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Supplemental Material to:

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MB-COMT promoter DNA methylation is associated with working-memory processing in schizophrenia patients and healthy controls

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Supplementary Materials

SM 1 Methods

SM 1.1 Clinical Measures

All study participants underwent an extensive clinical diagnostic assessment that included either a Structured Clinical Interview for DSM disorders (SCID-I/P or NP)[1] or the Comprehensive Assessment of Symptoms and History (CASH).[2] Severity of positive and negative symptoms was rated using the Scale for Assessment of Negative Symptoms (SANS)[3] and the Scale for Assessment of Positive Symptoms (SAPS).[4] Parental socioeconomic status (SES) was determined using the Hollingshead index.[5] Handedness was measured using the Annett Scale of Hand Preference.[6]

Antipsychotic history was collected as part of the psychiatric assessment using the *Psychiatric Symptoms You Currently Have* (PSYCH) instrument[7] and cumulative and current antipsychotic exposure was calculated using the chlorpromazine conversion (CPZ) conversion factors.[8] To calculate cumulative dose years, the following formulas were applied:

*Cumulative dose years = [(Dose in mg/day) * (Days on dose)] / [conversion factor * (365.25 days)]*

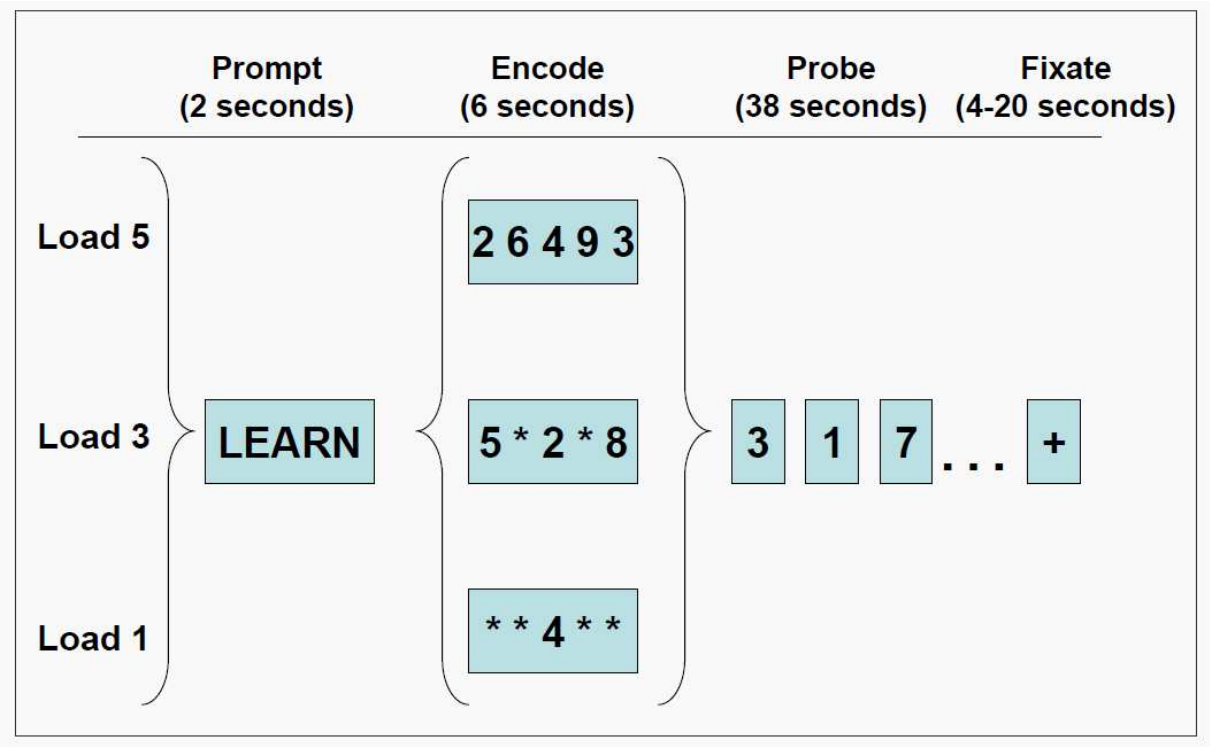
For current antipsychotic exposure, we converted all current doses into chlorpromazine units, using the formula below:

