Transcriptome Alterations in Prefrontal Pyramidal Cells Distinguish Schizophrenia from Bipolar and Major Depressive Disorders

Supplemental Information

Supplementary Methods

Laser microdissection. The right hemisphere of each brain was blocked coronally, immediately frozen, and stored at -80°C. For all procedures, samples from each subject in a given tetrad were prepared and processed together in order to control for experimental variance. Tissue sections (12 µm) containing DLPFC area 9 were cut on a cryostat, mounted on glass polyethylene naphthalate membrane slides (Leica Microsystems, Bannockburn, IL), which were blinded as to diagnosis, and stained with thionin for Nissl substance. Using a Leica laser microdissection system, pyramidal cell bodies with a characteristic triangular shape and prominent apical dendrite were identified and dissected from L3 or L5 as previously described and illustrated (1). For each subject, two pooled samples of 100 individually-dissected PCs were obtained from each layer of adjacent tissue sections. To prevent potential confounding effects of collection order, PCs were collected in the opposite order from each layer across the two sections per subject.

Data filtering. The Affymetrix control probesets were removed, as they have no biological relevance. In order to eliminate low expressing and non-informative probesets, we used a modification of a previously described filtering procedure based on a threshold determined by the contrast in expression levels of Y-chromosome genes between male and female subjects (2). For each Y-linked probe, the fold-enrichment in the male subjects relative to the female subjects was plotted against the average expression level in all male subjects (Supplementary Figure S1 and Supplementary Table S2). The inflection point in a lowess curve fitted to the data was determined, and probesets with expression values below the inflection point were eliminated because these expression values cannot be discriminated from noise. This procedure, which generated filtering thresholds at 3.5 and 3.2 for layers 3 and 5, respectively, yielded 23,937 and 27,173 probesets for these layers. We chose to keep their union (i.e., 27,298 probesets) for analysis in order to detect potentially informative probesets in either layer.

Detection of differentially-expressed transcripts within each diagnostic group. We followed a previously reported procedure (1) to fit a random intercept model (3) for each diagnosis separately in order to account for the matched design and the potential impact of covariates including sex, age, RIN, brain pH, PMI, death by suicide, presence of psychosis, presence of mood diagnosis, use of antidepressant, antipsychotic, or benzodiazepines and/or anticonvulsant medications or tobacco at time of death. To retain statistical power, only covariates present in at least 5 but not more than 13 subjects in a diagnostic group were included. For example, mood was included as a covariate in the SZ subject group due to the presence of 6 subjects with schizoaffective disorder but was not included in the BP or MDD subject groups. Conversely, psychosis was included for both BP and MDD subject groups but was not included in the SZ group. Based on these criteria, the following covariates were included: 10 covariates for SZ (sex, age, RIN, brain pH, PMI, death by suicide, presence of mood diagnosis, antidepressant, benzodiazepine and/or anticonvulsant, or tobacco use at time of death); 11 covariates for BP (sex, age, RIN, brain pH, PMI, death by suicide, presence of psychosis, antidepressant, antipsychotic, benzodiazepines and/or anticonvulsants, or tobacco use at time of death); 9 covariates for MDD (sex, age, RIN, brain pH, PMI, death by suicide, presence of psychosis, antidepressant or tobacco use at time of death). For each of the three diagnostic groups, we adopted the following random intercept model:

$$Y_{gik} = \gamma_{gi} + \sum_{l=1}^{L} \beta_{gl} X_{lik} + \alpha_{gk} + \varepsilon_{gik}$$

where Y_{gik} is the expression intensity of probe g, group i ($i = 0$ for control group, $i = 1$ for diagnosis group) and diagnosis-control pair k, γ_{g0} is the baseline expression level for gene g for control group and γ_{g1} is the expression level in diagnosis group. β_{gl} is the covariate effect of gene g and covariate l, and X_{lik} is the covariate value for group i, subject k and covariate l. α_{gk} is the random effect of diagnostic-control pair k for gene g such that $\alpha_{gk} \sim N(0, \sigma_g^2)$. For each model, we included ≤2 of L covariates where L is the number of covariates passing the inclusion criteria in each diagnosis group. Finally, ε_{gik} is the error term, $\varepsilon_{gik} \sim N(0, \sigma_{g0}^2)$. The best model was determined through the Bayesian Information Criterion (BIC) and the p-value of diagnosis effect was assessed via likelihood ratio test. We used permutation (by randomly permuting the subjects 500 times) to correct the p-value since the p-value from likelihood ratio test was biased from model selection. Since the p-value from likelihood ratio test (denoted by $\mathrm{p_{g0}}$, where g $(1 \leq \mathrm{g} \leq \mathrm{G})$ is probe index and 0 denotes the correct group label) was biased from model selection, we permuted group label $\mathrm{B}(= 500)$ times, and similarly calculated the permuted p-values $\mathrm{p_{gb}}$ $(1 \leq \mathrm{b} \leq \mathrm{B})$. We calculated the corrected p-value $\mathrm{p_g^*}$ by

$$\mathrm{p_g^*} = \frac{\sum_{\mathrm{b}=0}^{\mathrm{B}} \sum_{\mathrm{g}=1}^{\mathrm{G}} \mathrm{I}\left(\mathrm{p_{gb}} \leq \mathrm{p_{g0}}\right)}{\mathrm{G} \times (\mathrm{B} + 1)}$$

The resulting corrected p-value was adjusted for multiple comparisons using Storey's procedure (4). Given the small sample size of our study, the probe sets that were differentially expressed between a given diagnostic group and the UC group, were identified using a false discovery rate of 20%. The same probesets were used to perform pathway enrichment analyses.

