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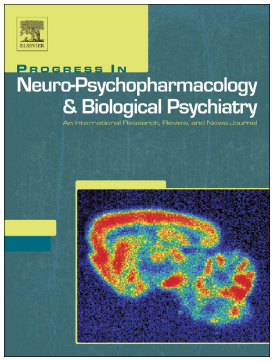

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Longitudinal DNA Methylation and Cell-Type Proportions Alterations in Risperidone Treatment Response in First-Episode Psychosis

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Running title: DNA methylation in risperidone response

Abstract (250 words)

Identifying biological markers to guide treatment decisions in first-episode psychosis (FEP) is essential for improving patient outcomes. This longitudinal study investigated DNA methylation (DNAm) patterns and DNAm-derived cell-type proportions (CTP) in blood and associated them with response to risperidone treatment, a second-generation antipsychotic drug, in antipsychotic-naïve FEP patients. We also explored longitudinal changes in DNAm associated with risperidone treatment. We profiled DNAm in 114 individuals before (anFEP) and after two months of risperidone treatment using microarrays. The main results were compared with 115 healthy controls and validated in an independent cohort of subjects with schizophrenia (n=26) with one-month follow-up data. We identified 302 differentially methylated positions (DMPs) associated with treatment response, measured by changes in the Positive and Negative Syndrome Scale score, of which 16 were validated in the independent cohort. Sixteen differentially methylated regions (DMRs) were associated with response, with one (in *SIPA1L3*) being validated. A decrease in B-cell proportions was correlated with symptom improvement in both cohorts. Additionally, four DMPs associated with risperidone treatment were identified: two related to the psychotic state and two specifically to risperidone treatment. DNAm-derived CTP showed alterations in anFEP compared with controls, particularly in the neutrophil-to-lymphocyte ratio, which normalized after treatment. These findings suggest that DNAm, particularly in B-cells, may be a promising marker for monitoring response to risperidone treatment in schizophrenia. Our longitudinal study revealed novel and known genes that may be regulated by risperidone and could be used as response markers to improve prognosis in schizophrenia and FEP.

1. Introduction

Schizophrenia (SCZ) is a severe psychiatric disorder characterized by a diverse symptom profile, imposing a substantial burden on patients, their families, and healthcare systems (Owen et al., 2016). While antipsychotic medications are effective in managing positive symptoms (e.g., delusions and hallucinations), predicting treatment response remains a significant challenge (Taylor et al., 2012). The current trial-and-error approach often leads to suboptimal outcomes, increased healthcare costs, and diminished quality of life for patients. Thus, identifying reliable biomarkers to guide treatment selection is imperative to improve patient care. However, most previous studies investigating biological predictors of antipsychotic response have been hampered by cross-sectional designs and patient populations with complex medication histories (Burghardt et al., 2020; Hannon et al., 2021; Lisoway et al., 2021; Rowbal et al., 2023). To address these limitations, research focusing on individuals at their first-episode psychosis (FEP) is essential for identifying biomarkers.

DNA methylation (DNAm), an epigenetic mechanism influenced by both genetic and environmental factors, has emerged as a promising candidate for monitoring antipsychotic response due to its stability and cost-effectiveness. By capturing the dynamic interplay between genes and environment, DNAm may provide insights into the molecular pathways underlying antipsychotic efficacy. Moreover, DNAm can serve as a basis for estimating different biological phenotypes that may be associated with antipsychotic response, such as white blood cell types. Several studies have reported an association between white blood cell count or the neutrophil-to-lymphocyte ratio (NLR) and SCZ (Sandberg et al., 2021). However, the etiology behind this association remains unknown, and it is unclear whether changes in white blood cell count and NLR are induced by antipsychotics or if these phenotypes are related to the diagnosis of schizophrenia.

Blood-based DNAm is particularly advantageous for biomarker development or understanding molecular mechanisms associated with antipsychotic efficacy due to its relatively noninvasive sampling process (Khavari and Cairns, 2020). Evidence shows that some associations between psychiatric disorders and DNAm are shared across blood and brain, likely reflecting molecular patterns arising in multiple tissues due to genetic, developmental, and environmental influences (Yousefi et al., 2022). For instance, two studies have examined DNAm in brain and blood samples from the same individuals, confirming a strong correlation in DNAm variation at individual CpG sites between these tissues (Braun et al., 2019; Smith et al., 2015). However, confounding factors (e.g. medication and smoking) and the potential for reverse causation pose challenges in interpreting DNAm data in blood (Hannon et al., 2021). To mitigate these limitations, our study employed a longitudinal epigenetic-wide association study (EWAS) design of an antipsychotic-naïve FEP cohort, considering that this approach minimizes the potential confounding effects of prior antipsychotic medication and allows for the examination of dynamic epigenetic changes in relation to treatment response over time. Moreover, only very few previous studies followed a similar design (Hu et al., 2022; Lokmer et al., 2023; Zong et al., 2023, 2022, 2021).

In this study, our aim was to identify differences in DNAm and in DNAm-derived cell-type proportions associated with the response to risperidone treatment, a second-generation antipsychotic drug, after short-term treatment in a cohort of antipsychotic-naïve FEP individuals. We also explored longitudinal changes associated with risperidone treatment itself. Our study consists of a discovery cohort of 114 anFEP individuals, from whom blood samples were collected before the initiation of risperidone treatment and again after two months of treatment (FEP-2M), as well as a validation cohort of 26 cases with a similar study design (Lokmer et al., 2023). Additionally, we included sex- and age-matched healthy controls (HC) to strengthen the conclusions drawn from the longitudinal data (Figure 1).

2. Materials and Methods

2.1. Discovery cohort

The discovery cohort comprises 125 antipsychotic-naïve first-episode psychosis (anFEP) individuals and a healthy control (HC) group of 126 age- and sex-matched individuals. All FEP patients were recruited from a psychiatric emergency unit in São Paulo, Brazil, and the full cohort is described elsewhere (Cavalcante et al., 2024). The diagnosis of a psychotic disorder was established by trained psychiatrists based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria using the Structured Clinical Interview of the DSM-IV (SCID-I). All patients fulfilled the criteria for one of the following psychotic diagnoses: schizophrenia, schizophreniform disorder, brief psychotic disorder, and psychotic disorder not otherwise specified. Upon enrollment in the research protocol, patients were prescribed a daily dose of risperidone, typically starting at 1–2 mg per day based on clinical assessment. Inclusion criteria were age between 16 and 40 years and no prior history of antipsychotic medication. Patients with psychotic episodes due to a general medical condition, substance-induced psychotic disorder, intellectual disability or psychotic episodes that were associated with bipolar or major depressive disorders were excluded. The HC subjects were age- and sex-matched volunteers who had no current or lifetime history of psychiatric diagnoses assessed based on DSM-IV-TR criteria or first-degree family history of severe psychotic disorder.

FEP patients underwent blood collection before (baseline) and two months after (FEP-2M) initiating risperidone treatment (mean 69.51 days, SD 32.32). The PANSS was administered at both timepoints to assess symptom severity and calculate response rate. The Research Ethics Committee of the Federal University of Sao Paulo (UNIFESP) approved the research protocol (CEP 0603/10, CAAE 89057218.7.0000.5505 and CAAE: 48242015.9.0000.5505), and all participants or family members provided written informed consent before enrollment. The project was also approved by the Research Ethic Committee of Norway (REK #686185)

2.2. Replication cohort

The replication cohort was composed of 28 individuals diagnosed with schizophrenia according to the DSM-IV and attending outpatient services in Puducherry, India. This cohort was selected as replication because it has also DNA methylation data and PANSS scores measured in two timepoints, similar to the the discovery cohort. The inclusion criteria were: schizophrenia diagnosis, age above 18, total PANSS score of at least 30 and newly prescribed risperidone. Patients who were prescribed antipsychotics other than risperidone, substance abusing patients and pregnant or lactating women were excluded from the study. Blood was collected before the initiation and after 1 month of risperidone treatment. From those 28, twelve (42%) individuals were antipsychotic naïve. Description of this cohort can be found in (Lokmer et al., 2023).