*Chlorpromazine units = [Dose drug (mg/day) / Conversion factor] * [100 CPZ units/mg]*

SM 1.2 The Sternberg Item Recognition Paradigm

In this study, the identical block-design SIRP paradigm was used across all 4 acquisition sites as shown in SM figure S1 and previously described.[9] Participants practiced the paradigm before scanning until they understood the task well enough to perform at a greater-than-chance level of accuracy. Each block began with a 2-

second prompt to begin (“learn”), followed by a 6-second presentation of a memory set, composed of one (load 1), three (load 3), or five (load 5) digits, constituting three levels of working memory load (Encode phase). The Encode phase was followed by a 38-second presentation of 14 digits, one at a time for 1.1 seconds each (the Probe phase). During the Probe phase, participants responded to each probe using a button box to indicate whether or not the probe digit was in the memory set. The participants responded using the thumbs of each hand, with the designated target thumb randomly assigned to the right or left hand. Each of the three runs included two 46-second blocks of each of the three load conditions (Encode-Probe sequence, 6 blocks total/run), presented in a pseudorandom order with the blocks of each condition alternating with fixation epochs (4-20 seconds).



SM Figure S1. Graphical illustration of the Sternberg Item Recognition paradigm.

SM 1.3 Image acquisition and processing

The T1-weighted structural brain scans at each of the four sites were acquired with a coronal gradient echo sequence: TR=2530 ms for 3T, TR=12ms for 1.5T; TE=3.79 for 3T, TE=4.76ms for 1.5T; TI=1100 for 3T; Bandwidth=181 for 3T, Bandwidth=110 for 1.5T; 0.625×0.625 voxel size; slice thickness 1.5 mm; FOV, 256×256×128 cm matrix; FOV=16 cm; NEX=1 for the 3T, NEX=3 for the 1.5T.

For all sites, functional images were acquired by using single-shot echo-planar imaging with identical parameters [orientation: AC–PC line; number of slices = 27;

slice thickness=4 mm, 1 mm gap; TR=2000 ms; TE=30ms (3T) or 40ms (1.5T), FOV=22cm; matrix 64×64; flip angle=90°; voxel dimen sions = 3.44×3.44×4 mm.

Cross-site calibration and reliability of these acquisition sequences for each scanner and the experimental set up for functional imaging as well as potential site and scanner differences were investigated prior to the study.[10,11] Additionally, test-retest reliability of functional and structural imaging data from ten MCIC subjects, who were all scanned at all four sites, was analyzed. Results showed that, even with different scanner models and field strengths, activation variability due to site differences is small compared to variability due to subject differences.[10–12]

Structural data, necessary for image registration purposes and dorsolateral prefrontal cortex (DLPFC) label generation, were analyzed using FreeSurfer. Segmentation and surface reconstruction quality were assured by manual inspection of all raw MRI volumes, segmented volumes in three planes and pial as well as inflated volumes.

Functional images were registered to a high-resolution T1 image of the same subject (using a new algorithm called Boundary-Based Registration)[13] and to the standard space defined by the MNI-152 atlas. We did this by first registering the T1 images to the standard brain using FLIRT[14,15] and then transposing the functional-to-T1 and T1-to-standard registrations in one step.

We fit a general linear model to the fMRI time course at each voxel in a whole-brain model to estimate the average activation during the three loads of the probe condition in all trials. Equal weight was given to all working memory loads. The magnitude of each ‘Contrast of Parameter Estimate’ (COPE), along with an estimate of its variability derived from model residuals, was passed to a second-level fixed effects analysis to combine COPE’s from separate runs, yielding a composite t-statistic map for each contrast of interest for each subject. The t-maps for each contrast were transformed into z-maps. A z-value of 2.3, corresponding to an uncorrected one-tailed voxel-wise p-value threshold of 0.01, was then used as a cluster-forming threshold to define contiguous clusters of activation. In the end, cluster significance was calculated based on Gaussian random field theory with a p-value of 0.05.

