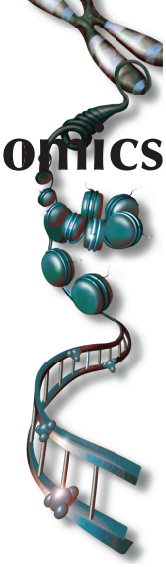
Systematic Review

Epigenomics



Epigenome-wide studies of antipsychotics: a systematic review and pathway meta-analysis

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Background & methods: Researchers have aimed to understand the mechanisms of antipsychotics through epigenetics to inform interindividual response rates. However, findings have widely varied across studies, making advancement in the field difficult. **Materials & methods:** A systematic review was performed to include all epigenome-wide studies of antipsychotic treatment in humans. Methylation sites were used for a pathway and enrichment map analysis was conducted. **Results & conclusion:** Seven studies were included and 82 methylation sites were used for the exploratory pathway meta-analysis that identified six pathway clusters. The findings here demonstrate that studies of the epigenome and antipsychotic treatment are highly heterogeneous in nature and could inform future work to target cross-cutting gene sets and pathways.

Plain language summary: Antipsychotics are a medication used for the treatment of individuals with schizophrenia and bipolar disorder. Antipsychotics work well, but some patients do not respond to treatment and some get side effects from the treatment, and we are not sure why. A possible reason could be how marks on our DNA interact with antipsychotics in a process called epigenetics. This area of research has been ongoing but requires a summary and report of the results together. This review aims to provide such a report so that one day antipsychotic treatment can be improved so that more patients respond and fewer get side effects.

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Keywords: antipsychotic • methylation • pathway • review • systematic

Severe mental illness consists of psychiatric disorders that cause serious functional impairment including schizophrenia and bipolar disorder. These mental illnesses are linked to increased mortality and decreased quality of life [1,2]. The primary pharmacotherapeutic modality for schizophrenia and bipolar disorder are antipsychotics, which are thought to work through the dopamine and serotonin systems. The response to antipsychotic treatment is highly disparate and, in addition, the side effect profile of these medications can be significant. Both lead to antipsychotic nonadherence rates of nearly 50% [3]. The mechanisms that lead to the observed efficacy or side effect differences between patients are not fully known.

Investigations into the mechanisms of antipsychotic efficacy and side effects have taken many forms. One of these research areas, the epigenome, has grown considerably in the last 10 years. The bulk of epigenomic research in antipsychotics has taken place, much like pharmacogenetics, by targeting a single or several genes of interest and has almost exclusively concentrated on DNA methylation (i.e., 5-methylcytosine). Technology has now enabled the growth of epigenome-wide assessments of DNA methylation with the most popular and cost-effective being array based. The advantages of an epigenome-wide approach are the potential identification of novel or multigene methylation changes that occur in the context of antipsychotic treatment. However, as with all 'omic' approaches, they tend to be weakened by low power, a lack of replication of findings and discordant findings between studies.

The current state of DNA methylation research of antipsychotics is diffuse and difficult to summarize due to the heterogeneity in the study designs, antipsychotics analyzed, methods used to analyze DNA methylation, clinical outcomes of interest (e.g., efficacy versus side effects) and the epigenetic findings themselves. The most recent reviews on the field indicate such in their reports. For example, Lisoway and colleagues conducted a narrative

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review that focused, in part, on DNA methylation changes with antipsychotic response in studies since 2014 [4]. The authors found seven studies investigating DNA methylation markers of antipsychotic response that utilized various approaches to analyze DNA methylation including global methylation by enzyme-linked immunosorbent assay, gene-specific methylation and epigenome-wide methylation. The authors describe that each study did find associations with DNA methylation and antipsychotic response. However, no study found changes in the same DNA methylation measure, gene or site. In this narrative, review each of the studies differed in their approaches such as the type of antipsychotic investigated and measurement time points (e.g., baseline, post, both, etc.). Another recent systematic review of all studies investigating DNA methylation associations with antipsychotic treatment in 2020 identified a total of 29 studies that, similar to the Lisoway review above, looked at various DNA methylation changes from global to gene specific [5]. This review found that of the 29 studies, eight measured global methylation, six measured epigenome-wide methylation and 18 looked at candidate epigenes (three studies used a combination of measurements). Again, the authors of the review detail that findings across the studies were not uniform due to the differing approaches, genes investigated, population-specific characteristics and methylation sites investigated. Of the epigenome-wide association studies included in the review, the populations, antipsychotic types and outcomes of interest greatly differed between the studies and no two studies identified an overlapping methylation site or gene. These reviews demonstrate that the field of antipsychotic DNA methylation research, and epigenome-wide methylation association studies of antipsychotics in particular, would benefit from a succinct summarization and an attempt to synthesize the results so that future research can be better informed and based on past work. This lack of summarization is ultimately inhibiting the ability of epigenomics to make an impact in antipsychotic treatment at the clinical level.

