# Altered Expression of CDC42 Signaling Pathway Components in Cortical Layer 3 Pyramidal Cells in Schizophrenia

## *Supplemental Information*

## Supplemental Methods

### Antipsychotic-exposed Monkeys

From each monkey in a triad, 150 individually-dissected pyramidal cells were collected from DLPFC deep layer 3. Total RNA was extracted (QIAGEN RNeasy microkit Plus), cDNA synthesized (QUANTA BioSciences qScript™ cDNA SuperMix) and microarray analysis conducted using GeneChip® Rhesus Macaque Genome Array (Affymetrix, Santa Clara, CA) with all samples from a given triad processed together. All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

## Statistical Analysis

*Microarray Sample Probe Set Filtering and Correction for Multiple Comparisons*

A detailed description of the statistical analyses used for the microarray data is provided in Arion *et al.* 2014 (1). Briefly, the Affymetrix CEL files were normalized and log2 transformed using RMA Express (2). Using a previously published method (3) to filter the probe sets, the microarray dataset was filtered to 1) remove low expression probe sets by eliminating the lowest 40% mean intensities across all samples, and 2) remove non-informative probe sets by eliminating probe sets with the lowest 40% standard deviation across all samples. The microarray data set was then analyzed using the Random Intercept Model with Bayesian Information Criterion variable selection (RIM-BIC). In order to combine the differential expression information for each transcript, an adaptively weighted Fisher's method was used

(4). The meta-analyzed p-values were then corrected for multiple comparisons using the Benjamini-Hochberg protocol to control false discovery rate (5). Finally, the potential influence of comorbid or confounding variables (sex, age, schizoaffective disorder diagnosis, suicide, RIN, PMI, pH, benzodiazepine or valproic acid use ATOD, antidepressant use ATOD and tobacco use ATOD) on differentially expressed probe sets was determined.

**Table S1. Demographic, postmortem, and clinical characteristics of human subjects used in this study**

| Subject Group[a] | | Case No. | S/R/A[b] | PMI[c] | pH | RIN | Storage Time[d] | Cause of Death[e] | DSM IV Diagnoses[f] Primary Substance[g] | | Anti-psychotics ATOD | Anti-depressants ATOD | BZ/VPA ATOD[h] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1• | C | 592 | M/B/41 | 22.1 | 6.7 | 9.0 | 203 | ASCVD | N | | | | |
| | S | 533 | M/W/40 | 29.1 | 6.8 | 8.4 | 213 | Accidental asphyxiation | US | | Y | N | N |
| 2• | C | 567 | F/W/46 | 15.0 | 6.7 | 8.9 | 208 | Mitral valve prolapse | N | | | | |
| | S | 537 | F/W/37 | 14.5 | 6.7 | 8.6 | 213 | Suicide by hanging | SA | | N | N | N |
| 3• | C | 1322 | M/W/62 | 16.5 | 6.8 | 8.6 | 73 | ASCVD | N | | | | |
| | S | 566 | M/W/63 | 18.3 | 6.8 | 8.0 | 193 | ASCVD | US | AAR | Y | Y | Y |
| 4• | C | 604 | M/W/39 | 19.3 | 7.1 | 8.6 | 201 | Hypoplastic coronary artery | N | | | | |
| | S | 581 | M/W/46 | 28.1 | 7.2 | 7.9 | 206 | Accidental combined drug overdose | PS | ADC; OAC | Y | N | Y |
| 5• | C | 546 | F/W/37 | 23.5 | 6.7 | 8.6 | 211 | ASCVD | N | | | | |
| | S | 587 | F/B/38 | 17.8 | 7.0 | 9.0 | 204 | Myocardial hypertrophy | US | AAR | Y | N | Y |
| 6• | C | 551 | M/W/61 | 16.4 | 6.6 | 8.3 | 210 | Cardiac tamponade | N | | | | |
| | S | 625 | M/B/49 | 23.5 | 7.3 | 7.6 | 198 | ASCVD | DS | AAC | Y | Y | N |
| 7• | C | 681 | M/W/51 | 11.6 | 7.2 | 8.9 | 191 | Hypertrophic cardiomyopathy | N | | | | |
| | S | 640 | M/W/49 | 5.2 | 6.9 | 8.4 | 196 | Pulmonary embolism | PS | | Y | Y | N |
| 8• | C | 806 | M/W/57 | 24.0 | 6.9 | 7.8 | 170 | Pulmonary embolism | N | | | | |
| | S | 665 | M/B/59 | 28.1 | 6.9 | 9.2 | 194 | Intestinal hemorrhage | PS | ADC | Y | Y | N |
| 9• | C | 822 | M/B/28 | 25.3 | 7.0 | 8.5 | 167 | ASCVD | N | | | | |
| | S | 787 | M/B/27 | 19.2 | 6.7 | 8.4 | 173 | Suicide by gun shot | SA | ODC | Y | N | N |
| 10• | C | 727 | M/B/19 | 7.0 | 7.2 | 9.2 | 184 | Trauma | N | | | | |
| | S | 829 | M/W/25 | 5.0 | 6.8 | 9.3 | 165 | Suicide by drug overdose | SA | ADC; OAR | N | N | Y |
| 11• | C | 871 | M/W/28 | 16.5 | 7.1 | 8.5 | 156 | Trauma | N | | | | |
| | S | 878 | M/W/33 | 10.8 | 6.7 | 8.9 | 156 | Myocardial fibrosis | DS | ADC | Y | Y | Y |
| 12• | C | 700 | M/W/42 | 26.1 | 7.0 | 8.7 | 188 | ASCVD | N | | | | |
| | S | 539 | M/W/50 | 40.5 | 7.1 | 8.1 | 212 | Suicide by combined drug overdose | SA | ADR | Y | Y | Y |
| 13• | C | 988 | M/W/82 | 22.5 | 6.2 | 8.4 | 135 | Trauma | N | | | | |
| | S | 621 | M/W/83 | 16.0 | 7.3 | 8.7 | 199 | Accidental asphyxiation | US | | N | N | N |
| 14• | C | 686 | F/W/52 | 22.6 | 7.0 | 8.5 | 190 | ASCVD | N | | | | |
| | S | 656 | F/B/47 | 20.1 | 7.3 | 9.2 | 195 | Suicide by gun shot | SA | ADC | Y | N | N |

