BMC Genetics

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

--Manuscript Draft--

Manuscript Number:	GTIC-D-15-00246
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DNA methylation in peripheral tissue of schizophrenia and bipolar disorder: a systematic review

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Word Counts: Abstract = 272; Text body = 3288

Tables= 4; Figures= 1

Abstract

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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.

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Conclusions: Moderate quality of available evidence shows some convergence of differential methylation at some common genetic loci in SZ and BD, 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 cytosines within CpG dinucleotides. These CpGs are clustered in 'CpG islands' that tend to be located in regulatory elements of the gene, such as promoters or enhancers [12]. Methylation at CpG islands usually results in transcriptional silence of the associated gene [12]. In recent studies of psychiatric phenomena, the functional impact of stress-related hypomethylation of genetic loci known to regulate stress responses (e.g., FKBP5) suggest that this process may be relevant to many stress-related disorders [13].

With the increasing use of peripheral tissue in studies of methylation in psychotic disorders, the aim of this study was to perform a systematic review of 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/, schizophreni$.tw, schizo$.tw, exp bipolar disorder/, bipolar disorder.tw, exp psychosis/, psychosis.tw, dna methylat$.tw, demethylat$.tw, hypomethylat$.tw and hypermethylat$.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 PCR based DNA methylation methods) [16].

Study selection

A flow-chart of the search and selection processes of the included studies is presented in Figure 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 [17], to assess the risk of bias within studies and across studies, methodological consistency and precision, as well as reporting transparency and comprehensibility. 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. Study characteristics are summarised in Tables 1-3 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 Figure 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 Figure 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.

*****Insert Figure 1 here*****

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 [18-39] (see Table 1), seven studies that compared BD to HC [40-46] (with three studies also comparing BD-I to BD-II; see Table 2), and four studies that compared HC to both SZ and BD [47-50] (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 1-3 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=185.7; range=1-750; see Tables 1-3) 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 1-3). The mean percentage of females per sample was 43.7% for SZ, 50.8% for BD and 42.4% for HC.

*****Insert Tables 1-3 here*****

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) [22, 34, 33, 36, 37, 48, 50, 32], while the most commonly reported genome-wide methods used were methyl-CpG-binding domain (MBD) protein-enriched genome sequencing (n=3) [18, 19, 30], 450K arrays (n=3) [24-26], and 27K arrays (n=3) [31, 35, 39]. Other methods for the study of candidate genetic loci were pyrosequencing (n=4) [23, 44-46], methylation specific polymerase chain reaction (PCR; n=3) [27-29], fluorescence-based real-time PCR (n=2) [42, 43], quantitative methylation specific PCR (n=2) [48, 50], methylation sensitive restriction enzyme (MSRE) quantitative PCR (n=1) [20], MethyLight protocol (n=1) [41] and high-resolution melt (HRM) method (n=1) [47]. Other methods used to measure genome-wide DNA methylation were radiolabelled [3H] cytosine-extension assay (n=2) [40, 21]; modified non-radioactive elongation assay (n=1) [20], luminometric methylation assay (n=1) [32], high-performance liquid chromatography (HPLC; n=1) [38] and methylated DNA immunoprecipitation (n=1) [31]. These inconsistencies in the way that methylation was quantified precluded meta-analysis.

Methylation analyses and genes investigated in SZ and BD

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 [21, 40], while one study found genome-wide hypomethylation in SZ [38]. 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 serotonin 1A receptor *(HTR1A)* (2 SZ/BD studies).

*****Insert Table 4 here*****

Evidence for DNA methylation in SZ and BD

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 (i.e., non-specified direction of difference). 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). For these genes, there was evidence of both hyper- and hypo- methylation 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*) [37]; hypermethylation of discs large homolog

4 (*DLG4*) and disrupted in schizophrenia 1 (*DISC1)* [30]; differential methylation of major histocompatibility complex class C (*HLA-C)* and calcium homeostatis modulator 1 *(CALHM1)* [39]. 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 1-3).

RELN

Differential methylation for *RELN* was reported for intron 1 in SZ [18], although another study also reported a lack of differential methylation of the *RELN* promoter in SZ [20].

BDNF

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 [42, 43], although one other study of BD (unspecified-type) reported lack of differential methylation at this site [41]. In SZ, the results were mixed with hypermethylation of *BDNF* promoter I [23], differential methylation of an unspecified *BDNF* promoter [28] and a lack of differential methylation of *BDNF* promoter IV [23].