With the state of the field of antipsychotic DNA methylation investigations being highly diverse, an exact epigenetic mechanism by which antipsychotics exert their efficacy or side effects has not emerged. Some have pointed to methylation (or demethylation), histone modifications and RNA-based mechanisms, yet this is still an active area of investigation [6–9]. The rationale for this review and pathway meta-analysis is that there is a need to both summarize the studies looking at the effects of antipsychotics on an epigenome-wide level for DNA methylation and to meta-analyze their findings to find common themes and connections. This review is an expanded effort, compared with the previous reviews described above, and aims to summarize and provide new information by combining the methylation outcomes of studies on antipsychotics in humans from any patient population. Thus, the objective of this study was to systematically identify and combine the results from all available epigenome-wide methylation association studies of antipsychotic treatment in humans to provide a summary of findings and identify potential pathways for future investigations so that the field can advance and mechanisms and biomarkers be elucidated to enhance the care of patients on antipsychotics.

Materials & methods

Systematic review sources search strategy & eligibility criteria

The systematic review was performed in Medline (Pubmed), Psychinfo and Embase through our institution's library information system. The search strategy included combinations of the following words: 'antipsychotic', 'epigenome', 'epigenetics', 'methylation', 'methylome' and 'array'. Further queries were performed with individual antipsychotic names such as 'olanzapine', 'quetiapine', 'risperidone', and so on. Searches were restricted to human and English-language studies. Search strategies are provided in the Supplementary data. The inclusion of studies were as follows: 1) study using human tissue source; 2) the study must utilize an epigenome-wide strategy such as array or whole genome bisulfite sequencing to assess DNA methylation; and 3) the study must report on an association between DNA methylation and antipsychotic treatment. Exclusion criteria included: 1) reviews; 2) abstracts without sufficient information (i.e., no list of top differentially methylated sites with antipsychotic use); 3) studies only reported on epigenome associations with psychiatric diagnosis (i.e., schizophrenia vs controls); 4) studies that used an epigenome-wide technology but restricted statistical analysis to a subset of genes; and 5) nonhuman studies including animal and cell line. Search results (final search conducted in December 2022) were downloaded from each respective source and uploaded to Covidence for screening and extraction.

Systematic review screening & extraction process

Imported records were first screened by title and abstract. Next, full texts were downloaded and reviewed for eligibility criteria. All screening was completed by two reviewers, with a third resolving disputes. Data that were extracted from each article included: title, authors, funding, study design, methylation analysis technology, outcome

of interest, statistical methodology for methylation analysis and study participant characteristics (mean age, sex proportion, psychiatric diagnosis, antipsychotic type). The differentially methylated sites (i.e., cg numbers for methylation arrays or gene names) along with their direction of effect based on antipsychotic were collected for the pathway analysis. The NHLBI Quality Assessment Tool was used to evaluate study quality.