| Subject Group[a] | | Case No. | S/R/A[b] | PMI[c] | pH | RIN | Storage Time[d] | Cause of Death[e] | DSM IV Diagnoses[f] Primary | Substance[g] | Anti-psychotics ATOD | Anti-depressants ATOD | BZ/VPA ATOD[h] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15● | C | 634 | M/W/52 | 16.2 | 7.0 | 8.5 | 197 | ASCVD | N | | | | |
| | S | 722 | M/B/45 | 9.1 | 6.7 | 9.2 | 185 | Upper GI bleeding | US | ODR; OAR | Y | N | N |
| 16● | C | 852 | M/W/54 | 8.0 | 6.8 | 9.1 | 159 | Cardiac tamponade | N | | | | |
| | S | 781 | M/B/52 | 8.0 | 6.7 | 7.7 | 174 | Peritonitis | SA | ADR | Y | Y | N |
| 17● | C | 987 | F/W/65 | 21.5 | 6.8 | 9.1 | 135 | ASCVD | N | | | | |
| | S | 802 | F/W/63 | 29.0 | 6.4 | 9.2 | 170 | Right ventricular dysplasia | SA | ADC; ODR | Y | N | Y |
| 18● | C | 857 | M/W/48 | 16.6 | 6.7 | 8.9 | 158 | ASCVD | N | | | | |
| | S | 930 | M/W/47 | 15.3 | 6.2 | 8.2 | 145 | ASCVD | DS | ADR; OAR | Y | N | Y |
| 19● | C | 739 | M/W/40 | 15.8 | 6.9 | 8.4 | 183 | ASCVD | N | | | | |
| | S | 933 | M/W/44 | 8.3 | 5.9 | 8.1 | 144 | Myocarditis | DS | | Y | Y | Y |
| 20● | C | 1047 | M/W/43 | 13.8 | 6.6 | 9.0 | 126 | ASCVD | N | | | | |
| | S | 1209 | M/W/35 | 9.1 | 6.5 | 8.7 | 107 | Diphenhydramine overdose | SA | | Y | N | N |
| 21● | C | 1086 | MW/51 | 24.2 | 6.8 | 8.1 | 120 | ASCVD | N | | | | |
| | S | 10025 | MB/52 | 27.1 | 6.7 | 7.8 | 99 | ASCVD | DS | OAR | N | N | N |
| 22● | C | 1092 | F/B/40 | 16.6 | 6.8 | 8.0 | 120 | Mitral valve prolapse | N | | | | |
| | S | 1178 | F/B/37 | 18.9 | 6.1 | 8.4 | 111 | Pulmonary embolism | SA | | Y | N | Y |
| 23● | C | 1336 | M/W/65 | 18.4 | 6.8 | 8.0 | 85 | Cardiac tamponade | N | | | | |
| | S | 1173 | M/W/62 | 22.9 | 6.4 | 7.7 | 111 | ASCVD | DS | ADR | Y | N | N |
| 24● | C | 1122 | M/W/55 | 15.4 | 6.7 | 7.9 | 116 | Cardiac tamponade | N | | | | |
| | S | 1105 | M/W/53 | 7.9 | 6.2 | 8.9 | 118 | ASCVD | SA | | Y | N | N |
| 25● | C | 1284 | M/W/55 | 6.4 | 6.8 | 8.7 | 95 | ASCVD | N | | | | |
| | S | 1188 | M/W/58 | 7.7 | 6.2 | 8.4 | 109 | ASCVD | US | AAR; OAR | Y | N | Y |
| 26● | C | 1191 | M/B/59 | 19.4 | 6.2 | 8.4 | 109 | ASCVD | N | | | | |
| | S | 1263 | M/W/62 | 22.7 | 7.1 | 8.5 | 98 | Asphyxiation | US | ADR | Y | Y | N |
| 27● | C | 970 | M/W/42 | 25.9 | 6.4 | 7.2 | 137 | ASCVD | N | | | | |
| | S | 1222 | M/W/32 | 30.8 | 6.4 | 7.5 | 105 | Combined drug overdose | US | AAC | Y | Y | N |
| 28● | C | 1247 | F/W/58 | 22.7 | 6.4 | 8.4 | 101 | ASCVD | N | | | | |
| | S | 1240 | F/B/50 | 22.9 | 6.3 | 7.7 | 101 | ASCVD | US | ADR | Y | N | N |
| 29● | C | 1324 | M/W/43 | 22.3 | 7.0 | 7.3 | 87 | Aortic dissection | N | | | | |