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*) [28], hypomethylation of solute carrier family 6 transporter member 3 (*SLC6A3*) [35] and differential methylation of vesicular monoamine transporter 2 (*SLC18A2*) [25]. 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 [50],

hypomethylation of *COMT* (isoform not specified) in SZ (but not BD) [49], hypermethylation of *S-COMT* in SZ [32], differential methylation of *COMT* domain containing 1 (*COMTD1*) promoter in SZ [35] and a lack of differential methylation of *S-COMT* promoter in SZ [34]. One global DNA methylation study also reported differential methylation of glial cell line-derived neurotrophic factor family receptor alpha 2 *(GFRA2)* in SZ [26], which indirectly affects dopaminergic neurons.

Serotonin

The reported results for serotonin were varied: hypermethylation of *5-HTR1A* in SZ and BD in two studies [47, 49], a lack of differential methylation of *HTR2A* in SZ and BD [48], differential methylation of serotonin 2A receptor (*SLC6A4*) in BD [46], differential methylation of 5-hydroxytryptamine serotonin receptor 1E G protein-coupled (*HTR1E*) in SZ [35] and a lack of differential methylation of *5-HTT* in SZ [32].

Glutamate

Methylation of glutamatergic receptors were reported only in SZ participants; in two studies, there was differential methylation of the glutamate receptor ionotropic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid 2 (*GRIA2*) [19] and hypermethylation of glutamate receptor ionotrophic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid 3 (*GRIA3*) and glutamate metabotrophic receptors 2, 5 and 8 (*GMR2, GMR5* and *GMR8*) [27].

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 [31]. Other notable findings, which were only reported in one study, include a significant association of gene expression with DNA methylation in SZ [39], and cellular heterogeneity of white blood cells as a major confounder in DNA methylation analyses also in SZ [24].

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 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. *FCAR, CREB1, CD224, LAX1*) related to immune system function and the inflammatory response in SZ [18, 31], consistent with recent evidence for shared genetic risk (for SZ and BD) in common variants of the major histocompatibility complex [51].

Genes implicated in SZ and BD

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 [52], for which peripheral blood levels have been found to be decreased in both SZ and BD patients [53, 54]. 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 [42, 43], while another study reported a lack of differential methylation at this site in BD patients with

a history of psychosis [41]. In SZ, there was evidence of differential methylation at several other *BDNF* sites, including promoter I [23], and an unspecified *BDNF* promoter [28], with one study reporting no difference in methylation of *BDNF* promoter IV [23].

On the *COMT* gene, there was consistent evidence for hypomethylation of *MB-COMT* in SZ and BD [50] and an unspecified *COMT* isoform in SZ only [49]; other SZ studies reported mixed findings including hypermethylation of *S-COMT* [32], differential methylation of *COMTD1* promoter [35] and a lack of significant differential methylation of *S-COMT* promoter [34]. The mixed evidence for methylation of dopamine transporter genes in SZ – including hypermethylation of *DAT1* [28], hypomethylation of *SCL6A3* [35] and differential methylation of *SLC18A2* [25], is interesting in the context of previous evidence of genome-wide differential methylation of *GFRA2* in SZ [26], a receptor for glial cell-derived neurotrophic factor (GDNF) which manages dopaminergic neuronal maintenance while also being implicated in SZ and BD [55, 56]. Non-specific, differential methylation of serotonin transporter sites were evident in BD (*SLC6A4*) [46], and SZ (*HTR1E*) [35], while hypermethylation of *5-HTR1A* was reported in two SZ and BD studies [47, 49]. 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 [57, 58]. However, there was also evidence for lack of differential methylation of *5-HTT* in SZ [32], and *HTR2A* in SZ and BD [48].

Finally, a number of glutamate receptor genes (*GRIA2, GMR2, GMR5, GMR8* and *GRIA3*) were found to be hypermethylated in SZ [19, 27] while in BD there was no such evidence. This is intriguing given that recent GWAS studies have implicated genes associated with glutamate neurotransmitter dysfunction as relevant to risk for both disorders [59, 60]. In SZ,

there was also a finding of hypermethylation of *DLG4* (Liao et al., 2014), a gene which has downstream regulatory effects on glumate receptors implicated in SZ pathophysiology [61]. The few studies of methylation in the promoter region of *RELN* in SZ should be mentioned as consistent with post-mortem evidence [62], while there were some other notable findings for hypermethylation of *DISC1* [30], differential methylation of *HLA-C* and *CALHM1* [39], and hypermethylation of *GABRB2* [37] which have each been identified as risk variants for SZ in previous work [63-65].

