Online-only material - Supplemental Information, figures and tables

Genome-Wide Epigenetic Regulation by Early-Life Trauma

Benoit Labonte, MSc[1,2]; Matt Suderman, PhD[3,5] ; Gilles Maussion, PhD[1]; Luis Navaro, MSc[8]; Volodymyr Yerko, PhD[1]; Ian Mahar, BSc[1,2]; Alexandre Bureau, PhD[6]; Naguib Mechawar, PhD[1,2,4]; Moshe Szyf, PhD[5]*; Michael J. Meaney, PhD[2,4,7]*; Gustavo Turecki, MD, PhD[1,2,4]*

[1] McGill group for suicide studies, Douglas Mental Health University Institute, McGill University, Montreal, Quebec, Canada.[2] Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada. [3] McGill Centre for Bioinformatics, McGill University, Montreal, Quebec, Canada. [4] Department of Psychiatry, McGill University, Montreal, Quebec, Canada.[5] Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada. [6] Centre de recherche Université Laval Robert-Giffard, Université Laval, Quebec City, Quebec, Canada. [7] Singapore Institute for Clinical Sciences, Singapore 177609. [8] Unitat de Genètica Molecular. Institut de Biomedicina de València (CSIC). Valencia, Spain

* These authors contributed equally

Reprint requests should be sent to Dr Gustavo Turecki
Address for correspondence:
Dr Gustavo Turecki
Douglas Mental Health University Institute
6875 LaSalle Boulevard, Verdun
H4H 1R3
Quebec
Canada
Tel. : +1 514 761 6131 (ext. 2369)
Fax : +1 514 762 3023
Email : gustavo.turecki@mcgill.ca

Online-only material - Supplemental Information, figures and tables

eMaterial and eMethods

Subjects

Brain tissue was obtained from the Quebec Suicide Brain Bank (QSBB; Douglas Mental Health Institute, Verdun, Québec). All subjects were Caucasians of French–Canadian descent, a population with a well identified founder effect [1]. All individuals were male and group-matched for age, pH and post-mortem intervals (PMI) (see eTable1). Inclusion criteria for both suicide completers and controls were the following: the subject had to be Caucasian and of French Canadian origin, the subject had to be male and die suddenly without prolonged agonal state. Forty-one subjects (25 SA and 16 CTRLs) were recruited for this study. An additional group of 20 non-abused suicide completers (SNA) was included in the validation experiments. Dentate gyrus fromthe left hemisphere (eFig. 1) was carefully dissected at 4˚C after having been flash-frozen in isopentene at -80˚C. Dissection of the HPC was performed by histopathologists using reference neuroanatomical maps [2, 3]. This study was approved by our IRB and signed informed consent was obtained from next of kin.

Psychological Autopsies

Information concerning early-life adversity, psychiatric history and socio-demographics was obtained by way of psychological autopsies performed by trained clinicians with the informants best-acquainted with the deceased as described elsewhere [4]. The process employed by our group in the collection of information used in psychological autopsies has been extensively investigated, producing valid information especially in the context of observable behaviors and major life events such as severe childhood abuse [4-10]. Both cases and controls were characterized by the same psychological autopsy methods, therefore avoiding the occurrence of systematic biases. Briefly, cases were selected on the presence of a history of severe early-life abuse while controls were individuals who died suddenly in work-related accidents, cardiovascular arrest or in car accidents with a negative history of early-life abuse.

Early-life history assessments

Characterization of early-life histories was based on adapted CECA (Childhood Experience of Care and Abuse) interviews assessing various dimensions of the childhood experience, including experiences of sexual and physical abuse [11]. We considered as severe early-life adversity reports of non-random major physical and/or sexual abuse during childhood (up to 15 years). Only cases with an abuse severity rating of 1 and 2 on a scale of 1 to 6 for sexual abuse, where 1 = sexual intercourse/rape and 6 = no abuse, were included. For physical abuse, we similarly only included cases with maximum ratings of 1 and 2 on a scale of 1 to 4, where 1=life-threatening injuries, 2=injuries with visible scars and 4=no abuse. The ratings were made by two different judges, with disagreements being brought to a consensus group. Concordance between siblings in CECA reporting is excellent, particularly for severe events, with values around 0.8 [5]. This information was then complemented with information available from medical charts and coroner records.

Psychopathology

Diagnoses were obtained using DSM-IV [12] criteria by means of SCID-I interviews [13] adapted for psychological autopsies. The frequency of major depressive disorder and substance abuse in the abused suicide group were, respectively, 52% and 16%. Most psychopathology did not have a significant effect on methylation differences, whereas substance disorders did. We adjusted for the effects of substance disorders in our analyses (see section methylation levels).

Relation of sample to other reports

Part of the sample used in this report has been previously used in studies investigating epigenetic changes in candidate genes [14-16]. However, the sample investigated in this study is substantially larger and these subjects have not been included in studies assessing genome-wide methylation patterns.

Methylated DNA Immunoprecipitation (meDIP), Labelling and Hybridization

MeDIP

Methylated DNA was extracted following an adaptation of a methylated DNA immunoprecipitation (meDIP) method developed previously [17]. Briefly, 6pg of green fluorescent protein (GFP) and methylated luciferase (mLUC) plasmids was combined with 3ug of genomic DNA and sonicated in order to create 300 to 800bp fragments. A representative fraction of genomic DNA (input) was immediately isolated. The remaining DNA was incubated with sepharose beads (GE Healthcare) and 5' methyl cytosine antibody (Calbiochem) overnight. The preparation was then spun on a SpinX column (Corning Inc) and the flow-through was recovered as the unmethylated DNA fraction. The remaining DNA was thoroughly washed and the methylated DNA fraction was recovered. Input, unmethylated and methylated fractions were then purified by phenol-chloroform and precipitated in ethanol. Enrichment in either methylated or unmethylated DNA was assessed by PCR using primers targeting external (GFP, mLUC) and internal controls (B-Actin, H19) for input, unbound and bound fractions.

Labelling

Input and methylated DNA fractions were amplified using the GenomePlex Complete Whole Genome Amplification (WGA) Kit (Sigma) and purified using the GenElute PCR Clean-Up Kit (Sigma) following manufacturer's instructions. DNA concentration and quality was assessed on a NanoDrop ND-1000 UV-VIS Spectrophotometer (NanoDrop Technologies, Rockland, DE, USA). Input and methylated fractions were labeled to Cyanine 3-dUTP (Cy3) and cyanine 5-dUTP (Cy5) using the Agilent Genomic Enzymatic Labeling Kit and then cleaned up using microcon Y-30 filters (Millipore). DNA yield and labeling specificity was assessed on Nanodrop. All samples reached the minimum Cy3 and Cy5 specific activity thresholds of 35 to 55pg and 25 to 40pg of DNA, respectively, and quantity ranges of 7 to 10µg of DNA.