Qualitative & meta-pathway analysis

The included study characteristics and results were combined and reported in a tabular format. The genes corresponding to the differentially methylated sites for each study were collected and combined into one list to be used for the meta-pathway analysis. The list of significant methylation sites was based on each individual study's definitions, since these definitions are tailored to an individual study's characteristics. If studies described many significant sites (i.e., >1000 sites), we chose to restrict the number entered into the pathway meta-analyses, as an overly broad approach would be more likely to find nonspecific, less meaningful results. The restrictions were based on p-value cut-offs and changes in methylation (beta change >10% or logFC >1.5) and are defined in the results [10]. The meta-pathway analysis then proceeded according to the methods of Reimand and colleagues [11]. Briefly, the full list of genes associated with the methylation sites was entered into g:Profiler to identify enriched pathways across all included studies with a false discovery rate (FDR) q-value less than 0.05. Data from g:Profiler was then entered into EnrichmentMap (FDR q-value < 0.01 and edge cut-off <0 .375) within Cytoscape to visualize the enriched pathways and their connections, and AutoAnnotate within Cytoscape was used to group and create clusters based on these pathways and their connectedness. The enriched pathways and visualization with annotations are included in the results. This systematic review protocol was not registered with a site such as PROSPERO. The materials and data used in this review are found in this report and its Supplementary Materials.

Results

Overview of studies

A total of seven studies were included after screening. The earliest study was published in the year 2014 and the latest in the year 2022 (see Supplementary Figure 1 for screening) [12–18]. The most common reason for exclusion of an article was the lack of antipsychotic association analysis with the epigenomic data [19]. Only one study did not use the Illuumina HumanMethylation array and all studies used blood as the source of DNA. Most of the studies utilized a case–control or pre–post design. Details of the included studies can be found in Table 1. The quality of the included studies varied depending on the study design and can be found in the Supplementary Tables 1–3. For example, the cross-sectional study either had most quality information missing for determination, while the case–control study by the same authors had most study quality information available and met the criteria [12,13].

Epigenome-wide methylation sites associated with antipsychotic use

The total number of significant methylation sites from all included studies was 82 and within each study the number of significant sites (definition of significant sites is detailed in Table 2) varied widely across the studies. A total of 40 of the sites were hypomethylated with the antipsychotic outcome of interest and 33 sites were within CpG islands. Two studies found a high number of statistically significant methylation sites associated with antipsychotic use (Adanty *et al.* [13] and Perez-Aldana *et al.* [17]), while the remaining sites found anywhere from one to seven significant sites. Two studies identified multiple sites in the same gene (Montano *et al.* and the *IFITM1* gene [16] and Kinoshita *et al.* and the *TRIM15* gene [15]). The significant methylation results for each study are described in Table 2 and this list was used for the input in the meta-pathway analysis.

Meta-pathway analysis

The pathway analysis was performed according to Reimand and colleagues and began with entry of the 82 significant genes in g:profiler [11]. Of the 82 genes, two duplicates were removed and ten entries could not be matched in the database, leaving a total of 70 genes for analysis. Enrichment was performed for Gene Ontology: Biological Pathways and Reactome databases. The Gene Ontology: Biological Pathways analysis found 596 enriched pathways with an FDR q-value < 0.05. The top pathway in this analysis was 'developmental process' where 32 of the entered genes matched in this pathway gave an FDR value of 1.56×10^{-11}. In the Reactome analysis, a total of 48 pathways had FDR q-values < 0.05, with the top reactome pathway being 'neuronal system' being associated with five of the entered genes (q-value < 0.006205). The full output for the g:profiler analysis can be found in Supplementary Table 4.

Table 1. Overview of studies included in systematic review.