Effects of clinical covariates. In order to explore whether any gene possessed an effect of suicide or psychosis, we used a linear regression model considering clinical variable (suicide or psychosis) as main effect and adjusting for diagnosis effect (SZ, BP and MDD) as covariates:

$$\mathrm{Y_{glik}} = \beta_{\mathrm{gl}} + \gamma_{\mathrm{gi}} + \varepsilon_{\mathrm{glik}},$$

where $\mathrm{Y}_{\mathrm{glik}}$ is the expression intensity of probe g, group l ($\mathrm{l} = 0$ for control group - clinical unaffected group, $\mathrm{l} = 1$ for clinical affected group, e.g. suicide group or psychosis group), diagnosis i, ($\mathrm{i} = 1,2,3$ representing SZ, BP and MDD group) and subject index k. $\beta_{\mathrm{g}0}$ is the baseline expression level for gene g for control group and $\beta_{\mathrm{g}1}$ is the expression level in clinical affected group. γ_{gi} is the diagnosis effect of gene g and diagnosis i. $\varepsilon_{\mathrm{glik}}$ is the error term, $\varepsilon_{\mathrm{glik}} \sim \mathrm{N}(0, \sigma_{\mathrm{g}}^{2})$. Note that UC subjects were not included since they do not have either suicide or psychosis. Also SZ subjects were excluded for psychosis analysis. P-values of clinical effect $\beta_{\mathrm{g}1} - \beta_{\mathrm{g}0}$ (suicide or psychosis) were adjusted for multiple comparisons using Benjamini-Hochberg procedure.

Supplementary Tables

Supplementary Table S1. Full demographic data for UC, SZ, BP and MDD subjects.

Tetrad	**HU#**	**Subject Group**	**DSM-IV Diagnoses**	**MOD**	**COD**	**Sex**	**Age**	**Race**	**PMI**	**pH**	**RIN**	**Storage Time (mo)**	**Hand Preference**	**Meds ATOD**	**Tob ATOD**
1	**10003**	UC	None	Accidental	Trauma	M	49	W	21.2	6.5	8.4	109.0	R	N	N
2	**1374**	UC	None	Natural	ASCVD	M	43	W	21.7	6.6	7.2	80.4	R	O	Y
3	**1282**	UC	None	Natural	ASCVD	F	39	W	24.5	6.8	7.5	95.7	R	N	N
4	**1298**	UC	None	Natural	ASCVD	M	48	W	24.5	6.5	7.9	92.7	R	N	N
5	**1047**	UC	None	Natural	ASCVD	M	43	W	13.8	6.6	9.0	126.7	R	O	N
6	**795**	UC	None	Natural	Ruptured aortic aneurysm	M	68	W	11.8	6.8	8.2	172.2	R	N	N
7	**1789**	UC	None	Natural	ASCVD	F	53	W	13.7	6.8	8.5	5.4	R	N	Y
8	**1324**	UC	None	Natural	Dissection of the aorta	M	43	W	22.3	6.7	7.3	87.6	L	N	N
9	**1444**	UC	None	Natural	Pulmonary embolism	M	46	W	22.0	6.3	8.4	66.7	R	N	N
10	**1086**	UC	None	Natural	ASCVD	M	51	W	24.2	6.6	8.1	120.7	R	N	Y

Tetrad	**HU#**	**Subject Group**	**DSM-IV Diagnoses**	**MOD**	**COD**	**Sex**	**Age**	**Race**	**PMI**	**pH**	**RIN**	**Storage Time (mo)**	**Hand Preference**	**Meds ATOD**	**Tob ATOD**
11	**1391**	UC	None	Natural	ASCVD	F	51	W	7.8	6.6	7.1	76.5	L	O	Y
12	**1196**	UC	None	Accidental	Asphyxiation	F	36	W	14.5	6.4	8.2	108.8	R	O	N
13	**1293**	UC	None	Accidental	Trauma	F	65	W	18.5	6.5	7.0	93.8	R	N	N
14	**1153**	UC	None	Natural	ASCVD	M	55	W	28.0	6.1	8.0	113.5	R	N	N
15	**789**	UC	None	Accidental	Asphyxiation	M	22	W	20.1	6.8	7.8	173.7	R	N	N
16	**686**	UC	None	Natural	ASCVD	F	52	W	22.6	7.1	8.5	191.0	R	O	Y
17	**1247**	UC	None	Natural	ASCVD	F	58	W	22.7	6.4	8.4	101.2	R	O	N
18	**1092**	UC	None	Natural	Mitral Valve Prolapse	F	40	B	16.6	6.8	8.0	120.1	R	O	N
19	**840**	UC	Adjustment Disorder with depressed mood, current; AAR (in remission 20 years)	Natural	ASCVD	F	41	W	15.4	6.6	9.1	162.5	R	N	Y
					mean		47.5		19.3	6.6	8.0	110.4			
					s.d.		10.5		5.3	0.2	0.6	43.4			

Tetrad	**HU#**	**Subject Group**	**DSM-IV Diagnoses**	**MOD**	**COD**	**Sex**	**Age**	**Race**	**PMI**	**pH**	**RIN**	**Storage Time (mo)**	**Hand Preference**	**Meds ATOD**	**Tob ATOD**
1	**1102**	BP	Bipolar NOS; ADR; ODC; OAC; OAR	Natural	ASCVD	M	50	W	12.1	6.7	8.3	119.0	L	B C D	Y
2	**1121**	BP	Bipolar I, PF; ADR	Natural	Pulmonary embolism	M	40	W	18.5	6.4	8.3	116.6	R	C P	N
3	**957**	BP	Bipolar I	Suicide	Drowning	F	39	W	22.2	6.7	8.4	139.7	R	D O	U
4	**886**	BP	Bipolar I; ADC	Suicide	Gunshot	M	45	W	27.2	7.1	8.3	155.6	R	N	Y
5	**1020**	BP	Bipolar I, PF; ADC; ODC	Accidental	Combined drug overdose	M	42	W	12.5	6.7	8.5	131.0	R	B C D O	Y
6	**1130**	BP	Bipolar I, PF; AAR	Natural	Esophageal cancer	M	65	W	8.9	6.7	8.0	115.7	R	B D O P	U
7	**1048**	BP	Bipolar I, PF	Suicide	Asphyxiation	F	51	W	21.5	6.7	7.7	126.1	R	D	Y
8	**697**	BP	Bipolar I, PF	Suicide	Incised wounds	M	39	W	24.2	6.6	7.8	189.9	R	C	N
9	**1069**	BP	Bipolar I; AAR	Natural	ASCVD	M	48	W	18.1	6.9	8.1	123.2	L	O	N
10	**1244**	BP	Bipolar I, PF; AAR; ODC; OAC	Accidental	Asphyxiation	M	52	W	23.5	6.7	8.0	101.4	R	B C D O P	Y
11	**10004**	BP	Bipolar I; ODC	Accidental	Combined drug overdose	F	50	W	11.7	6.4	8.5	108.5	R	B O P	U