2.3. Assessment of response to risperidone

To calculate the risperidone response rate, we used the formula described in Leucht et al. (2009) (Leucht et al., 2009) which consists of:

$$Percentage\ of response = 100 \times \frac{PANSS_{before\ treatment} - PANSS_{after\ treatment}}{PANSS_{before\ treatment} - 30}$$

We categorized the patients into responders and non-responders based on the response percent. Patients with 50% of response or more were allocated to the responder group (discovery cohort n=52; replication cohort n=17), while the others were allocated to the non-responder group (discovery cohort n= 54; replication cohort n=11). Eight individuals from the discovery cohort did not have PANSS data available for both timepoints, and were therefore excluded from this analysis.

2.4. Generation of DNA methylation data

In the discovery cohort, DNA was isolated from blood using the Gentra Puregene Kit (Qiagen, Maryland, USA) according to the manufacturer's protocol and converted with sodium bisulfite using the EZ DNA Methylation Kit (Zymo Research, CA, USA). Methylation levels were assessed using the Infinium HumanMethylation450K BeadChip (450K array, Illumina, CA, USA) for 61 anFEP, 59 FEP-2M and 62 HC; and the Infinium MethylationEPIC v1.0 BeadChip (EPIC array, Illumina) for 64 anFEP, 64 FEP-2M and 64 HC. We followed the manufacturer's protocol and their prerequisites to prepare the samples and randomized them before starting the protocol, keeping all paired samples (anFEP and FEP-2M) on the same beadchip to minimize batch effects.

In the replication cohort, DNA was extracted from blood cells using a QIAamp Blood Midi Kit (Qiagen, Hilden, Germany) and subsequently converted using the EpiTect Fast 96 Bisulfite Kit (Qiagen). Genome-wide methylation was assessed using the Infinium MethylationEPIC v1.0 BeadChip and all steps were performed following the manufacturers' protocols (Lokmer et al., 2023).

The raw files were processed separately for each batch using the minfi v.1.50.0 (Aryee et al., 2014; Fortin et al., 2016, 2014), meffil v1.1 (Min et al., 2018) and wateRmelon v.2.10.0 (Pidsley et

al., 2013) R packages. The batches consisted of (i) EPIC and (ii) 450K arrays in the discovery cohort, and (iii) EPIC arrays in the replication cohort. For all batches, we adopted a very stringent quality control pipeline, described in the Supplementary Information, ending up with: (i) discovery cohort 450K: 412,578 probes, and 62 HCs, 60 anFEP and 58 FEP-2M (56 pairs); (ii) discovery cohort EPIC: 743,082 probes, and 53 HCs, 54 anFEP and 51 FEP-2M (45 pairs); (iii) replication cohort EPIC: 738,999 probes, and 26 pairs of SCZ cases (52 samples). Then, we normalized our data using the preprocessFunnorm function from minfi (Aryee et al., 2014; Fortin et al., 2016, 2014), which is a between-array normalization method that removes unwanted variation by regressing out variability explained by the control probes. Finally, we extracted the M values for the statistical analyses using R v4.4.1 and RStudio v 2023.12.0.

We derived additional variables from the DNAm data: estimated proportions of six blood cell-types and DNAm smoking score. The estimated cell-types were neutrophils, monocytes, $CD4^+$ and $CD8^+$ T lymphocytes, B cells, and natural killer (NK) cells and were estimated using the estimateCellCounts2 function (Salas et al., 2018) from the FlowSorted.Blood.EPIC Bioconductor package v.2.8.0 (*FlowSorted.Blood.EPIC*, n.d.). The neutrophil-to-lymphocyte ratio (NLR) was calculated dividing the neutrophil proportion by the sum of the proportions of $CD4^+$T, $CD8^+$T, B cells, and NK cells. The DNAm smoking score was calculated as described in Elliott *et al*. (2014) (Elliott et al., 2014), using the weights from (Zeilinger et al., 2013) data and (Li et al., 2018).

2.5. Statistical analysis

To assess differences between groups (cases vs. controls or responders vs. non-responders) for demographics, we performed the chi-squared test for the categorical variables, and the Student's t test or Mann-Whitney U test for the continuous variables. The choice between these two tests was dependent on the normality of the data.

2.5.1 Differentially methylated positions (DMPs)

To identify differentially methylated positions (DMPs) associated with risperidone treatment, we conducted an EWAS using normalized M values as the outcome variable. We compared anFEP and FEP-2M (within-subject comparison) using a mixed linear regression approach, as implemented in the limma R package v3.60.6 (Ritchie et al., 2015). The duplicateCorrelation function was used to estimate the correlation between repeated measures on the same subject (Ritchie et al., 2015). We included technical artifacts (position on the microarray), DNAm smoking score, and estimated cell-type proportions as covariates. We used the metagen function from the meta package v8.0 (Schwarzer et al., 2015) to perform a meta-analysis and combine the 450K and EPIC summary statistics from the discovery cohort. Thus, in the meta-analysis, we focused on the 381,170 DNAm probes that EPIC and 450K microarrays had in common. The metagen function implements standard inverse variance meta-analysis where the pooled effect sizes are computed as the weighted average of effect sizes in meta-

analyzed EWAS. We computed effect sizes for fixed effects model and two-sided tests for the p-values. The DNAm values from CpG sites identified as DMPs in the meta-analysis were compared between anFEP and HCs, as well as between FEP-2M and HCs, using group as the main predictor while adjusting for technical artifacts (microarray ID and / or position on the microarray), DNAm smoking score, estimated cell-type proportions, sex, and age. These DMPs were further tested for association with one month of risperidone treatment in the replication cohort using the mixed linear model previously described. Given that 361,912 EPIC-only probes were excluded from the meta-analysis due to their lack of overlap with 450K probes, we also assessed their association by checking the EPIC EWAS results and validating the DMPs in the replication cohort.

To identify DMPs associated with risperidone response rate, we applied a mixed linear regression approach. We considered two models: a main effect model and an interaction model. In both models, we used normalized M values as the outcome variable. For the main effect model, we included risperidone response rate, treatment (baseline or follow-up), technical artifacts, DNAm smoking score, estimated cell-type proportions, sex, and age as predictors. For the interaction model, we included all the variables from the main effect model, along with an interaction term between risperidone response rate and treatment. We meta-analyzed response rate summary statistics from the 450K and EPIC batches as mentioned above, and DMPs associated with risperidone response rate or its interaction with treatment in the discovery cohort were validated using a mixed linear regression in the replication cohort. We also tested if the EPIC-only probes were associated with response rate and validated them in the replication cohort.

For DMPs associated with risperidone treatment and response rate in the discovery cohort, the significance threshold was set to a false discovery rate (FDR) < 0.05 using the Benjamini–Hochberg (BH) method. For replication, we considered a one-sided p-value < 0.05 as significant.

2.5.2 Differentially methylated regions (DMRs)

For differentially methylated regions (DMRs), we applied the dmrff method (v1.1.2) with default parameters (Suderman et al., 2018). Briefly, this method tests genomic regions spanned by sets of CpG sites (default parameters: 500 bp between consecutive sites, with nominal EWAS p-values < 0.05 and EWAS effect estimates with the same sign). As input, we included the EWAS summary statistics from the discovery cohort 450K and EPIC batches, as well as their normalized M values, and performed the meta-analysis using the dmrff.meta function, which uses variance-weighted fixed effects meta-analysis. Significant regions were considered as those with Bonferroni-adjusted p < 0.05 and with a minimum of two probes. To replicate the DMRs, we calculated the mean methylation of all CpGs within these regions in the replication cohort and checked if the mean methylation was associated with risperidone treatment, risperidone response rate or the interaction between response rate and treatment.

2.5.3 Estimated cell-type proportions (CTP)

Linear mixed-effects models were used to test the association between estimated cell-type proportions and risperidone treatment or response rate, using the lmer function from the lme4 v1.1 R package (Bates et al., 2015). In line with DMP and DMR analyses, models were run independently for each batch in the discovery cohort and meta-analyzed afterward using the metagen function from the meta R package.

For both treatment and response rate analyses, NLR and estimated cell-type proportions were the outcome variables. The model for risperidone treatment included treatment (baseline or follow-up) as the main predictor, adjusting for technical artifacts and smoking score, and individual ID was modeled as a random effect in the mixed linear model. For response rate analysis, we also considered two models, as described for DMPs. The predictors were risperidone response rate, treatment, and their interaction (only in the interaction model), along with technical artifacts, DNAm smoking score, sex, and age. The same models were applied in the replication cohort to assess the association of NLR and estimated cell-type proportions with risperidone treatment or response rate. Additionally, NLR and estimated cell-type proportions were compared between anFEP and HCs, and between FEP-2M and HCs in the discovery cohort using linear regression.