For functional images, quality assurance steps included checks for whole-brain coverage of brain masks, motion and global mean intensity outlier time points, alignment of structural and functional scans, and registration errors (Epi to T1 and T1

to template). Outlier time frames in each fMRI data time series (detected using the artefact detection tools (ART)[16] were defined by: *(a)* Global mean image intensity that differed by more than three standard deviations from the mean of the entire series of time frames in a scan, *(b)* Displacement due to motion by more than 1 mm in the x, y or z direction relative to the previous time frame, or *(c)* Rotation due to motion by more than 0.1 rad around any of the three axes relative to the previous time frame. We removed the outlier time frames through the use of nuisance regressors in the linear model. In the case of runs where more than 15% of the time frames were flagged as outliers, the entire run was dropped from the analysis or the subject had to be excluded.

SM 1.4 DNA methylation preprocessing

Approximately 200 ng of DNA was used to analyze each subject sample according to the manufacturer's protocol (Illumina, San Diego, CA). During bisulfite conversion of genomic DNA, unmethylated cytosines convert to uracil, while methylated cytosines remain unchanged. DNA was then subjected to whole-genome amplification. This step can introduce biases, especially when the amplification method is PCR-based as the decrease of Taq-like enzyme activity during denaturation cycles have been reported to affect amplification yield.[17] However, Illumina amplification methods are PCR-independent and instead based on random hexamer priming and Phi29 DNA polymerase, which has a proofreading activity resulting in error rates 100 times lower than the Taq polymerase. After whole-genome amplification and fragmentation, DNA samples were applied to the BeadChip and anneal to locus-specific 50mers during hybridization. Locus discrimination is provided by a combination of sequence-specific hybridization capture and allele-specific, single-base primer extension. Two probes are used to interrogate each CpG locus. The 3' end of the primers is positioned directly across from the CpG site. Allele-specific Extension of the primer incorporates a biotin nucleotide or a dinitrophenyl labeled nucleotide (C and G nucleotides are biotin labeled; A and T nucleotides are dinitrophenyl labeled.) Signal amplification of the incorporated label further improves the overall signal-to-noise ratio of the assay.

SM 1.5 Intensity data extraction

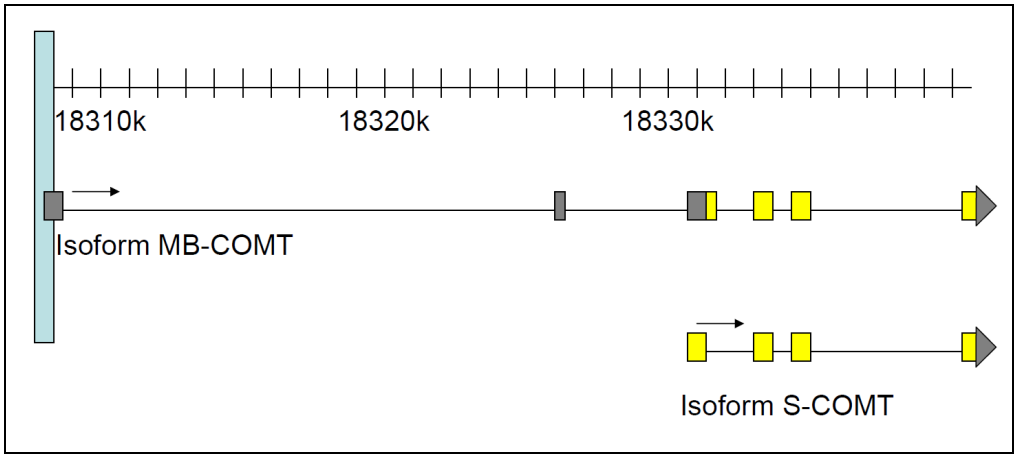
Data processing was performed according to Illumina's instructions. To estimate the methylation status, each CpG site is represented by two bead types: a methylated probe (M) and an unmethylated probe (U). The methylation level of a CpG site is then estimated based on the intensity of the M and U probes using the following formula:

$$\beta = \text{Max}(M,0) / [\text{Max}(M,0) + \text{Max}(U,0) + 100].$$

DNA methylation β-values vary between 0 and 1, representing the degree of methylation.

