DNA methylation in peripheral tissue of schizophrenia and bipolar disorder: a systematic review

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Abstract

Background: Increasing evidence suggests the involvement of epigenetic processes in the development of schizophrenia and bipolar disorder, and recent reviews have focused on findings in post-mortem brain tissue. A systematic review was conducted to synthesise and evaluate the quality of available evidence for epigenetic modifications (specifically DNA methylation) in peripheral blood and saliva samples of schizophrenia and bipolar disorder patients in comparison to healthy controls.

Methods: Original research articles using humans were identified using electronic databases. There were 33 included studies for which data were extracted and graded in duplicate on 22 items of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement, to assess methodological precision and quality of reporting.

Results: There were 15 genome-wide and 18 exclusive candidate gene loci investigations for DNA methylation studies. A number of common genes were identified as differentially methylated in schizophrenia/bipolar disorder, which were related to reelin, brain-derived neurotrophic factor, dopamine (including the catechol-O-methyltransferase gene), serotonin and glutamate, despite inconsistent findings of hyper-, hypo-, or lack of methylation at these and other loci. The mean STROBE score of 59% suggested moderate quality of available evidence; however, wide methodological variability contributed to a lack of consistency in the way methylation levels were quantified, such that meta-analysis of the results was not possible.

Conclusions: Moderate quality of available evidence shows some convergence of differential methylation at some common genetic loci in schizophrenia and bipolar disorder, despite wide variation in methodology and reporting across studies. Improvement in the clarity of reporting clinical and other potential confounds would be useful in future studies of epigenetic processes in the context of exposure to environmental and other risk factors.

Keywords: Epigenetics, Psychosis, Mood disorder, RELN, COMT, BDNF

Background

Schizophrenia (SZ) and bipolar disorder (BD) share some common genetic vulnerability [1, 2] and environmental risk factors [1, 3]. Only a small portion (approximately 23%) of the variance in risk for these disorders can be accounted for by common variation in the genome [4, 5]. The role of epigenetic processes (affecting gene expression) may thus account for substantial variation in the development of SZ and BD [6], and is consistent with evidence for non-genetic risk factors (e.g., obstetric complications [7, 8] and viral infections [9]) which may confer risk for these disorders via epigenetic processes.

Epigenetic modifications to the genome refer to changes in the physical structure of the chromatin, without a change in the DNA sequence itself [10]. The most widely studied epigenetic modification is DNA methylation, characterised by covalent linking of a methyl (CH3) group to a cytosine residue [11], almost exclusively occurring at CpG dinucleotides. These CpGs are clustered...
Evidence from observational case-control studies investigating differential DNA methylation in the peripheral tissues (blood or saliva) of SZ and/or BD patients, in comparison to a healthy control (HC) group. Assessment of the quality, consistency and strength of evidence reported across studies was undertaken for all studies using accepted criteria, using a validated tool for assessing methodological precision and quality of reporting.

Methods

Literature search: inclusion/exclusion criteria

Included are peer-reviewed, observational case-control studies investigating DNA methylation in the peripheral tissues (blood, saliva) of SZ (including schizoaffective disorder) and/or BD (type I and II) in comparison to a HC group. Excluded studies reported other types of epigenetic modifications (i.e. hydroxymethylation), mRNA gene products of the methylation pathway, or DNA methylation in germ line cells or post-mortem brain tissue, for which results have recently been reviewed elsewhere [14, 15].

Search strategy

Systematic searching of electronic databases MEDLINE, EMBASE, PsychINFO and PubMed identified studies published between 2000 and February 2015; further hand searching was conducted until April 2015. The following key terms were used: exp schizophrenia/, schizophrenia.tw, schizo.tw, exp bipolar disorder/, bipolar disorder.tw, exp psychosis/, psychosis.tw, dna methylation.tw, demethylation.tw, hypomethylation.tw and hypermethylation.tw.

Searches were limited to studies published in English, conducted in humans, and published after the year 2000 to minimise the methodological inconsistencies seen in the earliest studies of DNA methylation (e.g. improvements in polymerase chain reaction based DNA methylation methods) [18].

Study selection

A preferred reporting items for systematic reviews and meta-analysis (PRISMA) flowchart of the search and selection processes of the included studies is presented in Fig. 1. All decisions relating to study inclusion were completed independently by two authors (NT and LG) with any disagreements resolved by discussion with MG.