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 [21, 23, 32, 36, 43, 45, 47] (particularly in SZ studies [21, 23, 32, 36]), while a handful of other studies suggest that differential methylation increases with age [33, 50], [49] [48] [69]. There was also a trend for hypermethylation being more prevalent in females (see Tables 1-3). Previous studies have reported altered DNA methylation in SZ and BD following treatment with antipsychotics and mood stabilisers such as haloperidol [32], clozapine [66], lithium and valproate [42], 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. In addition, the lack of consistency in reporting the potential effects of clinical symptoms, age, sex, medication, and ethnicity, precluded adequate interpretation of inconsistencies in findings

across studies. Factors such as diet [67], exercise [68], smoking [69], trauma [70], emotional state [71] and ethnicity [72] 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 [33], 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 [24], despite associations between gene expression and DNA methylation in whole blood samples suggesting that differences are minimal.

However, the 16 different methods across the 33 reviewed studies are known to vary in efficacy 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 [73], with at least some incomplete conversions of cytosine to uracil resulting in a higher number of methylated CpGs being recorded [74]. 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 27k/450k arrays [19].

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). 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 [48, 50]. Consistent methylation results

across brain and blood tissues have been reported for particular promoter CpG islands in other studies not included in this review [75]. Notably, two of the included studies showed comparable methylation results for *HCG9* [44] and *SYNIII* [33] 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. Interpretation of this data thus remains cautious. Future research would benefit from addressing the impact of early life experiences (such as childhood trauma) on differential methylation in SZ and BD, in the context of other lifetime environmental exposures (e.g., birth complications, cannabis use), genome structure, and mRNA expression profiles to clarify the effects of environment on epigenetic processes in the development of psychosis.

List of abbreviations used

SZ, schizophrenia; BD, bipolar disorder; HC, healthy control; STROBE, strengthening the reporting of observational studies in epidemiology; MWAS, methylome-wide association study. For all gene name abbreviations please refer to text.

Competing interests

The authors declare they have no competing interest.

Authors' contributions

Author NT conducted the literature search, review of the literature, extraction and quality assessment of data, interpretation of results and preparation of 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, cross-checked data extracted and oversaw the preparation of the manuscript. All authors contributed and approved the final manuscript.

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Acknowledgements

This research was supported by two NHMRC Project Grants (#630471; #1081603) 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 provided by a NHMRC Career Development Fellowship (#1061875), CMS is an Australian Research Council Future Fellow (#FT120100097) and NT was supported by an Australian Postgraduate Award (APA).

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Table 1. Summary of studies reporting DNA methylation in schizophrenia