In the discovery cohort, associations with two-sided p-values < 0.017 were considered significant. This threshold was set after applying Bonferroni correction of multiple comparisons, accounting for three comparisons: anFEP *vs.* FEP-2M, anFEP *vs.* HC, and FEP-2M *vs.* HC. The significance threshold for replication was set at one-sided p-values < 0.05.

2.6. Enrichment analysis

We used the missMethyl package v1.38.0 (Phipson et al., 2016) in R and gometh and goregion functions to perform gene ontology and pathway analyses (considering Gene Ontology - GO terms and Kyoto Encyclopedia of Genes and Genomes - KEGG pathways) to test for association of the significant DMPs and DMRs with specific gene sets (adjusted $p < 0.05$). We considered significant pathways those with FDR < 0.05.

2.7. Sensitivity analyses

We acknowledge that factors such as benzodiazepine use prior to blood collection, risperidone dosage, and duration of untreated psychosis (DUP) may influence our results, particularly in models assessing risperidone response. However, data on benzodiazepine use were available for only 71.7% of patients, on risperidone dosage for 86.2%, and on DUP for 60.5%. To evaluate their potential impact, we examined their effects on DNAm within each discovery cohort batch (450K or EPIC) using models similar to the response rate analysis: M values ~ benzodiazepine use / risperidone dosage / DUP + technical artifacts + DNAm smoking score + estimated cell-type proportions + sex +

age. The results from both batches were combined using a meta-analysis, as described above. For the risperidone dosage model, we also included treatment (baseline or follow-up) as a predictor and performed the analysis on the entire sample. In contrast, for benzodiazepine use and DUP, the analysis was restricted to the FEP baseline samples. We then compared these results with those associated with risperidone treatment and response. For CpGs found in both sets, we conducted additional analyses by including these covariates in the main response rate model.

3. Results

3.1. Study overview and cohort characteristics

The cases were characterized by a higher proportion of males than females (Table 1). In the discovery cohort, FEP individuals, on average, presented higher DNAm smoking scores compared to controls. Smoking scores derived from DNAm were compared to the data available on self-reported smoking status and showed high correspondence (Figure S1). Responders and non-responders did not differ with regard to sex, age, and smoking score. However, in the replication cohort, non-responders tended to have a higher proportion of males and higher smoking scores at the baseline than responders (Table 1).

3.2. DNAm changes associated with 2-months of risperidone treatment

Our meta-analysis identified four DMPs associated with two months of risperidone treatment after correction for multiple comparisons (Tables S1-S2, Figure S2). Two of these CpGs (cg17931986: estimate= -0.245, adjP=0.016; and cg18058279: estimate=-0.132, adjP=0.016) showed decreased DNAm levels post-treatment, while the others (cg04287747: estimate= 0.109, adjP=0.016; and cg04732910: estimate= 0.115, adjP=0.041) showed increased levels (Figure S3). These CpGs are annotated to the *COL11A2* (cg17931986), *CAPN5* and *OMP* (cg18058279), *USP36* (cg04287747) and *MDM2* (cg04732910) genes.

Comparing these DMPs in the control group, both cg17931986 and cg18058279 showed hypermethylation in anFEP individuals at baseline compared to controls at a nominal significance threshold ($p<0.05$), while the methylation levels did not differ comparing FEP-2M and controls (Table S2, Figure S3). This suggests that these CpGs are associated with the psychotic state, reversing to control levels after two months of antipsychotic treatment. In contrast, cg04287747 and cg04732910 did not show baseline differences between anFEP and controls but had higher DNAm levels in the FEP-2M group compared to controls at a nominal significance threshold (Table S2, Figure S3). This pattern suggests their methylation changes are attributable to risperidone treatment, since DNAm values in FEP-2M are different from both groups.

The direction of effect for all these CpGs was consistent in the validation cohort, although they did not reach statistical significance (Table S3). A tendency was observed for cg04287747 (one-sided p-value=0.0725). We did not find any DMPs among the EPIC-only probes after adjusting for multiple tests and did not identify any DMRs associated with risperidone treatment.

3.3. Estimated cell-type proportions associated with 2-months of risperidone treatment

We identified an association between the neutrophil-to-lymphocyte ratio (NLR) and risperidone treatment, observing a decrease in the ratio after treatment (Table 2 and Figure S4). The NLR was significantly higher in anFEP individuals compared to controls (i.e., at baseline), but it did not differ when comparing the FEP-2M group with controls. This suggests that NLR is associated with the psychotic state and normalizes to levels comparable with controls after risperidone treatment. Similarly, the estimated cell-type proportions for neutrophils, $CD4^{+}$T, and NK cells followed the same pattern (Table 2), i.e., opposite direction of effects in the "anFEP vs. FEP-2M" and "controls vs. anFEP" comparisons, and no association between control and FEP-2M groups. However, none of these associations were replicated in the validation cohort (Table S4). Interestingly, although not significant after correction for multiple comparisons, B cell was decreased after 2 months of risperidone treatment (Table 2, Figure S5) and a similar decrease was observed in the validation cohort after 1 month of risperidone treatment in SCZ cases (one-sided p-value=0.005). This suggests that B cell proportions might be specifically influenced by risperidone treatment.

3.4. DNAm changes associated with response rate

After correcting for multiple comparisons, we identified 214 DMPs associated with risperidone response rate (all results in Table S5, Figure S6). Of these, 10 were validated in the replication cohort (Table 3, Supplementary Table S10). Half of the validated CpGs decreased their DNAm levels with symptom improvement, while the other half showed increased their levels (Table 3). Considering the EPIC-only probes, 88 DMPs were associated with response rate in the discovery cohort (Table S6), six of which were also found in the replication cohort (Table 3, Supplementary Table S11). We did not find any DMP to be associated with the interaction between response rate and treatment, and no significant pathway was identified considering the 302 DMPs found (Table S7).

In our sensitivity analyses, we identified 750, 57, and 9,434 CpG sites associated with benzodiazepine use, risperidone dosage, and DUP, respectively (Supplementary Tables S12, S13, S14). However, only one CpG site associated with benzodiazepine use (cg18137040) and seven CpG sites associated with DUP (cg06758350, cg11115431, cg08922308, cg12477880, cg24760414, cg00994804, cg26364947) were also among the 302 DMPs associated with risperidone response rate. None of these 8 CpG sites overlapped with the 16 validated CpGs. After adjusting for these variables

in the response rate model, we found that methylation at five of these CpGs remained significantly associated with the response rate: cg18137040 (p=0.039), cg08922308 (p=0.013), cg12477880 (p=0.023), cg00994804 (p=0.043), and cg06758350 (p=0.039).

We also evaluated whether the validated CpGs held an association using a categorical definition of response. Individuals who showed at least a 50% reduction in the response rate were classified as responders. All 10 validated meta-analysis derived-DMPs and 6 EPIC-only derived-DMPs were associated with this categorical definition of response (nominal p-value<0.05) in the discovery cohort, and the direction of effects was consistent across all these CpGs in the replication cohort, with six being significant (one-sided p-value < 0.05 – Table S8). The DMP with the highest effect size was cg17253517, located in the *ARB2A* gene. In the discovery cohort, the mean difference in DNAm for this CpG between responders and non-responders was over 3% at both baseline and follow-up, indicating that this CpG is hypermethylated in non-responders before the initiation of treatment, and the methylation state does not change after 2 months of treatment (Table 3, Figure S7). In the replication cohort, the mean DNAm difference was 2.1% at baseline and 5.2% after 1 month of treatment (Table 3, Figure S7).

We found 3 DMRs associated with risperidone response rate and 13 DMRs associated with the interaction between response rate and treatment (Table 4). Only one region on chromosome 19 was replicated, spanning 288 bp and located in the body of the *SIPA1L3* gene (one-sided p-value=0.0395).

3.5. B cell estimated proportions is correlated with symptoms improvement

We did not identify an association between the NLR and response rate or its interaction with treatment (Table 2). Although the effect sizes were very small, the estimated proportions of NK cells, B cells, and monocytes showed an interaction between response rate and treatment (Table 2).