Quality assessment and study characteristics

Information relating to data quality were graded in duplicate (authors NT and MG) using 22 items listed in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [19], to assess the risk of bias within studies and across studies, methodological consistency and precision, as well as reporting transparency and comprehensibility. The STROBE statement consists of a 22 item checklist which allows a systematic and critical assessment of the strengths and weaknesses of the study design, conduct and analysis [19]. Thresholds for determining study quality were determined by authors, as per the STROBE guidelines: scores on the STROBE checklist of ≥ 66% were considered to be reflective of high study quality, ≤ 33% of low quality reporting, and scores in between this range were of moderate quality. Thresholds for categorising significant change in methylation status for hypermethylation, hypomethylation and differential methylation (i.e. non-specified direction of difference), as well as no significant group difference in methylation were protocol dependent, but largely reflect convention of significance level being set at 0.05, with appropriate corrections applied according to the number of analyses being conducted. Study characteristics are summarised in Tables 1, 2 and 3 and include sample characteristics, methods of quantifying DNA methylation, as well as any relevant data (e.g., medication status) that could contribute to the investigation of subgroups.

Results

Search results: included and excluded studies

The systematic search strategy identified a total of 908 publications, of which 622 were duplicates (i.e., 286 unique studies); an additional 18 publications were found by hand searching reference lists and advance access publications (See Fig. 1). These 304 studies were screened for relevance by title and abstract, resulting in the removal of 178 studies. Full text screening of the remaining 126 studies excluded a further 75 studies which did not meet inclusion
criteria (see Fig. 1); of these, 41 were conference abstracts/reviews, 22 did not include SZ or BD participants, two did not have a comparison group consisting of healthy unrelated subjects, seven reported indirect measures of DNA methylation (i.e. mRNA expression of DNA methylation products), two investigated other types of epigenetic modification and one study did not investigate DNA methylation in the genome. An additional 18 studies were excluded which conducted DNA methylation analyses using only germ line cells or post-mortem tissue. A final total of 33 studies, which fulfilled inclusion criteria, were evaluated in this systematic review.

Study quality assessment
The STROBE ratings suggested that the available evidence for differential methylation in SZ and BD ranged from low (29.5 % minimum) to high quality (77 % maximum) with the mean of all scores at 59 % (SD: 2.36), suggesting moderate quality of available evidence and moderate probability of reporting bias.