Ref.	N		Mean age (SD)		Sex (F %)		Method	Subgroup analyses /covariates	RESULTS: methylation loci
	SZ	HC	SZ	HC	SZ	HC			
Aberg *et al.*, 2014 [18]	759	738	53 (12)	55 (12)	45	46	MBD protein-enriched genome sequencing *, %	Age, sex, smoking Ø, alcohol use Ø, medication Ø, autoimmune disorders Ø	- ■ **FAM63B** ~, **RELN** (first intron) ~, **FCAR** ~ and **8 other genes** ~ linked to hypoxia & the immune system - FAM63B top finding
Aberg *et al.*, 2012 [19]	750	750	-	-	-	-	MBD protein-enriched genome sequencing *, ǂ	Age, sex	- ■ (68 million reads) ~ : **GRIA2, HTRA3, CAMK2D, FNDC3B** and **DCTN** - GRIA2 top finding
Bonsch *et al.*, 2012 [20]	27	34	-	-	30	47	Modified non-radioactive elongation assay and MSRE-quantitative PCR *, %	Sex ∨, medication ∧, promoter methylation compared to global DNA methylation	- •, ~ : Lower methylation for SZ compared to HC (mainly due to male population) - **RELN** (promoter) Ø between groups; lower methylation for SZ females compared to males - **SOX10** (promoter) Ø - Lower methylation for SZ not on medication (SZ on medication had similar methylation levels to HC)
Bromberg *et al.*, 2008 [21]	28	26	39 (14)	42 (10)	64	62	Radiolabelled [3H] cytosine-extension assay *, %	Age Ø, sex, smoking ~, illness duration Ø, medication Ø	- •, Ø - Higher methylation in SZ non-smokers
Chen *et al.*, 2012 [22]	371	288	-	-	46	57	Bisulfite sequencing *, %	Sex ∧	- **MAOA** (promoter) Ø - Higher methylation in SZ females compared to males
Ikegame *et al.*, 2013 [23]	100	100	43 (13)	46 (12)	46	45	Pyrosequencing *, %	Age Ø, sex ∧	- **BDNF** (promoter I) ∧ for SZ CpG-72 compared HC, however methylation generally low in SZ/HC - **BDNF** (promoter IV) Ø between groups, higher methylation in SZ/HC females at all CpG sites
Kinoshita *et al.*, 2014 [24]	63	42	49 (10)	47 (10)	22	40	450K methylation array *, β	Age, sex, cell type heterogeneity ~	- • (485 764 CpG sites) ~ - Hypermethylation found in 1161 CpG sites (with 63% in promoter regions) when accounting for cellular heterogeniety - Cell type heterogeneity found to be a major confounder in DNA methylation analyses
Kinoshita *et al.*, 2013a [25]	42	42	52 (7)	52 (6)	0	0	450K methylation array *, %	Age, medication	- • (164 657 CpG sites including promoters, gene bodies and 3'-UTRs) ~ : **SLC18A2, GNAL, KCNH2** and **NTNG2** - Top findings were SLC18A2 and GNAL

Kinoshita *et al.*, 2013b [26]	24	23	31 (11)	31 (10)	54	57	450K methylation array *, %	Sex ~	- • (485 764 CpG sites) ~ : **B3GAT2, HDAC4, DGKI, PCM1, INSIG2, GFRA2** and **RAI1** - Differential methylation found in 94 CpGs, most sites located in promoter regions - Could not replicate published methylation findings in SZ for COMT, HTA1A and MAOA
Kordi-Tamandani *et al.*, 2013a [27]	81	71	48 (11)	47 (12)	25	20	Methylation specific PCR *, ε	-	- **GMR2** ∧**, GMR5** ∧**, GRIA3** ∧**, GMR8** ∧ (all promoter regions) - Methylation of GRIA3 increased the risk of SZ - Methylation of GRM2 and GRM5 decreased the risk of SZ
Kordi-Tamandani *et al.*, 2012 [28]	80	71	48 (11)	47 (12)	-	-	Methylation specific PCR *, ε	-	- **BDNF** (promoter) ~ increased the risk of SZ - **DAT1** ∧
Kordi-Tamandani *et al.*, 2013b [29]	94	99	48 (11)	47 (12)	29	27	Methylation specific PCR *, ε	Genotype ø	- **CTLA4** (promoter) ∧ increased the risk of SZ - CT genotype had a protective effect on the risk of SZ when compared to CC genotype
Liao *et al.*, 2014 [30]	2	1	25 (4)	31 (0)	100	100	MBD protein-enriched genome sequencing *, ×	Paranoid ∧/undifferentiated ~	- • ~ : **GRB2** ∧**, PRKCA** ∧**, DLG4** ∧**, MAPT-S1** ∧**, DISC1** ∧ and **16 other genes** mentioned - Differential methylation mostly found in intergenic and intronic regions
Liu *et al.*, 2013 [31]	98	108	34 (11)	-	25	36	27K methylation assay *, β	Age, sex, ethnicity, alcohol/nicotine/cannabis use, SZ symptoms ~, medication ∧, illness duration ∧, age of onset ~	- • (7562 CpG sites) ~ : **MS4A1** ∧**, MPG** ∨**, SLC25A10** ∧**, CBFA2T3** ∨ and **17 other genes ~** linked to inflammatory response, haematological development and cytotoxic reactions - Hypermethylation of MS4A1 associated with chlorpromazine dosage - Higher methylation of MPG and SLC25A10 associated with longer illness duration - Hypomethylation of CBFA2T3 associated with age of SZ onset
Melas *et al.*, 2012 [32]	177	171	52 (9)	-	51	-	Luminometric methylation assay and bisulfite sequencing *, %	Age ø, sex ø, smoking ø, alcohol use ø, medication ~, hospital admissions ø, length of hospital stay ø, familial absence of SZ ø, age of onset ∨	- •, ~ : **5-HTT** (8 CpG sites) ø**, S-COMT** (5 CpG sites) ∧ - Higher global methylation in SZ associated with haloperidol treatment compared to other antipsychotics
Murphy *et al.*, 2008 [33]	18	31	-	-	-	-	Bisulfite sequencing *, %	-	- **SYNIII** ø
Murphy *et al.*, 2005 [34]	20	31	-	-	-	-	Bisulfite sequencing *, ǂ	-	- **S-COMT** (promoter) ø