To better understand these interactions, we repeated the mixed-effects models and meta-analyses in the discovery cohort using the categorical definition of response (Figure 2A and Table S9). At baseline, the proportion of NK cells was higher in non-responders, with a mean difference of 1.1% compared to responders. After 2 months of risperidone treatment, NK cell proportions increased by 2.3% in responders and by 0.8% in non-responders, resulting in a mean difference of -0.4% (Table S9). For monocytes, non-responders had a lower proportion at baseline, with a mean difference of -0.8% compared to responders. After treatment, monocyte proportions increased by 0.1% in non-responders and remained unchanged in responders. At follow-up, the proportion of monocytes was higher in non-responders, with a mean difference of 0.2% (Table S9). Similarly, non-responders had a lower proportion of B cells at baseline, with a mean difference of -0.7% compared to responders. Following treatment, B cell proportions decreased by 0.8% in responders and remained unchanged in non-responders, resulting in a nearly null mean difference at follow-up (Table S9).

In the replication cohort, the association between response rate and estimated B cell proportion was confirmed (one-sided p-value=0.013, Figure 2B). After risperidone treatment, a lower B cell proportion was statistically correlated with a greater change in the response rate, indicating that the lower the proportion, the greater the improvement in symptoms. This aligns with the categorical response analysis in the discovery cohort, where it was observed that the B cell proportion decreased after treatment in responders but did not change in non-responders. These results suggest that, in addition to capturing variation in risperidone treatment, changes in estimated B cell proportions capture the variation in symptom improvement, and they are not associated with the psychotic state or schizophrenia diagnosis.

4. Discussion

This study presents the largest investigation to date exploring DNAm signatures and DNAm-derived cell-type proportions associated with short-term risperidone treatment and response rate in antipsychotic-naïve first-episode psychosis (anFEP) individuals, which was replicated in an independent cohort. Our study design offers several advantages. Firstly, we used blood samples from anFEP individuals, which eliminates the confounding effects of prior medication. Secondly, we included age- and sex-matched healthy controls from the same population, which helps disentangle the effects of the medication from the psychotic state itself. Thirdly, the inclusion of a replication cohort from a different population strengthens the generalizability of our findings. Lastly, by investigating DNAm and risperidone response longitudinally, we capture the dynamic changes at both phenotype and molecular levels associated with treatment effectiveness, a critical aspect overlooked in cross-sectional studies.

Our results highlight significant findings with important implications for understanding the role of DNAm in antipsychotic treatment. We identified two CpG sites associated with the risperidone treatment itself (cg04287747 and cg04732910), and two CpGs (cg17931986 and cg18058279) associated with the psychotic state. Intriguingly, our results showed increased methylation of cg17931986 in anFEP, whereas Li *et al.* (2021), using a cross-sectional design, observed hypomethylation at this CpG site in patients with a first episode of schizophrenia (FES) compared to controls (Li et al., 2021). Of note, FES patients may have been exposed to antipsychotic drugs and may have had up to three years of disease progression. This suggests that, although cg17931986 is hypermethylated in individuals experiencing their first episode of psychosis, DNAm levels at this CpG site could decrease as a result of medication exposure, as shown in our results, or psychosis progression, as might be indicated by Li *et al.* (2021). Furthermore, the gene annotated at this CpG, *COL11A2*, was among the most differentially methylated genes in eight previous schizophrenia EWAS from different tissues (Guo et al., 2023; Hannon et al., 2021; Li et al., 2021; Tesfaye et al., 2024; Vitale et al., 2017; Wockner et al., 2015, 2014). This convergence of evidence across various

research settings strengthens the candidacy of *COL11A2* as a potential therapeutic target or biomarker for schizophrenia and its treatment, with our findings specifically highlighting cg17931986 as a potential biomarker for predicting psychosis. The *COL11A2* (Collagen Type XI Alpha 2 Chain) encodes one of the two alpha chains of type XI collagen and lies within the major histocompatibility complex (MHC) region, which is a very complex region and is the second most gene-dense genomic region.

However, DNAm levels may capture more variation in response rate than in risperidone treatment itself, as302 DMPs and 16 DMRs associated with response to risperidone were found, indicating that future DNAm studies should focus more on response rate or the prognosis and symptom improvement. These results do not appear to be affected by benzodiazepine use before blood collection, risperidone dosage, or DUP, although further analyses focused on these specific variables should be conducted. The 16 validated CpG sites associated with risperidone response are annotated to genes that were differentially methylated in multiple EWAS. Among them, the *CD8A* stood out, being among the most associated genes in seven schizophrenia EWAS (Luo et al., 2021; Nie et al., 2021; Shen et al., 2021; van Eijk et al., 2015; Vitale et al., 2017; Wockner et al., 2015, 2014), and clozapine treatment in another study (Pérez-Aldana et al., 2022). This gene encodes the CD8α chain, a key component of the CD8 molecule expressed on the surface of CD8+ T cells (Ellmeier et al., 2013). The methylation levels at cg12606911, located in the CpG island near *CD8A* gene, were positively correlated with response rate and were validated in the replication cohort. This finding suggests a potential role for *CD8A* methylation in mediating response rate, warranting further investigation. Other convergent studies also identified that *RRM1* gene expression was downregulated in superior temporal cortex of SCZ cases using microarray (Sellmann et al., 2014), and single nucleotide polymorphisms in *SGIP1* were associated with major depressive disorder (Zhao et al., 2024), smoking and alcohol consumption (Saunders et al., 2022) and educational attainment (Okbay et al., 2022) in genome-wide association studies. Regarding the 16 DMRs, the one located in *SIPA1L3* was confirmed in the replication cohort and this gene had been among the most differentially methylated genes in schizophrenia EWAS (Nie et al., 2021; Wockner et al., 2014; Xiao et al., 2014) .

Our findings also suggest alterations in DNAm-derived cell-type proportions in anFEP individuals associated with both risperidone treatment and response rate. These results can be interpreted in two ways. The first possibility is that these alterations reflect a higher or lower number of immune cells in one comparison group compared to another at the time of blood collection. The second possibility is that these alterations indicate a widespread alteration in methylation of genes related to immune function, as previously proposed by (Luo et al., 2024).

We observed an increased estimated proportions of neutrophils and NLR, and decreased estimated proportions of $CD4^{+}$T, $CD8^{+}$T and NK cells in anFEP compared to controls. Notably, all estimated proportions except $CD8^{+}$T returned to control levels following treatment. These findings are consistent with previous DNAm studies reporting elevated estimated proportion of neutrophils and

reduced estimated proportions of CD8T, CD4T and NK cells in schizophrenia (Hannon et al., 2021; Kinoshita et al., 2014; Luo et al., 2024; Montano et al., 2016; Villar et al., 2023). Prior studies assessing blood cell counts directly (and not estimated from DNAm) have consistently reported increased neutrophils counts (Jackson and Miller, 2020; Steiner et al., 2019) and NLR in schizophrenia and FEP (Bioque et al., 2022; Karageorgiou et al., 2019). However, Karageorgiou et al. (2019) suggested antipsychotic treatment might influence these results (Karageorgiou et al., 2019), and, indeed, Steiner et al. (2019) observed a decrease in neutrophils and NLR in FEP after 6 weeks of antipsychotic treatment (Steiner et al., 2019). Our findings, aligned with these latter studies, suggest potential inflammatory alterations during acute psychosis that subside with treatment. This is further supported by our previous report showing risperidone treatment's suppressive effect on serum cytokine levels (Noto et al., 2015). Also plausible explanation is that acute psychotic episodes may elevate proinflammatory pathways related to stress response (Bioque et al., 2024), leading to altered circulating immune cells, and the use of antipsychotic medications in schizophrenia patients may further modulate the proportion of these cells. Thus, our findings may indicate alterations specific to anFEP that are modulated by antipsychotics.

