Exploring the mediation of DNA methylation across the epigenome between childhood adversity and First Episode of Psychosis—findings from the EU-GEI study

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Running title
Epigenetic mechanisms between adversity and psychosis

Abstract: 300
Main text: 5439

**Abtract**
Studies conducted in psychotic disorders have shown that DNA-methylation (DNAm) is sensitive to the impact of Childhood Adversity (CA). However, whether it mediates the association between CA and psychosis is yet to be explored. Epigenome wide association studies (EWAS) using the Illumina Infinium-Methylation EPIC array in peripheral blood tissue from 366 First-episode of psychosis and 517 healthy controls was performed. Adversity scores were created for abuse, neglect and composite adversity with the Childhood Trauma Questionnaire (CTQ). Regressions examining (I) CTQ scores with psychosis; (II) with DNAm EWAS level and (III) between DNAm and caseness, adjusted for a variety of confounders were conducted. Divide-Aggregate Composite-null Test for the composite null-hypothesis of no mediation effect was conducted. Enrichment analyses were conducted with *missMethyl* package and the KEGG database. Our results show that CA was associated with psychosis (Composite: OR = 1.68; p = <0.001; abuse: OR = 2.16; p<0.001; neglect: OR = 2.27; p=<0.001). None of the CpG sites significantly mediated the adversity-psychosis association after Bonferroni correction (p<8.1x10-8). However, 28, 34 and 29 differentially methylated probes associated with 21, 27, 20 genes passed a less stringent discovery threshold (p<5x10-5) for composite, abuse and neglect respectively, with a lack of overlap between abuse and neglect. These included genes previously associated to psychosis in EWAS studies, such as *PANK1*, *SPEG TBKBP1, TSNARE1 or H2R.* Downstream gene ontology analyses did not reveal any biological pathways that survived false discovery rate correction. Although at a non-significant level, DNAm changes in genes previously associated with schizophrenia in EWAS studies may mediate the CA-psychosis association. These results and associated involved processes such as mitochondrial or histaminergic disfunction, immunity or neural signalling requires replication in well powered samples. The lack of overlap between mediating genes associated with abuse and neglect suggests differential biological trajectories linking CA subtypes and psychosis.

## Introduction

Childhood adversity (CA), in the form of abuse and neglect, is associated with psychotic disorders[1]. Its effect is not limited to psychosis onset but also to a broad range of poor outcomes such as cognitive impairment, social cognitive deficits, and functional outcomes[2, 3] as well as poorer prognosis in various clinical dimensions[4]. Several biological processes have been implicated in the CA-psychosis dyad, such as alterations in neurogenesis[5], dopamine dysregulation[6], alterations in the hypothalamic pituitary adrenal axis[7] via its action in the hippocampus, or oxidative stress dysregulation[8]. However, evidence is often limited to correlational analyses between biomarkers involved in such pathways and CA in patients and controls, or regression analyses examining their association with psychosis as the outcome against healthy controls. Indeed, a recent literature review, examining formal mediation analyses using CA as exposure and psychosis as outcome, revealed very limited evidence at the time of the search in July 2019[9]. This shows that more research formally testing mediational pathways linking CA (in various forms) and psychosis is needed. This research should focus on large curated samples of patients in the early phase of disease in order to better understand this relationship[9].

In recent years, DNA methylation (DNAm), the most commonly studied epigenetic modification, has been proposed as a mechanism by which early adversities influence biological processes through the modulating of gene expression that can later exert negative pleiotropic effects of CA on mental health[10, 11]. A recent review on potential links between CA and DNAm in psychiatric conditions suggests that DNAm may be a potential mediator linking CA and various disorders, including psychosis[12]. Despite this suggestion of a mediating mechanism, a major limitation of available evidence is the lack of formal mediation models testing this hypothesis. Moreover, an epigenome-wide association study (EWAS) examining the influence of CA in people with psychotic disorders has not been conducted.

The traditional regression approach to mediation analysis proposed by Baron and Kenny et al.,1986[13], widely used in the social sciences, calculates mediation effect as the product of the effect of the exposure on the mediator and the effect of the mediator on the outcome. Tests, such as the Sobel test and the joint significant tests, [14] are commonly

used methods to detect mediation effects. However, it has been demonstrated that these commonly used tests perform poorly in genome wide analyses[15], for three reasons: (1) the association signals are generally weak and sparse with limited sample sizes; (2) the heavy multiple testing burden that needs to be adjusted for; (3) the composite null nature of the mediation effect testing that has not been taken into account[16]. In this context, the Divide-Aggregate Composite-null has been developed and validated, proving to be a powerful large-scale testing procedure that overcome such limitations and that can be applied efficiently to EWAS data[15, 17]. "