Hybridization

Hybridization, washing steps, scanning and data extraction were performed following Agilent's instructions. Every subject was hybridized on a separate microarray. For all samples, dye swaps were performed in order to control for dye integration bias. Hybridization was performed for 40 hours at 60˚C in rotating chambers. All microarrays were washed with acetonitrile, stabilization and drying solution in order to avoid ozone degradation of Cy5. Following washing steps, microarrays were scanned using an Agilent High-Resolution C Scanner (Agilent) at a resolution of 3µm under XDR mode. Data were extracted using Feature Extraction software (Agilent). For each sample, quality control (QC) reports were used in order to assess hybridization quality. Hybridization was assessed based on the following criteria: background noise <10, signal intensity >50, reproducibility <0.2 and derivative LR spread <0.3.

Microarray Analysis

Microarray design

A custom designed 400K promoter tiling array was used for this study (Agilent technologies). The array was designed using Agilent's array design platform eArray in July 2009. Probes were selected to tile all known gene promoters, i.e. intervals roughly 1200 bp upstream to 400 bp downstream of the transcription start sites of genes described in Ensembl (version 55) at 100 bp-spacing.

MeDIP microarray normalization

Extracted microarray intensities were processed and analyzed using the R software environment for statistical computing [18]. Log-ratios of the bound (Cy5) and input (Cy3) microarray channel intensities were computed for each microarray, and then microarrays were normalized to one another using quantile-normalization[19] under the assumption that all samples have identical overall methylation levels.

Methylation levels

Methylation levels were estimated from normalized probe intensities by applying a Bayesian deconvolution algorithm [20]. Promoter methylation levels were obtained taking the median estimated methylation level across each promoter. Promoters were defined as the region 2000 to -400bp of the transcription start sites of each gene.

Differential methylation

Differential methylation between groups of samples was determined in several stages to ensure both statistical significance and biological relevance. In the first stage, linear models implemented in the 'limma' package[21] of Bioconductor [22] were used to combine the two dye labeling schemes from the dye swaps and to compute a modified t-statistic for each probe. Models were adjusted for substance disorders, age and post-mortem interval (PMI). Among the extensive information collected for the individuals in this study, these variables were selected for adjustment based on a combination of domain knowledge and variance analysis of the microarray data. Eigen-R2 [23] was used to estimate the amount of variance in the microarray data explained by each variable. The estimate for each variable is similar to taking the average of the correlations between the variable and the intensities of each probe on the microarray. Correlation averages are vulnerable to technical artifacts such as stochastic noise of probes in regions with little or no methylation so Eigen-R2 uses principal component analysis to reduce the contribution of these and other problematic probes. Using Eigen-R2 we found that evidence of childhood abuse explained the most variance of any variable (eigen-R2 = 0.054). Our analyses were adjusted for substance disorders and PMI because these contributed to the variance according to the Eigen-R2 analyses, and we chose to adjust for age because DNA methylation is known to 'drift' with increasing age [24].

An individual probe was called differentially methylated if the p-value of its t-statistic was at most 0.01 (uncorrected for multiple testing) and the associated difference of means between the groups was at least 0.5. Given that the DNA samples were sonicated prior to hybridization, we assumed that probes within 500bp should approximately agree. Therefore, we partitioned the genome into regions of 1000bp and calculated the significance of enrichment for high or low t statistics of probes within each region (containing at least 1 probe). Significance was determined using the Wilcoxon rank-sum test comparing t statistics of the probes within the region against those of all the probes on the microarray and then adjusted to obtain false discovery rates for each region. A probe was then called differentially methylated if it satisfied each of the following:

1. it was called differentially methylated (i.e. the significance of its t-statistic was at most 0.01 and the difference of means between the groups was at least 0.5), and

2. it belonged to a region whose false discovery rate [25] was at most 0.1.

A promoter was called differentially methylated if it contained a probe called differentially methylated.

For genomic analyses (e.g. Fig. 2), methylation differences were summarized across 1MB regions. This was conducted by first computing a z-score for each promoter (-1200 to +400bp with respect to the transcription start site) indicating the enrichment of differentially methylated probes within the promoter. The z-score was calculated from the modified t-statistics of the probes in the promoter (described above using the limma package) Stouffer's method [26]: $\sum_{i=1}^{k} t_i/\sqrt{k}$ where k is the number of probes in the promoter. Strictly speaking, Stouffer's method requires z-scores but t-statistics are a reasonable approximation when they come from t-tests involving a large number of samples (in our case 40). We then computed a z-score for each 1MB region from Wilcoxon rank-sum statistics obtained by comparing z-scores of promoters within the region to those across the entire genome.

Site Selection for Validation

Regions were selected for validation by EPITYPER by applying a series of thresholds to probes and their surrounding DNA sequence until only a few probes remained. First, probes were required to have

a false discovery rate (see above) of at most 0.01, a p-value (see above) of at most 0.01, a mean normalized difference between SA and CTRL groups of at least 0.9, and at least 4 CpG sites within 200bp.

Expression microarray data

Microarray gene expression profiles were previously generated by our group [27]. The gene expression and methylation profiles include many but not all of the same individuals.Specifically, we have expression profiles for 9 controls and 13 SA individuals of those included in this study. No significant difference in age (SA: 30.9 ± 2.3, CTRL: 37.4 ± 4.2; t= 1.5, p= 0.15), pH (SA: 6.6 ± 0.1, CTRL: 6.7 ± 0.1; t= 1.0, p= 0.32) and PMI (SA: 23.2 ± 1.9, CTRL: 27.8 ± 3.2; t= -1.3, p= 0.21) were found between groups. Expression data was normalized as previously described [27], and expression differences obtained using linear models implemented in the 'limma' package [21] of Bioconductor [22] yielding a modified t-statistic for each probeset. As with the methylation microarray analysis, models were adjusted for drug use, age and post-mortem interval (PMI).

Expression differences were summarized across 500Kb or 1Mb regions by computing z-scores from Wilcoxon rank-sum statistics obtained by comparing promoter differential t-statistics within the region to those across the entire genome.

Neuronal and Non-Neuronal Isolation

Nuclei were isolated from hippocampal tissue as described previously [28]. Briefly, 150mg of tissue was thawed and dounced for one minute. Nuclei were then extracted by ultracentrifugation on a sucrose gradient (80%) for 2.5h at 24 000 RPM. Pelleted nuclei were resuspended and incubated with monoclonal human anti-NeuN (1:4000; Abcam), fluorescent Alexa488-labeled goat anti-mouse (1:4000; Invitrogen), blocking solution (10% BSA in normal goat serum) and 1X PBS for 1 hour at 4˚C on a rotating wheel. Nuclei were then filtered and sorted on a FACSVantage SE system (BD Bioscience, San Jose CA). Sorting specificity was assessed using an Olympus BX51 microscope with motorized stage under a 40X 0.75 NA UPlan FL N objective using both pre-sorted and sorted fractions. Neuronal and non-neuronal nuclei were coverslipped with Vectashield containing DAPI (1:4 with DAPI-free Vectashield) (Vector Lab). Sorted nuclei were pelleted by centrifugation in a sucrose solution (20%) for 15 minutes at 3 000 RPM. For subsequent DNA extraction, nuclei were first lysed at 56˚C for 10 minutes in a solution containing EDTA (50mM), proteinase QIAgen protease (1 unit) and 10% Sodium Dodecyl Sulfate (SDS). DNA was then purified using the DNAeasy extraction kit (QIAgen). Concentration and quality of DNA was assessed on Nanodrop.