While the majority of estimated cell-type proportions are associated with the psychotic state, B-cells are the only estimated proportion associated with the interaction between response rate and treatment, which was validated in the replication cohort. B-cells play a role in the adaptive immune system and are best known for antibody production; they can also secrete cytokines and chemokines. Few studies have identified a decrease in the number of B-cells in relation to clozapine (McAllister et al., 1989) and risperidone treatment (Müller et al., 2004). Interestingly, Steiner et al. (2010) found elevated B-cells during acute psychosis compared to controls, with a decline after antipsychotic treatment (Steiner et al., 2010). Moreover, different genetic studies have linked complex biological pathways of B-cell activation to schizophrenia. For example, enhancers active in CD19+ and CD20+ B-cells showed increased enrichment for genetic associations in the landmark GWAS on schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). There is also evidence from cell studies showing an association between B-cells and schizophrenia. Lago et al. (2020) observed abnormal cell signaling phenotypes in B-cells from patients with different neuropsychiatric diagnoses, including schizophrenia (Lago et al., 2020). Despite all this evidence suggesting an association between B-cells, schizophrenia and its treatment, a mechanistic framework explaining this association is still lacking (van Mierlo et al., 2019). Our results show that B-cell levels are associated with the interaction between short-term antipsychotic treatment and response rate, with changes in B-cell levels occurring over 1-2 months of treatment only in responders. This suggests that DNAm-derived B-cell estimated levels might serve as a proxy for the molecular mechanisms underlying treatment response. However, to better understand the implications of B-cells in antipsychotic treatment and response, new studies specifically focusing on B-cells in schizophrenia

and its treatment are warranted, particularly those using novel single-cell technologies, such as single-cell RNA sequencing, to perform a more in-depth phenotyping of B-cells.

While this is the largest EWAS to assess risperidone treatment and response rate, the sample size might be limited considering the vast number of CpG sites evaluated. To mitigate this, we validated our key findings in an independent cohort. Some differences between the cohorts (e.g. population background, disease stage, prior antipsychotic exposure and duration of treatment) might have limited broader validation. Despite these limitations, the validated results, particularly regarding B-cell response rate, suggest these response-related changes are independent of disease stage and associated with risperidone treatment, potentially changing within a month of treatment initiation.

We also need to interpret our results in the light of other limitations. First, blood cell type proportions were estimated based on methylation data and not absolute counts. Thus, a decrease in the proportion of one cell type could be a consequence of the increase of another or simply reflect differences in the accuracy of methylation estimates for various cell types, leading to variable sensitivity levels. Second, we did not account for potential confounding environmental exposures or somatic comorbidities, such as trauma, diet, stressful events, blood draw time, BMI or obesity, which can influence DNAm and may differ between populations. Third, our findings from blood samples might not fully capture disease-associated epigenetic changes occurring specifically in the brain. While we verified if similar results were reported in EWAS of brain tissues; it is worth noting that biomarkers do not need to reflect the underlying pathogenic process to be relevant. Fourth, although DNAm and cell type proportions are known to be influenced by genetic variation (Hannon et al., 2018, 2017, 2016a, 2016b; Kelly et al., 2024), we did not correct our results for it or population structure, although we validated findings in a different population. For the longitudinal analysis, this would likely not have a major impact. However, it could influence the observed correlations between DNAm and response rate.

Despite these limitations, our study offers groundbreaking insights into the dynamic interplay between DNAm, DNAm-derived cell-type proportions, and response to antipsychotic treatment in the early stages of schizophrenia. We report an association between DNAm-derived B-cell proportion and short-term treatment response in two independent longitudinal cohorts. These findings nominate B-cells as a potential biomarker for tailoring antipsychotic treatment strategies. Furthermore, by elucidating the connections between DNA methylation, immune function, and treatment response, this study paves the way for future investigations into the underlying mechanisms of schizophrenia pathophysiology. Additionally, our results can inform the interpretation of past and future case-control EWAS studies by disentangling the effects of antipsychotic medication and potentially revealing novel underlying mechanisms. However, further studies are needed to verify whether the results observed with DNAm-derived B-cell proportions replicate with B-cell counts in the blood of individuals at their first episode of psychosis and who are drug-naïve. Another question that should be

addressed is whether these results are specific to risperidone or can be reproduced by other antipsychotics as well.

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7. Contributors

VKO, SIB, LMS, AG, CSN, and SLH designed the study. VKO and LMS performed the data analyses and wrote the first draft of the manuscript. AKS and SLH supervised the data analyses. GOC, CSN, and MNN assisted with data collection. AVGB, CMC, SHL, and VKO conducted the biological experiments. AL, CGA, DGS, RPR, FB, SJ, and CMC collected data from the replication cohort and performed the initial analyses. RAB, GB, SIN, and SLH were the principal investigators of the funding grants. All the authors revised and approved the final manuscript.

8. Conflicts of interest

The authors have nothing to disclose

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Figure Legends

Figure 1: Study design for assessing DNA methylation (DNAm) to identify cell type proportions (CTP), differentially methylated positions (DMPs), differentially methylated regions (DMRs) (B) in relation to risperidone response rate and treatment in the discovery cohort (A) and replication cohort (C). After quality control, the discovery cohort was composed of 114 antipsychotic-naïve first-episode psychosis (FEP) individuals assessed at baseline and after two months of risperidone. The main results were compared to a sample of 115 healthy controls. The replication cohort was composed of 26 individuals with schizophrenia assessed before and after one month of risperidone treatment. Response was assessed using the Positive and Negative Symptoms Scale (PANSS).

Figure 2: (A) Violin and box plots for estimated B cells, NK cells and monocytes proportions. Red dots represent the mean within groups. Estimated proportions for the control group were plotted to estimate control levels. (B) Scatter plot of estimated B-cell proportions and percentage change in the total PANSS score after risperidone treatment in both discovery and validation cohorts.

Tables

Table 1: Characteristics of participants in discovery and replication cohorts.

	DISCOVERY COHORT				REPLICATION COHORT			
	Control (n=115)	**FEP (n=114)**	**Stats**	**p**	**SCZ cases (n=26)**			
Males (%)	60.9	66.7	χ^2 = 0.601	0.438	53.8			
Age (mean years ± SD)	25.8 ± 6.69	25.3 ± 6.92	U = 6159.5	0.430	32.8 ± 9.04			
Smoking score at baseline (mean ± SD)	-4.25 ± 2.85	-3.26 ± 3.96	U = 7448	0.075	-7.35 ± 3.01			
Smoking score at the follow-up (mean ± SD)	--	-3.04 ± 3.69	U = 7420 (*)	0.017	-7.54 ± 3.00			
	Responders (n=52)	**Non-responders (n=54)**	**Stats**	**p**	**Responders (n=16)**	**Non-responders (n=10)**	**Stats**	**p**
Males (%)	65.4	68.5	χ^2 = 0.019	0.892	37.5	80		0.051
Age (mean years ± SD)	24.7 ± 6.45	25.4 ± 7.30	U = 1355	0.759	32.3 ± 7.93	33.5 ± 11.0	U = 83	0.895
Smoking score at baseline (mean ± SD)	-3.70 ± 4.25	-3.18 ± 3.53	U = 1211	0.224	-8.29 ± 2.45	-5.86 ± 3.33	U = 44	0.060
Smoking score at the follow-up (mean ± SD)	-3.05 ± 4.12	-3.10 ± 3.47	U = 1252	0.758	-8.39 ± 2.33	-6.18 ± 3.56	U = 53	0.165
Total PANSS score at baseline (mean ± SD)	93.4 ± 24.1	89 ± 19.5	t = 1.041, df = 104	0.301	92.2 ± 12.1	89.5 ± 17.3	t = 0.477, df = 24	0.637
Total PANSS at the follow-up (mean ± SD)	48.6 ± 11.4	74.1 ± 15.2	t = -9.636, df = 100	< 0.001	49.4 ± 10.8	72.9 ± 13.4	t = -4.929, df = 24	< 0.001

FEP: first-episode psychosis. SCZ: schizophrenia. SD: standard deviation. U: statistics from the Mann-Whitney U test. χ^2 : statistics from the Chi-squared test. t: statistics from the Student's t test. (*) smoking scores of FEP individuals at the follow-up were compared with control scores.

Table 2: Meta-analysis results of the estimated cell-type proportions associated with 2-months of risperidone treatment, with psychotic state and response rate considering common effect model in the discovery cohort.

