What do DNA methylation studies tell us about depression? A systematic review

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Abstract
There has been a limited number of systematic reviews conducted to summarize the overview of the relationship between DNA methylation and depression, and to critically appraise the roles of major study characteristics in the accuracy of study findings. This systematic review aims to critically appraise the impact of study characteristics on the association between DNA methylation and depression, and summarize the overview of this association. Electronic databases and gray literatures until December 2017 were searched for English-language studies with standard diagnostic criteria of depression. A total of 67 studies were included in this review along with a summary of their study characteristics. We grouped the findings into etiological and treatment studies. Majority of these selected studies were recently published and from developed countries. Whole blood samples were the most studied common tissues. Bisulfite conversion, along with pyrosequencing, was widely used to test the DNA methylation level across all the studies. High heterogeneity existed among the studies in terms of experimental and statistical methodologies and study designs. As recommended by the Cochrane guideline, a systematic review without meta-analysis should be undertaken. This review has, in general, found that DNA methylation modifications were associated with depression. Subgroup analyses showed that most studies found BDNF and SLC6A4 hypermethylations to be associated with MDD or depression in general. In contrast, studies on NR3C1, OXTR, and other genes, which were tested by only few studies, reported mixed findings. More longitudinal studies using standardized experimental and laboratory methodologies are needed in future studies to enable more systematical comparisons and quantitative synthesis.

Introduction
A number of systematic reviews on susceptible genes and gene–environment interplay provide a comprehensive list of putative genetic and environmental risk factors for depression1–6. In contrast, there has been little compilation of our knowledge of DNA methylation modifications and depression.

To our knowledge, there are five reviews, including only one systematic review so far on the relationship between DNA methylation and depression7–11. Generally, they suggested that altered DNA methylations may be associated with the etiology of depression. Lockwood et al. in their narrative review of epigenetic findings in both animal and human studies concluded that epigenetics could play an important role in depression and suicide in humans1. Again, Uddin et al.8., using a similar approach, studied the role of sex in DNA methylation and post-traumatic stress disorder and major depressive disorder (MDD), and suggested that sex differences in DNA methylation among those genes known to influence brain development may explain the sexually dimorphic risk for developing post-traumatic stress disorder and MDD. Another narrative review found the inverse association between adverse environmental factors, i.e., early-life stress, and the epigenetic modification of gene expression9. A review examined the association between DNA methylation of seven candidate genes and depression, and found that brain-derived neurotropic factor (BDNF) and nuclear receptor subfamily 3 group C member 1 (NR3C1) gene methylation levels may be related to depression, whereas the relationship between serotonin transporter...
gene (SLC6A4; synonyms: 5-HTT and SERT) and depression was inconsistent. One recent systematic review assessed both animal and human studies and identified the correlation between burnout/depression and global and candidate-gene DNA methylation. However, this review did not examine the influence of experimental and statistical methodologies and analyses on findings.

Although a few reviews are published to explore the relationships between DNA methylation modifications and depression, there has been no review critically examining experimental methodologies and verification of laboratory testing in humans. The experimental methodologies and laboratory testing are closely linked with the accuracy of results. In addition, these reviews only focused on some aspects; for example, exploring the roles of sex and stress in this relationship. In this review, we aimed to (1) systematically synthesize the major findings on DNA methylation and depression, (2) compare the similarities and differences across different studies, including experimental and laboratory factors and statistical analyses used, which might partially explain some inconsistencies in the results, and (3) discuss the challenges and opportunities for future studies.

Materials and methods
The processing and reporting of the results of this systematic review were guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines 2009 revision. To ensure a thorough and systematic review of the literature, two methods were used to retrieve all the studies on relevant topics. We conducted a search of the computerized bibliographic databases PubMed, Web of Science, EMBASE, Medline, and Cochrane Library. The search strategy is detailed in Supplementary Appendix 1. The literature search comprised articles published until December 2017. A snowball technique was then applied to identify further studies. In addition, we manually searched other resources for other relevant studies. The reference lists of selected articles, review articles on relevant topics, and the gray literatures were screened. Figure 1 presents the process of study selection.

All suitable articles were evaluated with regard to their internal validity based on the four selection criteria as follows: (1) if they used a clear diagnosis criteria for depression (e.g., depression in general, major depressive disorder, depressive symptoms, or other types of depression), specifically the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) and its updates, and the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10) or other generally accepted diagnostic criteria; (2) if they examined the association between DNA methylation and depression; and (3) if they provided a statistical indicator (i.e., coefficient) or original data to estimate the relationship between DNA methylation and depression. Articles were excluded if (1) they did not specify a clear diagnosis for major depression, major depressive disorder, unipolar depression, or other types of depression or (2) they were not written in English.

Two authors (M. Li and X. Li) independently screened all the retrieved articles. Inconsistencies in interpretation were resolved through group discussions (X. Li, M. Li, and X. Meng). Endnote and RefWorks were the bibliographic softwares used. Data on author(s), year of publication, sample size, study design, study cohort, experimental methods, type of tissues, candidate genes or genome, DNA purification method, DNA methylation method, DNA methylation validation, genotyping, gene expression, experimental factors, statistical methods, and major findings were extracted independently. For those studies with multiple reports, a single record denoted one study with the information extracted from multiple reports. Group discussions dealt with all the inconsistent interpretations. The reviewers endeavored to contact the original authors of the studies for any missing information in order to gather complete and consistent study information. Open-ended questions were used to prevent misleading answers.

Because of the divergence of candidate genes and genomes, for example, some studies used the candidate-gene approach and others examined the whole genome, we grouped the summarized findings according to the number of studies available, including etiological studies and treatment studies. The etiological studies were then further divided into the following subgroups, including (1) BDNF, (2) SLC6A4, (3) NR3C1, (4) oxytocin receptor (OXTR), (5) other genes, and (6) genome-wide. Some articles were involved in multiple separate analyses as their data permitted.

Results
A total of 67 articles met our eligibility criteria. Figure 1 shows the detailed information of the process of study selection. Table 1 presents a summary of study characteristics of these selected studies. Supplementary Appendix 2 provides a list of the references for all the selected articles corresponding to their order in Table 1. Most of the reviewed articles were published between 2014 and 2017, especially in the past 4 years. The selected studies mainly focused on adults and seniors (58/67), covering a total number of 11,935 subjects worldwide (North America: 18/67, Asia: 21/67, Europe: 24/67, and Australia: 6/67). We also evaluated study quality, including design (study design, sample size, and subject characteristics), implementation (biological sample, DNA methylation method, purification of DNA extraction, and
validation of methylation), analysis (analytical method, batch effect, genotyping, and gene expression), and interpretation of results. Most studies in this review were case–control with hospital- or general population-based cohorts. There was a wide variety in terms of sample size, ranging from 11 to 1024. Whole blood was the most commonly used biological sample analyzed by generally accepted DNA methylation methods, such as bisulfite conversion with pyrosequencing. Both parametric and non-parametric statistics were used. Importantly, most of these studies did not analyze the influence of batch effect on their results (64/67), except the three studies targeted on genome-wide variations.