SM 1.6 Genotyping

Blood samples were obtained from 234 participants and sent to the Harvard Partners Center for Genetics and Genomics for DNA extraction. All DNA extraction and genotyping was done blind to group assignment. Genotyping was performed at the Mind Research Network Neurogenetics Core Lab using the Illumina HumanOmni-Quad BeadChip. Quality control steps included the following steps. SNPs on the X or Y chromosome, or those with a genotyping rate of less than 90% or a minor allele frequency of less than 5% were excluded from the analysis. We also removed participants with extreme heterozygosity values (+/- 3SD). The final data set consisted of 749,968 SNPs. Quality control steps were carried out with PLINK, 1.07.[18]



SM Figure S2. Location of the investigated genomic region (blue bar) based on Hapmap data (NCBI, build 36).

SM 2 Results

Site	Sample		Sex		Age	WRAT-IIIRT	Parental SES	Handedness
			(female)		(years)			
		N	N	%	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
UI	SCZ	21	3	14.3	31.38 (8.96)	48.30 (5.15)	2.30 (0.66)	0.86 (2.87)
	HC	49	22	44.9	30.29 (10.53)	50.08 (4.13)	2.84 (0.43)	0.73 (2.64)
MGH	SCZ	25	7	28.0	37.92 (9.81)	45.09 (8.49)	3.40 (1.12)	0.61 (1.92)
	HC	20	8	40.0	41.00 (9.33)	52.40 (3.36)	3.00 (1.03)	1.20 (3.12)
UMN	SCZ	21	5	23.8	31.38 (10.33)	46.29 (5.42)	2.52 (0.75)	2.19 (3.97)
	HC	16	7	43.8	31.88 (11.21)	50.88 (4.21)	2.38 (0.81)	0.50 (0.82)
UNM	SCZ	15	5	33.3	33.47 (13.10)	44.71 (7.55)	3.00 (1.18)	1.67 (3.37)
	HC	17	3	17.6	30.41 (12.68)	51.76 (3.36)	2.00 (0.61)	1.35 (2.69)

SM Table S1. Basic demographic characteristics by acquisition site. Abbreviations: WRAT-IIIRT, reading subtest of the Wide Range Achievement Test - III; SES, socio-economic status; handedness, Annett Handedness Scale; SCZ, schizophrenia patients; HC, healthy controls.

Site	Sample	Length of illness	Cumulative antipsychotic drug dose, atypical	Cumulative antipsychotic drug dose, typical	Current antipsychotic drug dose, atypical	Current antipsychotic drug dose, typical	Negative symptoms	Positive Symptoms
(Tesla)	(SCZ patients)	(years)	(dose years)	(dose years)	(CPZ units)	(CPZ units)	(SANS score)	(SAPS score)
	N	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
1.5	67	11.29 (9.96)	16.62 (28.36)	34.91 (134.52)	319.53 (365.76)	103.72 (280.31)	8.00 (3.97)	4.57 (2.92)
3	15	9.50 (11.34)	8.33 (9.70)	0.80 (2.18)	194.64 (229.59)	141.67 (548.67)	9.00 (3.72)	4.53 (2.56)
Total	82	10.96 (10.18)	15.10 (26.12)	28.44 (121.65)	297.12 (347.39)	110.84 (342.38)	8.18 (3.92)	4.56 (2.84)

SM Table S2. Clinical variables of schizophrenia patients. Means and standard deviations (SD) are given. We found no differences in length of illness, typical and atypical cumulative or current antipsychotic medication, negative and positive symptoms between the acquisition site-specific scanner field strengths. Abbreviations: CPZ, chlorpromazine; SANS, Scale for Assessment of Negative Symptoms [3]; SAPS, Scale for Assessment of Positive Symptoms [4].

Correlation with *MB-COMT* promoter methylation		
	Spearman's rho	*P*
only Schizophrenia patients		
Cumulative Antipsychotic drug dose, atypical	0.14	0.20
Cumulative Antipsychotic drug dose, typical	0.15	0.20
Current Antipsychotic drug dose, atypical	0.02	0.88
Current Antipsychotic drug dose, typical	-0.13	0.24
Length of illness	0.14	0.21
Negative symptoms	0.19	0.08
Positive symptoms	-0.12	0.30

SM Table S3. Spearman correlations between the *MB-COMT* methylation and typical and atypical cumulative or current antipsychotic drug dose, length of illness and negative or positive symptoms in the patients group only.

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