol. 102, no. s407, pp. 58–62, 2000.

[24] D. N. Allen, G. P. Strauss, K. A. Barchard, M. Vertinski, W. T. Carpenter, and R. W. Buchanan, “Differences in developmental changes in academic and social premorbid adjustment between males and females with schizophrenia,” *Schizophrenia Research*, vol. 146, no. 1-3, pp. 132–137, 2013.

[25] A. Mendrek and A. Mancini-Marie, “Sex/gender differences in the brain and cognition in schizophrenia,” *Neuroscience & Biobehavioral Reviews*, vol. 67, pp. 57–78, 2016.

[26] H. Hafner, “Gender differences in schizophrenia,” *Psychoneuroendocrinology*, vol. 28, pp. 17–54, 2003, Suppl 2.

[27] R. A. Waterland and R. L. Jirtle, “Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases,” *Nutrition*, vol. 20, no. 1, pp. 63–68, 2004.

[28] A. S. Brown and E. S. Susser, “Prenatal nutritional deficiency and risk of adult schizophrenia,” *Schizophrenia Bulletin*, vol. 34, no. 6, pp. 1054–1063, 2008.

[29] E. W. Tobi, L. H. Lumey, R. P. Talens et al., “DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific,” *Human Molecular Genetics*, vol. 18, no. 21, pp. 4046–4053, 2009.