DNA Bisulfite treatment and Epityper

Neuronal and non-neuronal DNA fractions extracted from FACS-sorted nuclei were treated with sodium bisulfite (Na-BIS) using the Epitech Bisulfite kit (QIA). BIS DNA was then sent to the Innovation Center of Genome Quebec, where Epityper[29] was performed. Results were analyzed by two way-mixed model ANOVA with groups as a fixed factor and CpGs as a repeated measure followed by LSD post-hoc tests. Level of significance was fixed at 0.05.

Luciferase assays

ALS2 full length and truncated promoters were amplified by PCR from human genomic DNA. The methylated full length construct was obtained by mean of inverse PCR using a primer containing a methyl moiety on the exact location of interest. The unmethylated full length construct was generated using the same primer without methylation. PCR products were digested with BglII and HindIII enzymes, and then ligated into the pGL3 vector independently. Be(2)c cells were plated in 24 well plates at 40,000 cells/well in growth medium. 24 hours after plating, cells were transfected with 400 nanogram/well pGL3 constructs and 50 nanogram/well renilla construct. A renilla luciferase construct was used as a control for transfection efficiency. After 24 hours the luciferase reporter activity was assayed. Activity tests were performed in six replicates for each construct tested. To correct for variations in transfection efficiencies, luciferase activities were normalized to renilla activity. Data were analyzed by t-test. Levels of significance was fixed at 0.05.

Quantification of gene expression using qRT-PCR

Total RNA was extracted using the RNeasy lipid tissue extraction kit (Qiagen) and was followed by Dnase I treatment, and cDNA conversion was performed using oligo(dT) primers (IDT). The same subjects used for methylation analysis were studied for expression analyses using quantitative RT-PCR. The expression of ALS2 transcripts was quantified using quantitative RT-PCR in an ABI 7900HT (Applied Biosystems, Foster City, CA, USA). Amplification of cDNA was performed using multiplex custom designed Taqman probes in quintuplets. The cycle threshold values for all replicates were pooled to obtain a mean value. Replicates with a standard deviation greater than 0.3 were excluded from the analysis. For each replicate, the quantity of cDNA was extrapolated from a standard curve, composed of a mix of cDNA from all subjects, including 6 dots, each of which was 5 times diluted (5:1). Mean quantities from all sample replicates were normalized to the reference gene GAPDH. Results were analyzed by one way ANOVA followed by LSD post-hoc tests. Levels of significance were fixed at 0.05.

eResults

Validation of Differential Methylation in Neuronal and Non-neuronal Cellular Fractions

According to the criteria previously described (see methods section), we selected for validation genomic regions in the promoter of DGKZ, HIST2H2AB, RGS3, NR1D1 and TAF5L. In addition, to rule out the effect of suicide, a group of suicide completers with a negative history of childhood abuse was included. This group was matched with SA and CTRL groups for age, pH and PMI.*DGKZ.* A region of 272 bp including 13 CpGs was covered in the promoter of DGKZ. In the neuronal cell fraction, two-way ANOVA revealed a significant main effect of group ($F_{(2, 59)}$= 3.1, p= 0.046), SA group being significantly hypermethylated compared to CTRL (p= 0.023) and SNA (p= 0.013) with no difference between SNA and CTRL (eFig. 2A). Two-way ANOVA also revealed a significant main effect of CpG sites ($F_{(10, 59)}$=88.5, p= $1.38E^{-46}$) and a trend for group by CpG sites interaction ($F_{(20, 59)}$= 1.5, p= 0.082). Post hoc comparisons showed that methylation levels at CpG 4 and CpG 5 were significantly higher in the SA group compared to the SNA (CpG4: p= 0.002; CpG5: p= 0.035) and CTRL (CpG4: p= 0.016; CpG5: p= 0.002; eFig.2C) samples. Other significant differences across groups are summarized in eFigure 2C.

There was not a group effect in the non-neuronal cell fraction (eFig. 2B). Results from gene expression microarrays performed on a subset of the subjects used in the validation experiments suggest that DGKZ is upregulated in SA compared to CTRL (log fold change (LFC): 0.5).

HIST2H2AB. A region of 128 bp including 9 CpG sites was covered in the promoter of HIST2H2AB. In the neuronal cell fraction, two-way ANOVA revealed a significant main effect of group ($F_{(2, 39)}$= 10.0, p= $6.67E^{-6}$; eFig. 2D). Post-hoc analyses revealed a significant difference between SA and CTRL (p=$5.9E^{-6}$) SA and SNA (p= 0.03) and SNA compared to CTRL (p= 0.004). We also found a significant main effect of CpG sites ($F_{(6, 39)}$= 51.3, p= 3.05E-^{26}) and a trend toward a significant group by CpG sites interaction ($F_{(12, 39)}$= 1.7, p= 0.076). Post hoc comparisons revealed a significant effect of abuse at CpG sites 4 and 8.9, showing a significant difference in SA compared to CTRL (CpG4: p= 0.001; CpGs8.9: p= 0.075) and SNA (CpG4: p= 0.003; CpGs8.9: p= 0.019) with no difference between SNA and CTRL (eFig. 2F). Other significant differences across groups are summarized in eFigure 2F.

Two-way ANOVA did not reveal a significant main effect of group in the non-neuronal fraction, but a trend was observed ($F_{(2, 39)}$= 2.7, p= 0.071; eFig. 2E).

RGS3. A region of 254 bp including 6 CpG sites in RGS3 promoter was investigated. In the neuronal cell fraction we did not find evidence of a main effect of group, but a trend was observed ($F_{(2, 34)}$= 2.7, p= 0.077; eFig. 2G).

Similarly, no main effect of group was observed in the non-neuronal cell fraction (eFig. 2H). These negative results in both the neuronal and non neuronal cell fraction suggest that the region assessed by epiTYPER was possibly different from the region identified in the microarrays. Results from gene expression microarrays performed on a subset of the subjects used in the validation experiments suggest that RGS3 is downregulated in SA compared to CTRL (LFC: 0.1).

NR1D1. A region of 153 bp including 15 CpG sites was assessed in the promoter of NR1D1. In the neuronal cell fraction, two-way ANOVA revealed a significant main effect of both group ($F_{(2, 44)}$= 7.5, p= 0.001) and CpG sites ($F_{(14, 44)}$=73.8, p= $2.02E^{-31}$). Post hoc analyses showed that methylation levels were significantly higher in the SA group compared to SNA (p= 0.0015) and CTRL (p= 0.0005; eFig. 2I). We also found a trend toward a significant group by CpG site interaction ($F_{(14, 44)}$= 1.7, p= 0.08). Post hoc analyses revealed a significant effect of abuse at CpG11, methylation levels being significantly higher in SA compared to CTRL (p= 0.036) and SNA (p= 0.032) with no difference between SNA and CTRL (eFig. 2K). Moreover, a significant hypermethylation in SA compared to CTRL (p= 0.017) and a trend when compared to SNA (p= 0.06) was also found at CpG1. Other significant differences are summarized in eFigure 2K.