This review was designed to apply evidence-based approaches to summarize the findings between DNA methylation and depression. High heterogeneity was identified among the studies reviewed. The Cochrane guidelines do not recommend using quantitative methods, such as meta-analysis, to synthesize the research findings. Thus, qualitative methods were then used to summarize
| ID | First author | Publication year | Country | Sample size | Sample characteristics | Study design | Diagnoses of depression | DNA methylation methods/kits | Targeted genetic locations | Markers found in genome-wide studies/Cpg sites for candidate-gene studies | Major findings |
|----|--------------|------------------|---------|-------------|-----------------------|-------------|------------------------|----------------------------|---------------------------|---------------------------------|-----------------|
| 1  | Bostrom et al. | 2017             | Sweden  | 223         | Population-based adolescent cohort | Case-control | Depression in general | Illumina 450k | Genome-wide | The promoter region of NR4A4 and TS of ZSWIM | Two Cpg sites (cg1927623 and cg6103084) predicted depression in adolescents. cg0462364 was hypermethylated |
| 2  | Roy et al.    | 2017             | USA     | 34          | Hospital-based cohort | Case-control | MDD | Peripheral blood mononuclear cells | Immunoprecipitation (S-methylcytosine-enriched and qPCR) | BDNF, PRPS, CRHRP, CRHR1, NRIC1 | Promoters, Cpg islands | BDNF, PRPS, CRHRP, CRHR1, NRIC1 gene promoters were significantly hypermethylated in MDD |
| 3  | Meng et al.   | 2017             | China   | 162         | Hospital-based cohort | Case-control | MDD | White blood cells | Bisulfite conversion, pyrosequencing | NET, SLC6A4, GABA-A, BDNF, CRHBP, CRHR1 | Promoters, other Cpg sites | There were no significant differences in DNA methylation of the NET gene promoter between healthy controls and patients with MDD |
| 4  | Kaut et al.   | 2017             | Germany | 12          | Senior cases and controls | Case-control | MDD | Brain tissue | Bisulfite conversion, pyrosequencing | BDNF, CRHBP, CRHR1, NR3C1 | Promoters, Cpg islands | There were no significant differences in DNA methylation of BDNF and CRHBP between controls and cases |
| 5  | Ryan et al.   | 2017             | Australia | 380        | Late-life MDD and controls | Case-control | MDD | Bucal cells | Bisulfite conversion, pyrosequencing | IL-6 and treatment responses | Promoters, Cpg islands | There were no significant differences in DNA methylation of the IL-6 gene promoter and response between controls and cases with MDD |
| 6  | Shi et al.    | 2017             | China   | 161         | Hospital-based cohort | Case-control | MDD | Whole blood | Bisulfite conversion, pyrosequencing | S-HTR, S-CGAA | Promoters, Cpg islands | Methylation (hyper- and hypomethylated) at positions 4 and 5 was significantly associated with MDD |
| 7  | Han et al.    | 2017             | South Korea | 145        | Hospital-based cohort | Case-control | MDD | Whole blood | Bisulfite conversion, pyrosequencing | TESC | Gene body, other Cpg sites | MDD had significantly higher methylation on Cpg2 position of TESC gene-regulating downstate variants (rs2994198) than controls |
| 8  | Takeuchi et al.| 2017             | Japan   | 30          | Cases with best and worst treatment responses | Case-control | MDD | Whole blood | Bisulfite conversion, pyrosequencing | Genome-wide | Promoters, Cpg islands | Retent's DNA methylation profile at specific gene, such as PPF1A4 and HS3ST7 was associated with individual variations in therapeutic responses |
| 9  | Ciucanu et al. | 2016             | Canada  | 32          | White Caucasians, cases and controls | Case-control | MDD | PPC brain tissue | Bisulfite conversion, quantified with EpiTYPER | H1N2 | Promoters, and gene body, Cpg islands | Hypomethylation of synapsins (H1N2) was linked to depression |
| 10 | Won et al.    | 2016             | South Korea | 74         | Antidepressant-free cases and controls | Case-control | MDD | Whole blood | Bisulfite conversion, pyrosequencing | S-CGAA | Promoter region, other Cpg sites | Significant inverse correlations were observed between SLC6A4 DNA methylation and fractional amyotrophy. SLC6A4 DNA methylation was significantly higher at Cpg2 in MDD |
| 11 | Walker et al. | 2016             | Scotland | 29          | Members of a large family multiply affected by BD and MDD | Case-control | MDD | Whole blood | Sodium bisulfite using the EZ-96 DNA Methylation Kit, bead array using the Infinium HumanMethylation450 BeadChip | Genome-wide | Three Cpg sites (promoter region of NCAM2 and hypermethylated, 5' end and promoter-first exon of CDK5 and ITPB for hypermethylation) | Nominal significant differences in DNA methylation were observed, altered DNA methylation was a potential mechanism for mood disorders |
| 12 | Osborne et al. | 2016             | USA     | 291         | Derived from two prospective cohorts designed to study PPD and two cohorts from which DNA was taken long after pregnancy | Case-control | PDD | Whole blood | Illumina Human Methylation 450 (HM450) bead array for 91 women with mood disorders (including data), bisulfite conversion pyrosequencing using the PyroMark MD system for the rest of the samples | Genome-wide | No site identified | Epigenetic variation at PPD biomarker loci was likely to be associated with expression |
| 13 | Bustamante et al. | 2016 | USA | 147 | Late-life MDD and controls | Case-control | MDD | Whole blood | Bisulfite conversion using EpiTect Bisulfite Kit, pyrosequencing using PyroMark Q2A Assay Design Software | Genome-wide | No site identified | DNA methylation was significantly lower over Cpg sites 5-13 in those with vs. without MDD |
| 14 | Na et al.     | 2016             | South Korea | 117        | Recurrent MDD and controls | Case-control | MDD | Whole blood | Bisulfite conversion, pyrosequencing, using PyroMark MD system with the Pyro Gold reagents kit | BDNF and treatment response | Promoters, Cpg islands | Retents with MDD had significantly higher rates of methylation at Cpg2 and Cpg4 than healthy controls. No difference was found in naive or on-medication patients |
| ID  | First author          | Publication year | Country          | Sample size | Sample characteristics | Study design   | Diagnoses of depression | Biological samples | DNA methylation methods/kits | Targeted genetic locations | Markers found in genome-wide studies/CGP sites for candidate-gene studies | Major findings                                                                                           |
|-----|-----------------------|------------------|------------------|-------------|------------------------|----------------|-------------------------|---------------------|-----------------------------|-------------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|
| 15  | Kimmel et al.         | 2016             | USA              | 352         | Caucasian women         | Cohort         | PPD                     | Whole blood          | Bisulfite conversion by EZ DNA Methylation-Gold Kit and pyrosequencing using PyroMark MD system | DNA methylation rate | S-UTR, CpG islands | CGP (cg12695986) positioned in the middle of SP1 transcription factor binding site; its methylation had a negative correlation with PPD |
| 16  | Kahl et al.           | 2016             | Germany          | 70          | Treated MDD in patients and university announcements for controls | Case-control   | MDD                     | Whole blood          | Bisulfite conversion, PCR and sequencing, Sodium-bisulfite conversion using the EpiTect Bisulfite Kit | GLU1, GLU4             | Promoters, CpG islands | Increased methylation of GLU1 in MDD                                              |