In the current study, we will address for the first time whether CA, measured as a composite cumulative measure of adversity, is associated with DNAm changes in individuals with a First Episode of Psychosis (FEP) and whether these changes mediate the CA-psychosis association. We will use the DACT method to test for the mediation effects between CA and psychosis at an EWAS scale[17]. Second, given recent evidence of a differential effect of abuse and neglect on various clinical dimensions[4] suggesting a different biological underpinning, similar analyses will be conducted for abuse and neglect separately. This will allow to test whether this differential effect also relates to biological underpinnings in the form of DNAm Supplementary material (SM) (S. **Figure 1**). Lastly, enrichment analyses will be conducted to explore whether the CA and psychosis-associated differential methylated positions (DMP) cluster onto any relevant biological pathway for psychosis aetiopathogenesis.

Methods

**Study design and participants**

The sample was drawn from the EU-GEI (European Network of National Schizophrenia Networks Studying Gene–Environment Interactions) multi-centre study[18]. The EU-GEI study is a multicentre incidence and case–sibling–control study of genetic and environmental determinants of psychotic disorders[18]. The current study was based on participants from work the 'Incidence and first-episode'[18] work package of the EU-GEI study. For the analyses presented in this paper, only participants who had complete data on DNAm and CA using the CTQ were included, with no restriction on site or

ethnicity. Patients and controls were recruited from 17 different sites. Cases and controls were not related. During the case ascertainment period, participants, aged 18–64 years, were invited to take part in the study if they presented to mental healthcare services with a FEP.

Patients were identified by clinically trained researchers who carried out regular checks across the 17 catchment areas. Exclusion criteria included previous treatment for psychosis, a diagnosis of organic psychosis (ICD-10: F09) or transient psychotic symptoms resulting from acute intoxication (ICD- 10: F1X.5), and language barriers. The diagnosis was confirmed by the Operational Criteria Checklist for Psychotic and Affective Illness within the EU-GEI consortium[19, 20]. As described by Gayer-Anderson et al. 2020[18], research teams were overseen by a psychiatrist with experience in epidemiological research and included trained research nurses and clinical psychologists. Control participants without a lifetime diagnosis of psychotic disorder were recruited from the same population as the cases using guided random and quota sampling strategies. Exclusion criteria for both controls and cases included intelligence quotient <70 and language barriers. Written informed consent was obtained and an institutional review board (IRB) approval was obtained from all centres. Teams received training in epidemiological principles and incidence study design to minimise non-differential ascertainment bias across different local and national health care systems.

**Socio-demographic characteristics**

Socio-demographic data were collected using the Medical Research Council (MRC) Socio-demographic Schedule modified version[21], and supplemented with additional information from medical records on educational attainment, employment, marital and living status. Ethnicity was self-ascribed using categories employed by the 2001 UK Census (http://www.ons.gov.uk/ons/guide-method/census/census-2001/index.html).

**Childhood Trauma Questionnaire (CTQ)**

CTQ[22, 23] was used to measure the exposure to past experiences of abuse (sexual, physical, and emotional), neglect (physical and emotional) and to calculate a composite

measure of cumulative exposure of the five types of experiences. This self-reported instrument enquires about such types of events occurring prior to the age of 18, with answers ranging from 'never true', through 'rarely true', 'sometimes true', 'often true', to 'very true'. This yields a total score and five sub-scores for physical abuse, emotional abuse, sexual abuse, physical neglect, and emotional neglect. The reliability and validity of the CTQ have been demonstrated previously[22]. For this study, data were dichotomised for each childhood adversity domain (0 = 'absent' and 1 = 'present'), based on the moderate to severe cut-off score from the CTQ Manual[23] as follows: ≥13 for emotional abuse; ≥10 for physical abuse; ≥8 for sexual abuse; ≥15 for emotional neglect; and ≥10 for physical neglect. We used a composite category involving the cumulative exposure score to any form of the five adversities (score ranging 0-5), to abuse subtypes (score ranging 0-2; "0" being no abuse, "1" being one abuse and "2" being two or three exposures to different abuse experiences), and to neglect subtypes (score ranging 0-2; "0" being no neglect, "1" being one neglect and "2" being two different neglect exposures). We have used this approach of cumulative score rather than adding the total raw CTQ scores given meta-analytic evidence of a dose-response effect of cumulative trauma in psychosis using similar methods[1], also observed in a larger EUGEI sample[24]; and given previous reports examining the same method when exploring the DNAm signature of trauma[12, 25, 26]. Additionally, exploratory analyses have revealed that the effect of CTQ in psychosis (path *c*) is greater when using the cumulative score based on the predefined severity threshold, rather than the raw original total score SM ("*CTQ operationalisation analyses*").