Study (year)	Study design	Population	Study size (n)	Female (%)	Description of antipsychotics in study	Outcome of interest for methylation association	Variables controlled for in methylation analysis	Type of technology used for epigenome-wide assessment	Statistical cutoff for identifying 'significant sites'	Study funding source	Ref.
Adanty *et al.* (2022a)	Cross-sectional	Schizophrenia patients	136	35	LAIA = 17.6% (rest oral); average CPZE = 529.7 ± 257.2	Antipsychotic dose in CPZE	Age, cell composition, sex	450K	FDR < 0.05	G, O	[12]
Adanty *et al.* (2022b)	Case–control	Schizophrenia patients and healthy controls	45	21	Clozapine	Clozapine treatment versus healthy control	Patients matched to healthy controls on cell composition and demographics[§]	450K	$p < 2.4 \times 10^{-7}$	G, I	[13]
Hu *et al.* (2022)	Pre–post	Schizophrenia patients and healthy controls	38	33	Risperidone 6–8 mg/day for 8 weeks	Changes with risperidone treatment	Age, cell composition, drinking status, sex, smoking status	450K	$p < 1.00 \times 10^{-6}$	G	[14]
Kinoshita *et al.* (2017)	Pre–post	Schizophrenia patients	21	62	All clozapine (mean dose 91.3 mg/day and mean duration 182.7 days)	Changes with clozapine treatment	None detailed	450K	$\Delta\beta > 0.05$ and $p < 0.001$	G, O	[15]
Montano *et al.* (2016)	Case–control	Schizophrenia patients and healthy controls	Discovery (1334) Replication (497)	44	70% AAP; 12% typical; 10% both; 8% none	Atypical antipsychotic treatment versus nonatypical antipsychotic treatment	Age, cell composition, ethnicity/race, smoking status, principal components of negative control probes	450K	$p < 0.05$[†]	G	[16]
Pérez-Aldana *et al.* (2022)	Case–control	Clozapine-treated patients and drug-naive patients	87	60	All clozapine with a mean dosage of 207.76 mg/day	Clozapine versus drug-naive patients	Batch effects and cell composition	EPIC	$p < 1 \times 10^{-8}$	G, O	[17]
Rukova *et al.* (2014)	Pre–post	Schizophrenia patients and healthy controls	20	60	Atypical and typical	Changes with treatment efficacy	Patients matched to healthy controls on age and sex	Agilent microarray	Statistically significant[‡]	G	[18]

[†]Within this study significant hits must have a FDR < 0.2 in discovery set and a FDR <0.05 in replication, and were based on schizophrenia versus healthy control analyses. A secondary analysis of these 172 significant sites based on antipsychotic used a cut-off of $p < 0.05$.
[‡]Not defined in paper.
[§]Demographics used in matching not defined.
Δβ: Beta change; CPZE: Chlorpromazine equivalent; FDR: False discovery rate; G: Government; I: Internal; O: Organization.

Table 2. Extracted methylation sites for included studies.

Study (year)	Gene of methylation site and CpG location				Ref.
	CpG island	Shore	Shelf	Open Sea	
Adanty *et al.* (2022)	↓*RPS6KA2*				[12]
Adanty *et al.* (2022)‡	↓*AK3* ↑*AKT1S1* ↓*API5* ↓*ARHGAP5* ↓*ARHGAP29* ↓*ARHGEF7*† ↓*FAM150A* ↑*FIGNL2*† ↓*HMGN3* ↓*LINC00511*† ↓*NXN* ↓*SALL1*† ↓*SLC6A4* ↓*SOX17*† ↓*ST8SIA3* ↓*SVIL* ↓*TEAD1* ↓*ZNF445*	↓*DACH1* ↑*NUFIP2* ↑*RGMA* ↓*SLC38A2*	↓*ABCD2* ↑*CC2D1B*† ↓*POU3F3*†	↓*HMP19* ↓*MUCL1* ↓*ZNF764*	[13]
Hu *et al.* (2022)	↑*LOC389906* ↑*SDHAP3*			↑*SNTG2*	[14]
Kinoshita *et al.* (2017)		↑*HIVEP3*	↓*PCGF3*	↑*TRIM15*¶	[15]
Montano *et al.* (2016)	↓*MARCH11*	↓*SATB1*	↑*IFITM1*¶		[16]
Pérez-Aldana *et al.* (2022)§	↓*ACBD3* ↓*GPR19* ↓*LINC03010*† ↓*SLC6A20*	↑*ADAMTS17*† ↓*BCL6* ↓*BRUNOL5* ↓*GOLGA8B* ↓*HEBP1* ↑*NUMBL* ↓*NAT9* ↓*RHCG*† ↑*ZBTB47*	↑*CORO1C* ↓*GRHL3* ↑*TRAK1*	↑*ATP11B*† ↑*ATXN1* ↑*CALML3*† ↑*CNGA4*† ↑*CNR1*† ↑*COMT* ↑*CRHR1* ↓*DENND2A* ↑*FNDC3A* ↑*GAS7* ↑*JAKMIP3* ↑*MAPKAPK3* ↑*MTFR2* ↑*MYO18A* ↑*NMUR2*† ↑*PLEKHA6* ↑*SAMD4A* ↑*WAPAL* ↑*ZMYND17*	[17]
Rukova *et al.* (2014)	↑*AP1S3* ↑*BCOR* ↑*C16orf59* ↓*KCNK15* ↑*LOC146336* ↑*MGC16384* ↑*XRN2*				[18]