| 17  | Iga et al.            | 2016             | Japan            | 57          | Unmediated cases and controls | Case-control   | MDD                     | Leukocytes           | Bisulfite conversion, pyrosequencing, EpiTect Plus DNA Bisulfite Kit (Qiagen) | Promoters, CpG islands | SLC5A4                           | Mean methylation level was significantly increased in patients compared with controls, p = 0.04. No significant difference was found in single CpG site |
| 18  | Oh et al.             | 2015             | Peripheral blood samples from Australia, The Netherlands, and UK; prefrontal cortex and sperm samples from Canada | 260         | Cases and matched controls | Case-control   | MDD                     | Peripheral blood, prefrontal cortex, and sperm | Bisulfite conversion, pyrosequencing, using Gold Q96 reagents, and Pyromark Q24 | Genome-wide      | No site identified       | Hypomethylated loci were found in the white blood cells of MDD twins. The brain and the sperm showed higher proportions of hypomethylated regions in MDD patients compared with the controls |
| 19  | Nagy et al.           | 2015             | Canada           | 121         | Cases with MDD and died from suicide, and controls, not died from suicide and with no MDD | Case-control   | MDD                     | Brain tissue         | Bisulfite conversion using EpiTect Bisulfite Kit from Qiagen, PCR, and sequencing | Genome-wide      | 115 DMRs                         | Significant differences (decrease) in the methylation patterns specific to astrocytic dysfunction associated with depressive psychopathology |
| 20  | van der Knapp et al.  | 2015             | The Netherlands  | 954         | Adolescents cohort      | Case-control   | Depression in general   | Whole blood          | Methylation levels, analyzed using EpiTYPER method, bisulfite conversion using EZ-96 DNA Methylation Kit, followed by PCR | NR1C1 and SLC5A4 | Promoters, CpG islands | NR1C1 methylation levels at NR1C1/L1 were positively associated with the risk of a depressive disorder and were positively associated with depressive symptom scores at follow-up, but became non-significant when accounted for depressive symptom scores at the baseline |
| 21  | Melas et al.          | 2015             | Sweden           | 44          | Female cases and controls | Case-control   | Depression in general   | Saliva               | Bisulfite conversion using EZ-96 DNA Methylation-Gold Kit, PCR, and sequencing | MAOA                  | Gene body, other CpG sites | Subjects with a history of depression were hypomethylated, compared to controls. Female individuals were hypermethylated at the MAOA region compared to males |
| 22  | Hohne et al.          | 2015             | Germany          | 116         | Remitted MDD and healthy controls | Case-control   | Peripheral blood cells  | MDD                  | Bisulfite conversion, PCR, and sequencing using EpiTYPER assay | DNA methylation rate | SLC5A4 and SLC5A4 | Subjects with TT genotype and a lifetime history of MD had a 10X higher DNA methylation rate than healthy controls with the same HINT2 genotype |
| 23  | Choi et al.           | 2015             | South Korea      | 113         | MDD with a mixed history of treatment | Case-control   | MDD                     | Whole blood          | Bisulfite conversion, pyrosequencing was performed on a PyroMark ID system using the PyroGold reagent kit (Qiagen) | DNA methylation rate | SLC5A4 and SLC5A4 | There were no significant differences in the 100k DNA methylation status at CpG1, CpG2, CpG3, and CpG4 between patients with MDD and healthy controls |
| 24  | Domschke et al.       | 2015             | Germany          | 94          | Caucasian case cohort with antidepressants | Cohort         | MDD                     | Whole blood          | Sodium bisulfite converted using EZ-96 DNA Methylation-Gold Kit, PCR and sequencing using BigDye Terminator | DNA methylation rate | Promoters and gene expression for CpG sites | The study did not find a major influence of MAOA DNA methylation on antidepressant treatment response. However, the presently observed trend towards CpG-specific MAOA gene hypomethylation might potentially drive impaired antidepressant treatment response in females—larger pharmacogenetic studies are needed |
| 25  | Córdova-Palomera et al.| 2015             | Spain            | 34          | Caucasian MZ twins      | Twin study     | Depression in general   | Whole blood          | Bisulfite conversion, bead array using The Illumina Infinium Human Methylation450 (450K) beadChip | Gene body, other CpG sites | DPPD7                             | A hypomethylated loci were found in the white blood cells of MDD twins. The brain and the sperm showed higher proportions of hypomethylated regions in MDD patients compared with the controls |
| ID | First author | Publication year | Country | Sample size | Sample characteristics | Study design | Diagnoses of depression | Biological samples | DNA methylation methods/kits | Targeted genetic locations | Markers found in genome-wide studies/CGP sites for candidate-gene studies | Major findings |
|----|--------------|------------------|---------|-------------|------------------------|-------------|------------------------|-------------------|-----------------------------|--------------------------|---------------------------------|------------------|
| 26 | Renner et al. | 2015 | Germany | 85 | Female inpatients and controls | Case–control | Depression and/or dysphoria | Leukocytes | Biallelic conversion using EpiTect Bisulfite Kit, PCR, and sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit | MDD | Gene body, other CGP sites | Depressed female patients had decreased OXTR exon 1 DNA methylation compared to non-depressed women. Exon 1 methylation appears to be associated with depressive phenotypes whereas exon 2 methylation was influenced by genotype rs6876 |
| 27 | Hughes et al. | 2015 | USA | 120 | Age- and sex-matched cases and controls | Case–control | MDD | Buffy coat of blood | Biallelic conversion using EpiTect Bisulfite Kit, presequencing using PyroMark Q96 MD | FAI2L, FAI2U, and ELOVL3 | S’UTR, CGP islands, and shores | MDD patients had a lower methylation in FAI2S, but higher in ELOVL3 |
| 28 | Chagnon et al. | 2015 | Canada | 43 | Women aged 65 years and plus | Case–control | Depression (major and minor) and/or anxiety | Saliva | Biallelic conversion, presequencing using PyroMark Q96 MD, except for APOE analyzed on Illumina BeadChip | MDD | Gene body, other CGP sites | A higher BDNF and OXTR DNA methylation was observed in subjects with anxiety/depression compared to controls |
| 29 | Cordoba-Palomera et al. | 2015 | Spain | 34 | Twin pairs with MDD and healthy controls | Case–control twin study | MDD | Whole blood | Biallelic conversion using Illumina Infinium HumanMethylation450Beadchip | Genome-wide | cg001122889 (WDR26) | Hypomethylation in WDR26 gene was associated with a lifetime diagnosis of depression |
| 30 | Bell et al. | 2015 | USA | 545 | Nested case-control study in a longitudinal cohort | Nested case-control | PPD | Whole blood | Biallelic conversion using PyroMark Gold Q24 | OXTR | Gene body, other CGP sites | Methylation was not significantly associated with postpartum depression |