**Genome-wide DNA methylation analysis**

*Illumina EPIC chip processing*

Genomic DNA was extracted using standard protocols[27, 28]. Whole-blood genomic DNA diluted with water (50 ng/µl) was treated with sodium bisulfite using the EpiTect® Bisulfite Kit from QIAGEN® following the manufacturer's protocol. DNAm was assessed using the Illumina Infinium HumanMethylationEPIC BeadChip kit (Illumina, Inc., San Diego, California) and quantified on an Illumina HiScan System (Illumina, Inc.). The level of methylation is expressed as a 'beta' value (β-value), ranging from 0 (no cytosine methylation) to 1 (complete cytosine methylation). [29]. Data pre-processing and downstream statistical analyses were conducted using R version 3.6.0[30].

*Quality control procedures*

Quality control of the data in cases and controls was performed by applying the following steps using the *watermelon* R package[31]: (i) checking the signal intensity and removing probes with a signal below 1500 (N=25); (ii) removing duplicates (N=26); (iii) checking and removing probes when they had less than 80% of conversion with bisulfite (N=5); (iv) gender check by using the clusterGender' function (k-means clustering of principal components highly correlated with gender r > 0.5 to form 2 clusters) and removing those that did not match their reported gender (N=8); (v) sample methylation data was compared to their genotyped data using the 15 SNPs common to both arrays, removing those with a correlation < 0.9 (N=4); (vi) potential confounding effect of batch effects was checked; (vii) CpG sites with a detection p-value of>0.05 in 1% of the samples identified by the filter function within the *watermelon* R package[31] were removed (N=1); (viii) bead count per CpG was performed; (ix) testing case control differences in terms of epigenetic age, cell composition, smoking status; comparing the signal between blood and buccal samples. At a later stage, using a previous EPIC quality control pipeline https://github.com/PGC-PTSD-EWAS/EPIC_QC, it was detected that an additional 3330 CpG probes corresponded to probes located in SNPs, and these were removed prior to final analyses. DNAm was explored in 614719 probes at EWAS level after quality control procedures.

From the initial EU-GEI sample that had data on DNAm, 49 participants were excluded because of missing or unreliable data on CTQ, 1 participant because of missing smoking score, and another 1 due to ambiguous information on age. A further 3 cases were excluded because they were taking clozapine which can modulate DNAm in those with schizophrenia (SCZ) [32]. This final data set was comprised of 883 participants (366 FEP cases and 517 controls). All data pre-processing and downstream statistical analyses were performed using R version 3.6.0. QQplots and regional Manhattan plots were generated using the R packages *qqman*[33].

*Confounders*

Cell-type composition (including monocytes, CD8T, CD4T, natural killers, B cells and granulocytes), was estimated using the Houseman algorithm[34] to adjust for the

potential differential cellular heterogeneity. Smoking has been shown to affect the DNAm signature, as described in a recent review[12], therefore smoking was accounted for with a calculated smoking score. This continuous variable consists of a weighted sum of effect sizes of DNAm values on 183 established CpG sites, subtracted from the mean of non-smokers. This score has been shown to be significantly higher in smokers compared to non-smokers and highly correlate with smoking dosage in previous studies[35]. It has been developed in order to capture the real epigenetic impact of smoking; avoiding the common tendency to underreport smoking habits[36, 37] and also allowing to capture passive smoking which is very difficult to assess[38]. To rule out possible confounding effects of medication, a binary category including current use of antipsychotics (yes or no) was included in analyses. Given heterogeneity in populations, 10 principal components (PC) were calculated and included in the models. Other covariates such as batch effects, site (ascertainment area where the study was conducted), gender and age were included in the analyses. Lastly, we conducted a set of preliminary analyses examining whether educational attainment (EA) was a significant confounder that should be included in our models. First, we examined the possible confounding effect of EA on the association between CTQ scores and psychosis status and our results revealed that in our sample the addition of EA to the model (along with age sex, country and CTQ score) did not change the effect of CTQ score on psychosis (CTQ composite coefficient 1.674; p-value 0.000 with EA and 1.680; p-value = 0.000 without EA). Moreover, we conducted a correlation analysis between CTQ composite, and EA and the correlation proved to be non-existent (r= -0.073; p-value= 1.000). Therefore, given these two sets of analyses, we concluded that the possible confounding effect of EA on CTQ exposure was not major, and we decided not to include it in the final models described below. Details can be found in SM ("*Additional analyses section*").