Arrows indicate the directionality of methylation change of a given gene with that study's outcome of interest. The outcome of interest can be found in Table 1.
†Intergenic methylation site listed, nearest gene used for purposes of meta-pathway analysis.
‡Study found >1000 significant sites so sites with a logFC > ±1.5 were used for meta-pathway analysis (all sites had $p < 7.40 \times 10^{-19}$).
§Study found >44,000 significant sites based on genome-wide cut-off of $p < 1 \times 10^{-8}$; therefore, we used sites where false discovery rate was <0.05 and with a beta change of >±10%.
¶*IFITM1* and *TRIM15* genes had two separate probes that were significant within their respective studies.

Next, the results from g:profiler were entered into the Cytoscape Enrichment Map application to look at the clustering and interconnectivity of the pathways from g:profiler. The output from this analysis is shown in Figure 1 where each node (or circle) represents a distinct pathway and the lines represent the number of overlapping genes between the pathways. The major themes identified using the auto annotate function include: 1) developmental and regulatory processes; and (2) transport. The full table of enrichment results is provided in Supplementary Table 5 and the high-resolution EM PDF of the network figure is provided in Supplementary Figure 2.

Many enriched pathways were found in the pathway meta-analysis that were interconnected by various genes, as seen in the enrichment map in Figure 1. The top two clusters of pathways that came from the enrichment

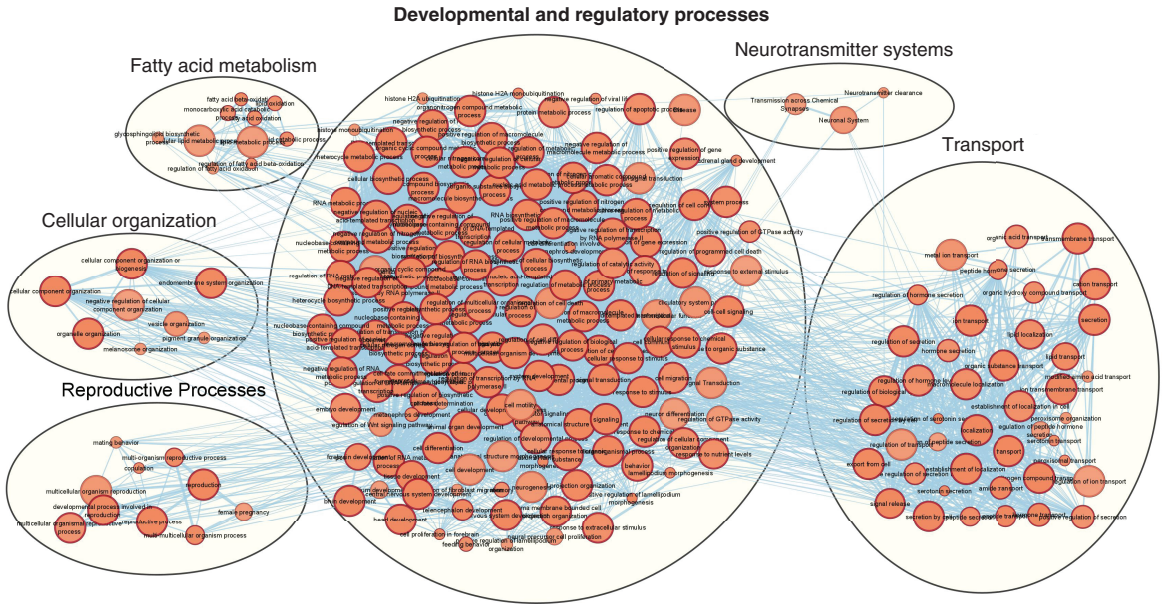

Figure 1. Cytoscape enrichment map of g:profiler gene ontology analysis. Pathway meta-analysis results that utilized findings from studies in systematic reviews. Each node (or circle) represents a distinct pathway and the lines represent the number of overlapping genes between the pathways.