In contrast, no significant main effect of group (eFig. 2J) was found in the non neuronal fraction. Results from gene expression microarrays performed on a subset of the subjects used in the validation experiments suggest that NR1D1 is downregulated in SA compared to CTRL (LFC: 0.04).

TAF5L. A region of 80 bp including 4 CpG sites was investigated in the promoter of TAF5L gene. Two-way ANOVA revealed a significant main effect of group ($F_{(2, 24)}$= 3.3, p= 0.042) in the neuronal cell fraction (eFig. 2M). Post hoc analyses showed that methylation levels were significantly higher in SA compared to SNA (p= 0.011) and CTRL (p= 0.034) with no difference between SNA and CTRL. Moreover, a significant main effect of CpG sites was found ($F_{(3, 24)}$= 114.9, p= $1.03E^{-25}$) but no significant group by CpG sites interaction. Data are summarized in eFigure 2L.

Similarly, a significant main effect of group was found in the non-neuronal cell fraction ($F_{(2, 24)}$= 4.3, p= 0.016) with significantly higher methylation levels in SA compared to SNA (p= 0.033) and CTRL (p= 0.003; eFig. 2O). No difference was found in SNA compared to CTRL. In addition, a significant main effect of CpG sites ($F_{(3, 24)}$= 149.6, p= $2.58E^{-36}$) and a trend toward a significant interaction between group and CpG sites ($F_{(6, 24)}$= 1.9, p= 0.097) were observed. Interestingly, higher levels of methylation were found at CpG2 and CpG3 in SA compared to CTRL (CpG2: p= 0.01; CpG3: p= 0.015) and SNA (CpG2: p= 0.028; CpG3: p= 0.042) while no difference between SNA and CTRL was observed at these sites (eFig. 2N). Results from gene expression microarrays performed on a subset of the subjects used in the validation experiments suggest that TAF5L is downregulated in SA compared to CTRL (LFC: 0.04).

Overall, these results suggest that, at least for the genes validated, methylation differences observed in the microarrays are primarily accounted by methylation differences in the neuronal cell fraction, although some effects were also found in the non-neuronal cell fraction. Indeed, all genes but RGS3 showed hypermethylation in the neuronal cell fraction, while methylation differences were found in the non-neuronal cell fraction only for HIST2H2AB and TAF5L.

eTable 1 Subjects information

	SA	SNA	CTRL
Gender (Male/Female)	25/0	21/0	16/0
Age	37.3±10.6	40.6±12.2	40.9±14.3
pH	6.5±0.3	6.6±0.3	6.5±0.3
PMI	28.5±12.9	34.8±15.1	32.6±15.1
Medication	24% (6/25)	29% (6/21)	31% (5/16)

Legend: Values are given as mean ± SD. Medication included: acetaminophen, lidocain, diphenhydramine, codeine, amitriptyline, nortriptiline, sertaline, venlafaxine, temazepam, diazepam, flurazepam, lorazepam, olanzapine, doxepine, clomipramine, valproic acid, lithium, phenytoin, caramiphen.

eTable 2. Complete list of significantly differentially methylated gene promoters subjected to multiple testing correction (FDR) ranked by corrected p-value (q-value).

Hypermethylated in SA				
Chrm	**FDR**	**LFC**	**Gene**	**TSS**
11	0,0015	0,970	DGKZ	642
1	0,0015	0,966	HIST2H2AB	836
17	0,0052	1,266	NR1D1	314
17	0,0052	1,078	NR1D1	358
9	0,0052	0,981	RGS3	878
1	0,0055	0,901	TAF5L	-968
1	0,0055	0,853	TAF5L	-518
1	0,0055	0,819	TAF5L	-957
11	0,0056	0,980	ABCG4	208
20	0,0057	1,338	HCK	564
2	0,0057	1,101	MIR10B	-96
20	0,0057	0,811	HCK	383
16	0,0057	0,893	HYDIN	377
11	0,0061	0,855	C2CD3	-548
14	0,0066	1,173	C14orf174	-739
3	0,0066	0,815	FHIT	294
1	0,0068	1,056	C1orf51	1199
2	0,0068	1,217	ALS2	596
1	0,0068	1,203	PPFIA4	68
1	0,0068	0,984	PPFIA4	84
2	0,0068	0,969	ALS2	352
11	0,0072	0,980	PELI3	881
15	0,0081	1,222	SNORD116-20	-423
15	0,0081	0,982	SNORD116-21	522
17	0,0083	0,975	TOP2A	-14
3	0,0088	1,092	FYTTD1	-519
10	0,0102	1,342	SVIL	223
10	0,0102	1,025	SVIL	513
10	0,0102	0,942	SVIL	419
12	0,0102	1,120	DYRK2	1132
12	0,0102	1,113	DYRK2	753
14	0,0102	1,077	CHD8	436
2	0,0107	1,073	DCTN1	241
2	0,0107	0,980	DCTN1	881
19	0,0114	1,311	KLK2	950
12	0,0116	1,103	PRMT8	4054
12	0,0127	1,267	FOXM1	859
11	0,0141	1,033	SNORD31	-150
2	0,0148	1,476	SPTBN1	1200

2	0,0148	1,403	SPTBN1	1192
2	0,0148	0,968	SPTBN1	1102
7	0,0150	1,308	FAM131B	518
7	0,0150	1,110	FAM131B	509
2	0,0158	1,439	HDLBP	101
14	0,0161	0,924	KIAA1737	893
17	0,0162	0,987	ACLY	953
16	0,0171	1,203	UBE2I	-905
15	0,0174	1,349	PKM2	467
14	0,0174	1,192	JDP2	1247
15	0,0174	1,149	PKM2	407
2	0,0176	1,332	DCTN1	-256
15	0,0177	1,426	FAM96A	459
15	0,0177	1,107	FAM96A	446
1	0,0177	0,786	PMF1	731
1	0,0177	1,150	ELAVL4	304
12	0,0183	0,829	STAT6	473
1	0,0186	1,163	DSTYK	516
11	0,0192	0,969	NRXN2	683
11	0,0192	0,960	NRXN2	630
12	0,0194	0,867	KDM2B	616
10	0,0194	0,891	SYNPO2L	298
17	0,0199	0,937	RPL19	1094
12	0,0203	1,096	PRICKLE1	835
9	0,0209	0,995	STOML2	-136
5	0,0215	1,190	PCDHGC5	862
3	0,0215	0,882	GSK3B	-71
14	0,0219	0,754	PTGR2	404
10	0,0231	0,870	SFMBT2	664
X	0,0232	1,004	AR	1095
1	0,0239	1,046	MIR555	1254
X	0,0239	1,010	TRAPPC2	-792
1	0,0239	0,956	MIR555	1255
19	0,0244	1,587	TFPT	46
19	0,0244	1,339	TFPT	-94
14	0,0245	1,128	RNASE1	-178
6	0,0257	1,167	PLAGL1	597
16	0,0257	0,959	NOL3	666
19	0,0259	1,595	ZNF565	410
1	0,0262	1,140	NHLH1	916
15	0,0272	1,071	USP50	213
12	0,0273	1,118	SSH1	1017
22	0,0275	0,947	PHF5A	-28
2	0,0278	1,214	C2orf56	-654