Tetrad	**HU#**	**Subject Group**	**DSM-IV Diagnoses**	**MOD**	**COD**	**Sex**	**Age**	**Race**	**PMI**	**pH**	**RIN**	**Storage Time (mo)**	**Hand Preference**	**Meds ATOD**	**Tob ATOD**
12	**1180**	BP	Bipolar I, PF	Suicide	Jump	F	28	W	22.3	6.3	7.5	111.2	R	D	N
13*	**10006**	BP	Bipolar I, PF; ADR	Suicide	Gunshot	F	55	W	17.5	6.4	8.1	107.3	R	N	Y
14	**716**	BP	Bipolar I; ADC; OAC	Suicide	Gunshot	M	58	W	27.7	6.8	8.3	186.3	R	O	U
15	**1181**	BP	Bipolar I, PF; ODC; OAR	Accidental	Morphine overdose	M	28	W	27.4	6.2	8.0	111.2	L	B C D O	Y
16	**1328**	BP	Bipolar NOS	Natural	ASCVD	F	49	W	21.5	6.7	7.5	86.9	R	B C D L O	N
17	**1044**	BP	Bipolar I, PF	Natural	ASCVD	F	56	W	24.5	6.1	7.1	126.9	L	B D O P	N
18	**984**	BP	Bipolar I, PF	Accidental	Combined drug overdose	F	42	W	31.2	6.5	8.0	136.0	L	D L P	Y
19	**945**	BP	Bipolar I; AAC	Suicide	Asphyxiation	F	43	W	31.9	6.7	7.2	141.9	R	B D O P	Y
					mean		46.3		21.3	6.6	8.0	128.1			
					s.d.		9.5		6.6	0.2	0.4	26.4			

Tetrad	**HU#**	**Subject Group**	**DSM-IV Diagnoses**	**MOD**	**COD**	**Sex**	**Age**	**Race**	**PMI**	**pH**	**RIN**	**Storage Time (mo)**	**Hand Preference**	**Meds ATOD**	**Tob ATOD**
1	**10010**	MDD	MDD, recurrent, PF; AAR	Suicide	Amitriptyline overdose	M	42	W	14.3	6.4	7.6	106.0	R	C D O	N

Tetrad	HU#	Subject Group	DSM-IV Diagnoses	MOD	COD	Sex	Age	Race	PMI	pH	RIN	Storage Time (mo)	Hand Preference	Meds ATOD	Tob ATOD
2	**1226**	MDD	MDD, recurrent; ODC; ODR; OAC; OAR	Natural	ASCVD	M	44	W	19.3	6.5	7.5	104.4	R	N	Y
3	**967**	MDD	MDD, recurrent; ADC	Natural	ASCVD	F	40	W	22.2	6.6	7.4	138.3	R	N	Y
4	**1053**	MDD	MDD	Natural	ASCVD	M	47	W	24.0	6.6	8.1	125.4	R	N	N
5	**1215**	MDD	MDD; ADR	Natural	ASCVD	M	44	W	11.0	6.5	7.9	106.9	R	B O	Y
6	**698**	MDD	MDD, PF	Suicide	Hanging	M	59	W	13.0	6.6	9.0	189.5	R	D O P	N
7	**1190**	MDD	MDD, recurrent; ADC	Suicide	Asphyxiation	F	47	W	22.3	6.6	8.0	109.5	R	N	Y
8	**668**	MDD	MDD, PF; AAR	Suicide	Hanging	M	34	W	24.4	6.6	8.1	194.1	R	N	N
9	**863**	MDD	MDD	Natural	ASCVD	M	51	W	28.3	7.3	8.4	158.2	R	N	N
10	**1312**	MDD	MDD, recurrent; ADR; ODC	Accidental	Combined drug overdose	M	51	W	24.6	6.5	8.1	90.0	R	O	N
11	**986**	MDD	MDD, recurrent	Natural	Asthma	F	53	W	11.9	6.7	8.7	135.9	R	D O	N
12	**1157**	MDD	MDD, recurrent	Suicide	Hanging	F	26	W	13.4	6.4	7.8	113.3	R	D	N

Tetrad	**HU#**	**Subject Group**	**DSM-IV Diagnoses**	**MOD**	**COD**	**Sex**	**Age**	**Race**	**PMI**	**pH**	**RIN**	**Storage Time (mo)**	**Hand Preference**	**Meds ATOD**	**Tob ATOD**
13	**1041**	MDD	MDD, recurrent, PF; AAC; ODC	Accidental	Combined drug overdose	F	52	W	10.3	6.5	8.4	127.5	R	B D O P	Y
14	**1071**	MDD	MDD; AAR	Suicide	Gunshot	M	62	W	26.0	6.5	8.1	122.5	R	O	Y
15*	**1131**	MDD	MDD, recurrent	Suicide	Gunshot	M	29	W	26.6	6.9	8.5	115.7	R	N	N
16	**1143**	MDD	MDD, recurrent; ADR; ODC	Accidental	Combined drug overdose	F	49	W	23.4	6.4	8.1	114.5	R	B D O	Y
17	**934**	MDD	MDD, recurrent, PF	Natural	ASCVD	F	54	W	17.9	6.2	8.2	144.9	R	D O	N
18	**1289**	MDD	MDD	Natural	ASCVD	F	46	W	25.0	6.3	7.3	94.8	R	U	N
19	**1221**	MDD	MDD, recurrent	Natural	Pulmonary embolism	F	28	B	24.8	6.6	7.2	105.7	R	N	N
					mean		45.2		20.1	6.6	8.0	126.2			
					s.d.		10.1		6.0	0.2	0.5	28.8			