| Subject Group[a] | | Case No. | S/R/A[b] | PMI[c] | pH | RIN | Storage Time[d] | Cause of Death[e] | DSM IV Diagnoses[f] Primary Substance[g] | | Anti-psychotics ATOD | Anti-depressants ATOD | BZ/VPA ATOD[h] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | 10020 | M/W/38 | 28.8 | 6.6 | 7.4 | 101 | Salicylate overdose | PS | AAC; OAC | Y | Y | Y |
| 30• | C | 1099 | F/W/24 | 9.1 | 6.5 | 8.6 | 119 | Cardiomyopathy | N | | | | |
| | S | 10023 | F/B/25 | 20.1 | 6.7 | 7.4 | 100 | Suicide by drowning | DS | | Y | Y | Y |
| 31• | C | 1307 | M/B/32 | 4.8 | 6.7 | 7.6 | 90 | ASCVD | N | | | | |
| | S | 10024 | M/B/37 | 6.0 | 6.1 | 7.5 | 99 | ASCVD | PS | | N | N | N |
| 32• | C | 1391 | F/W/51 | 7.8 | 6.6 | 7.1 | 76 | ASCVD | N | | | | |
| | S | 1189 | F/W/47 | 14.4 | 6.4 | 8.3 | 109 | Combined drug overdose | SA | AAR | Y | Y | Y |
| 33• | C | 1282 | F/W/39 | 24.5 | 6.8 | 7.5 | 95 | ASCVD | N | | | | |
| | S | 1211 | F/W/41 | 20.1 | 6.3 | 7.8 | 107 | Sudden unexpected death | SA | | Y | Y | N |
| 34• | C | 1159 | M/W/51 | 16.7 | 6.5 | 7.6 | 113 | ASCVD | N | | | | |
| | S | 1296 | M/W/48 | 7.8 | 6.5 | 7.3 | 93 | Pneumonia | US | | Y | Y | N |
| 35• | C | 1326 | M/W/58 | 16.4 | 6.7 | 8.0 | 87 | ASCVD | N | | | | |
| | S | 1314 | M/W/50 | 11.0 | 6.2 | 7.2 | 89 | ASCVD | US | | Y | Y | Y |
| 36• | C | 902 | M/W/60 | 23.6 | 6.7 | 7.7 | 152 | ASCVD | N | | | | |
| | S | 1361 | M/W/63 | 23.2 | 6.4 | 7.7 | 82 | Cardiomyopathy | SA | ODC | Y | N | Y |
| 37 | C | 1374 | M/W/43 | 21.7 | 6.6 | 7.2 | 79 | ASCVD | N | | | | |
| | S | 904 | M/W/33 | 28.0 | 6.2 | 7.1 | 150 | Pneumonia | SA | | Y | N | Y |
| 38 | C | 1555 | M/W/17 | 15.1 | 6.9 | 7.9 | 44 | Trauma | N | | | | |
| | S | 1649 | M/B/17 | 21.4 | 6.9 | 8.1 | 29 | Hanging | US | | Y | Y | N |
| 39 | C | 1268 | M/B/49 | 19.9 | 7.1 | 7.9 | 96 | ASCVD | N | | | | |
| | S | 1230 | M/W/50 | 16.9 | 6.6 | 8.2 | 102 | Doxepin overdose | US | | Y | Y | N |
| 40 | C | 1466 | F/B/64 | 20.0 | 6.7 | 8.8 | 61 | Trauma | N | | | | |
| | S | 1341 | F/W/44 | 24.5 | 6.6 | 8.8 | 83 | Trauma | SA | ODC | Y | N | Y |
| 41 | C | 1518 | M/W/50 | 20.7 | 6.4 | 7.7 | 50 | ASCVD | N | | | | |
| | S | 1367 | M/W/47 | 28.9 | 6.6 | 7.2 | 80 | Combined drug overdose | SA | ADC; ODR | N | N | N |
| 42 | C | 1386 | M/W/46 | 21.2 | 6.7 | 8.3 | 75 | ASCVD | N | | | | |
| | S | 1420 | M/W/47 | 23.4 | 6.8 | 8.2 | 69 | Jump | SA | AAR; ODC; OAR | Y | Y | N |
| 43 | C | 1472 | M/W/61 | 23.8 | 6.5 | 8.0 | 60 | Pulmonary embolism | N | | | | |