Nishioka *et al.*, 2013 [35]	17	15	23 (5)	23 (4)	59	33	27K methylation assay *, β	Sex ø, SZ symptoms, GAF score, duration of untreated psychosis, age at onset	- • (603 CpG sites), ~ : **COMTD1** (promoter) ~, **SLC6A3** ∨**, HTR1E** ~ and **7 other genes ~** related to the nuclear lumen, transcription factor binding and nucleotide binding
Ota *et al.*, 2014 [36]	51	51	25 (8)	26 (8)	37	37	Bisulfite sequencing *, ǂ	Age ø, sex ∧	- **GCH1** ∧ (CpG13, CpG15, CpG16 and CpG21 only) in first episode psychosis - Only CpG21 ∧ associated with SZ males
Pun *et al.*, 2011 [37]	30	30	-	-	50	37	Bisulfite sequencing *, %	Single-nucleotide polymorphism	- **GABRB2** (CpG sites 1-26) ∧
Shimabukuro *et al.*, 2007 [38]	210	237	-	-	41	54	HPLC *,β	Age ~, sex ∨, subtypes of SZ ø	- •, ∨ - Lower methylation for SZ males with decreasing age
Van Eijk *et al.*, 2014 [39]	264	252	-	-	-	-	27K methylation assay *, β	Age, sex, gene expression associations ~	- • (11 320 CpG sites), ~ : **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; HTRA3, HtrA serine peptidase 3; CAMK2D, calcium/calmodulin-dependent protein kinase 2 delta; FNDC3B, fibronection type 3 domain containing 3B; DCTN, dynactin; SOX10, sex determining region Y box 10; MAOA, monoamine oxidase A; BDNF, brain-derived neurotrophic factor; SLC18A2, vesicular monoamine transporter 2; GNAL, guanine nucleotide binding protein G alpha activating polypeptide olfactory type; KCNH2, potassium voltage-gated channel subfamily H member 2; NTNG2, netrin G2; B3GAT2, beta-1 3-glucuronyltransferase 2; HDAC4, histone deacetylase 4; DGKI, diacylglycerol kinase iota; PCM1, pericentriolar material 1; INSIG2, insulin induced gene 2; GFRA2, glial cell line-derived neurotrophic factor family receptor alpha 2; RAI1; retinoic acid induced 1; GMR2, glutamate metabotrophic receptor 2; GMR5, glutamate metabotrophic receptor 5; GRIA3, glutamate receptor ionotrophic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid 3; GMR8, glutamate metabotrophic receptor 8; DAT1, dopamine active transporter 1; CTLA4, cytotoxic T-lymphocyte-associated protein 4; GRB2, growth factor receptor-bound protein 2; PRKCA, protein kinase C alpha; DLG4, discks large homolog 4; MAPT-S1, microtubule-associated protein tau; DISC1, disrupted in schizophrenia 1 protein; GCH1, GTP cyclohydrolase 1; PRRT1, proline-rich transmembrane protein 1; HLA-C, human leukocyte antigen receptor C; MRPL41, mitochondrial ribosomal protein L41; MS4A1, membrane-spanning 4-domains subfamily A member 1; MPG, N-methylpurine-DNA glycosylase; SLC25A10, solute carrier family 25 member 10; CBFA2T3, core-binding factor alpha subunit 2 translocated to 3; 5-HTT, serotonin 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, 5-hydroxytryptamine serotonin receptor 1E G protein-coupled; GABRB2, gamma-aminobutyric acid A receptor beta 2. Study type: • global DNA methylation; ■ methylome-wide association study; tissue type: * blood; measure of methylation: % percentage; β beta-value; ξ odds ratio; × peak score; ǂ no reported; results (relative to SZ): ∧ significant hypermethylation; ∨ significant hypomethylation; ~ differentially methylated; ø no significant difference.