Tetrad	**HU#**	**Subject Group**	**DSM-IV Diagnoses**	**MOD**	**COD**	**Sex**	**Age**	**Race**	**PMI**	**pH**	**RIN**	**Storage Time (mo)**	**Hand Preference**	**Meds ATOD**	**Tob ATOD**
1	**1314**	SZ	US	Natural	ASCVD	M	50	W	11.0	6.5	7.2	89.8	R	C D O P	N

Tetrad	HU#	Subject Group	DSM-IV Diagnoses	MOD	COD	Sex	Age	Race	PMI	pH	RIN	Storage Time (mo)	Hand Preference	Meds ATOD	Tob ATOD
2	**1195**	SZ	SA	Natural	Ischemic ileitis	M	46	W	15.8	6.6	7.0	108.8	U	B C O P	Y
3	**1211**	SZ	SA	Natural	Sudden unexpected death	F	41	W	20.1	6.5	7.8	107.2	L	D O P	Y
4	**1367**	SZ	SA; ADC; ODR	Accidental	Combined drug overdose	M	47	W	28.9	6.6	7.2	81.9	R	N	N
5	**10024**	SZ	PS	Natural	ASCVD	M	37	B	6.0	6.1	7.5	100.0	L	O	N
6	**1188**	SZ	US; AAR; OAR	Natural	ASCVD	M	58	W	7.7	6.3	8.4	109.9	R	C O P	Y
7	**1010**	SZ	US	Natural	Sudden unexpected death	F	44	B	18.7	6.2	8.1	132.7	L	C D P	N
8	**10020**	SZ	PS; AAC; OAC	Suicide	Salicylate overdose	M	38	W	28.8	6.6	7.4	101.2	R	C D P	Y
9	**10025**	SZ	DS; OAR	Natural	ASCVD	M	52	B	27.1	6.7	7.8	99.8	R	N	Y
10	**1088**	SZ	US; ADC; OAC	Accidental	Combined drug overdose	M	49	W	21.5	6.5	8.1	120.5	R	D O P	Y
11	**1189**	SZ	SA; AAR	Suicide	Combined drug overdose	F	47	W	14.4	6.4	8.3	109.7	R	B C D O P	Y
12	**10023**	SZ	DS	Suicide	Drowning	F	25	B	20.1	6.7	7.4	100.4	R	B D P	N
13	**843**	SZ	DS	Suicide	Jump	F	41	W	17.1	7.3	9.4	162.0	R	P	Y

Tetrad	HU#	Subject Group	DSM-IV Diagnoses	MOD	COD	Sex	Age	Race	PMI	pH	RIN	Storage Time (mo)	Hand Preference	Meds ATOD	Tob ATOD
14	**1263**	SZ	US; ADR	Accidental	Asphyxiation	M	62	W	22.7	7.1	8.5	98.8	R	D P	Y
15	**1222**	SZ	US; AAC	Suicide	Combined drug overdose	M	32	W	30.8	6.3	7.5	105.0	R	D P	N
16	**656**	SZ	SA; ADC	Suicide	Gunshot	F	47	B	20.1	7.2	9.2	195.3	R	O P	Y
17	**1240**	SZ	US; ADR	Natural	ASCVD	F	50	B	22.9	6.3	7.7	101.9	R	O P	Y
18	**1341**	SZ	SA; ODC	Accidental	Trauma	F	44	W	24.5	6.6	8.8	84.3	R	B O P	N
19	**10026**	SZ	US	Suicide	Thermal injuries	F	46	W	23.8	6.6	7.6	99.6	R	D O P	Y
					mean		45.1		20.1	6.6	7.9	111.0			
					s.d.		8.5		6.9	0.3	0.7	27.0			

Data for schizophrenia subjects 10024 and 10020 (in gray in the table) from pairs 5 and 8 respectively were lost during sample processing and were therefore not used for analysis.

DSM-IV Substance-Related Disorder Diagnoses: ADC (Alcohol Dependence, current at time of death); ADR (Alcohol Dependence, current at time of death); AAC (Alcohol Abuse, current at time of death); AAR (Alcohol Abuse, in remission at time of death); ODC (Other substance Dependence, current at time of death); ODR (Other substance Dependence, in remission at time of death); OAC (Other substance Abuse, current at time of death); OAR (Other substance Abuse, in remission at time of death).

Hand Preference: L (Left); M (Mixed); R (Right); U (Unknown).

Prescribed medications at time of death: B (Benzodiazepines); C (Anticonvulsants); D (Antidepressants); L (Lithium); N (No medications); O (Other medications); P (Antipsychotic); U (Unknown).

Tobacco use at time of death: N (No tobacco); U (Unknown); Y (Yes, chew, cigarette, pipe or other).

DSM-IV Psychiatric Diagnoses: DS (Disorganized Schizophrenia); MDD (Major Depressive Disorder); PS (Paranoid Schizophrenia); RS (Residual Schizophrenia); SA (Schizoaffective Disorder); US (Undifferentiated Schizophrenia).

DSM-IV Specifiers: PF (Psychotic Features).

Supplementary Table S2. Probesets/genes used in the Y-linked filtering.

