Supplemental Information

5-hydroxymethylcytosine alterations in the human postmortem brains of autism spectrum disorder

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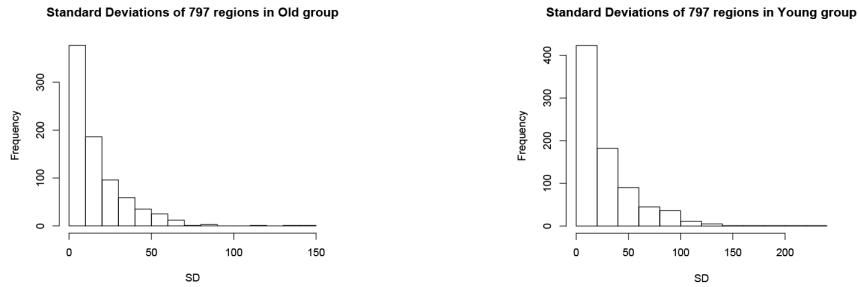[3]The Children's Hospital and Institute of Translational Medicine, School of Medicine, Zhejiang University, Hangzhou, China 310029;
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Patient ID	Total Reads #	% of Alignment	Age	Gender	Group
Control group					
451	44,668,515	95.67	4	M	**Young**
4327	75,105,434	92.82	5	F	
616	38,340,897	61.97	12	M	
2149	57,972,549	73.19	16	M	
1409	37,454,869	67.25	18	M	
4271	34,232,319	70.61	19	M	**Middle**
5251	47,087,627	71.99	19	M	
1442	26,703,654	80.54	24	M	
1455	25,334,592	77.55	25	M	
1981	30,662,106	71.66	25	M	
5873	29,502,164	70.01	28	M	
1324	31,411,904	66.33	29	M	
5079	117,444,657	94.91	33	M	
3253	19,437,149	83.64	40	F	**Old**
7333	33,741,537	76.61	41	M	
4781	31,233,175	94.13	45	M	
7119	56,579,404	71.54	48	M	
6259	26,660,508	65.98	50	M	
6206	20,206,052	64.36	54	M	
ASD group					
4671	46,075,726	92.23	4	M	**Young**
5308	56,995,682	92.08	4	M	
7014	64,538,681	82.34	11	M	
4899	16,622,004	41.88	14	M	
3924	30,660,924	63.69	16	F	
4099	34,801,879	39.36	19	M	**Middle**
7436	55,858,514	88.07	24	M	
4166	41,910,193	80.99	24	M	
6994	22,589,918	64.06	29	M	
3845	42,874,575	67.38	30	M	
5297	65,721,856	85.99	33	M	
1977	22,764,279	75.52	38	M	**Old**
5115	46,074,219	94.47	46	M	
7085	35,918,642	86.97	49	F	
7109	55,327,128	66.24	51	M	
7376	22,853,932	76.37	52	F	
7459	16,573,163	86.94	54	M	

Figure S1. Sample information of 19 Controls and 17 ASD patients.

A

Standard Deviations of 797 regions in Old group

Standard Deviations of 797 regions in Young group

B

Autosome

Mean (51.60 ± 67.6)

Sex chromosome

Mean (17.76 ± 26.3)
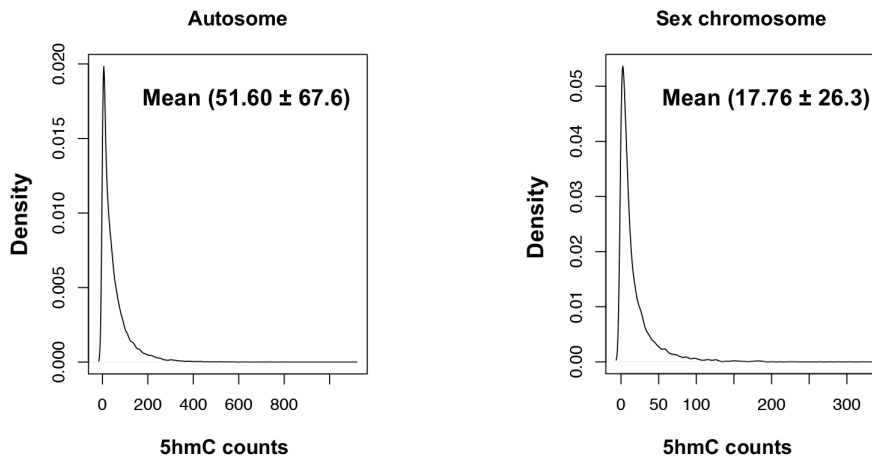
C

MARKER	MARKER LOCATION	ASSOCIATED REGION (CLUMP)	GENES IN REGION
rs4392770	chr7:72213451	chr7:72022225..72417063	MIR4650-1 MIR4650-2 POM121 SBDSP1 SPDYE7P TYW1B
rs7024761	chr9:115096346	chr9:114918772..115147463	HSDL2 MIR3134 PTBP3 SUSD1
rs2564899	chr9:114857260	chr9:114832028..114888773	MIR3134 SUSD1

Figure S2. Characterization of 797 DhMRs in young group ASD cerebellum.