**Statistical analyses**

To statistically test for mediation, a set of regressions where conducted to examine whether the changes in DNAm EWAS level are at least partially responsible for the effect of CA (cumulative score, abuse, and neglect scores) on psychosis, using data on the 883 participants (cases and controls together): (i) the total effect of CA on psychotic disorder versus controls (path *c* – Scenario 1 in **Figure 1**), was calculated using a

logistic regression model using the Glm function (glm(case/control~ CTQ +age+sex+country, family = binomial(link = "logit"); (ii) the effect of CA on DNAm level on each of the 614719 EWAS probes (path *a* – **Figure 1**) was calculated using the linear regression model: lm(DNAm in each probe~CTQ +age+sex+cell types+smoking score+batch+10 PC+ antipsychotics+country); (iii) disease status (case-control) was regressed on DNAm and the confounders with logistic regression (path *b*) by the model glm(case/control~ (DNAm in each probe)+age+sex+cell types+smoking score+batch+10 PC+ antipsychotics+country, family = binomial(link = "logit") (codes are available upon request).

Then, to test each DNAm probe for mediation of the CA-psychosis association, the Divide-Aggregate Composite-null Test (DACT) was performed. As illustrated in **Figure 1**, mediation effect is present if paths *a* and *b* are both non-zero for the same probe. The null hypothesis of no mediation is a composite of 3 scenarios: that either path *a* or path *b* pathways, or both, are absent (**Figure 1**). DACT uses the EWAS epigenetic data to estimate the frequencies of these three scenarios among the DNAm probes and use this information to create a composite p-value for testing whether paths *a* and *b* are both non-zero for each given probe. This test has been recently validated[16] and shown to be more powerful than more traditional tests such as the Sobel test and the joint significance test or Max P[14]. Details on the development and validation of this method can be found in Liu et al., 2021[16]. Separate mediation analyses were conducted with childhood adversity (composite), abuse and neglect as the outcome variable.

(paste Figure 1 here)

We used Bonferroni corrected p-value significance threshold of p<8.1x10-8 (as calculated by 0.5/614719: total number of probes that passed stringent QC)), and a suggestive nominally-significant *P*-value threshold of *P* < 5e-05 based on others studies using EPIC array[39, 40]) was established to identify DMPs mediating CA with psychosis,

**Gene ontology pathway analysis**

Illumina UCSC gene annotation was used to create a test gene list from the mediating DMPs (*P* < 5e-05). Gene ontology and pathway analysis were performed, using the

*missMethyl* package[41], which takes into account the variable number of EPIC probes associated with each gene. Downstream KEGG pathways analyses were also performed to provide further insights into potential relevant biological processes associated with the significant DMPs (*P* < 5e-05), according to previous studies[42]. Independent pathways with FDR <0.05 were considered to be significantly associated with CA subtypes, and pathways with p<0.05 were also reported.

**Results**

Characteristics of the cases and controls of the samples used for the current study are presented in S. **Table 1** (SM), and demographic and baseline characteristics comparison between the cases include in this study and the whole EU-GEI sample can be found in SM **Table 2**. FEP patients taking part in this study tended to be more often of white ethnic background compared to the others but did not differ in terms of age, gender, adversity scores, diagnosis of non-affective psychosis and years of education.

**Path *a*: the influence of CTQ on DNA methylation in cases and controls**

For analyses on the composite measure of adversity, none of the DMP passed EPIC-array Bonferroni threshold significance; however, we identified 35 nominally-significant (*P* < 5e-5) severe adversity-associated involving 24 genes DMPs (S. **Table 3** and **SM Figure 9, SM**). Among these, the top 5 ranking probes were associated with genes including *C1orf168, EVPL, CHRNB4, PLAT, and HLA-J.*

For analyses restricted to abuse subtype, although none of the DMP passed EPIC-array Bonferroni threshold significance, we identified 35 severe adversity-associated DMPs that passed the nominally-significant threshold (*P* < 5e-5; S. **Table 4** and **Figure 10, SM**) spanning across 25 genes, among which the top 5 genes were *EVPL, PTPRS, WAC, NHS, and RFX3.*

For the neglect subtype, we reported one DMP passed EPIC-array Bonferroni threshold significance, called cg11476306 and located on chr 19 and in gene *PIP5K1C*; in addition, we identified 20 nominally-significant psychosis-associated DMPs (*P* < 5e-5; **S. Table 5** and SM **Figure 11, SM**) spanning across 15 genes. The top 5 genes were *PIP5K1C, APLP1, MEGF11, PDE4D,* and *CD276.*

**Path *b*: examining the influence of DNAm in cases controls status**

None of the probes reached Bonferroni significance, however our data revealed 34 nominally-significant severe adversity-associated DMPs (*P* < 5e-5; **S. Table 6** and **S. Figure 12**) spanning across 21 genes, including *APLN, TFEB, DPYSL3, PRAME,* and *MUC6*.