Sample characteristics
The 33 included studies examining differential DNA methylation in peripheral tissues comprised 22 studies that compared SZ to HC [20–41] (see Table 1), seven studies that compared BD to HC [42–48] (with three studies also comparing BD-I to BD-II; see Table 2), and four studies that compared HC to both SZ and BD [49–52] (SZ/BD; see Table 3). The most common tissue for methylation was blood (n = 31; SZ: 22, BD: 7, SZ/BD: 2), however two studies reported the use of saliva (both were SZ/BD studies). Tables 1, 2 and 3 summarise sample characteristics for the 33 included studies. Sample sizes varied considerably across studies (for SZ, M = 130.6; SD = 203.4; range = 2-759; for BD, M = 75.6, SD = 103.2, range = 3-370; for HC, M = 125.3; SD =
| Ref.                          | N  | Mean age (SD) | Sex (F %) | Method                                      | Subgroup analyses /covariates                                                                 | RESULTS: methylation loci                                                                 |
|------------------------------|----|---------------|-----------|---------------------------------------------|---------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| Aberg et al., 2014 [20]      | 759 | 738           | 53 (12)   | MBD protein-enriched genome sequencing b, d, e | Age, sex, smoking m, alcohol use m, medication m, autoimmune disorders m                    | Differential methylation 1 of FAM63B, RELN (first intron), FCAR and 8 other genes linked to hypoxia & the immune system |
| Aberg et al., 2012 [21]      | 750 | 750           | - -       | MBD protein-enriched genome sequencing b, d, i | Age, sex                                                                                 | Differential methylation 1 of GRIA2, HTRA3, CAMK2D, FNDG38 and DCTN                         |
| Bonsch et al., 2012 [22]     | 27  | 34            | 30 47     | Modified non-radioactive elongation assay and MSRE-quantitative PCR a, c, d, e | Sex h, medication l, promoter methylation compared to global DNA methylation               | Global methylation differences 1; Lower methylation of RELN and SOX10 promoters c, m in SZ; SZ on medication had similar methylation levels to HC |
| Bromberg et al., [23]        | 28  | 26            | 39 (14)   | Radiolabelled [3H] cytosine-extension assay a, d, e | Age m, sex, smoking j, illness duration m, medication m                                     | No global methylation differences m; Higher methylation in SZ non-smokers           |
| Chen et al., 2012 [24]       | 371 | 288           | - -       | Bisulfite sequencing c, d, e                  | Sex i                                                                                       | No differential methylation of MAOA (promoter m) Greater methylation in SZ females compared to males |
| Ikegame et al., 2013 [25]    | 100 | 100           | 43 (13)   | Pyrosequencing c, d, e                       | Age m, sex                                                                                 | Hypermethylation of BDNF (promoter I) j for SZ CpG-72 compared HC; however methylation generally low in SZ/HC; No differential methylation of BDNF (promoter M) m between groups; higher methylation in SZ/HC females at all CpG sites |
| Kinoshita et al., 2014 [26]  | 63  | 42            | 49 (10)   | 450 K methylation array a, d, f              | Age, sex, cell type heterogeneity 1                                                          | Global methylation differences (485 764 CpG sites) 1 Hypermethylation in SZ found in 1161 CpG sites when controlling for cellular heterogeneity |
| Kinoshita et al., 2013a [27] | 42  | 42            | 52 (7)    | 450 K methylation array a, d, e              | Age, medication                                                                            | Global methylation differences (164 657 CpG sites) 1 including SLC18A2, GNAL, KCNH2 and NTNG2 |
| Kinoshita et al., 2013b [28] | 24  | 23            | 31 (11)   | 450 K methylation array a, d, e              | Sex l                                                                                      | Global methylation differences in SZ (486 764 CpG sites) 1 including B3GAT2, HDAC4, DGKI, PCMT1, INSIG2, GFRA2 and RA1; Did not replicate published methylation findings in SZ for COMT, HTA1A and MAOA |
| Kordi-Tamandani et al., 2013a [29] | 81  | 71            | 48 (11)   | Methylation specific PCR c, d, g              | -                                                                                          | Hypermethylation of GMR2, GMR5, GRIA3, GMR8 (all promoter regions)                      |
| Study                                      | Sample Size | Controls | Studies | Treatment | Genotype | Comparator | Differential Methylation |
|--------------------------------------------|-------------|----------|---------|-----------|-----------|------------|--------------------------|
| Kordi-Tamandani et al., 2012 [30]          | 80          | 71       | 48 (11) | 47 (12)   | -         | Methylation specific PCR | -                        |
| Kordi-Tamandani et al., 2013b [31]         | 94          | 99       | 48 (11) | 47 (12)   | Genotype  | -          | Methylation specific PCR |
| Liao et al., 2014 [32]                     | 2           | 1        | 25 (4)  | 31 (0)    | -         | MBD protein-enriched genome sequencing | -                        |
| Liu et al., 2013 [33]                      | 98          | 108      | 34 (11) | -         | 25        | 36         | K methylation assay      |
| Melas et al., 2012 [34]                    | 177         | 171      | 52 (9)  | -         | 51        | Luminometric methylation assay and bisulfite sequencing | -                        |
| Murphy et al., 2008 [35]                   | 18          | 31       | -       | -         | -         | Bisulfite sequencing | No differential methylation of SYNIII |
| Murphy et al., 2005 [36]                   | 20          | 31       | -       | -         | -         | Bisulfite sequencing | No differential methylation of S-COMT (promoter) |
| Nishioka et al., 2013 [37]                 | 17          | 15       | 23 (5)  | 23 (4)    | 59        | 33         | K methylation assay      |
| Ota et al., 2014 [38]                      | 51          | 51       | 25 (8)  | 26 (8)    | 37        | 37         | Bisulfite sequencing     |
| Pun et al., 2011 [39]                      | 30          | 30       | -       | -         | 50        | 37         | Bisulfite sequencing     |
| Ota et al., 2014 [38]                      | 51          | 51       | 25 (8)  | 26 (8)    | 37        | 37         | Bisulfite sequencing     |
| Pun et al., 2011 [39]                      | 30          | 30       | -       | -         | 50        | 37         | Bisulfite sequencing     |
| Pun et al., 2011 [39]                      | 30          | 30       | -       | -         | 50        | 37         | Bisulfite sequencing     | Hypermethylation of GABBR2 in SZ (CpG sites 1–26) |

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Table 1  Summary of studies reporting DNA methylation in schizophrenia (Continued)

| Study | Sample Number | Controls | Study Type | Tissue Type | Measure of Methylation | Results |
|-------|----------------|----------|------------|-------------|------------------------|---------|
| Shimabukuro et al., 2007 [40] | 210 | 54 | Global | Blood | Percentage | Increase in SZ in males, decrease with age |
| Van Eijk et al., 2014 [41] | 264 | 27 | Candidate Gene | Blood | Percentage | Significant methylation differences in SZl: including PRRT1, HLA-C, MRPL41, CALHM1; Significant association between DNA methylation and gene expression |