Gene	Gene Name	U219 probe set
AMELY	amelogenin, Y-linked	11735923_x_at
AMELY	amelogenin, Y-linked	11735922_a_at
AMELY	amelogenin, Y-linked	11763047_x_at
BPY2 /// BPY2B /// BPY2C	basic charge, Y-linked, 2 /// basic charge, Y-linked, 2B /// basic charge, Y-linked, 2C	11735950_s_at
CDY1 /// CDY1B	chromodomain protein, Y-linked, 1 /// chromodomain protein, Y-linked, 1B	11740640_s_at
CDY1 /// CDY1B	chromodomain protein, Y-linked, 1 /// chromodomain protein, Y-linked, 1B	11737714_s_at
CDY1 /// CDY1B /// CDY2A /// CDY2B	chromodomain protein, Y-linked, 1 /// chromodomain protein, Y-linked, 1B /// chromodomain protein, Y-linked, 2A /// chromodomain protein, Y-linked, 2B	11735917_s_at
CSPG4P1Y	chondroitin sulfate proteoglycan 4 pseudogene 1, Y-linked	11753487_at
DAZ1 /// DAZ2 /// DAZ3 /// DAZ4	deleted in azoospermia 1 /// deleted in azoospermia 2 /// deleted in azoospermia 3 /// deleted in azoospermia 4	11742214_s_at
DAZ1 /// DAZ2 /// DAZ3 /// DAZ4	deleted in azoospermia 1 /// deleted in azoospermia 2 /// deleted in azoospermia 3 /// deleted in azoospermia 4	11741801_s_at
DAZ1 /// DAZ2 /// DAZ3 /// DAZ4	deleted in azoospermia 1 /// deleted in azoospermia 2 /// deleted in azoospermia 3 /// deleted in azoospermia 4	11726143_s_at
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	11724077_x_at
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	11749841_x_at
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	11748424_x_at
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	11724076_a_at
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	11724075_a_at
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	11734664_x_at
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	11734665_x_at
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	11756863_a_at
EIF1AY	eukaryotic translation initiation factor 1A, Y-linked	11720807_x_at
HSFY1 /// HSFY2	heat shock transcription factor, Y-linked 1 /// heat shock transcription factor, Y linked 2	11761710_at
HSFY1 /// HSFY2	heat shock transcription factor, Y-linked 1 /// heat shock transcription factor, Y linked 2	11732660_s_at
HSFY1 /// HSFY2	heat shock transcription factor, Y-linked 1 /// heat shock transcription factor, Y linked 2	11755570_s_at
KDM5D	lysine (K)-specific demethylase 5D	11726814_x_at
KDM5D	lysine (K)-specific demethylase 5D	11726813_a_at
KDM5D	lysine (K)-specific demethylase 5D	11745012_a_at
KDM5D	lysine (K)-specific demethylase 5D	11755369_a_at
KDM5D	lysine (K)-specific demethylase 5D	11761654_a_at
LOC100509646 /// PRY /// PRY2	PTPN13-like protein, Y-linked-like /// PTPN13-like, Y-linked /// PTPN13-like, Y-linked 2	11734626_s_at
LOC100509646 /// PRY /// PRY2	PTPN13-like protein, Y-linked-like /// PTPN13-like, Y-linked /// PTPN13-like, Y-linked 2	11742500_s_at
NCRNA00185	non-protein coding RNA 185 /// testis-specific transcript, Y-linked 14	11745579_at

Gene	**Gene Name**	**U219 probe set**
/// TTTY14	(non-protein coding)	
NLGN4Y	neuroligin 4, Y-linked	11744945_a_at
NLGN4Y	neuroligin 4, Y-linked	11759789_x_at
NLGN4Y	neuroligin 4, Y-linked	11738620_a_at
NLGN4Y	neuroligin 4, Y-linked	11748414_a_at
PRKY	protein kinase, Y-linked, pseudogene	11729036_x_at
RBMY1A1 /// RBMY1B /// RBMY1D /// RBMY1E /// RBMY1F /// RBMY1J	RNA binding motif protein, Y-linked, family 1, member A1 /// RNA binding motif protein, Y-linked, family 1, member B /// RNA binding motif protein, Y-linked, family 1, member D /// RNA binding motif protein, Y-linked, family 1, member E /// RNA binding motif protein, Y-linked, family 1, member F /// RNA binding motif protein, Y-linked, family 1, member J	11737478_s_at
RBMY1A1 /// RBMY1B /// RBMY1D /// RBMY1E /// RBMY1F /// RBMY1J	RNA binding motif protein, Y-linked, family 1, member A1 /// RNA binding motif protein, Y-linked, family 1, member B /// RNA binding motif protein, Y-linked, family 1, member D /// RNA binding motif protein, Y-linked, family 1, member E /// RNA binding motif protein, Y-linked, family 1, member F /// RNA binding motif protein, Y-linked, family 1, member J	11741444_s_at
RBMY1A1 /// RBMY1B /// RBMY1D /// RBMY1E /// RBMY1F /// RBMY1J	RNA binding motif protein, Y-linked, family 1, member A1 /// RNA binding motif protein, Y-linked, family 1, member B /// RNA binding motif protein, Y-linked, family 1, member D /// RNA binding motif protein, Y-linked, family 1, member E /// RNA binding motif protein, Y-linked, family 1, member F /// RNA binding motif protein, Y-linked, family 1, member J	11752599_s_at
RPS4Y1	ribosomal protein S4, Y-linked 1	11716411_x_at
RPS4Y2	ribosomal protein S4, Y-linked 2	11738932_x_at
RPS4Y2	ribosomal protein S4, Y-linked 2	11738931_at
SRY	sex determining region Y	11738437_at
TBL1Y	transducin (beta)-like 1, Y-linked	11734646_a_at
TBL1Y	transducin (beta)-like 1, Y-linked	11746673_x_at
TMSB4Y	thymosin beta 4, Y-linked	11736926_at
TSPY1 /// TSPY10 /// TSPY2 /// TSPY3 /// TSPY4 /// TSPY8	testis specific protein, Y-linked 1 /// testis specific protein, Y-linked 10 /// testis specific protein, Y-linked 2 /// testis specific protein, Y-linked 3 /// testis specific protein, Y-linked 4 /// testis specific protein, Y-linked 8	11741782_s_at
TTTY10	testis-specific transcript, Y-linked 10 (non-protein coding)	11751103_at
TTTY11	testis-specific transcript, Y-linked 11 (non-protein coding)	11751807_at
TTTY12	testis-specific transcript, Y-linked 12 (non-protein coding)	11744771_at
TTTY13	testis-specific transcript, Y-linked 13 (non-protein coding)	11753556_at
TTTY5	testis-specific transcript, Y-linked 5 (non-protein coding)	11745588_at
TTTY6 /// TTTY6B	testis-specific transcript, Y-linked 6 (non-protein coding) /// testis-specific transcript, Y-linked 6B (non-protein coding)	11753641_at
TTTY6 /// TTTY6B	testis-specific transcript, Y-linked 6 (non-protein coding) /// testis-specific transcript, Y-linked 6B (non-protein coding)	11753753_s_at
TTTY6 /// TTTY6B	testis-specific transcript, Y-linked 6 (non-protein coding) /// testis-specific transcript, Y-linked 6B (non-protein coding)	11753642_x_at
TTTY6B	testis-specific transcript, Y-linked 6B (non-protein coding)	11763105_at
TTTY6B	testis-specific transcript, Y-linked 6B (non-protein coding)	11763106_x_at
TTTY8 /// TTTY8B	testis-specific transcript, Y-linked 8 (non-protein coding) /// testis-specific transcript, Y-linked 8B (non-protein coding)	11763063_at
TTTY8 /// TTTY8B	testis-specific transcript, Y-linked 8 (non-protein coding) /// testis-specific transcript, Y-linked 8B (non-protein coding)	11753633_s_at