**Path *c*: examining the influence of DNAm in cases controls status**

The composite cumulative score of CTQ, as well as abuse and neglect scores were positively associated with psychosis risk (OR = 1.68; p = <0.000) abuse score OR = 2.16; p<0.001; neglect: OR = 2.27; p=<0.001). Results were adjusted by age, sex and country.

**The mediating role of DNAm between CA (composite, abuse and neglect) and cases/control status**

For analyses with the composite measure of adversity, none of the DMP passed EPIC-array Bonferroni threshold significance, however, we identified 28 nominally-significant severe adversity-associated DMPs (*P* < 5e-5) for mediation, spanning across 21 genes, whose characteristics can be found in **Table 1. Figure 2** is a Manhattan plot displaying the EWAS results:

(paste table 1 and Figure 2 here)

For analyses limited to abuse, none of the DMP passed EPIC-array Bonferroni threshold significance, nevertheless, we identified 34 nominally-significant severe adversity-associated DMPs for mediation(*P* < 5e-5) spanning across 27 genes **(Table 2).** Interestingly, six of these 27 genes overlapped with the genes found for composite measure of adversity (*HRH2, HDAC5, HDAC5, NEK6, DMD, BSND,* and *LRRC27*), **S Figure 13 (SM)** is a Manhattan plot displaying the EWAS results:

(paste table 2 here)

Analyses limited to neglect revealed that none of the DMP passed EPIC-array Bonferroni threshold significance. However, 29 severe adversity-associated DMPs passed the "discovery" P-value of $P < 5e\text{-}5$ for mediation, spanning across 20 genes, that are displayed in **S. Table 7 (SM)**. Of these 20 genes, 10 overlapped with the genes found for composite measure of adversity (*PANK1, SPEG, TBC1D16, ANKFY1, C9orf24, ZHX2, TTC7B, ITGAX, HNRNPUL2,* and *TTC9C*). **S Figure 14** (SM) shows a Manhattan plot displaying the EWAS results.

As can be seen in **Figure 3**, none of the mediating genes involved in the abuse psychosis path overlap with those involved in the neglect psychosis path, while some overlap was present between the composite measure and these two adversity subtypes.

(paste figure 3 here)

**Gene ontology enrichment analyses**

The genes associated with differentially methylated probes that passed the nominally significant threshold ($P < 5e\text{-}5$) (for composite adversity, abuse and neglect) were identified. Exploratory downstream enrichment analyses were performed in those genes using *missMethyl* package with the KEGG data set. The top 10 ranked biological pathways based on DMPs located in the gene body are summarised in the SM (**S. Tables 8**, **9** and **10** respectively). None of the pathways survived the FDR adjustment.

(paste table 3 here)

**Discussion**

To our knowledge, this study represents the first analysis that utilised a genome-wide approach to explore whether DNAm mediates the relationship between CA, and for the subtypes abuse and neglect in people with a FEP. We used the DACT approach, specifically developed to address mediation effects in EWAS data[16], that allowed us to interrogate mediating effects of DNAm in 614719 CpG sites across the entire genome.