Table 2. Summary of studies reporting DNA methylation in bipolar disorder

Ref.	N		Mean age (SD)		Sex (F %)		Method	Subgroup analyses /covariates	RESULTS: methylation loci
	BD	HC	BD	HC	BD	HC			
Bromberg *et al.*, 2009 [40]	49	27	39 (13)	42 (10)	41	37	Radiolabeled [3H] cytosine-extension assay *, %	Medication (valproate) Ø, sex Ø, smoking Ø, duration of illness Ø, family history of BD Ø	- •, Ø
Carlberg *et al.*, 2014 [41]	60	278	42 (15)	32 (4)	45	62	MethyLight *, %	Age ~, gender Ø, clinical variables, genotype Ø	- **BDNF** (exon I promoter) Ø in BD compared to HC
D'Addario *et al.*, 2012 [42]	94	52	52 (12)	-	60	-	Fluorescence-based real-time PCR *, %	Medication ∧, BD-I compared to BD-II Ø, mood state Ø	- **BDNF** (exon I promoter) ∧ only in BD-II - Higher methylation for treatment with mood stabiliser and antidepressants, but lower for lithium and valproate
Dell'Osso *et al.*, 2014 [43]	111	44	-	-	-	-	Fluorescence-based real-time PCR *, %	Age Ø, sex Ø, mood state ∧, medication, BD-I compared to BD-II	- **BDNF** (exon I promoter) ∧ in BD-I/BD-II; BD-II higher levels than BD-I - Higher methylation in depressed compared to manic/mixed states - Higher methylation in BD-II males with increasing age
Kaminsky *et al.*, 2012 [44]	370	382	43 (11)	42 (6)	58	55	Pyrosequencing *, %	Age ∧, sex, genotype ∨, medication (mood stabiliser) ∧	- **HCG9** (first exon extending into the first intron) ∨ in BD (when controlling for age and genotype) - Higher methylation in BD/HC with increasing age - Lower methylation in GG allele compared to GA allele carriers - Higher mood stabiliser dose increases methylation towards HC levels
Kuratomi *et al.*, 2008 [45]	23	18	57 (11)	46 (12)	52	33	Pyrosequencing *, %	Age Ø, sex ∧, medication (valproate) Ø, BD-I compared to BD-II ∨	- **SMS** (5'region) ~, higher methylation for females in BD-I/II group compared to HC - **PPIEL** (promoter and 5'region) ∨ for BD-II compared to BD-I - **PIP5KL1** Ø, **ARMC3** Ø
Sugawara *et al.*, 2011 [46]	20	20	39 (13)	39 (9)	60	20	Pyrosequencing *, %	-	- **SLC6A4** (promoter) ~, higher methylation in CpG 3 and 4 for BD compared to HC

BD, bipolar disorder; HC, healthy control; N, sample number; F, female; SD, standard deviation; PCR; polymerase chain reaction. Genes: BDNF, brain-derived neurotrophic factor; HCG9, human leukocyte antigen complex group 9; SMS, spermine synthase; PPIEL, peptidylprolyl isomerase E-like; PIP5KL1, phosphatidylinositol-4-phosphate 5-kinase-like 1; ARMC3, armadillo repeat containing 3; SLC6A4, serotonin transporter solute carrier family 6 member 4. Study type: • global DNA methylation; tissue type: * blood; measure of methylation: % percentage; results (relative to BD): ∧ significant hypermethylation; ∨ significant hypomethylation; ~ differentially methylated; Ø no significant differences.