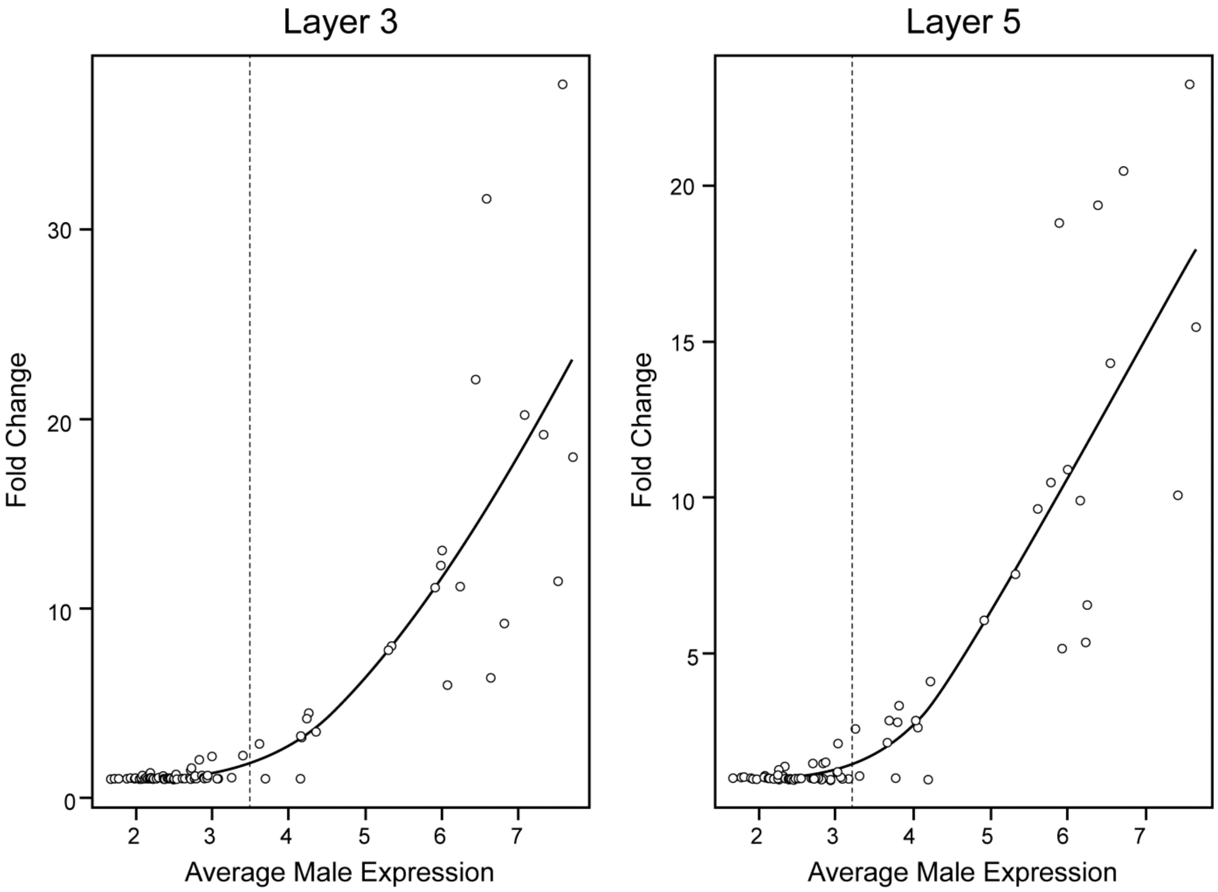
Gene	**Gene Name**	**U219 probe set**
TTTY9A /// TTTY9B	testis-specific transcript, Y-linked 9A (non-protein coding) /// testis-specific transcript, Y-linked 9B (non-protein coding)	11750283_at
USP9Y	ubiquitin specific peptidase 9, Y-linked	11725294_at
USP9Y	ubiquitin specific peptidase 9, Y-linked	11725295_s_at
USP9Y	ubiquitin specific peptidase 9, Y-linked	11725293_at
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	11763812_at
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	11739844_at
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	11735625_a_at
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	11763734_at
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	11763858_a_at
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	11735945_x_at
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	11735626_x_at
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	11761675_at
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	11763837_s_at
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	11763859_x_at
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	11763792_a_at
VCY /// VCY1B	variable charge, Y-linked /// variable charge, Y-linked 1B	11722961_s_at
XKRY /// XKRY2	XK, Kell blood group complex subunit-related, Y-linked /// XK, Kell blood group complex subunit-related, Y-linked 2	11738554_s_at
ZFY	zinc finger protein, Y-linked	11729084_a_at
ZFY	zinc finger protein, Y-linked	11740673_a_at
ZFY	zinc finger protein, Y-linked	11729085_a_at

Supplementary Table S3. Complete list of significant DEGs related to suicide.