Estimated Proportions	anFEP (ref) vs. FEP-2M			Controls (ref) vs. anFEP			Controls (ref) vs. FEP-2M			Response			Response*Time point Interaction		
	Estimate	SE	p	Estimate	SE	p	Estimate	SE	p	Estimate	SE	p	Estimate	SE	p
NLR	-0.651	0.176	**<0.001**	0.883	0.222	**<0.001**	0.141	0.126	0.262	0.0003	0.0049	0.956*	0.0032	0.002	0.64
Neutrophils	-0.036	0.011	**0.001**	0.063	0.015	**<0.001**	0.018	0.015	0.239	< -0.0001	0.0004	0.958*	0.0001	0.004	0.86
$CD4^+$T cells	0.012	0.004	**0.006***	-0.025	0.007	**<0.001**	-0.004	0.006	0.559	0.0001	0.0002	0.517	0.0001	0.002	0.75
$CD8^+$T cells	0.015	0.003	**<0.001***	-0.030	0.005	**<0.001**	-0.014	0.006	**0.016**	-0.0001	0.0001	0.259*	-0.0001	0.001	0.61
NK cells	0.014	0.003	**<0.001**	-0.013	0.004	**0.004***	-0.002	0.005	0.758	-0.0001	0.0001	0.375	0.0003	0.001	**0.02**
B-cells	-0.006	0.003	0.035	-0.001	0.004	0.879	-0.004	0.003	0.199	0.0001	0.0001	0.215*	-0.0002	0.001	**0.07**
Monocytes	0.002	0.002	0.459	0.001	0.003	0.694	0.004	0.003	0.249	0.0001	0.0001	0.151	-0.0002	0.001	**0.06**

Estimated cell proportions associated with the outcome after correction for multiple comparisons (Bonferroni correction for 3 comparisons – $p < 0.017$) are highlighted in bold. anFEP: antipsychotic-naïve first-episode of psychosis. FEP-2M: first-episode of psychosis after 2-months of risperidone treatment. Ref: reference level. (*) Between-study heterogeneity measure I^2 statistic > than 40%

Table 3: Results of differentially methylated positions (DMPs) associated with the risperidone response rate in the discovery and replication cohorts. Response rate was defined as the percentage change in total PANSS score. For the discovery cohort, statistics were derived from a meta-analysis using a common effect model, except for EPIC-only probes, which used mixed-effects regression models. Statistics for the replication cohort were also derived from mixed-effects regression models.

			Discovery Cohort						Replication Cohort				
CpG ID	chr	Gene	Mean Diff. Baseline	Mean Diff. Follow-up	Estimate	SE	p-value	I2	Mean Diff. Baseline	Mean Diff. Follow-up	Estimate	SE	One-sided p
cg12606911	2	*CD8A*	-0.010	0.010	0.0093	0.0018	2.42 E-07	0	-0.014	-0.011	0.0004	0.0002	0.048
cg13052034	1	*SGIP1*	0.001	0.015	-0.0055	0.0011	1.64 E-06	86	0.031	0.011	-0.0013	0.0008	0.038
cg17253517	5	*ARB2A*	0.035	0.037	-0.0047	0.0010	4.73 E-06	0	0.021	0.052	-0.0012	0.0006	0.022
cg26312920	7	*NEUROD6*	-0.005	0.003	0.0040	0.0009	4.89 E-06	21	-0.004	-0.016	0.0006	0.0003	0.001
cg22315844	6		-0.016	-0.014	0.0051	0.0011	5.44 E-06	0	-0.069	-0.060	0.0014	0.0006	0.011
cg08289130	16		0.038	0.028	-0.0082	0.0018	7.73 E-06	0	0.045	0.039	-0.0019	0.0009	0.016
cg18678763	11	*RRM1*	-0.008	-0.005	0.0052	0.0012	1.07 E-05	73	-0.014	-0.005	0.0005	0.0003	0.036
cg27401945	10		0.012	0.012	-0.0049	0.0011	1.09 E-05	0	0.067	0.049	-0.0015	0.0009	0.042
cg08713568	1		0.016	0.016	-0.0033	0.0008	2.67 E-05	0	0.021	0.022	-0.0008	0.0004	0.018
cg03316587	8		-0.010	-0.021	0.0031	0.0008	2.75 E-05	43	0.009	-0.005	0.0008	0.0004	0.038
cg12188634*	5		-0.040	-0.034	0.0066	0.0012	3.91 E-07	--	-0.043	-0.004	0.0017	0.0006	0.002
cg21250689*	2		0.027	0.023	-0.0064	0.0012	5.02 E-07	--	0.028	0.046	-0.0012	0.0006	0.024
cg22083301*	11	*YIF1A*	-0.020	-0.022	-0.0041	0.0008	9.39 E-06	--	-0.020	-0.024	0.0007	0.0004	0.030
cg24662224*	2	*RFX8*	0.006	0.006	-0.0034	0.0007	6.71 E-06	--	0.005	< 0.001	-0.0002	0.0001	0.032
cg03959141*	20		-0.044	-0.036	0.0087	0.0017	7.38 E-07	--	-0.041	-0.047	0.0013	0.0007	0.033
cg03335213*	20	*EYA2*	-0.030	-0.023	0.0063	0.0011	5.48 E-07	--	-0.048	-0.003	0.0010	0.0006	0.039

I2: degree of heterogeneity. (*) Epic-only probes. Mean differences were calculated as [average of beta values in non-responders] – [average of beta values in responders]

Table 4: Differentially methylated regions (DMRs) associated with risperidone response rate and with the interaction between response rate and time point in the discovery cohort. The DMR validated in the validation cohort is highlighted in bold.

Outcome	Chr	Start BP	End BP	n	Genes	Estimate	SE	Adj-pval
Response	2	3933739	3934010	2	---	-0.019	0.003	1.55E-03
	17	73824354	73824620	3	*UNC13D*	-0.016	0.003	1.90E-02
	17	171006	171257	4	*RPH3AL*	-0.016	0.003	4.75E-02
Interaction	1	93646459	93646505	2	*TMED5; CCDC18*	-0,011	0,002	2.79E-02
	19	39881719	39881847	2	*PAF1; MED29*	-0.010	0.001	1.29E-07
	11	93474015	93474146	3	*C11orf54; TAF1D*	-0.011	0.002	4.55E-02
	3	44626453	44626538	3	*ZNF660*	-0.010	0.002	2.66E-02
	17	41856489	41856518	3	*DUSP3; C17orf105*	-0.009	0.002	2.25E-04

13	45563688	45563889	4	*KIAA1704; NUFIP1*	-0.009	0.001	2.02E-04
19	**38572847**	**38573135**	**4**	***SIPA1L3***	**0.006**	**0.001**	**1.93E-03**
17	80050646	80051063	4	*FASN*	0.010	0.002	8.29E-04
22	29702810	29702866	5	*GAS2L1*	-0.010	0.002	8.74E-03
5	153418426	153418565	5	*MFAP3; FAM114A2*	-0.008	0.002	1.78E-02
5	176074358	176074720	5	*TSPAN17*	-0.008	0.001	1.81E-03
3	126422945	126423272	8	*CHCHD6*	-0.007	0.001	7.07E-03
3	47844180	47844655	10	*DHX30*	-0.006	0.001	4.35E-03