Table 3. Summary of studies reporting DNA methylation in bipolar disorder and schizophrenia in the same study

Ref.	N			Mean age (SD)			Sex (F %)			Method	Subgroup analyses /covariates	RESULTS: methylation loci
	SZ	BD	HC	SZ	BD	HC	SZ	BD	HC			
Carrard *et al.*, 2011 [47]	40	58	67	32 (8)	42 (10)	42 (12)	40	57	27	HRM assay *, %	Age Ø, sex Ø, symptoms Ø	- **5-HTR1A** ∧ for SZ/BD compared to HC and for SZ compared to BD
Gradirivasf *et al.*, 2011 [48]	24	24	24	-	-	-	-	-	-	Bisulfite sequencing and qMSP +, %	Age ∨, sex Ø, genotype ∨, medication (antipsychotics) ∨, marital status Ø, smoking Ø, alcohol abuse Ø, education Ø	- **HTR2A** (promoter) Ø for most CpG sites except for -1438A/G, -1420 and -1223 polymorphic sites - ∨ of T120C site in SZ/BD, CC allele carriers in SZ/BD and TC allele carriers in SZ - Lower methylation in SZ CC allele carriers with increasing age
Li *et al.*, 2014 [49]	6	3	1	24 (7)	47 (11)	-	67	33	-	Methylated DNA immunoprecipitation *, ×	Age ~, sex ~	- ■ **ADRB1** ∧**, HTR1A** ∧**, NPAS1** ∧ and **COMT** ∨ in SZ; **HNRNPA1 and 56 other genes** in SZ/BD - **11 genes** were differentially methylated in SZ and BD
Nohesara *et al.*, 2011 [50]	20	20	25	-	-	-	-	-	-	Bisulfite sequencing and qMSP +, %	Age ∧, sex, marital status Ø**,** genotype Ø	- **MB-COMT** (promoter) ∨ - Higher methylation in SZ with increasing age, especially for over 40 years of age

SZ, schizophrenia; BD, bipolar disorder; HC, healthy control; N, sample number; F, female; SD, standard deviation; HRM, high resolution melt; qMSP, quantitative methylation specific polymerase chain reaction. Genes: 5-HTR1A, serotonin 1A receptor; HTRA2A, serotonin 2A receptor; ADRB1, adrenoreceptor beta 1; NPAS1, neuronal PAS domain-containing protein 1; HNRNPA1, heterogenous nuclear ribonucleoprotein A1; MB-COMT, membrane-bound catechol-O-methyltransferase.

Study type: ■ methylome-wide association study; Tissue type: * blood; + saliva; measure of methylation: % percentage, × peak score; results (relative to SZ and BD): ∧ significant hypermethylation; ∨ significant hypomethylation; ~ differentially methylated; Ø no significant differences.

Table 4. Most commonly identified genes in schizophrenia and bipolar methylation analyses

	Gene loci	Methylation status				Group
		Candidate loci			Global DNA	
Serotonin	5-HTR1A	↑				SZ, BD
	HTR1A	↑		■		SZ, BD
	HTR2A		Θ			SZ, BD
	SLC6A4			■		BD
	HTR1E			■		SZ
	5-HTT				Θ	SZ
Glutamate	GRIA2			■		SZ
	GMR2 GMR5 GMR8 GRIA3	↑				SZ
BDNF	BDNF exon 1 promoter	↑				BD-I and BD-II
	BDNF exon 1 promoter		Θ			BD
	BDNF promoter I	↑				SZ
	BDNF promoter			■		SZ
	BDNF promoter IV		Θ			SZ
Dopamine	DAT1	↑				SZ
	SLC6A3		↓			SZ
	SLC18A2			■		SZ
COMT	MB-COMT		↓			SZ, BD
	S-COMT	↑				SZ
	COMT		↓	■		SZ, BD
	COMTD1 promoter			■		SZ
	S-COMT promoter		Θ			SZ
RELN	RELN intron 1			■		SZ
	RELN promoter		Θ			SZ

SZ, schizophrenia; BD, bipolar disorder; 5-HTR1A, serotonin 1A receptor; HTRA2A, serotonin 2A receptor; SLC6A4, serotonin transporter solute carrier family 6 member 4; HTR1E, 5-hydroxytryptamine serotonin receptor 1E G protein-coupled; 5-HTT, serotonin transporter; GRIA2, glutamate receptor ionotropic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid 2; GMR2, glutamate metabotrophic receptor 2; GMR5, glutamate metabotrophic receptor 5; GMR8, glutamate metabotrophic receptor 8; GRIA3, glutamate receptor ionotrophic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid 3; BDNF, brain-derived neurotrophic factor; DAT1, dopamine active transporter 1; SLC6A3, solute carrier family 6 transporter member 3; SLC18A2, vesicular monoamine transporter 2; MB-COMT, membrane-bound catechol-O-methyltransferase; S-COMT, soluble catechol-O-methyltransferase; COMTD1, catechol-O-methyltransferase domain containing 1; RELN, reelin.