| 31 | Zhang et al. | 2015 | China | 125 | MDD only, with or without suicide attempts | Case–control | MDD | Whole blood | Biallelic conversion, methylation-specific PCR | TPH2 | Promoters, other CGP sites | The TPH2 promoter was methylated in 36.0% (38/105) of MDD + suicide patients, as compared with that in 13.0% (10/75) of MDD patients |
| 32 | Nantharat et al. | 2015 | Thailand | 62 | Untreated MDD and controls | Case–control | MDD | Whole blood | Biallelic presequencing PyroMark UNE-1 kit (Bioneer, Uppsala, Sweden) | NR3C1 | Promoters, CGP islands | Hypermethylation levels at CGP7 were found in MDD in females but not in males |
| 33 | Kleiman et al. | 2015 | Germany | 11 | Treatment-resistant cases Perspective cohort | MDD | Whole blood | Biallelic conversion using EpiTect Bisulfite Kit, PCR, and sequencing using BigDye Terminator Cycle Sequencing Kit | Treatment responses on BDNF | Promoters, CGP islands | Remitters had a significantly lower promoter methylation rate than non-remitters, especially exon 1 |
| 34 | Kim et al. | 2015 | South Korea | 969 | Patients with recent acute coronary syndrome | Longitudinal | Mix of major and minor depression | Leukocytes | Biallelic conversion using EpiTect Bisulfite Kit, presequencing using PyroMark Q96 MD | BDNF | Promoters, CGP islands | At baseline, a higher methylation percentage in MDD compared with no depression. Higher BDNF methylation independently associated with prevalent depressive disorder at baseline and follow-up |
| 35 | Kaut et al. | 2015 | The Netherlands | 12 | Recurrent MDD and controls | Pilot-replication | MDD | Postmortem brain, HP, PFC tissue | Biallelic conversion with a SimCookResearch bisulfite kit and Illumina Human Methylation 450K bead arrays | Genome-wide, selected genes for replication | GRIN2A, three CGP sites on GRIN2A | 11 genes in the hippocampus and 20 genes in the prefrontal cortex revealed differential methylation. In replication, GRIN2A was found hypermethylated in both tissues and single CGP level |
| 36 | Kang et al. | 2015 | South Korea | 631 | Aged 65 years and plus for cases and controls | Longitudinal | Depression in general | Leukocytes | Biallelic conversion using EpiTect Bisulfite Kit, presequencing using the PSQ 96W System | BDNF | Promoters, CGP islands | Higher BDNF methylation was independently associated with depression and severe depressive symptoms |
| 37 | Kang et al. | 2015 | South Korea | 309 | Hospital-based, all women with breast cancer undergoing breast surgery | Longitudinal | Mix of major and minor depression | Leukocytes | Biallelic conversion using EpiTect Bisulfite Kit, presequencing using the PSQ 96W System | BDNF | Promoters, CGP islands | A higher methylation percentage at CGP9 with depression, both 1 week and 1 year after breast cancer |
| 38 | Januar et al. | 2015 | France | 1024 | Aged 65 years and plus for cases and controls | Case–control | MDD | Buccal cells | Biallelic conversion, PCR, and sequencing. Sodium-bisulfite conversion using the EpiTect Bisulfite Kit, sequencing was | BDNF | Promoters, CGP islands | Depression at baseline and chronic life was associated with higher BDNF methylation |
| ID | First author | Publication year | Country | Sample size | Sample characteristics | Study design | Diagnoses of depression | Biological samples | DNA methylation methods/kits | Targeted genetic locations | Markers found in genome-wide studies/CGP sites for candidate-gene studies | Major findings |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 39 | Frodl et al. | 2015 | Ireland | 60 | Cases had experienced acute depressive episodes, matched on age and sex with controls | Case–control | MDD | Whole blood | Bisulfite conversion, pyrosequencing, PyroMark Q24 | SLC6A4 | Promoters, CpG islands | MDD was not significantly associated with methylation |
| 40 | Booij et al. | 2015 | Canada | 69 | Adults, matched on sex and gender between cases and controls, cases not taking antipsychotics or mood stabilizers | Case–control | MDD | Whole blood | Bisulfite conversion, pyrosequencing, PyroMark Q24 Software (Qiagen) for methylation percentage at each site | SLC6A4, treatment response on BDNF | Gene body, CpG islands | MDD diagnosis was not significantly associated with DNA methylation. Patients with SSRIs had greater methylation |
| 41 | Numata et al. | 2015 | Japan | 63 | Hospital-based cases and matched controls | Case–control | MDD | Whole blood | Bisulfite conversion using EZ DNA methylation Kit, bead array using Infinium Human Methylation 450 Beadchips | Genome-wide | 363 (Of 313 CGIs) | 363 CpG sites demonstrated lower DNA methylation in MDD patients than in controls. 18 MDD-associated DNA methylation markers to discriminate cases from controls |
| 42 | Haghighi et al. | 2015 | USA | 53 | MDD and suicide cases and controls | Case–control | MDD | Whole blood | Bisulfite conversion using Illumina Infinium Human Methylation 25K BeadChips | Genome-wide | Not mentioned | Increased age-related DNA methylation perturbations in the prefrontal cortex in major depression suicide compared with nonsuicidal controls |
| 43 | Tadic et al. | 2014 | Germany | 39 | MDD inpatients | Cohort | MDD | Leukoocytes | Bisulfite conversion, PCR, and sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit | Treatment response on BDNF | Promoters, CpG islands | Hypomethylation was associated with depressive symptoms. The results supported that DNA methylation differences may be important in the pathogenesis of psychiatric disease |
| 44 | Khulan et al. | 2014 | Finland | 166 | Senior cases and controls | Case–control | Depressive symptoms | Whole blood | Bisulfite conversion using EZ DNA methylation Kit, bead array using Illumina methylation 450k beadchip and Infinium chemistry | Genome-wide | HP1BP3 and TTC9B | Hypomethylation of the 5-MT transcripational control region might impair antidepressant treatment response in Caucasian patients with MDD |
| 45 | Domschke et al. | 2014 | Germany | 94 | Caucasian cases with antidepressants | Cohort | MDD | Whole blood | Sodium bisulfite converted using EZ-96 DNA methylation Kit, PCR, and sequencing using BigDye Terminator | Treatment response on 5-HTT | Gene body, CpG islands | Hypomethylation of the 5-MT transcripational control region might impair antidepressant treatment response in Caucasian patients with MDD |
| 46 | Kaminsky et al. | 2014 | USA | Not mentioned | Not mentioned | Longitudinal | PPD | Whole blood | Not mentioned | HPBP3 and TCTC8 | Not mentioned | HPBP3 and TCTC8 (hypomethylation) predicted PPD with an area under the receiver operator characteristic curve (AUC) of 0.8. CpG methylation levels at two loci within the HPBP3 and TCTC8 genes were identified as biomarkers predictive of PPD |
| 47 | Guanxiang et al. | 2014 | USA | 93 | Caucasian women | Longitudinal | PPD | Whole blood | Illumina’s Infinium Human Methylation 450K BeadChip Kit | Genome-wide | Two loci within the HPBP3 and TCTC8 genes | Lower levels of 5-fmc and 5-mc in severe MDD than controls, no difference among severe and remitted patients |
| 48 | Tseng et al. | 2014 | China (Taiwan) | 74 | MDD cases and controls | Case–control | Leukoocytes | ELISA-based for global DNA methylation profiling and MethylationFlash methylated DNA quantification kit (for 5-mc) | Genome-wide | Global methylation levels, no site mentioned | Global methylation levels, no site mentioned |
| 49 | Okada et al. | 2014 | Japan | 100 | Untreated cases or cases without a history of | Case–control | MDD | Whole blood | Bisulfite conversion using EZ DNA methylation kits, SLC6A4 | Promoters, CpG islands | The pre-treatment-methylation rates (CpG3) of SLC6A4 is associated with therapeutic responses to antidepressants in unmedicated patients with MDD |
Table 1 continued