analysis were: 1) developmental and regulatory processes; and 2) transport. Gene ontology pathways within the developmental and regulatory processes cluster include regulation of metabolic process, regulation of gene expression, DNA-templated transcription, regulation of biosynthetic process and tissue development. This cluster's enrichment was driven by 59 of the genes entered from the included studies. The transport cluster was enriched by 43 gene ontology categories made up of 31 entered genes. Of the 31 genes in this cluster, all but three overlapped with the developmental and regulatory processes cluster (which is evident from the interconnections or lines between the clusters in Figure 1).

Discussion

Overview

The systematic review and pathway meta-pathway analysis presented here identified seven studies investigating associations between DNA methylation and antipsychotic treatment. These studies were similar in their approach to analyzing DNA methylation but did differ greatly in the antipsychotics studied and their study designs. Furthermore, their findings differed widely as well, with no single methylation site being replicated across the studies. These heterogeneous findings further evidence our premise of the need to systematically review epigenome-wide studies and perform a meta-pathway analysis that can potentially make sense of hundreds of methylation targets identified. Nevertheless, this heterogeneity should be considered when interpreting the pathway meta-analysis results.

Top clusters identified from enrichment analysis

The primary developmental and regulatory processes cluster identified in the enrichment analysis is wide ranging, and its identification here with antipsychotic use (especially of the nervous system gene ontology categories) seems unsurprising given antipsychotics' role both in brain activity and the high number of side effects seen with these drugs. For example, one gene entered from our analysis, *CNR1*, is a gene that has been linked to antipsychotic-induced weight gain and tardive dyskinesia [20,21]. In addition, it has been correlated to antipsychotic response but not disease susceptibility [22]. Another example of a gene in the meta-pathway analysis cluster of developmental and regulatory processes with evidence in the antipsychotic literature is *COMT*. This gene is located within the folate cycle and has a role in the activation of folate and also the production of methyl donors for cellular processes such as DNA methylation. This gene has been studied intensively, allowing for meta-analyses identifying a relationship between *COMT* variation and antipsychotic response and side effects [23,24].

As with the developmental and regulatory processes cluster, the transport cluster is large and varied, including gene ontology categories such as regulation of ion transport, localization, serotonin transport and transmembrane transport with various connected genes. Included in these overlapping genes are the previously discussed *CNR1* and *COMT* genes. The nonoverlapping genes include *ATP11B*, *KCNK15* and *SLC6A20*. Research on various transporter associations has also occurred with respect to antipsychotic efficacy and side effects [25,26]. This is exemplified by the corpus of research on SLC transporters and the blood–brain barrier [27,28]. The *SLC* gene is part of the solute carrier family and has a role in blood–brain barrier transport of several antipsychotic-associated neurochemicals such as serotonin and dopamine. To date, the subtypes of *SLC* genes studied have not found strong associations with antipsychotic response [29].

Within these two large clusters can be seen the considerable work that has been done in antipsychotic research; nevertheless, many of the genes in this cluster have not been investigated with antipsychotic use. The findings here could help researchers to target their hypotheses from entire genomes to clusters of pathways or targeted gene sets that have shown enrichment. This could be essential to understanding the polygenic and multipathway effects that antipsychotics are likely to exhibit and may help to define future mechanisms or complex biomarker profiles.