| Subject Group[a] | | Case No. | S/R/A[b] | PMI[c] | pH | RIN | Storage Time[d] | Cause of Death[e] | DSM IV Diagnoses[f] Primary Substance[g] | | Anti-psychotics ATOD | Anti-depressants ATOD | BZ/VPA ATOD[h] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | 1453 | M/W/62 | 11.1 | 6.4 | 8.2 | 63 | Trauma | PS | ADR | Y | N | Y |
| 44 | C | 1026 | M/W/59 | 19.8 | 6.3 | 7.4 | 128 | ASCVD | N | | | | |
| | S | 1454 | M/W/59 | 24.1 | 6.1 | 7.6 | 62 | Trauma | PS | AAR; ODC | Y | Y | N |
| 45 | C | 694 | M/W/38 | 20.7 | 7.0 | 7.7 | 189 | Subarachnoid hemorrhage | N | | | | |
| | S | 1455 | M/W/42 | 8.2 | 6.4 | 7.7 | 62 | Peritonitis | PS | AAR; OAC | Y | N | Y |
| 46 | C | 1350 | M/W/21 | 24.2 | 6.4 | 7.3 | 82 | Trauma | N | | | | |
| | S | 1474 | M/W/37 | 39.9 | 6.7 | 7.0 | 60 | Hanging | SA | ADR | N | N | N |
| 47 | C | 1792 | F/W/36 | 28.1 | 6.5 | 7.5 | 5 | Pulmonary embolism | N | | | | |
| | S | 1506 | F/W/47 | 14.1 | 6.6 | 7.5 | 55 | Combined drug overdose | SA | ADC | Y | Y | N |
| 48 | C | 1524 | M/W/66 | 9.4 | 6.4 | 8.1 | 48 | Intestinal infarction | N | | | | |
| | S | 1542 | M/W/65 | 17.4 | 6.7 | 7.8 | 45 | Combined drug overdose | PS | | Y | Y | Y |
| 49 | C | 1270 | F/W/73 | 19.7 | 6.7 | 7.7 | 96 | Trauma | N | | | | |
| | S | 1579 | F/W/69 | 16.1 | 6.7 | 7.7 | 39 | ASCVD | SA | ADR; ODC | Y | N | Y |
| 50 | C | 1372 | M/W/37 | 20.5 | 6.6 | 9.0 | 79 | Asphyxiation | N | | | | |
| | S | 1581 | M/W/32 | 18.4 | 6.8 | 9.0 | 39 | ASCVD | PS | ODC; OAC | Y | Y | N |
| 51 | C | 1543 | F/W/45 | 17.9 | 6.8 | 7.4 | 45 | Subarachnoid hemorrhage | N | | | | |
| | S | 10026 | F/W/46 | 23.8 | 6.6 | 7.6 | 98 | Thermal injuries | US | | Y | Y | N |
| 52 | C | 1583 | M/W/58 | 19.1 | 6.8 | 8.2 | 39 | Trauma | N | | | | |
| | S | 1686 | M/B/56 | 14.1 | 6.2 | 8.3 | 22 | ASCVD | PS | AAR | Y | Y | Y |
| 53 | C | 1554 | M/W/50 | 23.2 | 6.5 | 7.6 | 44 | ASCVD | N | | | | |
| | S | 1691 | M/W/51 | 31.9 | 6.6 | 7.7 | 20 | Combined drug overdose | PS | ADR; ODC | Y | N | Y |
| 54 | C | 1635 | M/W/66 | 25.3 | 6.8 | 8.2 | 31 | Cardiac tamponade | N | | | | |
| | S | 1706 | M/B/60 | 28.1 | 6.8 | 8.4 | 17 | Sepsis | SA | AAR; ODC; OAR | Y | N | N |
| 55 | C | 1384 | M/W/67 | 21.9 | 6.6 | 7.0 | 77 | ASCVD | N | | | | |
| | S | 1712 | M/W/63 | 15.1 | 6.2 | 7.1 | 15 | ASCVD | SA | ADR; ODC | Y | Y | Y |
| 56 | C | 1558 | M/W/54 | 24.4 | 6.9 | 7.7 | 43 | ASCVD | N | | | | |
| | S | 1734 | M/W/54 | 28.6 | 6.1 | 7.7 | 12 | Pneumonia | US | AAR; ODC; OAR | Y | N | N |