| ID | First author | Publication year | Country | Sample size | Sample characteristics | Study design | Diagnoses of depression | DNA methylation methods/kits | Targeted genetic locations | Markers found in genome-wide studies/CGP sites for candidate-gene studies | Major findings |
|----|--------------|------------------|---------|-------------|------------------------|--------------|-------------------------|----------------------------|-----------------------------|--------------------------------|---------------|
| 50 | Na et al.    | 2014             | South Korea | 117         | Major depressive episodes, untreated cases (no history of antidepressants) | Case-control | MDD, Whole blood         | Analyzed using a MassARRAY, methyltransferase sequencing with the Pyro Gold reagent kit (Quagen, Valencia, CA, USA) | NR3C1, BDNF, ZBTB20 | Methyltransferase sites (CGP sites) | MDD had significantly lower methylation than healthy controls at two CGP sites (CGP sites (4)) |
| 51 | Davies et al.| 2014             | UK       | 454 (50 twins, 354 case-control) | Monozygotic twins, discordant for depression | Twin study and case-control, Whole blood | MDD | Methylation analysis using a MassARRAY, 5′-nterminated nucleotide incorporation using a PyroMark ID system with the Pyro Gold reagent kit (Quagen, Valencia, CA, USA) | Genome-wide region | Coding region of 281800 gene | Both AU and UK did not identify DMR of genome-wide significance. MDD was associated with hypermethylation on the coding region of 281820 |
| 52 | Carlberg et al.| 2014             | Austria  | 554         | Unrelated in- and outpatients of White European origin | Case-Control | MDD, Peripheral blood mononuclear cells (PBMCs) | Methyltransferase analysis using a MassARRAY, 5′-nterminated nucleotide incorporation using a PyroMark ID system with the Pyro Gold reagent kit (Quagen, Valencia, CA, USA) | BDNF, treatment response | Promoters, CGP islands | BDNF exon I promoter was significantly increased in MDD. Current antidepressant therapy was associated with increased methylation |
| 53 | De Filosso et al. | 2014               | Italy    | 87          | Stable, pharmacologically treated MDD and matched controls | Case-control | MDD, Peripheral blood mononuclear cells (PBMCs) | Methyltransferase analysis using a MassARRAY, 5′-nterminated nucleotide incorporation using a PyroMark ID system with the Pyro Gold reagent kit (Quagen, Valencia, CA, USA) | BDNF, treatment response | Promoters, CGP islands | Overall lithium and valproate tend to decrease the DNA methylation level in BDNF gene promoters, when compared to other classes of medications. However, within each different disorder, mood stabilizers did not seem to affect DNA methylation, suggesting that such an alteration was likely not due to treatment use |
| 54 | Zhao et al. | 2013             | USA      | 84          | MZ twins (male veterans) for lifetime and concurrent MDD | Twin study | MDD, Leukocytes | Methyltransferase analysis using a MassARRAY, 5′-nterminated nucleotide incorporation using a PyroMark ID system with the Pyro Gold reagent kit (Quagen, Valencia, CA, USA) | SLC6A4 | Promoters, CGP islands | Variation in methylation levels within the promoter region of SLC6A4 was associated with variations in depressive symptoms. A 10% increase in the difference in DNA methylation level was associated with a 4.4-fold increase in the difference in BDI scores. The SLC6A4 promoter did not modulate this association. The use of antidepressants did not affect the relationship between SLC6A4 methylation and depressive symptoms |
| 55 | Melas et al.| 2013             | Sweden   | 174         | Female cases and controls | Case-control | MDD, Peripheral blood mononuclear cells (PBMCs) | Methyltransferase analysis using a MassARRAY, 5′-nterminated nucleotide incorporation using a PyroMark ID system with the Pyro Gold reagent kit (Quagen, Valencia, CA, USA) | SLC6A4, MOA | Promoters, CGP islands | Overall MOA methylation levels were decreased in depressed females compared to controls |
| 56 | Byrne et al. | 2013             | Australia | 48          | Queensland twin study (discordant MDD and concordant no MDD) | Twin study | MDD, Peripheral blood mononuclear cells (PBMCs) | Methyltransferase analysis using a MassARRAY, 5′-nterminated nucleotide incorporation using a PyroMark ID system with the Pyro Gold reagent kit (Quagen, Valencia, CA, USA) | BDNF, treatment response | Promoters, CGP islands | The difference in mean methylation was significant in females within discordant pairs, but not in males |
| 57 | Kim et al. | 2013             | South Korea | 286        | Patients with a recent ischemic stroke | Longitudinal | MDD, Peripheral blood mononuclear cells (PBMCs) | Methyltransferase analysis using a MassARRAY, 5′-nterminated nucleotide incorporation using a PyroMark ID system with the Pyro Gold reagent kit (Quagen, Valencia, CA, USA) | SLC6A4, MOA | Promoters, CGP islands | Higher SLC6A4 methylation status was independently associated with a major post-stroke depression at both baseline and followup |
| 58 | Kim et al. | 2013             | South Korea | 286        | Patients with a recent ischemic stroke | Longitudinal | MDD, Peripheral blood mononuclear cells (PBMCs) | Methyltransferase analysis using a MassARRAY, 5′-nterminated nucleotide incorporation using a PyroMark ID system with the Pyro Gold reagent kit (Quagen, Valencia, CA, USA) | BDNF, treatment response | Promoters, CGP islands | Prevalent, persistent, and incident PSD had a higher BDNF methylation status. The CGP site 6 was significantly associated with incident post-stroke depression |