Although none of the analyses survived to Bonferroni correction, we report nominally significant ($P$ < 5e-5) DMPs, located in multiple genes of interest, mediating the association between composite measure of adversity, abuse, and neglect with psychosis. Some of these have been previously associated with various phenotypes, including SCZ, as shown in **Table 3**. These included *PANK1, SPEG, TTC7B, ZHX2, HDAC5, NEK6*, *PKNOX2, TSNARE1; TMEM114, SORT1, PPP2R2D*, *VARS, NMB* and *LRRC27.* One of the replicated genes, *PANK1*, is the top ranked gene mediating both the composite measure of adversity and neglect with psychosis, which has previously been associated to SCZ in an EWAS study[43]. It codes a protein belonging to the pantothenate kinase family, which is a key regulatory enzyme in the biosynthesis of coenzyme A (CoA) in mammalian cells, important for oxidation of fatty acids, and the oxidation of pyruvate in the citric acid cycle[44]. This enzyme has a crucial role in mitochondrial functioning, which has recently appeared as key biological process involved in psychosis aetiopathogenesis[45]. *SPEG* was the second top ranked gene for both analyses with composite adversity and neglect. Two previous EWAS studies have found that increased methylation at this level was associated with SCZ[32, 43], and another found an association with Alzheimer Disease[46]. This gene encodes a protein with similarity to members of the myosin light chain kinase family, essential for myocyte cytoskeletal development. The top ranked DMP (cg08457495) for analyses of abuse lies within a CpG island at the level of the TSS1500 within the gene *TBKBP1*, which is an important gene regulating immunity. *TBKBP* and its homologue IKKε are serine–threonine kinases that mediate the induction of type I Interferon in antiviral innate immunity[47]; although its molecular role in psychiatric conditions remain unknown, a recent GWAS study in frontotemporal dementia patients found that a genetic variant within that loci was associated with the condition[48]. Abundant evidence supports the role of neuroinflammation and altered immune processes in the aetiopathogeneses of psychosis[49, 50]. Various EWAS studies having found DMPs located in genes involved in the immune system in those with SCZ [51-54], as well as related to CA in different conditions such as borderline personality disorder and post traumatic stress disorder[55-57]. Exploring the specific implications of DNAm changes in *TBK1* in immune processing involved with the disorder is a necessary target for future research.

(paste Table 3 here)

Another important gene that appeared among the top ranked DMPs mediating composite adversity and psychosis is *TSNARE1.* As shown in **Table 3,** it has also been associated to SCZ [32], and with child abuse in EWAS studies[58]. Importantly, a GWAS study involving SCZ patients of Caucasian ancestry identified that two SNPs within *TSNARE1* were associated with SCZ[59], which was later replicated in another whole exome sequency study[60]. A follow-up study in a Chinese population with SCZ confirmed that the minor allele of the SNPs (rs10098073) within *TSNARE1* was associated with a reduced risk of SCZ. Furthermore, gene expression data also points to a dysregulation of *TSNARE1* in SCZ, and another study in pluripotent stem cells showed synergistic effects between *TSNARE1* with other common variants associated to SCZ in altering pre and post synaptic neuronal deficits[61]. *TSNARE1* plays key roles in docking, priming, and fusion of synaptic vesicles with the presynaptic membrane in neurons, thus synchronizing neurotransmitter release into the synaptic cleft[62], and experimental preclinical studies suggest that it is a negative regulator of endosomal trafficking in cortical neurons[63]. Altogether, strong genetic, epigenetic, gene expression and stem cells in vitro evidence point at a possible involvement of *TSNARE1* in SCZ, with epigenetic evidence suggesting a concurrent involvement of CA in DNAm changes in this gene, which provides a possible pathway in the CA-SCZ association. Other genes involved in the neurodevelopment of the central nervous system that require further exploration are the *ITGAX,* involved in the modulation of neural differentiation through its action on microglia[64]; *ADGRG1*, involved in myelinisation processes[65, 66] and extracellular matrix[67, 68]; and *MFF*, involved in oligodendrocytes formation[69]. All these processes are important for SCZ aetiopathogenesis[70-72], and therefore are of interest for future research.

None of our gene ontology enrichment analyses passed the FDR threshold, therefore drawing conclusions on the potential biological pathways that stem from our enrichment analyses is not possible. Taking this limitation into consideration, a preliminary exploration of the top 10 biological pathways that survived a more conservative p-value of < 0.05 could still provide some insights, as previously done by others[42, 73]. The top 1 and top 5 pathways for composite measure of adversity and abuse respectively was the histamine-induced gastric acid secretion, led by *HRH2,* (coding for the histamine receptor H2), which was the top 4 gene for composite adversity and

abuse. Action on the histaminergic system alongside serotoninergic (5-HT), norepinephrinergic modulation putatively underlies the beneficial effect of atypical antipsychotics in mood and anxiety disorders [74]. The *H2R* is a G-protein coupled receptor located post-synaptically that has high expression in the central nervous system (CNS), as well as the heart and stomach[75, 76]. Within the CNS, *H2R* has a high density of expression in the cortex, basal ganglia, amygdala, and hippocampus[77]. *H2R* is commonly co-expressed with *H1R* which may account for their similar excitatory function, such as memory consolidation, inflammation, and motor function[78-81]. Clinically, *H2R* antagonists are widely used to inhibit gastric acid secretion[82]. However, following a case report detailing the resolution of acute psychotic symptoms of a patient treated with famotidine, 7 RCTs have explored treatment of SCZ with *H2R* antagonists. These studies have had mixed findings on improvement of positive and negative symptoms. Nevertheless, the impact on cognition was not included in their analyses, which should be considered for future research given preclinical findings suggesting the role of *H2R* on cognitive processes[83-89]. Genetic variations of *H2R* in SCZ have been studied and subsequently identified *H2R* 64949G allele, the presence of which was 80% more frequent in patients with SCZ compared to controls, while homozygous manifestations of the allele were found to be 180% more frequent in patients[90, 91]. Given the above, future studies examining the implications of *HR2* DNAm on clinical outcomes, and its modulating effect in cognitive processes could be an important target for future research.