Figures

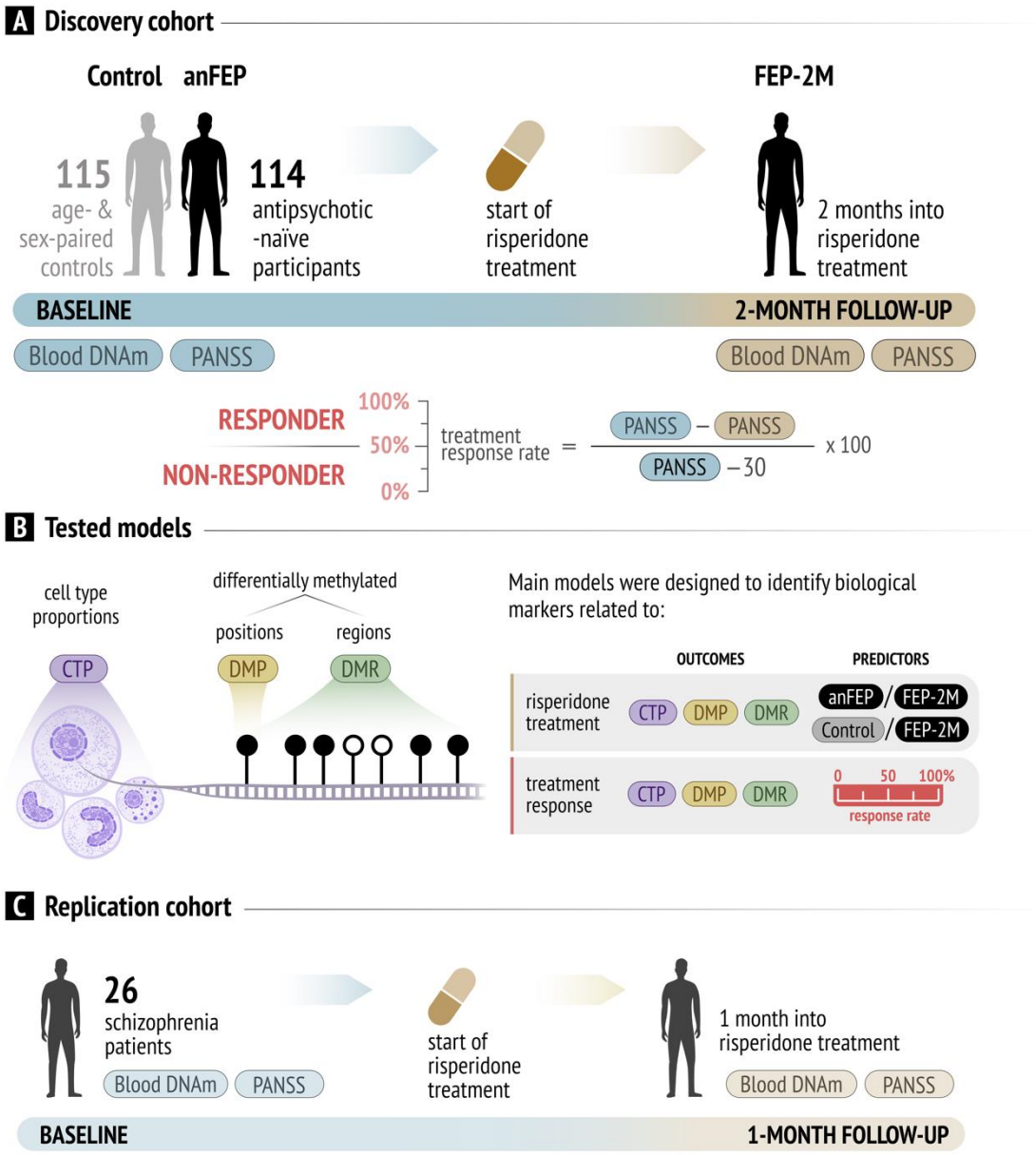

Figure 1: Study design for assessing DNA methylation (DNAm) to identify cell type proportions (CTP), differentially methylated positions (DMPs), differentially methylated regions (DMRs) (B) in relation to risperidone response rate and treatment in the discovery cohort (A) and replication cohort (C). After quality control, the discovery cohort was composed of 114 antipsychotic-naïve first-episode psychosis (FEP) individuals assessed at baseline and after two months of risperidone. The main results were compared to a sample of 115 healthy controls. The replication cohort was composed of 26 individuals with schizophrenia assessed before and after one month of risperidone treatment. Response was assessed using the Positive and Negative Symptoms Scale (PANSS).

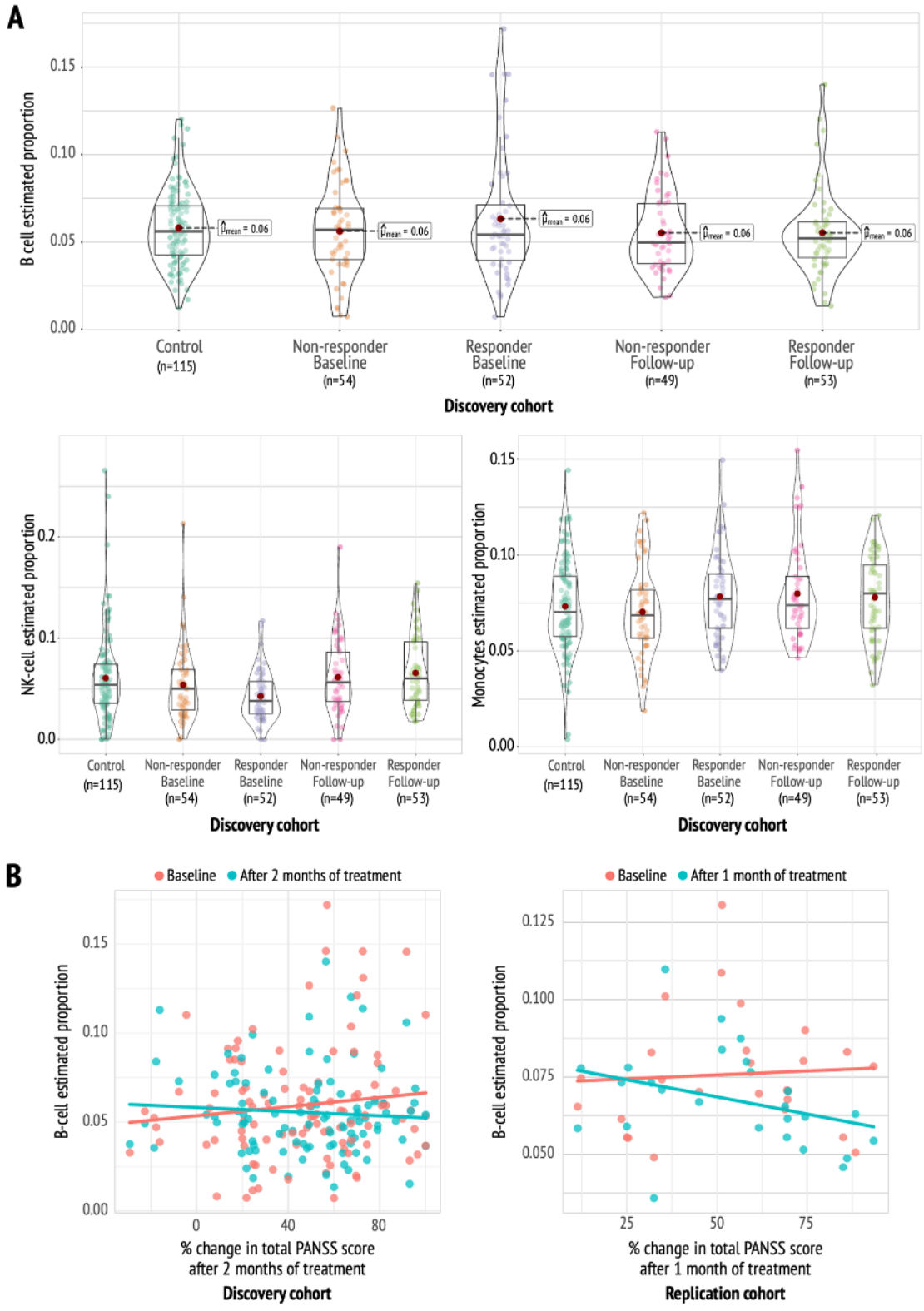

Figure 2: (A) Violin and box plots for estimated B cells, NK cells and monocytes proportions. Red dots represent the mean within groups. Estimated proportions for the control group were plotted to estimate control levels. (B) Scatter plot of estimated B-cell proportions and percentage change in the total PANSS score after risperidone treatment in both discovery and validation cohorts.