SZ schizophrenia, HC healthy control, N sample number, F female, SD standard deviation, MBD methyl-CpG-binding domain, MSRE methylation specific restriction enzymes, HPLC high performance liquid chromatography, UTR untranslated regions, PCR polymerase chain reaction, GAF global assessment of functioning, Genes: FAM63B family with sequence similarity 63 member B, RELN reelin, FCAR Fc fragment of IgA receptor, GRIA2 glutamate receptor ionotropic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid 2, HTRA1 HTRA serine peptidase 3, CAMK2D calcium/calmodulin-dependent protein kinase 2 delta, FND3B fibronectin type 3 domain containing 3B, DCTN dynactin, SOX10 sex determining region Y box 10, MAOA monoamine oxidase A, BDNF brain-derived neurotrophic factor, SLC25A10 solute carrier family 25 member 10, CBFA2T3 core-binding factor alpha subunit 2 translocated to 3, 5-HTT serotonin neurotransmitter transporter, SYNIII synapsin 3, S-COMT soluble catechol-O-methyltransferase, COMTD1 catechol-O-methyltransferase domain containing 1, SLC6A3 solute carrier family 6 transporter member 3, HTR1E serotonin receptor 1E G protein-coupled, GABRB2 gamma-aminobutyric acid A receptor beta 2

Study type
- global DNA methylation
- methylome-wide association study
- candidate gene study

Tissue type
- blood

Measure of Methylation
- percentage
- beta-value
- odds ratio
- peak score
- no reported

Results
- significant hypermethylation
- significant hypomethylation
- significant differentially methylated
- no significant difference

In SZ males hypomethylation decreases with age
185.7; range = 1-750; see Tables 1, 2 and 3) with the mean age being 39.1 years (SD = 11.3, range = 23-53 years) for SZ, 45.3 years (SD = 7.4; range = 39-57 years) for BD, and 40.9 years (SD = 8.9; range = 23-12 years) for HC (see Tables 1, 2 and 3). The mean percentage of females per sample was 43.7 % for SZ, 50.8 % for BD and 42.4 % for HC.

Methodological variability

There were 16 different methods reported in the 33 included studies, with four studies using more than one method to determine methylation status. The most commonly used methodology for candidate gene loci was bisulfite sequencing of candidate genetic loci (n = 8) [24, 34–36, 38, 39, 50, 52], while the most commonly reported genome-
wide methods used were methyl-CpG-binding domain (MBD) protein-enriched genome sequencing (n = 3) [20, 21, 32], 450 K arrays (n = 3) [26–28], and 27 K arrays (n = 3) [33, 37, 41]. Other methods for the study of candidate genetic loci were pyrosequencing (n = 4) [25, 46–48], methylation specific polymerase chain reaction (PCR; n = 3) [29–31], fluorescence-based real-time PCR (n = 2) [44, 45], quantitative methylation specific PCR (n = 2) [50, 52], methylation sensitive restriction enzyme (MSRE) quantitative PCR (n = 1) [22], MethyLight protocol (n = 1) [43] and high-resolution melt (HRM) method (n = 1) [49]. Other methods used to measure genome-wide DNA methylation were radiolabelled [3H] cytosine-extension assay (n = 2) [23, 42]; modified non-radioactive elongation assay (n = 1) [22], luminometric methylation assay (n = 1) [34], high-performance liquid chromatography (HPLC; n = 1) [40] and methylated DNA immunoprecipitation (n = 1) [33]. These inconsistencies in the way that methylation was quantified precluded meta-analysis.

### Methylation analyses and genes investigated in schizophrenia and bipolar disorder

Genome-wide DNA methylation analyses (including three methylome-wide association study; MWAS) were conducted in 15 out of 33 studies (comprising 13 SZ studies, one BD sample, and one combined SZ/BD sample). Two of these 15 genome-wide DNA methylation studies (one SZ and one BD) reported no difference in DNA
methylation status between clinical cases and controls [23, 42], while one study found genome-wide hypomethylation in SZ [40]. Of the 15 genome-wide studies, only four reported estimates of ‘global’ methylation changes across the entire genome (i.e., % differential methylation without reference to specific genes). The remaining 18 studies focused exclusively on candidate gene loci (9 SZ, 6 BD and 3 SZ/BD studies). There was a total 163 different genes investigated, with four genes investigated in more than one study. These included reelin (RELN) (2 SZ studies), brain-derived neurotrophic factor (BDNF) (3 SZ and 3 BD studies), catechol-O-methyltransferase (COMT) (1 SZ/BD, 3 SZ studies) and hydroxytryptamine serotonin 1A receptor (HTR1A) (2 SZ/BD studies). Out of the 33 studies, 3 provided a raw results database for download [32, 41, 51].