9	0,0278	0,989	SNHG7	-134
2	0,0278	1,444	AC007358.2	231
22	0,0279	0,974	ARVCF	2984
X	0,0279	0,914	TCEAL1	91
6	0,0287	1,494	ECHDC1	72
15	0,0292	0,848	C15orf61	744
14	0,0293	1,298	MOAP1	-1219
14	0,0293	0,891	MOAP1	-1212
13	0,0314	1,139	USPL1	-316
1	0,0314	1,061	CD53	2517
X	0,0314	0,855	ZFX	279
X	0,0320	1,306	USP27X	-581
7	0,0324	1,002	C7orf55	-114
2	0,0332	1,231	CGREF1	253
2	0,0332	1,115	CGREF1	312
9	0,0333	0,966	AKNA	1073
11	0,0334	0,843	ST3GAL4	-874
8	0,0336	0,909	LACTB2	-1065
8	0,0336	0,885	LACTB2	-928
17	0,0337	0,968	NME1-NME2	14
1	0,0338	1,202	HS2ST1	-722
3	0,0343	0,936	TADA3	-101
9	0,0345	1,014	C9orf23	388
6	0,0346	1,021	VTA1	1146
15	0,0362	0,850	SPATA5L1	-127
1	0,0370	0,991	CEP350	337
6	0,0373	0,940	BTN2A1	976
7	0,0380	1,134	NOBOX	195
7	0,0380	0,963	NOBOX	259
4	0,0392	1,033	GAK	-999
11	0,0392	0,959	OR51E2	51
8	0,0398	0,877	LRRC14	-592
20	0,0420	1,282	PLCB1	68
20	0,0420	0,989	PLCB1	-185
1	0,0421	1,096	ACOT11	874
1	0,0433	0,892	SPSB1	950
3	0,0434	1,034	SST	223
3	0,0434	0,882	SST	331
3	0,0440	0,975	PSMD2	-44
17	0,0451	1,088	SPAG9	1029
18	0,0457	1,062	RAB31	1183
18	0,0457	1,037	RAB31	1218
15	0,0459	1,082	ADPGK	1082
22	0,0463	0,825	CSNK1E	292

2	0,0463	0,816	PRPF40A	-487
1	0,0464	1,117	SMAP2	-196
2	0,0465	0,847	EFR3B	-27
22	0,0470	1,106	HSCB	-31
1	0,0470	0,849	FCER1G	882
14	0,0473	1,122	HOMEZ	335
12	0,0475	1,170	LMBR1L	-195
19	0,0493	1,202	WIZ	519
17	0,0493	0,898	SPAG5	361
4	0,0494	1,053	FGFR3	1731
4	0,0495	1,283	CXCL10	945
3	0,0497	1,289	RFT1	884
3	0,0497	1,159	RFT1	812
8	0,0500	0,900	ZNF251	1020
15	0,0513	1,088	SNORD115-22	-833
20	0,0526	0,982	SGK2	442
2	0,0533	0,919	C2orf48	-33
6	0,0535	0,983	AL035696.1	-118
3	0,0539	1,084	C3orf19	113
2	0,0542	0,996	DQX1	-134
4	0,0546	1,126	WHSC1	964
4	0,0546	1,067	WHSC1	967
17	0,0550	1,116	NEK8	319
16	0,0550	0,924	MTHFSD	573
X	0,0552	0,982	FOXP3	672
X	0,0552	0,949	FOXP3	667
1	0,0560	0,914	C1orf113	822
1	0,0565	1,016	ERI3	873
20	0,0576	1,180	SNHG11	930
13	0,0584	1,079	SPERT	-456
6	0,0585	1,143	RIMS1	499
9	0,0594	0,861	DENND1A	763
1	0,0597	1,103	ILDR2	1272
12	0,0603	1,234	RPL41	165
19	0,0603	1,084	PNMAL2	1142
11	0,0607	1,743	ZNF259	945
19	0,0611	1,056	SYT5	177
11	0,0617	1,123	SRPR	-402
1	0,0618	1,032	NOS1AP	545
18	0,0626	1,191	MYO5B	681
12	0,0639	0,967	KIAA1602	867
5	0,0642	0,968	SLC45A2	263
20	0,0646	1,008	ZNF133	-164
12	0,0651	1,112	CAND1	122

12	0,0651	1,045	CAND1	288
12	0,0651	0,831	CAND1	92
2	0,0652	1,207	ACYP2	1157
19	0,0659	1,106	LSM4	215
1	0,0667	1,060	SNORA61	-380
17	0,0676	1,019	PCTP	605
17	0,0676	0,946	PCTP	674
1	0,0677	0,902	EBNA1BP2	360
3	0,0677	0,889	SCN10A	202
13	0,0688	1,156	POMP	1229
15	0,0697	0,795	SNORD115-7	-49
22	0,0698	1,043	LGALS2	-89
13	0,0700	0,827	F10	748
10	0,0712	0,853	CUL2	59
15	0,0716	1,002	SNORD115-43	-810
2	0,0720	0,950	CTNNA2	451
1	0,0722	1,437	EYA3	31
11	0,0722	1,270	USP47	-241
1	0,0729	1,092	MEF2D	781
2	0,0731	0,931	ACVR1	378
1	0,0733	0,851	ECE1	807
14	0,0742	1,163	TTC6	1253
14	0,0742	1,060	TTC6	1269
4	0,0745	0,916	RPL21P44	795
7	0,0758	1,042	ZNF767	1105
19	0,0767	1,532	TCF3	731
12	0,0781	0,844	MSI1	962
19	0,0787	1,144	ZNF136	303
2	0,0789	1,020	CGREF1	18
8	0,0794	1,084	SULF1	1257
13	0,0805	1,140	RNF6	-147
1	0,0810	1,119	DPH2	282
17	0,0811	1,156	VTN	519
19	0,0814	1,306	GATAD2A	13
11	0,0816	1,034	C11orf55	90
10	0,0818	0,945	DNMBP	1200
10	0,0818	0,831	DNMBP	1193
9	0,0820	1,180	MSMP	1101
1	0,0822	1,025	RP1-21O18.1	1119
3	0,0823	0,926	FYCO1	1085
3	0,0826	1,229	C3orf30	-9
15	0,0833	0,816	C15orf34	736
20	0,0835	0,956	LOC284798	738
7	0,0836	0,850	C7orf20	755