(**A**) Histogram plot of the standard deviations (SD) of the 797 regions in old group (left, mean of SDs: 16.7) and young group (right, mean of SDs: 27.9).

(**B**) Numbers of 5hmC reads are higher in autosome (Mean ± SD = 51.60 ± 67.6) than in sex chromosome (Mean ± SD = 17.76 ± 26.3).

(**C**) ASD risk SNPs that overlapped with DhMRs.

A

Pathway Name	Statistics (BH corrected)
Cell-Cell communication	rawP=0.0044;adjP=0.0476
heparan sulfate biosynthesis (late stages)	rawP=0.0009;adjP=0.0476
heparan sulfate biosynthesis	rawP=0.0019;adjP=0.0476
IL8- and CXCR2-mediated signaling events	rawP=0.0041;adjP=0.0476
IL8-mediated signaling events	rawP=0.0051;adjP=0.0476
Transmembrane transport of small molecules	rawP=0.0044;adjP=0.0476

B

Gene Name	GOTERM_BP_DIRECT (selected)	KEGG_PATHWAY (selected)
FMR1	GO:0006397~mRNA processing GO:0006974~cellular response to DNA damage stimulus GO:0007215~glutamate receptor signaling pathway	hsa03013:RNA transport
DPP6	GO:0072659~protein localization to plasma membrane	
GSTM1	GO:0042178~xenobiotic catabolic process	hsa00982:Drug metabolism-cytochrome P450
DDX53	GO:0010501~RNA secondary structure unwinding	
EPHA6	GO:0048013~ephrin receptor signaling pathway	hsa04360:Axon guidance
GNA14	GO:0007165~signal transduction	hsa04020:Calcium signaling pathway
ARHGAP11B	GO:0021987~cerebral cortex development	
ARHGAP15	GO:0007165~signal transduction	
CDH8	GO:0007155~cell adhesion GO:0035249~synaptic transmission, glutamatergic	
CACNB2	GO:0007268~chemical synaptic transmission	hsa04010:MAPK signaling pathway
CNTNAP3	GO:0007155~cell adhesion	
DGKK	GO:0006979~response to oxidative stress	hsa00561:Glycerolipid metabolism
NELL1	GO:0007399~nervous system	
NXPH1		
SDK1	GO:0007416~synapse assembly	
UTRN	GO:0007528~neuromuscular junction development	

C

D

	# of gene with significant 5hmC change (ASD vs. Control)	# of total gene	Ratio	Normalized to Reference
ASD	17	845	0.0201	10.58
SCZ	9	572	0.0157	8.26
ID	6	483	0.0124	6.53
Reference	52	27023	0.0019	1

**Figure S3. Gene ontology analysis of DhMRs-associated genes.**

(**A**) Pathway analysis of 181 DhMRs-associated genes.

(**B**) 16 ASD risk genes with intragenic DhMRs involve in different biological process and pathways.

(**C**) 5hmC distribution patterns of DPP6, GSTM1 and FMR1 in young group and old group.

(**D**) 5hmC changes in the ±10kb region of psychiatric genes. By using DSS, we compared 5hmC counts (ASD vs. Control samples) in the ±10kb region of psychiatric genes., including ASD, schizophrenia (SCZ) and intellectual disorders (ID) risk genes.