Evidence for DNA methylation in schizophrenia and bipolar disorder

Across all studies of SZ and/or BD, there were 21 sites reported as hypermethylated, seven sites of hypomethylation, and 135 genetic loci reported as differentially methylation. The most common genes identified as differentially methylated in SZ/BD were different receptors, transporters and neurotransmitters related to RELN, BDNF, dopamine, serotonin and glutamate (see Table 4); this consisted of 14 candidate gene loci studies (one RELN, five BDNF, five dopamine, two serotonin and one glutamate) and 10 genome-wide studies (one RELN, one BDNF, three dopamine, four serotonin and one glutamate). For these genes, there was evidence of both hyper- and hypomethylation in both SZ and BD, as well as some evidence for lack of differential methylation. There were also several studies reporting DNA methylation of genes previously linked to SZ, including: hypermethylation of gamma-aminobutyric acid receptor beta-2 (GABRB2) [39], discs large homolog 4 (DLG4) and the gene disrupted in schizophrenia 1 (DISC1) [32], as well as differential methylation of major histocompatibility complex class C (HLA-C) and calcium homeostasis modulator 1 (CALHM1) [41]. The results of specific genetic loci reported in more than one study are discussed in further detail below. In addition, 11 studies (7 SZ, 3 BD and 1 SZ/BD study) reported no differences in methylation in a number of genes (see Tables 1, 2 and 3).

Reelin

Differential methylation for RELN was reported for intron 1 in SZ [20], although another study also reported a lack of differential methylation of the RELN promoter in SZ [22].

Brain-derived neurotrophic factor

Methylation investigations for BDNF in SZ and BD were reported only for promoter regions. In BD (both type I and II) there was consistent reporting of hypermethylation of the BDNF exon 1 promoter in two studies [44, 45], although one other study of BD (unspecified-type) reported lack of differential methylation at this site [43]. In SZ, the results were mixed with hypermethylation of BDNF promoter I [25], differential methylation of an unspecified BDNF promoter [30] and a lack of differential methylation of BDNF promoter IV [25].

Dopamine

There was mixed evidence for methylation status of genes associated with dopamine transporters in SZ, which included hypermethylation of dopamine active transporter 1 (DAT1) [30], hypomethylation of solute carrier family 6 transporter member 3 (SLC6A3) [37] and differential methylation of vesicular monoamine transporter 2 (SLC18A2) [27]. The other inconsistent results for genes associated with dopamine were for COMT with studies reporting hypomethylation of membrane-bound (MB-) COMT in SZ and BD [52], hypomethylation of COMT (isoform not specified) in SZ (but not BD) [51], hypermethylation of soluble (S-) COMT in SZ [34], differential methylation of COMT domain containing 1 (COMTD1) promoter in SZ [37] and a lack of differential methylation of S-COMT promoter in SZ [36]. One global DNA methylation study also reported differential methylation of glial cell line-derived neurotrophic factor family receptor alpha 2 (GFRα2) in SZ [28], which indirectly affects dopaminergic neurons.

Serotonin

The reported results for serotonin were varied: hypermethylation of 5-hydroxytryptamine serotonin 1A receptor (5-HT1A) in SZ and BD in two studies [49, 51], a lack of differential methylation of 5-hydroxytryptamine serotonin 2A receptor (HTR2A) in SZ and BD [50], differential methylation of serotonin 2A receptor (SLC6A4) in BD [48], differential methylation of 5-hydroxytryptamine serotonin receptor 1E G protein-coupled (HTR1E) in SZ [37] and a lack of differential methylation of serotonin neurotransmitter transporter (5-HTT) in SZ [34].

Glutamate

Methylation of glutamatergic receptors were reported only in SZ participants; in two studies, there was differential methylation of the glutamate receptor ionotrophic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid 2 (GRIA2) [21] and hypermethylation of glutamate receptor ionotrophic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid 3 (GRIA3) and glutamate metabotropic receptors 2, 5 and 8 (GMR2, GMR5 and GMR8) [29].
Subgroup analyses

Subgroup analyses and/or the study of covariates were reported in 28 out of 33 studies. However, only 20 studies reported significant effects of age, sex, pharmacological (antipsychotic/antidepressant) treatment, symptom severity, and/or smoking/alcohol abuse. Further analyses of ethnicity effects on DNA methylation was absent in all but one study [33]. Other notable findings, which were only reported in one study, include a significant association of gene expression with DNA methylation in SZ [41], and cellular heterogeneity of white blood cells as a major confounder in DNA methylation analyses also in SZ [26].