Other enrichment clusters of interest

The enrichment analysis also identified a few minor clusters including 'neurotransmitter systems' and 'fatty acid metabolism', among others. Of these, the cluster of fatty acid metabolism has been of great interest in antipsychotic research, particularly as it pertains to antipsychotic side effects [30,31]. The investigation of fatty acid metabolism and other metabolic processes is a critical layer to add to the genomic-based investigations that will help to identify the mechanisms and impactful biomarkers behind antipsychotic efficacy and side effects. Similarly, neurotransmitter systems seem like a natural cluster fitted to antipsychotic epigenomic research given the primary effects of antipsychotics on the brain. The gene ontology pathways in this cluster included 'transmission across chemical synapses', 'neuronal system' and 'neurotransmitter clearance', and the genes from the entry associated with this cluster included *COMT*, *RPS6KA2*, *SLC38A2* and *SLC6A4*. As already discussed, the *SLC* gene family has a connection with the transport cluster and is present here for its role in blood–brain barrier transport. Likewise, the already discussed *COMT* gene has connections with antipsychotic efficacy and cognition. Other genes have a connection to antipsychotic research, although not directly studied. For example, the *RPS6KA2* gene, which encodes for a member of a serine/theonine kinase is active in phosphorylation of protein substrates including in MAPK signaling. Although the associated gene has not been investigated in targeted genetic antipsychotic studies, the MAPK pathway has, and furthermore, proteomics is an active area of antipsychotic research including protein activity (e.g., phosphorylation, acetylation, etc.) [32,33].

Limitations

A few limitations should be considered in this systematic review and pathway meta-analysis. First, the overall number of studies was small and highly heterogeneous in nature, which should be considered when interpreting the combined analyses. For example, the studies differed in their methodology, antipsychotics analyzed and outcomes of interest. Furthermore, there was no replication of a methylation site or gene among the studies. Nevertheless, the purpose of this work was to provide researchers in the field of epigenomic antipsychotic research with an easily referenced and up-to-date synthesis of the state of the field as it pertains to DNA methylation. Given this heterogeneity and the novel nature of the pathway meta-analysis approach performed here, the results of the pathway analysis should be interpreted with caution. Previous work has combined datasets (generally publicly available) to perform 'meta-epigenomic' studies. Although these studies are highly useful and increase the power to detect findings, we believe one limitation of such an approach is combining data from heterogeneous studies (e.g., design, timing, etc.), which could make the findings less reliable. Our approach here takes the significant findings from each individual study, which accounts for individual study designs and attempts to synthesize them in terms of pathway connections. Yet, our approach is employed at the 'gene level', which removes many of the details and important features that individual methylation sites provide such as their location and potential effect on gene regulation. It should also be noted that epigenome-wide methylation studies utilizing arrays (which were all the studies included here) only have methylation sites covering a fraction of the genome's methylation sites. Although these arrays have sites in almost all genes of the genome, it should still be considered only a minor representation of the genome's DNA methylation landscape. Our qualitative synthesis in Table 2 attempts to assist researchers by summarizing the hyper or hypomethylation observed along with the location of the CpG site associated with the

gene. Finally, the overall quality of the studies was moderate, so future work should aim to perform epigenomic studies of antipsychotics in the form of randomized controlled studies.

Conclusion

The findings here suggest that epigenome-wide studies of antipsychotic treatment have identified methylation differences in a variety of major areas that have considerable crosstalk. This review and synthesis of epigenome-wide findings provides future data for researchers to perform targeted studies of connected pathways and genes and their products such as proteins and lipids in an effort to better define the mechanisms underlying antipsychotic efficacy and side effects.

The investigations into epigenomic mechanisms will continue to expand in the next 5–10 years and will likely include targeted, deep sequencing with whole-gene bisulfite sequencing. These investigations, which should consider the state of the field presented here, will begin to develop multigene, combinatorial epigenetic signatures of antipsychotic efficacy and side effects. Future work could also consider the use of full methylome sequencing to better understand the methylation changes beyond what can be captured by methylation arrays as well as the utilization of two-stage, Mendelian randomization to assist in understanding the role of DNA methylation in antipsychotic treatment.

Summary points

- Epigenome-wide studies into antipsychotic response have been ongoing for the past 10 years.
- The findings from these studies often widely vary in methodology, with no study finding the same epigenome associations.
- This study aimed to systematically review this literature, extract significant methylation sites from each study, and combine them in a pathway and enrichment meta-analysis.
- Seven studies were identified that investigated antipsychotic effects at the epigenome-wide level.
- A total of 82 methylation sites were extracted from this study and used for a pathway and enrichment meta-analysis.
- Gene ontology analysis of these 82 sites found 596 enriched pathways with a false discovery rate q-value <0.05.
- Enrichment analysis of the gene ontology results produced six pathway clusters with significant interconnectedness.
- The pathway meta-analysis interpretation should be moderated by the limitations of the current epigenome-wide association studies of antipsychotics, which are highly heterogeneous in methods and results.
- Several genes within these pathway clusters already have evidence from targeted epigenomic or genomic studies.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/epi-2023-0222

Author contributions

All authors made substantial contributions to the work including conception, design, acquisition and interpretation. All authors contributed to drafting and gave final approval of the work including accountability for all aspects of the work.