Ethical statements

The Research Ethics Committee of the Federal University of Sao Paulo (UNIFESP) approved the research protocol (CEP 0603/10, CAAE 89057218.7.0000.5505 and CAAE: 48242015.9.0000.5505), and all participants or family members provided written informed consent before enrollment. The project was also approved by the Research Ethic Committee of Norway (REK #686185)

Highlights

- Blood DNA methylation predicts risperidone response
- 302 CpG sites and 16 regions linked to risperidone response
- B cell changes predict risperidone response
- Risperidone alters DNA methylation
- Risperidone normalizes immune cell levels

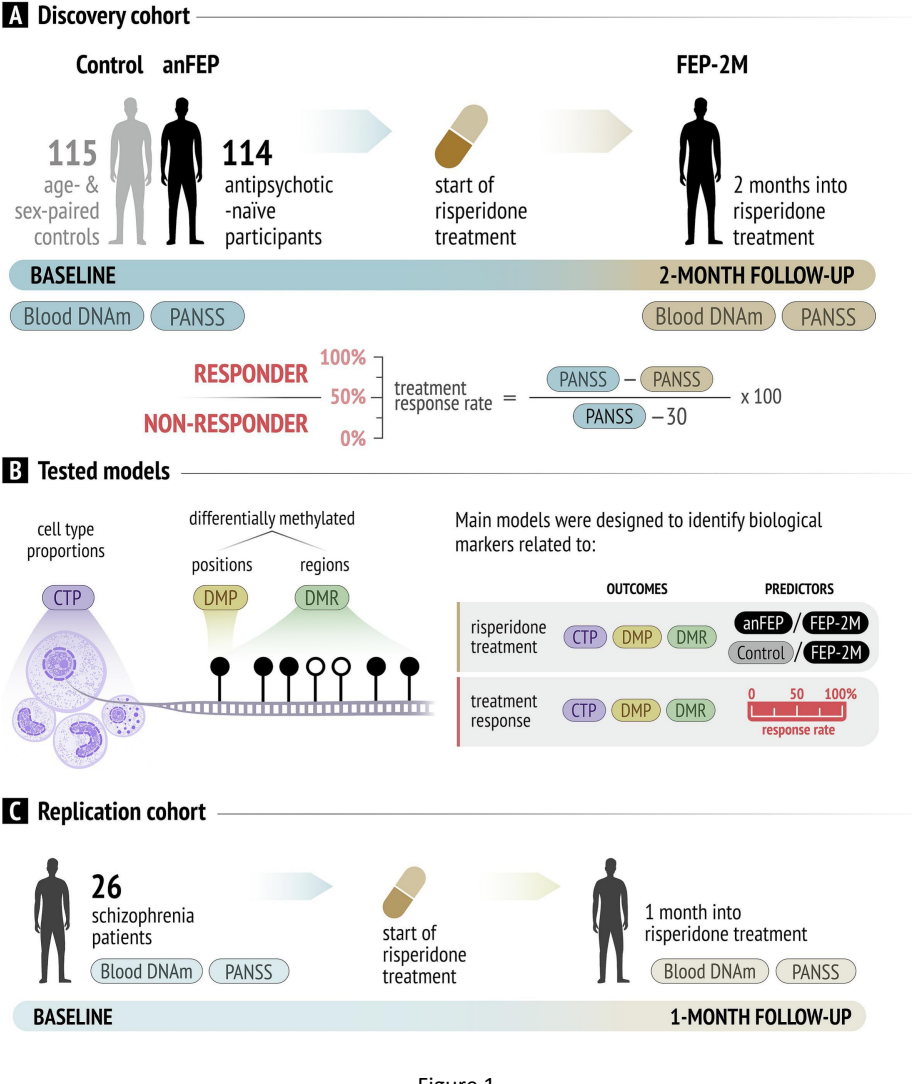
A Discovery cohort
Control
anFEP
FEP-2M
115
age- & sex-paired controls
114
antipsychotic -naïve participants
start of risperidone treatment
2 months into risperidone treatment
BASELINE
2-MONTH FOLLOW-UP
Blood DNAm
PANSS
Blood DNAm
PANSS
RESPONDER
NON-RESPONDER
100%
50%
0%
treatment response rate
PANSS − PANSS / PANSS − 30
x 100
B Tested models
cell type proportions
differentially methylated
positions
regions
CTP
DMP
DMR
Main models were designed to identify biological markers related to:
OUTCOMES
PREDICTORS
risperidone treatment
CTP
DMP
DMR
anFEP / FEP-2M
Control / FEP-2M
treatment response
CTP
DMP
DMR
0
50
100%
response rate
C Replication cohort
26
schizophrenia patients
Blood DNAm
PANSS
start of risperidone treatment
1 month into risperidone treatment
Blood DNAm
PANSS
BASELINE
1-MONTH FOLLOW-UP

Figure 1

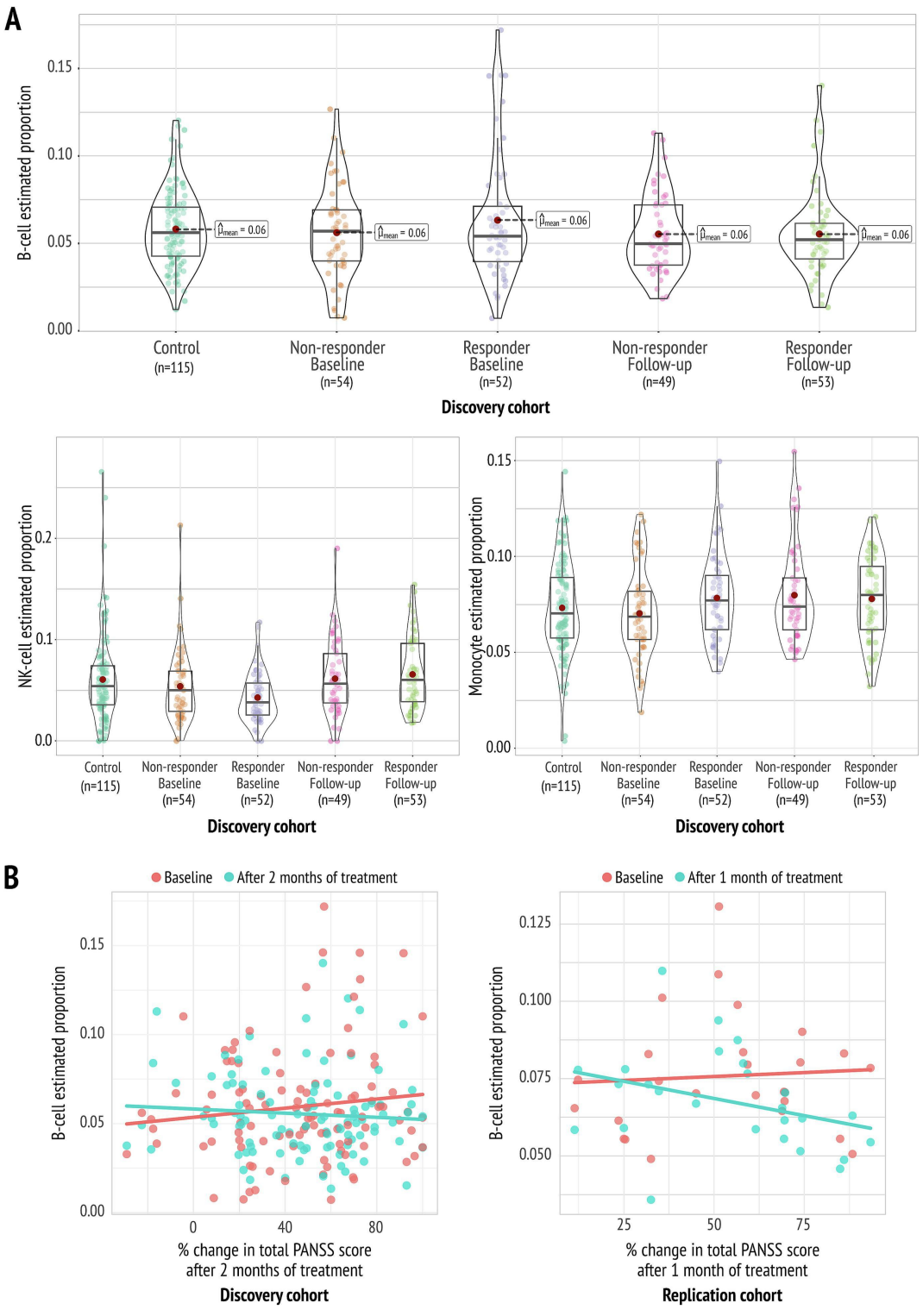
A
B-cell estimated proportion
0.15
0.10
0.05
0.00
$\hat{\rho}_{mean}$ = 0.06
Control (n=115)
Non-responder Baseline (n=54)
Responder Baseline (n=52)
Non-responder Follow-up (n=49)
Responder Follow-up (n=53)
Discovery cohort
NK-cell estimated proportion
0.2
0.1
0.0
Monocyte estimated proportion
0.15
0.10
0.05
0.00
B
Baseline
After 2 months of treatment
B-cell estimated proportion
0.15
0.10
0.05
0.00
0
40
80
% change in total PANSS score after 2 months of treatment
Discovery cohort
Baseline
After 1 month of treatment
0.125
0.100
0.075
0.050
25
50
75
% change in total PANSS score after 1 month of treatment
Replication cohort

Figure 2