Disease association analysis

q-value (−log$_{10}$ value)
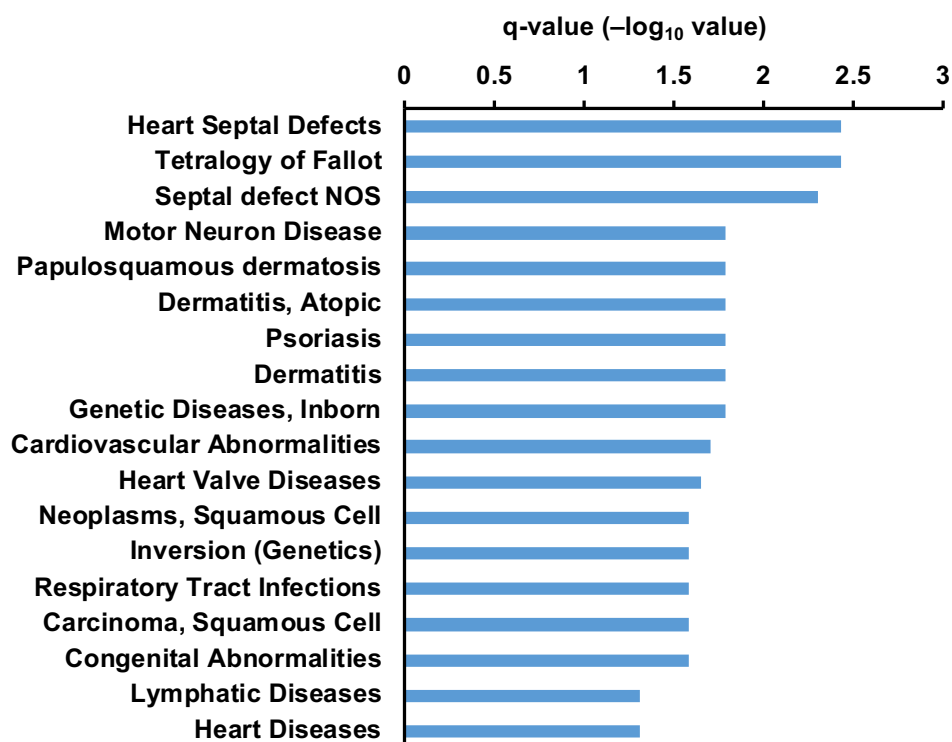
Figure S4. Disease association analysis of protein-coding genes (with enhancer in 100kb from TSS).

Disease association analysis

q-value (–log$_{10}$ value)

Pathway analysis

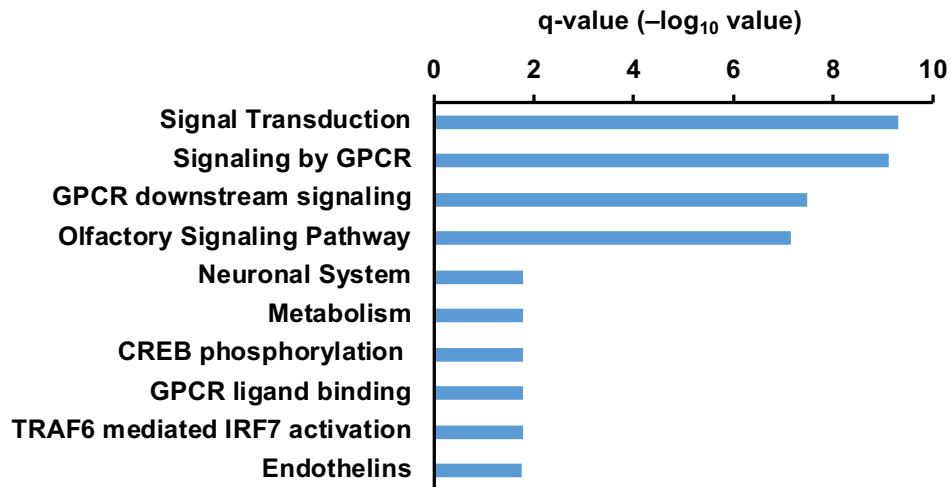**q-value (–log$_{10}$ value)**
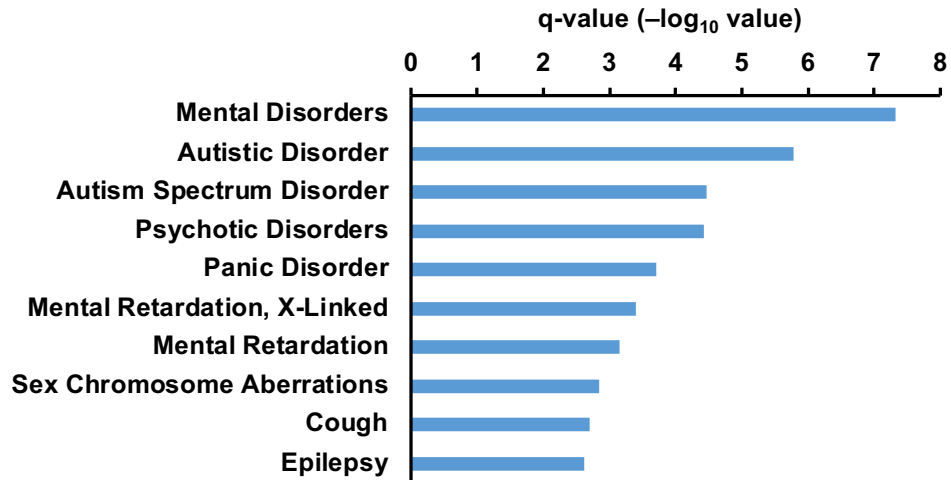
Figure S5. Disease association and Pathway analysis of 329 intergenic DhMR-associated genes identified from TAD Pathway analysis.