In the current study, we conducted analyses separately for the subtypes of abuse and neglect in order to explore whether DNAm changes related to abuse overlapped or differed with those related to neglect. This question stems from recent findings showing a differential impact of both type of adversities on psychopathological outcomes in those with psychosis, with abuse being specifically related to the positive symptoms of psychosis, while neglect appears to be specifically linked to the negative dimension, suggesting different trajectories[4]. Our results, (illustrated in **Figure 3**) on DNAm show a lack of overlap in the genes that passed the discovery P-value between abuse and neglect, while some overlap was present between the composite measure and these two adversity subtypes: 6/27 DMP associated genes were related to abuse and the

composite measure, while this was the case for 10/20 of those from neglect analyses. Although we could not relate the DNAm changes to the psychopathological domains, as described in the limitations, our results suggest different biological signatures of DNAm according to the type of traumatic experience, in the onset of psychotic disorder. Future studies exploring the DNAm relationship between these two adversities with positive and the negative dimensions of psychosis respectively will pose an interesting research area.

This study presents various strengths and limitations. A first relevant strength is the use for the first time in psychiatric populations of the DACT[16] a novel approach specifically designed to explore mediation by DNAm epigenome wide, which takes into account the composite nature of the null hypothesis of no mediation, improving the power of more traditional methods such as the Sobel test and the joint significance test[14]. A second strength is the sample of FEP with a relatively young population of cases (30.5 year old sample mean age), which limits the influence of chronicity of the psychotic disease on epigenetic changes, the impact of age itself[92], as well antipsychotic medication, which is known for being an important modulator of DNAm[93]. Third, we controlled for known important confounders in epigenetic studies including smoking [94], cell type composition in blood[95], PCs as well as antipsychotic medication, thereby, disentangling their confounding effects on DNAm. Fourth, DNAm was quantified using the Illumina EPIC array, which to date is the most robust and highly reliable, currently the best high-throughput platform with content spanning regulatory regions associated with the majority of known annotated genes, allowing us to explore 614719 CpG sites across the entire epigenome. Lastly, we used various forms of adversity measures, not only limiting our analyses to the broader composite measure where the specific biological underpinnings are diluted hampering the study of specific effects.
Nonetheless, the results from our study should be considered with care in light of some limitations. First, we could not map our findings on DNAm related to abuse and neglect with positive and negative dimension of psychosis respectively, as the sample EU-GEI was not assessed with an instrument that could capture symptom severity. This would have been interesting, given previous findings showing a differential effect of those adversities in the respective dimensions[4], as mentioned above. Second, the direction of the mediation is difficult to interpret with the information presented in the study.

Whereas a positive mediation could suggest that hypermethylation associated to CA may increase the risk of psychosis, and inversely; previous work shows that such direction (hyper or hypomethylation) is strongly dependent on the genotype, which has not been accounted for in this study. Furthermore, although increased methylation is generally associated to repression of gene expression, this is not always the case[96], and without measures of gene expression, it is not possible to elucidate the impact of DNAm at a molecular level in each specific gene, therefore limiting the understanding of the downstream implication of our results. Therefore, our results remain exploratory and deserve future attention with more specific hypotheses related to specific genes, where the effect of genotype is accounted for, and the functional consequences of DNAm are explored molecularly. Third, despite the current study being the largest to date interrogating the epigenetic signature of DNAm in relation to CA in those with psychosis, it is still underpowered, and a larger sample may be needed in future, to replicate our results and test the same hypotheses in other sources such as saliva or post-mortem brains, given the relatively low blood-brain correlation of DNAm markers[97]. Fourth, different biological processes operate differently across various developmental stages, therefore considering the timing of trauma could give important insights into which processes operate at different ages. Unfortunately, CTQ does not report the age of exposure to CA, which prevents us examining this in the current work. We hope that this will be the object of future attention. Fifth, we have conducted mediation analyses in a cross-sectional study, and although traumatic events were recorded before psychosis onset, we cannot exclude that psychosis itself may lead to changes in the DNAm, and not the other way around as we assume, therefore, posing a reverse causation issue. Therefore, longitudinal studies examining the outcome after the DNAm changes are required. Lastly, we could not account for maternal education which in multiple studies have shown to been used as general cognitive index and has shown to be associated with CA exposure. Although our preliminary analyses revealed that educational attainment (another proxy of cognition) was not related to adversity exposure in our sample, we cannot exclude that other socio-economic variables may have played a confounding role in our results.