Key: Hyper-methylation: ↑; hypo-methylation: ↓; differential methylation: ■; no difference in methylation: Θ

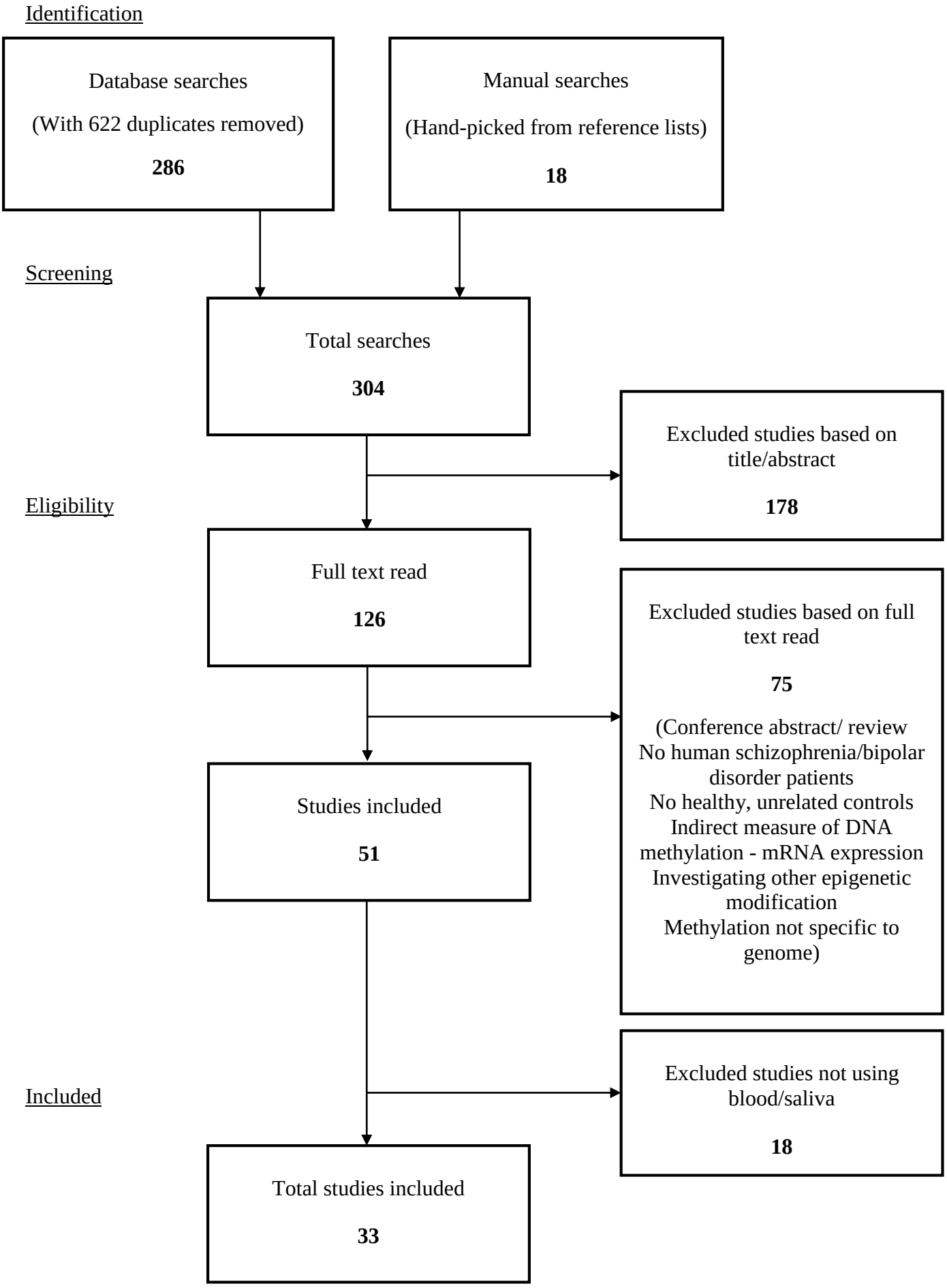

Figure. 1. Systematic selection process for included and excluded studies of this review

Cover Letter