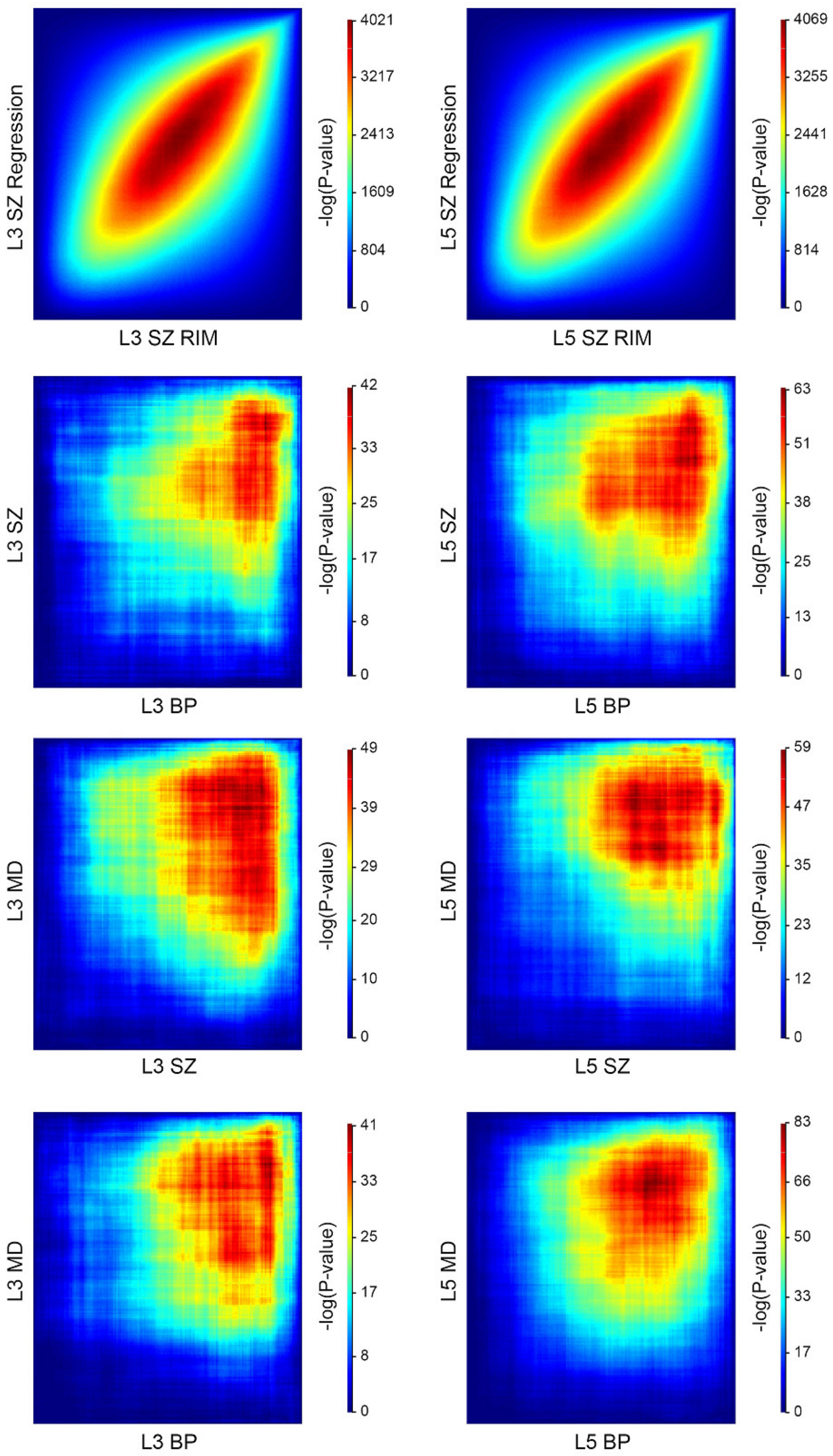
Layer 3			Layer 3			Layer 5			Layer 5		
DEG	% change	q-value	DEG	% change	q-value	DEG	% change	q-value	DEG	% change	q-value
PLCH1	**-52.7**	0.0941	KLF10	**89.3**	0.0759	TNRC6A	**-52.4**	0.0762	RIOK1	**116.0**	0.0762
PRDM8	**-52.5**	0.0159	ADIPOR1	**84.5**	0.0478				PRPS2	**63.3**	0.0762
CMIP	**-40.8**	0.0362	WDR77	**71.2**	0.0825						
ATG13	**-37.6**	0.0113	RPS29	**69.3**	0.0759						
SH3PXD2A	**-36.4**	0.0583	UBE2A	**66.8**	0.0703						
UVRAG	**-35.0**	0.0776	TIMM8B	**65.0**	0.0781						
ZNF652	**-34.7**	0.0478	GPATCH11	**63.2**	0.0581						
EPB41L1	**-34.5**	0.0478	PSMB5*	**59.2**	0.0362						
CAMK2G	**-32.0**	0.0998	RGL1	**57.9**	0.0734						
NFASC	**-31.7**	0.0627	UBE2D3	**56.0**	0.0833						
DGKZ	**-31.0**	0.0581	PSMB5	**55.0**	0.0428						
CACNA1I	**-29.3**	0.0794	ASPHD2	**50.5**	0.0766						
YPEL3	**-29.0**	0.0036	TM9SF3	**47.6**	0.0998						
CCDC92*	**-28.4**	0.0159	UBE2A	**42.3**	0.0759						
CYFIP2	**-27.4**	0.0811	BNIP3L	**42.2**	0.0478						
DDX24	**-24.2**	0.0759	SMNDC1	**41.7**	0.0825						
MAST1	**-23.9**	0.0744	MRPL51	**34.3**	0.0509						
SORT1	**-23.6**	0.0408	PSMB5	**33.5**	0.0766						
SPTAN1	**-22.6**	0.0362	BZW1	**30.9**	0.0478						
ANKRD17	**-20.7**	0.0759	DLD	**29.0**	0.0736						
GNAO1	**-16.4**	0.0962	UQCRB	**26.2**	0.0817						
TRIM2	**-15.2**	0.0811	GMFB*	**25.7**	0.0817						
RAB7A	**-14.0**	0.0535	RGS4	**22.2**	0.0817						
			FBXW7	**12.5**	0.0773						
			BEX1	**11.9**	0.0362						