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Competing interests disclosure

The authors have no competing interests or relevant affiliations with any organization or entity with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Writing disclosure

No writing assistance was utilized in the production of this manuscript.

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• **Included in the systematic review. Investigation of epigenome-wide associations with antipsychotic dosage.**

13. Adanty C, Shakeri A, Strauss J, Graff A, De Luca V. Head-to-head comparison of various antipsychotic agents on genome-wide methylation in schizophrenia. *Pharmacogenomics* 23(1), 5–14 (2022).

• **Included in the systematic review. Investigation of epigenome-wide associations with several antipsychotic included compared to one another and healthy control group.**

14. Hu M, Xia Y, Zong X *et al.* Risperidone-induced changes in DNA methylation in peripheral blood from first-episode schizophrenia patients parallel changes in neuroimaging and cognitive phenotypes. *Psychiatry Res.* 317, 114789 (2022).

• **Included in the systematic review. Investigation of epigenome-wide associations with risperidone in pre–post format.**

15. Kinoshita M, Numata S, Tajima A *et al.* Effect of clozapine on DNA methylation in peripheral leukocytes from patients with treatment-resistant schizophrenia. *Int. J. Mol. Sci.* 18(3), (2017).

• **Included in the systematic review. Investigation of epigenome-wide associations with clozapine treatment in naturalistic, pre–post study.**

16. Montano C, Taub MA, Jaffe A *et al.* Association of DNA methylation differences with schizophrenia in an epigenome-wide association study. *JAMA Psychiatry* 73(5), 506–514 (2016).

• **Included in the systematic review Investigation of epigenome-wide associations with antipsychotic treatment that includes discovery and replication cohort. Within the replication cohort the analysis of association with antipsychotic treatment was performed for the genes from the discovery cohort.**

17. Pérez-Aldana BE, Martínez-Magaña JJ, Mayén-Lobo YG *et al.* Clozapine long-term treatment might reduce epigenetic age through hypomethylation of longevity regulatory pathways genes. *Front. Psychiatry* 13, 870656 (2022).

• **Included in the systematic review. Investigation of epigenome-wide associations with clozapine. Reports thousands of sites associated with antipsychotic treatment.**

18. Rukova B, Staneva R, Hadjidekova S, Stamenov G, Milanova V, Toncheva D. Whole genome methylation analyses of schizophrenia patients before and after treatment. *Biotechnol. Biotechnol. Equip.* 28(3), 518–524 (2014).

- **Included in the systematic review. Investigation of epigenome-wide associations with antipsychotic treatment in pre–post format. The oldest of the studies included the study utilized a unique microarray.**

19. Nishioka M, Bundo M, Koike S *et al.* Comprehensive DNA methylation analysis of peripheral blood cells derived from patients with first-episode schizophrenia. *J. Hum. Genet.* 58(2), 91–97 (2013).
20. Tiwari AK, Zai CC, Likhodi O *et al.* A common polymorphism in the cannabinoid receptor 1 (*CNR1*) gene is associated with antipsychotic-induced weight gain in Schizophrenia. *Neuropsychopharmacology* 35(6), 1315–1324 (2010).
21. Tiwari A, Zai C, Likhodi O *et al.* Association study of cannabinoid receptor 1 (*CNR1*) gene in tardive dyskinesia. *Pharmacogenomics J.* 12(3), 260–266 (2012).
22. Hamdani N, Tabeze J-P, Ramoz N *et al.* The *CNR1* gene as a pharmacogenetic factor for antipsychotics rather than a susceptibility gene for schizophrenia. *Eur. Neuropsychopharmacol.* 18(1), 34–40 (2008).
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