3	0,0838	1,109	CHST2	813
21	0,0839	1,056	USP25	926
5	0,0839	1,049	FAM151B	-793
7	0,0839	0,959	CRHR2	542
2	0,0844	1,011	KIAA1841	203
4	0,0844	1,094	ZNF518B	769
3	0,0846	1,377	HEG1	807
X	0,0846	1,146	FGF13	-810
3	0,0861	0,898	NUDT16	-403
22	0,0862	1,027	TXNRD2	2296
19	0,0863	1,438	ZC3H4	-142
19	0,0863	1,219	ZC3H4	-115
15	0,0864	0,937	CYFIP1	255
12	0,0866	0,926	GPR133	464
1	0,0866	1,137	C1orf25	27
9	0,0867	1,209	C9orf126	444
2	0,0870	0,948	ASB3	195
11	0,0880	0,884	SERPING1	-1625
8	0,0892	1,060	SNX16	-795
6	0,0895	1,051	GJA1	465
20	0,0913	1,087	TP53INP2	325
X	0,0913	1,390	ZNF185	1070
4	0,0913	1,118	CASP3	79
1	0,0923	0,976	UROD	139
X	0,0923	0,905	HDAC6	94
15	0,0944	1,027	SPRED1	1181
8	0,0944	1,012	TATDN1	-824
1	0,0945	0,798	SRP9	510
8	0,0951	0,935	C8orf45	23
1	0,0952	1,298	DAB1	1116
1	0,0957	0,995	OR14A16	0
5	0,0987	1,504	HMGCR	56
12	0,0991	1,197	LHX5	707
Hypomethylated in SA				
Chrm	**FDR**	**LFC**	**Gene**	**TSS**
14	8,05E-04	0,78	SNORD114-14	950
14	8,05E-04	0,81	SNORD114-14	884
9	1,48E-03	1,03	AL449083.1	505
15	3,13E-03	0,96	SNRPN	535
11	7,18E-03	1,06	OR10AB1P	241
6	1,02E-02	0,75	BPHL	1145
11	1,02E-02	1,19	OR51B2	-970
3	1,07E-02	1,09	AC024158.1	824
3	1,07E-02	1,41	AC024158.1	785

2	1,16E-02	1,08	FAP	18
21	1,16E-02	1,05	NCRNA00110	1142
6	1,29E-02	1,01	PKIB	407
9	1,41E-02	1,21	MAPKAP1	-37
X	1,50E-02	0,92	SERPINA7	1052
X	1,50E-02	1,24	SERPINA7	941
1	1,58E-02	0,80	PDE4B	824
11	1,58E-02	1,21	OR5AR1	393
2	1,58E-02	1,22	REG1B	172
12	1,62E-02	0,91	RIC8B	55
12	1,62E-02	1,13	RIC8B	-104
12	1,87E-02	1,26	AC008013.3	-87
1	1,91E-02	1,06	HIPK1	801
10	1,93E-02	1,06	HABP2	143
15	1,93E-02	1,10	TGM7	549
15	1,93E-02	1,32	TGM7	429
5	1,95E-02	1,00	PCDHB9	1000
12	1,96E-02	1,02	CLEC12B	708
6	1,96E-02	1,05	TAAR2	938
12	1,99E-02	0,93	ALDH1L2	50
3	2,03E-02	1,16	PLCH1	749
8	2,07E-02	0,92	DEFA6	534
7	2,09E-02	0,97	DNAH11	433
1	2,16E-02	1,12	OR10X1	341
1	2,39E-02	0,94	VAV3	1210
11	2,45E-02	1,10	BDNFOS	-3418
9	2,53E-02	1,10	CD274	1002
6	2,57E-02	0,79	GPR110	722
3	2,57E-02	0,98	ANKRD28	17
4	2,57E-02	1,02	SCOC	381
4	2,57E-02	1,04	SCOC	380
2	2,59E-02	0,89	FAP	-594
2	2,59E-02	1,01	FAP	-573
8	2,97E-02	1,09	CA3	1534
10	2,97E-02	1,25	FBXW4	1041
14	3,14E-02	1,22	BATF	1073
22	3,16E-02	0,87	SLC25A18	507
3	3,19E-02	1,09	KLHDC6	85
1	3,24E-02	0,95	TGFB2	-669
11	3,42E-02	1,20	CYP2R1	463
7	3,45E-02	0,94	U66059.56	101
2	3,46E-02	1,02	MYO3B	98
6	3,55E-02	1,08	GJA1	2874
9	3,58E-02	0,69	TTC39B	-173

1	3,64E-02	0,98	LOC339535	390
21	3,70E-02	1,21	NCAM2	1148
2	3,80E-02	0,96	GIGYF2	-595
1	3,86E-02	0,91	EVI5	570
21	3,97E-02	1,11	CLDN17	1006
X	4,04E-02	0,83	MIR548I4	757
3	4,06E-02	1,11	ARMC8	-16
14	4,16E-02	1,01	SNORD114-7	583
12	4,17E-02	1,21	PLCZ1	-790
4	4,22E-02	0,97	PCDH7	1173
4	4,69E-02	1,18	STOX2	637
6	4,72E-02	1,04	RUNX2	-126
2	4,78E-02	0,70	LIMS1	479
1	4,81E-02	1,32	RGS5	862
X	4,88E-02	1,09	ITM2A	415
18	4,93E-02	1,61	SLC14A1	-2028
2	5,08E-02	1,38	HTR2B	812
22	5,23E-02	1,04	GRAP2	-77
6	5,42E-02	0,93	TAGAP	402
17	5,44E-02	1,45	AC005863.1	342
21	5,46E-02	0,98	C21orf62	236
1	5,52E-02	0,80	RASAL2	189
1	5,52E-02	1,19	RASAL2	687
11	5,70E-02	0,96	C11orf52	346
10	5,71E-02	0,86	EXOC6	1111
10	5,71E-02	0,88	EXOC6	682
5	5,76E-02	1,08	RAD1	-283
X	6,01E-02	0,99	FRMD7	151
8	6,27E-02	0,92	C8orf39	1196
4	6,32E-02	1,22	TRIML2	165
15	6,36E-02	0,98	NDNL2	1070
12	6,49E-02	1,00	MYF6	299
X	6,80E-02	0,80	SRPX2	117
X	6,96E-02	0,93	DMD	1110
17	7,04E-02	1,07	MYH8	-93
17	7,15E-02	1,04	H3F3B	960
17	7,15E-02	1,28	H3F3B	902
5	7,20E-02	1,06	EDIL3	214
5	7,29E-02	0,88	PCDHA2	-356
17	7,33E-02	0,98	KRT37	843
4	7,37E-02	1,27	C4orf21	47
7	7,45E-02	1,02	FAM180A	244
9	7,59E-02	1,28	AL137019.1	964
22	7,67E-02	1,01	C22orf27	562

7	8,00E-02	1,59	IKZF1	312
11	8,18E-02	1,02	MMP12	-155
7	8,23E-02	1,13	CCDC129	886
15	8,29E-02	1,23	CHRNA5	247
6	8,31E-02	0,97	PRL	509
11	8,42E-02	0,77	OR8D2	1254
X	8,42E-02	1,11	CCNB3	-755
X	8,57E-02	0,93	AGTR2	362
17	9,00E-02	1,28	SOX9	2918
13	9,05E-02	1,13	DCT	390
11	9,13E-02	1,12	SCGB1D2	436
11	9,13E-02	1,19	SCGB1D2	440
2	9,18E-02	0,76	ABCA12	28
14	9,44E-02	0,86	ESR2	4676
14	9,64E-02	1,24	DPF3	895
1	9,67E-02	1,34	F13B	169
13	9,80E-02	1,30	CLDN10	14

Legend: Chrm: Chromosome; LFC: Log fold change; TSS: Distance from the transcription start site (positive: upstream, negative: downstream).

eTable 3. List of the 5 most significantly enriched functional annotation clusters.