*Indicates DEG with more than one significant DEP; data for the DEP with the greatest percentage difference is shown at FDR 0.1.

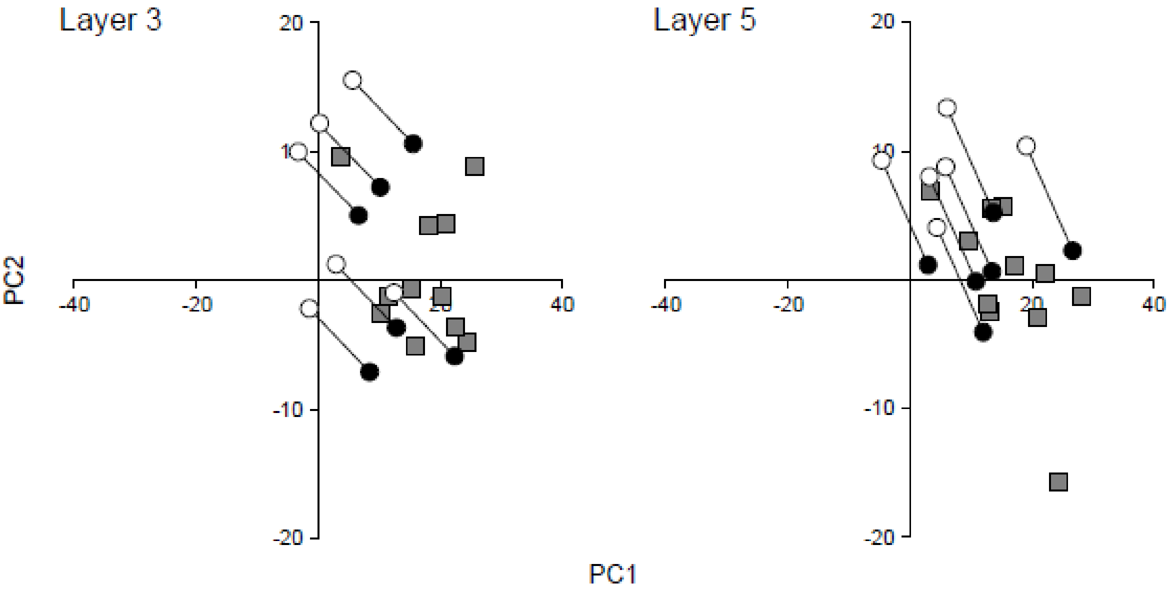
Supplementary Figures



Supplementary Figure S1. Y chromosome-linked filtering of non-informative probesets. The x axis represents the average expression for a subset of Y chromosome-linked probesets for male subjects. The y axis represents the enrichment in fold change of the expression of the Y chromosome-linked probesets in male subjects compared to female subjects. The inflection point of a lowess curve fitted to the scatter plot was then determined. The mean (MEAN) and standard deviation (SD) of fold enrichment (y-axis) was calculated for probes with average male expression (x-axis) below 3. The x-axis cut-point (filtering threshold) was chosen to correspond to the fold enrichment at MEAN+3*SD (y-axis) on the fitted curve.



Supplementary Figure S2: Rank-rank hypergeometric overlap (RRHO) plot of all Y-chromosome filtered probesets (n= 27,298). X-axis (left to right) and Y-axis (bottom to top) are ranked by differential expression p-value from 0 to 1. Each dot in the heatmap represents an enrichment level via $\log_{10}$ (p-value) of a hypergeometric test and the corresponding X-axis and Y-axis represent varying p-value cutoffs. Increasing enrichment levels are depicted using colors from blue to red. The color scale legend is indicated on the right hand side of each comparison. Please note that the scale differs for each comparison due to variations in the p-value distribution for SZ, BP and MDD. First row: unpaired (regression and paired (RIM) analysis comparison. Following rows: transcriptome-wide comparisons of SZ vs BP, SZ vs MDD and BP vs MDD for L3 and L5 PCs through RRHO.



Supplementary Figure S3. Principal component analysis for DEPs in L3 and L5 PCs for SZ subjects with or without a diagnosis of schizoaffective disorder (SA). The data points for SA subjects are shown before and after correction by the statistical analysis model used in this study. The subjects with a diagnosis of pure SZ are represented by gray squares; the subjects with a diagnosis of SA before correction for the effect of mood are represented by open circles and after correction by black circles. Note that after correction, SA subjects cluster more tightly with all other SZ subjects.

Supplementary References

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2. Galfalvy HC, Erraji-Benchekroun L, Smyrniotopoulos P, et al. Sex genes for genomic analysis in human brain: internal controls for comparison of probe level data extraction. *BMC Bioinformatics.* 2003;4:37.
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4. Storey JD. A direct approach to false discovery rates. *J R Stat Soc.* 2002;64(Series B):479-498.