| 59 | Kang et al. | 2013             | South Korea | 108        | Patients with MDD only | Longitudinal | MDD, Peripheral blood mononuclear cells (PBMCs) | Methyltransferase analysis using a MassARRAY, 5′-nterminated nucleotide incorporation using a PyroMark ID system with the Pyro Gold reagent kit (Quagen, Valencia, CA, USA) | SLC6A4, MOA | Promoters, CGP islands | SLC6A4 methylation status as a marker for childhood adversities among MDD, but was not associated with treatment outcomes |
| 60 | Bayles et al.| 2013             | Australia | 106         | Newly diagnosed or currently untreated and have not been receiving antidepressants | Case-control | MDD, Leukocytes | Methyltransferase analysis using a MassARRAY, 5′-nterminated nucleotide incorporation using a PyroMark ID system with the Pyro Gold reagent kit (Quagen, Valencia, CA, USA) | MOA | Promoters, CGP islands | There were no significant differences between MDD cases and controls in terms of the pattern of methylation of the SLC6A4 promoter. Antidepressant treatment did not change the result |
| ID  | First author          | Publication year | Country | Sample size | Sample characteristics | Study design | Diagnoses of depression | Biological samples | DNA methylation methods/kits | Targeted genetic locations | Markers found in genome-wide studies/ CpG sites for candidate-gene studies | Major findings                                                                 |
|-----|-----------------------|------------------|---------|-------------|------------------------|--------------|-------------------------|-------------------|-----------------------------|----------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| 61  | Zill et al.           | 2012             | Germany | 162         | Caucasian cases and controls | Case-control | MDD                     | Leukocytes      | Bisulfite conversion, PCR, and sequencing, EpiTect Bisulfite Kit | ACE                        | Promoters, CpG islands                                                                 | MDD patients showed a hypermethylation pattern at all the CpG sites compared to healthy controls |
| 62  | Sabuncyan et al.      | 2012             | USA     | 154         | MDD and controls          | Replication  | MDD                     | Postmortem frontal cortex, lymphoblasted cell lines, postmortem brain | CHARM assay platform     | Genome-wide                                                                 | No site identified                                                                 | PRIMA1 significantly increased the methylation in MDD in pilot, but not in replication      |
| 63  | Uddin et al.          | 2011             | USA     | 100         | Lifetime depression cases and non-depressed controls | Case-control | Depression in general   | Whole blood     | Bisulfite conversion using EZ-96 DNA Methylation Kit, bead array using HumanMethylation12 (8-HM-27) DNA Analysis BeadChip | Genome-wide                                                                 | 21 uniquely methylated and 107 uniquely unmethylated sites with depression | Uniquely unmethylated gene sets distinguished between those with versus without lifetime depression. In particular, some processes (e.g., brain development, tryptophan metabolism) showed patterns suggestive of increased methylation among individuals with depression whereas others (e.g., lipoprotein) showed patterns suggestive of decreased methylation among individuals with depression |
| 64  | Fuchikami et al.      | 2011             | Japan   | 38          | Japanese adults           | Case-control | MDD                     | Whole blood     | Bisulfite conversion using EZ DNA methylation kit, BeadChip Sequenom MassARRAY EpiTyper | SLC6A4                      | Promoters, CpG islands                                                                 | Significant methylation difference was found in CpG1 but not in IV                              |
| 65  | Olsson et al.         | 2010             | Australia | 150        | Australian adolescents (cases and controls) | Case-control | MDD                     | Buccal cells    | Bisulfite conversion, Sequenom MassARRAY EpiTyper | SLC6A4                      | Promoters, CpG islands                                                                 | There was no association between depressive symptoms and either buccal cell 5-HTT methylation or 5-HTTLPR. Depressive symptoms were more common among those with elevated buccal cell 5-HTT methylation who carried a 5-HTTLPR short allele |
| 66  | Alt et al.            | 2010             | The Netherlands | 12        | Depression and control groups matched for sex, age, brain weight, and postmortem delay | Case-control | MDD                     | Brain tissues   | Bisulfite conversion, pyrosequencing using PyroMark ID | NR3C1                      | Promoters, CpG islands                                                                 | No significant difference in methylation pattern was found between case and control groups |
| 67  | Philibert et al.      | 2008             | USA     | 192         | Lifetime MDD and controls | Longitudinal  | MDD                     | Lymphoblasted cell lines | Bisulfite conversion, methylation ratios calculated by usingMassARRAY | SLC6A4                      | Promoters, CpG islands                                                                 | Greater amounts of methylation in females vs males, and a trend of higher methylation was associated with greater vulnerability of lifetime MDD |