Category	**Enrichment Score: 2,106**	**Count**	**%**	**P-value**	**Fold Enrich**
Protein Dimerization	protein homodimerization activity	13	4.39	5.43E-03	2.53
	protein dimerization activity	17	5.74	9.27E-03	2.04
	identical protein binding	19	6.42	9.54E-03	1.93
Category	**Enrichment Score: 1,683**	**Count**	**%**	**P-value**	**Fold Enrich**
Immune System	negative reg. of immune response	4	1.35	6.17E-03	10.51
	negative reg. of immune system process	6	2.03	8.50E-03	4.75
	negative reg. of response to stimulus	6	2.03	1.80E-02	3.94
	reg. of immune effector process	4	1.35	1.97E-01	2.60
Category	**Enrichment Score: 1,625**	**Count**	**%**	**P-value**	**Fold Enrich**
Cell Adhesion	cell-cell adhesion	11	3.72	9.47E-03	2.62
	cell adhesion	18	6.08	3.73E-02	1.69
	biological adhesion	18	6.08	3.78E-02	1.69
Category	**Enrichment Score: 1,545**	**Count**	**%**	**P-value**	**Fold Enrich**
Cell Plasticity	cell soma	8	2.70	9.14E-03	3.40
	cell projection	18	6.08	1.74E-02	1.84
	neuron projection	11	3.72	2.15E-02	2.30
	dendrite	5	1.69	1.93E-01	2.19
Category	**Enrichment Score: 1,481**	**Count**	**%**	**P-value**	**Fold Enrich**
Gene Expression	positive reg. of biosynthetic process	21	7.09	4.45E-03	1.98
	positive reg. of macromolecule biosynthetic process	19	6.42	1.06E-02	1.91
	transcription activator activity	14	4.73	1.06E-02	2.22
	positive reg. of macromolecule metabolic process	23	7.77	1.07E-02	1.76

	positive reg. of nitrogen compound metabolic process	18	6.08	1.86E-02	1.84
	positive reg. of transcription	16	5.41	2.48E-02	1.86
	transcription factor activity	24	8.11	2.54E-02	1.60
	positive reg.of transcription from RNA pol. II promoter	12	4.05	2.58E-02	2.12
	positive reg. of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	17	5.74	2.80E-02	1.79
	positive reg. of transcription, DNA-dependent	14	4.73	2.99E-02	1.93
	positive reg. of gene expression	16	5.41	3.11E-02	1.81
	positive reg. of cellular biosynthetic process	18	6.08	3.13E-02	1.73
	positive reg. of RNA metabolic process	14	4.73	3.16E-02	1.91
	transcription regulator activity	33	11.15	3.61E-02	1.42
	sequence-specific DNA binding	16	5.41	4.66E-02	1.71
	reg. of transcription	49	16.55	7.68E-02	1.24
	reg. of transcription from RNA polymerase II promoter	17	5.74	8.60E-02	1.54
	reg. of transcription, DNA-dependent	35	11.82	8.63E-02	1.30
	transcription	40	13.51	9.99E-02	1.25
	reg. of RNA metabolic process	35	11.82	1.09E-01	1.27
	DNA binding	42	14.19	1.86E-01	1.17

eLegends

eTable 1. Subject information

Legend: Values are given as mean ± SD. Medication included: amitriptyline, nortriptiline, sertaline, venlafaxine, temazepam, diazepam, flurazepam, lorazepam, olanzapine, doxepine, clomipramine, valproic acid, lithium, phenytoin, caramiphen.

eTable 2. Complete list of significantly differentially methylated gene promoters subjected to multiple testing correction (FDR) ranked by corrected p-value (q-value).

Legend: Chrm: Chromosome; FDR: False Discovery Rate; LFC: Log fold change; TSS: Distance from the transcription start site (positive: upstream, negative: downstream).

eTable 3. List of the 5 most significantly enriched functional annotation clusters.

eFigure 1. Anatomical sketch of the hippocampus. DG: dentate gyrus, Sub: Subiculum

eFigure 2. Methylation levels in gene promoters selected for validation in abused suicide completers (SA; black), non abused suicide completers (SNA; grey) and controls (CTRL; white). **A** Total % methylation in all CpGs for DGKZ in the neuronal cell fraction. **B** Total % of methylation in all CpGs in DGKZ promoter in the non-neuronal cell fraction.**C** Individual CpG methylation levels in the promoter of DGKZ in the neuronal cell fraction. Neuronal fraction N= SA, 21; SNA, 19; CTRL, 14; Non Neuronal fraction N= SA,27, SNA, 20; CTRL, 17. For DGKZ, methylation values for CpGs 7 and 8 and 12, and 13 are pooled together. **D** Total % of methylation in all CpGs in HIST2H2AB in the neuronal cell fraction. **E** Total % of methylation in all CpGs in HIST2H2AB promoter in the non-neuronal cell fraction. **F** Individual CpG methylation levels in the promoter of HIST2H2AB in the neuronal cell fraction. Neuronal fraction N= SA, 24; SNA, 20; CTRL, 16; Non Neuronal fraction N= SA,27, SNA, 20; CTRL, 17. For HIST2H2AB, methylation values for CpGs 1 and 2 and 8 and 9 are pooled together. **G** Total % methylation in all CpGs in RGS3 in the neuronal cell fraction. **H** Total % methylation in all CpGs for RGS3 promoter in the non-neuronal cell fraction. Neuronal fraction N= SA, 19; SNA, 19; CTRL, 15; Non Neuronal fraction N= SA, 26; SNA, 20; CTRL, 16. **I** Total % of methylation in all CpGs in NR1D1 in the neuronal cell fraction. **J** Total % methylation in all CpGs for NR1D1 promoter in the non-neuronal cell fraction. **K** Individual CpG methylation levels in the promoter of NR1D1 in the neuronal cell fraction. Neuronal fraction N= SA, 22; SNA, 18; CTRL, 15; Non Neuronal fraction N= SA, 27; SNA, 20; CTRL, 17. For NR1D1, methylation values for CpGs 4 and 5, 6 and 7, 8 and 9, and 12 to 15 are pooled together. **L** Individual CpG methylation levels in the promoter of TAF5L in the neuronal cell fraction. **M** Total % methylation in all CpGs in TAF5L promoter in the non-neuronal cell fraction. **N** Individual CpG methylation levels in the promoter of TAF5L in the neuronal cell fraction. **O** Total % methylation in all CpGs in TAF5L in the non-neuronal cell fraction. Neuronal fraction N= SA, 24; SNA, 18; CTRL, 15; Non Neuronal fraction N= SA, 27; SNA, 20; CTRL, 17. Values are given as mean % of methylation ± SEM. * $p<0.05$; # $p<0.01$.