In conclusion, the present study was underpowered to identify putative mediation of the impact of CA on psychosis by DNA methylation. Although none of our DMP reached statistical significance based on Bonferroni correction, we reported a number of nominally significant DMPs ($p < 5e-05$) that are associated with genes that have been previously implicated in the pathogenesis of SCZ in genetic and epigenetic studies, as well as a number of novel candidate genes. For example, we reported differential DNAm in genes associated in immune and neurodevelopmental process, as well as the dopaminergic and glutamatergic pathways, which is in line with recent literature in the field suggesting a mediating role of such pathways between CA and psychosis[12]. Although none of our enrichment analyses revealed pathways surviving the FDR correction, the top ranked biological process involved the histaminergic function, which can be an important target for future research, given the direct link with psychopathology and medication therapeutic effects in patients with SCZ. Lastly, low overlap between mediating genes and pathways according to abuse and neglect suggests that biological trajectories between CA and psychosis are distinct depending on the type of adversity.

**Aknowlegments**

We thank all the contributors to the EU-GEI (WP2 group) study for their hard work: Kathryn Hubbard, Stephanie Beards, Simona A. Stilo, Mara Parellada, Pedro Cuadrado, José Juan Rodríguez Solano, Angel Carracedo, David Fraguas, Álvaro Andreu-Bernabeu, Gonzalo López, Bibiana Cabrera, Esther Lorente-Rovira, Paz Garcia-Portilla, Javier Costas, Estela Jiménez-López, Mario Matteis, Marta Rapado-Castro, Emiliano González, Covadonga M. Díaz-Caneja, Emilio Sánchez, Manuel Durán-Cutilla, Nathalie Franke, Fabian Termorshuizen, Daniella van Dam, Elsje van der Ven, Elles Messchaart, Marion Leboyer, Franck Schürhoff, Stéphane Jamain, Grégoire Baudin, Aziz Ferchiou, Baptiste Pignon, Jean-Romain Richard, Thomas Charpeaud, Anne-Marie Tronche, Flora Frijda, Giovanna Marrazzo, Crocettarachele Sartorio, Fabio Seminerio, Camila Marcelino Loureiro, Rosana Shuhama, Mirella Ruggeri, Chiara Bonetto, Doriana Cristofalo, Domnico Berardi, Marco Seri, Elena Bonora, Giuseppe D'Andrea, Laura Ferraro, Giada Tripoli, Silvia Amoretti, Gisela Mezquida

## Conflicts of interest

M Bernardo has been a consultant for, received grant/research support and honoraria from, and been on the speakers/advisory board of AB-Biotics, Adamed, Angelini, Casen Recordati, Janssen-Cilag, Menarini, Rovi and Takeda. Dr. Arango. has been a consultant to or has received honoraria or grants from Acadia, Angelini, Gedeon Richter, Janssen Cilag, Lundbeck, Minerva, Otsuka, Roche, Sage, Servier, Shire, Schering Plough, Sumitomo Dainippon Pharma, Sunovion and Takeda. Dr Peter B. Jones declare to have consulted for Ricordati and Janssen. Dr Murray has received payments for non-promotional seminars from JANSSEN, SUNOVIAN, LUNDBECK AND OTSUKA

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## Figures legends

### **Figure 1** Interpreting the output of mediation analyses

*Footnote: Rejection of the hypothesis that DNA methylation level mediates the effect of childhood adversity on psychosis requires both paths "a" and "b" to be non-0. Thus, the null hypothesis is not rejected if path "a" (scenario 2), or path "b" (scenario 3), or both paths (scenario 1), are 0. An estimate of the overall mediation effect is obtained by multiplying the estimates for the effects of paths "a" and "b".*

**Figure 2.** Manhattan plot showing the DNAm mediating DMP between composite cumulative measure of adversity and psychosis.

Footnote: *Red line: array-wide multiple testing threshold (p<5.8x10-8); blue line: "discovery" p-value threshold (P < 5e-5).*

**Figure 3** Diagram showing the mediating overlapping genes between composite, abuse and neglect analyses with psychosis.

Footnote: *while 6 mediating genes (22.2%) from the composite analyses where common with the abuse analyses, and 10 (50%) were common with the neglect analyses, no overlap was observed between the abuse and neglect analyses.*