MDD = major depressive disorder, PPD = postpartum depression, PFC = prefrontal cortex, BD = bipolar disorder, HIP = hippocampus, SSRI = selective serotonin reuptake inhibitors, DMR = differentially methylated regions, PSD = poststroke depression
the overview of the research findings. We present the results in two categories based on the research objectives of these selected studies, namely etiological (genome-wide and candidate-gene) and treatment studies. Supplementary Appendix 3 provides a detailed description of each subgroup and its results.

**Etiological studies: genome-wide**

Although all genome-wide studies found significant methylation modifications associated with depression, both hyper- and hypomethylation correlations were reported. Inconsistent results were also noted. For instance, in one study, hypermethylation was previously found in a pilot study, but was not present on its replication; a significant decrease in mean methylation was observed among females, but not among males; lower methylation levels were found among severe MDD patients vs healthy controls, but no difference between severe vs remitted patients; and one study found both hypermethylation in some processes (e.g., brain development and tryptophan metabolism), and hypomethylations in other tissues (e.g., lipoprotein). Generally, sample sizes were not associated with study designs or major findings. However, studies with large sample sizes were more likely to use DNA purification methods and examine gene expression than those with smaller samples. Results from studies with large sample sizes are considered to be more reliable.

**Etiological studies: candidate-gene**

Generally, most studies found BDNF and SLC6A4 hypermethylation to be associated with MDD or depression. Studies on NR3C1, OXTR, and the rest of candidate genes, which were tested by only a few studies, reported mixed findings (hyper- and hypomethylation modifications and non-significant differences). The promoter regions and CpG islands were frequently targeted in these studies. The sample size in each group varied dramatically from 12 to 1024. Some of these studies also had gene expression for significant findings. Replications of findings were better in BDNF and SLC6A4 than in other studied genes. Studies with a longitudinal study design, reliable laboratory arrays, and statistical analyses were more likely to provide robust results.

**Treatment studies**

Findings in this group are more inconsistent compared to those in etiological studies. Half of the studies did not identify any significant methylation sites associated with antidepressant responses, and the rest had mixed significant findings (hyper- and hypomethylations) on different candidate genes. Again, the promoter regions and CpG islands were the major targets. This group of studies had a higher level of heterogeneity compared to other subgroups, as treatment history and stages of treatments may influence methylation modifications.

**Discussion**

This review firstly explored the role of DNA methylation in depression considering both the laboratory and analytic factors that could potentially confound the findings. A total of 67 articles were included in this review. The majority of the selected studies were recently published and were from developed countries. Whole blood was the most common tissue used in these analyses. Bisulfite conversion, along with pyrosequencing, was widely used to test DNA methylation level. There was a high heterogeneity among the studies in terms of the laboratory and statistical methodologies used and study designs. Large sample size and laboratory verification (DNA purification and DNA methylation validation) are the major characteristics important for accurate results.

The findings of our study are as follows. (1) For studies using candidate-gene approaches, BDNF, NR3C1, SLC6A4, and OXTR genes were the most frequently studied genes. Promoters and CpG islands were the common targeted regions. Overall, most of the studies found that BDNF and SLC6A4 hypermethylations were associated with depression. Studies on NR3C1, OXTR, and other candidate genes reported mixed findings in terms of methylation modification and depression. Again, promoters and CpG islands still were the focus. (2) All genome-wide studies found significant methylation sites, including hyper- and hypomethylations. (3) For studies that explored antidepressant treatment responses, their results were inconsistent as they targeted on a number of different genes and different stages of treatment. (4) Large-sample size studies were more likely to use DNA purification methods, examine gene expression in their analyses, and provide more reliable results.

**Findings on etiological genome-wide studies**

All genome-wide studies reported that DNA methylation was significantly associated with depression. Hypermethylations were observed in six studies on the following genes: zinc finger and BTB domain containing 20 (ZBTB20), heterochromatin protein 1-binding protein 3 (HP1BP3), tetratricopeptide repeat domain 9B (TTC9B), and glutamate ionotropic receptor NMDA type subunit 2A (GRIN2A).

ZBTB20 exists in the hippocampal neurons and cerebellum granule cells, and plays a role in many processes, including neurogenesis, glucose homeostasis, and postnatal growth. It may also have an impact on the development and regionalization of the human hippocampus, which has been found to be related to depression. Both HP1BP3 and TTC9B are linked to estrogen signaling. HP1BP3 is highly expressed in the brain and is
related to a number of physical and behavioral phenotypes in mice, such as dwarfism, impaired bone mass, impaired maternal behavior, and anxiety\textsuperscript{30,31}. Lower \textit{HP1BP3} has been found to be associated with postpartum depression and Alzheimer’s disease in humans\textsuperscript{21,32}. \textit{TTC9B} has been identified to be related to gonadal hormones\textsuperscript{33} and may be linked to hippocampal synaptic plasticity, which is critical for hippocampal long-term potentiation and depression\textsuperscript{34}. These markers in peripheral blood may indicate estrogen-mediated epigenetic changes in the hippocampus and in turn, potentially, raise the vulnerable phenotypes based on their actions in brain\textsuperscript{21}.

The \textit{GRIN2A} gene provides the instructions for making a protein called glutamate receptor subunit epsilon-1 in human encoded GluN2A, which is one of the components (subunit) of a subset of \textit{N}-methyl-D-aspartate (NMDA) receptors. They are involved in normal brain development and are responsible for changes in the brain in response to experience (synaptic plasticity), learning, and memory\textsuperscript{26}. Methylation modifications in \textit{GRIN2A} may play a key role in determining the function of NMDA receptors. Generally, gene promoter-region methylation could repress the gene expression, but the methylation on gene body can be positively correlated with expression activity\textsuperscript{35}. This suggests that the hypermethylation of the \textit{GRIN2A} gene body may result in the overexpression of NR2A and, thus, promote vulnerability for MDD via up-regulating NMDA receptor-dependent glutamatergic signaling\textsuperscript{36}.

Hypomethylations were also observed among depression patients on the following genes: WD repeat domain 26 (\textit{WDR26}), the promoter region of miRNA4646, 5-hydroxymethylcytosine (5-hmc), and 5-methylcytosine (5-mc)\textsuperscript{17,23,37–41}. Consistent with our findings on \textit{WDR26}, previous studies have found that the hypomethylation of \textit{WDR26} in depressed individuals may be related to lower gene-expression levels\textsuperscript{42}. Additionally, the decreased blood transcription levels of \textit{WDR26} were associated with depression-related phenotypes\textsuperscript{42–45}. 5-mc is a methylated form of the DNA base cytosine, which could be involved in the regulation of gene transcription. Its presence is important for the maintenance of the active chromatic state and for neurogenesis at non-promoter CpG islands\textsuperscript{46}, and is associated with stable and long-term transcriptional silencing of promoters\textsuperscript{47}. 5-mc is also found to be involved in the critical mechanism mediating genomic imprinting. This process has been identified as a key for normal development, and its abnormal imprinting can result in disorders such as Prader–Willi, Angelman, and Beckwith–Wiedemann syndrome\textsuperscript{47}.

5-hmc is a product of conversion of 5-mc. It is related to the regulation of gene expression, prompting DNA demethylation. The three ten-eleven translocation (TET) enzymes oxidize each step in the demethylation of 5-mc. 5-mc is first converted to 5-hmc, then to 5-formylcytosine (5fC), and then to 5-carboxylcytosine (5caC), each by TET1\textsuperscript{34}. Reduced levels of TET1 and, subsequently, 5hmC cause impaired self-renewal of stem cells\textsuperscript{19}.