Discussion

This review highlights findings of moderate quality, showing mixed evidence of hyper- and hypomethylation of several common genetic loci in 22 studies of schizophrenia and/or bipolar disorder, from a total of 33 reviewed studies. Differential methylation converged on

Table 4 Most commonly identified differentially methylated genes and related systems in schizophrenia and bipolar disorder studies

| Loci   | Summary | Genetic loci | Results | Gene expression status | Group          |
|--------|---------|--------------|---------|------------------------|----------------|
| Serotonin | [49]    | 5-HT1A       | a       | -                      | SZ, BD         |
|         |         | HTR1A        | c, a    | Decrease (in SZ)        | SZ, BD         |
|         |         | HTR2A        | d       | Increase (SZ & BD)      | SZ, BD         |
|         |         | SLC6A4       | c       | Decrease (S/S genotype only) | BD             |
|         |         | HTR1E        | c       | -                      | SZ             |
|         |         | S-HTT        |         | -                      | SZ             |
| Glutamate | [21]    | GRIA2        |         | -                      | SZ             |
|         |         | GMR2         | a       | Increase (GRM2, GRM5 & GRIA3 only) | SZ             |
|         |         | GMR5         |         | -                      |                |
|         |         | GMR8         |         | -                      |                |
|         |         | GRIA3        |         | -                      |                |
| BDNF    | [44]    | BDNF exon 1 promoter | a | - | BD-II |
|         |         | BDNF exon 1 promoter | a | Decrease (BD-II only) | BD-II |
|         |         | BDNF promoter I | a | - | SZ |
|         |         | BDNF promoter II | c | - | SZ |
|         |         | BDNF promoter IV | d | - | SZ |
| Dopamine | [30]    | DAT1         | a       | No difference           | SZ             |
|         |         | SLC6A3       | b       | Increase (SZ & BD)      | SZ, BD         |
|         |         | SLC18A2      | c       | -                      | SZ             |
| COMT    | [52]    | MB-COMT      | b       | Increase (SZ & BD)      | SZ, BD         |
|         |         | S-COMT       | a       | -                      | SZ             |
|         |         | COMT         | c, b    | -                      | SZ, BD         |
|         |         | COMTD1 promoter | c | - | SZ |
|         |         | S-COMT promoter | d | - | SZ |
| RELN    | [20]    | RELN intron 1 | a       | -                      | SZ             |
|         |         | RELN promoter | d       | -                      | SZ             |
five candidate genes (RELN, BDNF, COMT, 5-HTT and glutamate receptor genes) which have each been previously implicated in the neuropathology of SZ and/or BD. Differential methylation was also reported in several genes (e.g. Fc fragment of IgA (FCAR), cyclic AMP-responsive element-binding protein 1 (CREB1), lymphocyte transmembrane adaptor 1 (LAX1)) related to immune system function and the inflammatory response in SZ [20, 33], consistent with recent evidence for shared genetic risk (for SZ and BD) in common variants of the major histocompatibility complex [53].

**Genes implicated in schizophrenia and bipolar disorder**

The most commonly reported sites of epigenetic changes were in regions known to regulate the availability of neurotrophins, dopamine and serotonin. For example, BDNF is a neurotrophin involved in neuroplasticity and dopaminergic neuron survival [54], for which peripheral blood levels have been found to be decreased in both SZ and BD patients [55, 56]. However, in BD, there were two studies reporting hypermethylation of the BDNF gene (exon 1 promoter) that was associated with pharmaceutical treatment and mood states [44, 45], while another study reported a lack of differential methylation at this site in BD patients with a history of psychosis [43]. In SZ, there was evidence of differential methylation at several other BDNF sites, including promoter I [25], and an unspecified BDNF promoter [30], with one study also reporting no difference in methylation of BDNF promoter IV [25].

On the COMT gene, there was consistent evidence for hypomethylation of MB-COMT in SZ and BD [52] and an unspecified COMT isof orm in SZ only [51]; other SZ studies reported mixed findings including hypermethylation of S-COMT [34], differential methylation of COMTD1 promoter [37] and a lack of significant differential methylation of S-COMT promoter [36]. The mixed evidence for methylation of dopamine transporter genes in SZ – including hypermethylation of DAT1 [30], hypomethylation of SLC6A3 [37] and differential methylation of SLC18A2 [27], is interesting in the context of previous evidence of genome-wide differential methylation of GFRA2 in SZ [28], a receptor for glial cell-derived neurotrophic factor (GDNF) which manages dopaminergic neuronal maintenance while also being implicated in SZ and BD [57, 58]. Non-specific, differential methylation of serotonin transporter sites were evident in BD (SLC6A4) [48] and SZ (HTR1E) [37], while hypermethylation of 5-HTR1A was reported in two SZ and BD studies [49, 51]. These results converge with the numerous reports of variation in serotonin transporter gene (5-HTT or SLC6A4) interacting with stressful life events to result in psychiatric (usually mood) disorder [59, 60]. However, there was also evidence for lack of differential methylation of 5-HTT in SZ [34], and HTR2A in SZ and BD [50].