eFigure 3. Labelling of neuronal nuclei by NeuN antibody conjugated with Alexa488. (**A, B**) NeuN labelling before FACS. Neuronal nuclei labelled by DAPI (**A**) are also collabelled with Alexa-488 (**B**) while a proportion of nuclei are labelled only with DAPI (**A**). (**C, D**) After FACS, the neuronal fraction is enriched in neuronal nuclei. Neuronal nuclei labelled with DAPI (**C**) are co-labelled by Alexa-488 (**D**), and no nuclei are labelled with only DAPI. Circles denote neuronal nuclei. **A** and **C**: DAPI staining. **B** and **D**: Alexa-488 staining.

eReferences

1. Labuda M, Labuda D, Korab-Laskowska M, Cole DE, Zietkiewicz E, Weissenbach J, Popowska E, Pronicka E, Root AW, Glorieux FH. Linkage disequilibrium analysis in young populations: pseudo-vitamin D-deficiency rickets and the founder effect in French Canadians. *Am J Hum Genet.* Sep 1996;59(3):633-643.

2. Nolte J. *The Human Brain: An Introduction to Its Functional Neuroanatomy.* 5th ed. St-Louis, MO: Mosby-Year Book Inc.; 2002.

3. Haines D. *Neuroanatomy: An Atlas of Structures, Sections, and Systems.* 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2000.

4. Dumais A, Lesage AD, Alda M, Rouleau G, Dumont M, Chawky N, Roy M, Mann JJ, Benkelfat C, Turecki G. Risk factors for suicide completion in major depression: a case-control study of impulsive and aggressive behaviors in men. *Am J Psychiatry.* Nov 2005;162(11):2116-2124.

5. Brewin CR, Andrews B, Gotlib IH. Psychopathology and early experience: a reappraisal of retrospective reports. *Psychol Bull.* Jan 1993;113(1):82-98.

6. Conner KR, Conwell Y, Duberstein PR. The validity of proxy-based data in suicide research: a study of patients 50 years of age and older who attempted suicide. II. Life events, social support and suicidal behavior. *Acta Psychiatr Scand.* Dec 2001;104(6):452-457.

7. Hawton K, Appleby L, Platt S, Foster T, Cooper J, Malmberg A, Simkin S. The psychological autopsy approach to studying suicide: a review of methodological issues. *J Affect Disord.* Sep 1998;50(2-3):269-276.

8. Kelly TM, Mann JJ. Validity of DSM-III-R diagnosis by psychological autopsy: a comparison with clinician ante-mortem diagnosis. *Acta Psychiatr Scand.* Nov 1996;94(5):337-343.

9. McGirr A, Tousignant M, Routhier D, Pouliot L, Chawky N, Margolese HC, Turecki G. Risk factors for completed suicide in schizophrenia and other chronic psychotic disorders: a case-control study. *Schizophr Res.* May 2006;84(1):132-143.

10. Brent DA, Perper JA, Moritz G, Allman CJ, Roth C, Schweers J, Balach L. The validity of diagnoses obtained through the psychological autopsy procedure in adolescent suicide victims: use of family history. *Acta Psychiatr Scand.* Feb 1993;87(2):118-122.

11. Bifulco A, Brown GW, Harris TO. Childhood Experience of Care and Abuse (CECA): a retrospective interview measure. *J Child Psychol Psychiatry.* Nov 1994;35(8):1419-1435.

12. Association AP. *Diagnostic and statistical manual of mental disorders*. 4th ed. Washington DC; 1994.

13. Spitzer RL, Williams JB, Gibbon M, First MB. The Structured Clinical Interview for DSM-III-R (SCID). I: History, rationale, and description. *Arch Gen Psychiatry.* Aug 1992;49(8):624-629.

14. Ernst C, Deleva V, Deng X, Sequeira A, Pomarenski A, Klempan T, Ernst N, Quirion R, Gratton A, Szyf M, Turecki G. Alternative splicing, methylation state, and expression profile of tropomyosin-related kinase B in the frontal cortex of suicide completers. *Arch Gen Psychiatry.* Jan 2009;66(1):22-32.

15. McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M, Turecki G, Meaney MJ. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci.* Mar 2009;12(3):342-348.

16. McGowan PO, Sasaki A, Huang TC, Unterberger A, Suderman M, Ernst C, Meaney MJ, Turecki G, Szyf M. Promoter-wide hypermethylation of the ribosomal RNA gene promoter in the suicide brain. *PLoS One.* 2008;3(5):e2085.

17. Keshet I, Schlesinger Y, Farkash S, Rand E, Hecht M, Segal E, Pikarski E, Young RA, Niveleau A, Cedar H, Simon I. Evidence for an instructive mechanism of de novo methylation in cancer cells. *Nat Genet.* Feb 2006;38(2):149-153.

18. R Development Core Team. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing; 2007.

19. Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics.* Jan 22 2003;19(2):185-193.

20. Down TA, Rakyan VK, Turner DJ, Flicek P, Li H, Kulesha E, Graf S, Johnson N, Herrero J, Tomazou EM, Thorne NP, Backdahl L, Herberth M, Howe KL, Jackson DK, Miretti MM, Marioni JC, Birney E, Hubbard TJ, Durbin R, Tavare S, Beck S. A Bayesian deconvolution

strategy for immunoprecipitation-based DNA methylome analysis. *Nat Biotechnol.* Jul 8 2008;26(7):779-785.

21. Smyth GK. Limma: linear models for microarray data. In: R. Gentleman VC, S. Dudoit, R. Irizarry, W. Huber ed. *Bioinformatics and Computational Biology Solutions using R and Bioconductor.* Vol 1. New York: Springer,; 2005: 397-420.

22. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.* 2004;5(10):R80.

23. Chen LS, Storey JD. Eigen-R2 for dissecting variation in high-dimensional studies. *Bioinformatics.* Oct 1 2008;24(19):2260-2262.

24. Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suner D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu YZ, Plass C, Esteller M. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci U S A.* Jul 26 2005;102(30):10604-10609.

25. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B-Methodological.* 1995;57(1):289-300.

26. Stouffer SA. *The American soldier*. Princeton,: Princeton University Press; 1949.

27. Sequeira A, Klempan T, Canetti L, ffrench-Mullen J, Benkelfat C, Rouleau GA, Turecki G. Patterns of gene expression in the limbic system of suicides with and without major depression. *Mol Psychiatry.* Jul 2007;12(7):640-655.

28. Matevossian A, Akbarian S. Neuronal nuclei isolation from human postmortem brain tissue. *J Vis Exp.* 2008(20).

29. Coolen MW, Statham AL, Gardiner-Garden M, Clark SJ. Genomic profiling of CpG methylation and allelic specificity using quantitative high-throughput mass spectrometry: critical evaluation and improvements. *Nucleic Acids Res.* 2007;35(18):e119.