Notably, inconsistent results were identified within the same studies among different subgroups; for example, different sexes\textsuperscript{16}, processes (e.g., brain development, tryptophan metabolism, and lipoprotein)\textsuperscript{18}, tissues (white blood cells, brain, and sperm)\textsuperscript{30}, or between pilot and replication studies\textsuperscript{15}.

**Findings on etiological candidate-gene studies**

For candidate-gene studies, the majority (11/12) of studies on \textit{BDNF} found \textit{BDNF} hypermethylation were associated with cases suffering from depression. Most of the studies had relatively large sample sizes and examined DNA purification. This is consistent with the recent reviews on \textit{BDNF} and depression. Chen et al. indicated that more than half of the studies showed an increased \textit{BDNF} methylation in depressed patients. Bakusc et al. concluded in their review that hypermethylation was consistently found in MDD subjects across the three studies selected\textsuperscript{10}. The \textit{BDNF} gene provides the instructions for making a protein found in the brain and spinal cord, and promotes the survival of nerve cells (neurons). It is actively involved in the growth, maturation, and maintenance of these neurons, and in the regulation of synaptic plasticity, which is important for learning and memory\textsuperscript{26,51}. It is reported that changes in the methylation level of the \textit{BDNF} promoter are associated with its lower expression in the prefrontal cortex\textsuperscript{52} and its activity in the hippocampus in animal studies\textsuperscript{53}. A similar decrease in \textit{BDNF} levels was also found in the serum and plasma of MDD patients; thus, it is hypothesized that MDD is related to impaired neuronal plasticity\textsuperscript{53}.

Positive associations between \textit{SLC6A4} methylation modifications and depression have also been identified in many studies in this review and previous reviews\textsuperscript{10,11}. All longitudinal studies in this review and studies with more comprehensive considerations of lab and statistical work have consistently found that depression patients had \textit{SLC6A4} hypermethylation compared to controls. \textit{SLC6A4} gives the instructions for making a protein in the brain that is involved in the regulation of serotonergic signaling by transporting serotonin or 5-hydroxytryptamine (5-HT) from synaptic spaces into presynaptic neurons\textsuperscript{54} and in the regulation of emotional behaviors\textsuperscript{55}. The alterations of \textit{SLC6A4} play an important role in brain development and function in humans\textsuperscript{56}. It has been hypothesized that DNA hypermethylation may result in the reduction of \textit{SLC6A4} expression and 5-HT reuptake, which in turn may increase the vulnerability to affective disorders at critical stages of development\textsuperscript{57,58}.

Findings on \textit{NR3C1}, \textit{OXTR}, and other genes were less coherent. Both hypo- and hypermethylation levels were
noted in depressive patients compared to controls. No significant associations between DNA methylation on these genes and depression were also reported by some studies. Similar findings were also found by recent reviews10,11. NR3C1 is the receptor to which cortisol and glucocorticoids bind. It regulates gene transcriptions and is linked to development, metabolism, and immune response69,70. OXTR is a receptor of the hormone and neurotransmitter oxytocin61,62. It presents in the central nervous system and plays an important role in modulating various behaviors, such as stress and anxiety, social memory and recognition, sexual and aggressive behaviors, bonding/affiliation, and maternal behavior63–65. We found that some of the selected studies had certain limitations in terms of the type of study design, sample size, and range of laboratory work and statistical analyses. Due to the high heterogeneity across the selected studies, this review could not provide more conclusive results on these genes in terms of relationships between DNA methylation modifications on these genes and depression.

Findings on treatment studies

Findings of this subgroup were less consistent than those of the other two subgroups analyzed. However, this is in line with another recent review on DNA methylation, and clinical response to antidepressants in MDD patients was inadequate to provide any consistent support for such a relationship66. Both the increased and decreased DNA methylation levels on SLC6A4 and BDNF genes were associated with the use of antidepressant medications, whereas MAOA methylation modification was not linked to antidepressant response. The relationship between antidepressant treatment and DNA methylation of certain genes has been reported, i.e., BDNF DNA methylation modification was associated with decreased gene expression, which can lead to MDD67. The use of antidepressants can restore the decreased BDNF to the normal level and alleviate depressive symptoms53,67. Inconsistencies across all these findings may be explained by different ethnicities, duration of treatments, and pharmacogenetic heterogeneities68,69. Investigations on antidepressant response should cover all the different treatment stages, since the level of DNA methylation may be altered during the treatment70.

Strengths and limitations

This review synthesizes the findings on DNA methylation associated with depression and critically appraised the major study characteristics that can significantly impact this association, including study design, study population, targeted genetic variations, methylation arrays, types of tissues, DNA purification, methylation validation, appropriate statistical methods, and the consideration of downstream analyses, e.g., genotyping and gene expression.

However, there are several limitations to be noted. First, this review was designed to provide an overview of the relationship between DNA methylation and depression. Therefore, all eligible studies with a wide range of genomics coverage, i.e., targeted genes or whole genome, and different types of study designs were included. As many study characteristics were heterogeneous, no pooled results were made to simply estimate this relationship. Second, although we used subgroup analyses to synthesize homogeneous studies, different types of tissues, study designs, phenotypes of the outcome, comparison groups, analytic methods, and sample sizes can still lead to inconsistent results. Third, most of studies were cross-sectional. DNA methylation level is dynamic and potentially reversible, and can be affected by a number of environmental factors. Findings from these cross-sectional studies may not be able to reveal the true nature of this complex relationship. Finally, only English databases were searched, which may limit the comprehensiveness of eligible studies.

Overall, we found that hyper- and hypomethylations on promoter regions and CpG islands of a number of genes were significantly associated with the disease. Most of the studies applied the widely acceptable laboratory techniques and statistical analyses, which made the pooled results more likely to reach a consistent finding. Future studies should adopt longitudinal study designs to explore the dynamic change of methylation levels. To allow for a systematic comparison of studies, there should be an agreement upon the consistent set of standards involving a minimum set for the items for the execution and reporting of methylation studies similar to what is required for the reporting of clinical trials, systematic reviews and meta-analysis12,71. Gene expression should also be routinely added into the research to uncover how, when, and what underlying mechanisms link these identified methylation sites to depression. This would advance the field and provide a firm base for the evidence on the relationship between DNA methylation and depression.

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X.L. and M.L. conducted the search and, together with XM, reviewed the articles returned by the search for eligibility, reviewed all data extraction, and
prepared the draft of this manuscript. X.M. and C.D. designed this review. T.Z. and R.J. assisted with the interpretation of the results. X.M. oversaw the project, provided feedback on all steps of the search, data extraction, and interpretation. All authors contributed to the writing and editing of the manuscript.

Conflict of interest
The authors declare that they have no conflict of interest.

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