Finally, a number of glutamate receptor genes (GRIA2, GMR2, GMR5, GMR8 and GRIA3) were found to be hypermethylated in SZ [21, 29] while in BD there was no such evidence. This is intriguing given that recent genome-wide association studies (GWAS) have implicated genes associated with glutamate neurotransmitter dysfunction as relevant to risk for both disorders [61, 62]. In SZ, there was also a finding of hypermethylation of DLG4 [33], a gene which has downstream regulatory effects on glutamate receptors implicated in SZ pathophysiology [63]. The few studies of methylation in the promoter region of RELN in SZ should be mentioned as consistent with post-mortem evidence [64], while there were some other notable findings for hypermethylation of DISCI [32], differential methylation of HLA-C and CALHM1 [41], and hypermethylation of GABRB2 [39] which have each been identified as risk variants for SZ in previous work [65–67].

**Associations with demographic and clinical variables**

Only 20 of 33 studies examined the effects of age, sex, medication, symptom severity, and/or smoking/alcohol abuse on methylation patterns, with mixed findings. However, there were consistent trends emerging for no significant associations between methylation status of various genes and age [23, 25, 34, 38, 45, 47, 49] (particularly in SZ studies [23, 25, 34, 38]), while a handful of other studies suggest that differential methylation increases with age [50–52]. There was also a trend for hypermethylation being more prevalent in females (see Tables 1, 2 and 3). Previous studies have reported altered DNA methylation in SZ and BD following treatment with antipsychotics and mood stabilisers such as haloperidol [34], clozapine [68], lithium and valproate [44], but these variables were inconsistently reported in the studies reviewed here.

**Limitations**

There are a number of limitations to this review. The most obvious was the inability to conduct a meta-analysis owing to the diversity of experimental protocols (there were 16 different methods reported across 33 studies). Methodological variability also precludes interpretation of results for the most commonly reported genes across these studies. In addition, the lack of consistency in reporting the potential effects of clinical symptoms, age, sex, medication, and ethnicity, precluded adequate interpretation of findings across studies. For example, factors such as diet [69], exercise [70], smoking [71], trauma [72], emotional state [73] and ethnicity [74] are known to effect DNA methylation status, but were not adequately reported in many studies. Variability in
DNA extraction methods and blood cell composition may have also affected the results of included studies [35], for which details are not included in this review. For example, the cellular heterogeneity of white blood cells has been considered to confound DNA methylation analyses [26], despite associations between gene expression and DNA methylation in whole blood samples suggesting that differences are minimal. However, methods for conducting methylation analyses are known to vary in efficacy and sensitivity, and may have affected the pattern of results revealed here. For example, bisulfite sequencing (conducted in eight of 33 included studies), is prone to PCR amplification bias [75], with at least some incomplete conversions of cytosine to uracil resulting in a higher number of methylated CpGs being recorded [76]. Moreover, methods for determining the appropriate significance threshold to determine differential methylation status was dependent on the experimental protocol employed in each study, such that the strength of the results reported in these studies may be equivocal. Another potential bias lies in the sample size differences between studies of candidate gene and global DNA methylation studies, of which the latter require larger sample sizes with respect to multiple testing issues. In addition, the results of global DNA methylation studies are simply not comparable with approaches such as MBD protein-enriched genome sequencing which is more sensitive than 27 k/450 k arrays [21].

Finally, this study did not directly compare the methylation status of particular genes arising from studies of post-mortem versus peripheral tissue, but included studies using DNA derived from blood or saliva (only two studies used saliva). While methylation patterns in saliva cells may be affected by oral hygiene, we note that similar patterns of methylation were reported in saliva and post-mortem tissue in both these studies [50, 52]. There are certain advantages and limitations to using both post-mortem and peripheral tissues for DNA methylation studies. While the brain is the primary organ of pathology in SZ and BD, methylation analyses using post-mortem tissue may be affected by pH, post-mortem interval and variability of different neuronal cell types and brain regions [77]. On the other hand, peripheral tissue is easily accessible in a minimally invasive and of low cost procedure, thus allowing for collection of larger sample sizes to overcome cellular heterogeneity of methylation patterns and facilitating longitudinal studies. Consistent methylation results across brain and blood tissues have been reported for particular promoter CpG islands in other studies not included in this review [17]. Notably, two of the included studies showed comparable methylation results for major histocompatibility complex 9 (HCG9) [46] and synapsin 3 (SYNIII) [35] in both post-mortem brain tissue as well as a blood-derived DNA.

Conclusions

Moderate quality evidence shows differential DNA methylation in peripheral tissue of SZ and BD participants, with some common genes affected despite the direction of methylation at common sites not always being consistent. While it remains questionable as to whether the differences in statistical thresholds between genome-wide to candidate gene loci studies are too great to compare results from such studies, we could not systematically address the effects of these methodological difference on general patterns of findings across disorders because of the limited data available for some methods in some groups. We also note that the role of DNA methylation in modifying gene expression has only been explored in the last 20 years [78], and it is likely that the inconsistency of the results reported here reflects the numerous methods available for determining DNA methylation alterations in peripheral tissues of these disorders, which can vary according to cell type. Reliable patterns in methylation alterations specific to SZ or BD are likely to emerge with improved, cost-effective and standardized technology that also account for cellular heterogeneity. Apart from methodological issues, another potential explanation for variability among findings is that none of the studies of psychotic samples to date have addressed the impact of early life experiences (such as childhood trauma) on differential methylation patterns in SZ and BD; more consistent methylation patterns may be revealed in the context of common lifetime environmental exposures (e.g., childhood maltreatment, birth complications, cannabis use), genomic structure, and/or mRNA expression profiles. The consideration of these additional factors will be necessary in future research to clarify the contribution of environmental effects on epigenetic processes in the development of psychosis.

Abbreviations

SZ: Schizophrenia; BD: Bipolar disorder; HC: Healthy control; STROBE: Strengthening the reporting of observational studies in epidemiology; PRISMA: Preferred reporting items for systematic reviews and meta-analyses; MWAS: Methyome-wide association study; GWAS: Genome-wide association study; DNA: Deoxyribonucleic acid; mRNA: Messenger ribonucleic acid; PCR: Polymerase chain reaction; MBD: Methyl-CpG binding domain; MSRE: Methylation sensitive restriction enzyme; HRM: High-resolution melt; HPLC: High-performance liquid chromatography; MB-: Membrane-bound; S-: Soluble; GNDF: Gial cell-derived neurotrophic factor.

Genes mentioned in text

RELN, Relnlin; BDNF: Brain-derived neurotrophic factor; COMT: Catechol-O-methyltransferase; COMT-D1: Catechol-O-methyltransferase domain containing 1; FKBP5: FK506 binding protein 5; HTR1A 5-HT1A: 5-hydroxytryptamine serotonin 1A receptor; HTR2A: 5-hydroxytryptamine serotonin 2A receptor; HTR1E: 5-hydroxytryptamine serotonin receptor 1E; S-HTT: Serotonin neurotransmitter transporter; GABBR2: Gamma-aminobutyric acid receptor beta-2;
DLG4: Discs large homolog 4; DISC1: Disrupted in schizophrenia 1; HLA-C: Major histocompatibility complex class C; CALHM1: Calcium homeostasis modulator 1; DAT1: Dopamine active transporter 1; SLC6A3: Solute carrier family 6 transporter member 3; SLC6A4: Solute carrier family 6 transporter member 4; SLC18A2: Vesicular monoamine transporter 2; GPRRA2: Glial cell line-derived neurotrophic factor family receptor alpha 2; GRIA3: Glutamate receptor ionotropic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid 2; GRIA3: Glutamate receptor ionotropic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid 3; GMR2: GMR5 and GMRF, glutamate metabotropic receptors 2, 5 and 8 respectively; FCTR: Fc fragment of IgA; CREB1: Cyclic AMP-responsive element-binding protein 1; LAX1: Lymphocyte transmembrane adaptor 1; HCG9: Major histocompatibility complex 9; SYNLII: Synaptin 3.

Competing interests

The authors declare they have no competing interest.

Authors’ contributions

Authors MG and NT conceived of the study; NT conducted the literature search, undertook the first review of the literature, the extraction and quality assessment of data, and prepared the first draft of the manuscript. Author LG independently reviewed the literature identified by NT and conducted quality assessment of the data. Author CMS contributed to the background and preparation of the manuscript. Author MJG contributed to decisions about study inclusion, data quality and interpretation of results, and oversaw the preparation of the manuscript. All authors contributed and approved the final manuscript.

Acknowledgements

This research was supported by two NHMRC Project Grants (#630471; #1081603) held by MG, and a grant from the Netherlands Institute of Advanced Studies (NIAS, Royal Dutch Academy for Arts and Sciences). Salary support for MG was provided by a NHMRC Career Development Fellowship (#1001870). CMS is an Australian Research Council Future Fellow (#FT120100097) and NT was (NIAS, Royal Dutch Academy for Arts and Sciences). Salary support for MG was held by MJG, and a grant from the Netherlands Institute of Advanced Studies (NIAS, Royal Dutch Academy for Arts and Sciences). Salary support for MG was awarded to support the preparation of the manuscript. All authors contributed and approved the final manuscript.

Received: 29 September 2015 Accepted: 13 January 2016

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