● Subject pairs used for microarray study.

[a] C, normal comparison; S, schizophrenia;

[b] A, age in years; B, black; F, female; M, male; R, race; S, sex; W, white;

[c] PMI, postmortem interval (hours);

[d] Storage time (months) at -80°C;

[e] ASCVD, arteriosclerotic cardiovascular disease; GI, gastrointestinal;

[f] DS, disorganized schizophrenia; N, none; PS, paranoid schizophrenia; SA, schizoaffective disorder; US, undifferentiated schizophrenia;

[g] ADC, alcohol dependence, current at time of death; ADR, alcohol dependence, in remission 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;

[h] BZ, benzodiazepines; VPA, sodium valproate; ATOD, at time of death;

Y, yes; N, no.

**Table S2. Sequences and priming efficiency for all human qPCR primer sets used in this study**

| Symbol | Forward Primer | Sequence | Reverse Primer | Sequence | Primer Efficiency % |
|---|---|---|---|---|---|
| *ACTB* | ACTB-Hu-F1 | GATGTGGATCAGC AAGCA | ACTB-Hu-R1 | AGAAAGGGTGTAA CGCAACTA | 100 |
| *PPIA* | Cyclo-F2 | GCAGACAAGGTCC CAAAG | Cyclo_R2 | GAAGTCACCACCC TGACAC | 98 |
| *GNAS* | GNAS-Hu-F1 | AGAGGCGATTGAA ACCATTG | GNAS-Hu-R1 | GTCAAAGTCAGGC ACGTTCA | 99 |
| *ARHGDIA* | ARHGDIA-Hu-F2 | GCCAAATTGCCA AAACTCAA | ARHGDIA-Hu-R2 | GCCCGCTGAGACA GAAAA G | 95 |
| *CDC42* | CDC42-F2 | CAAGGACATTTGTT TGCCATT | CDC42-R1 | TTTGGTGCATTTCA AAGGTG | 100 |
| *CDC42EP4* | CDC42EP4-F4 | AGGAAGGCCAGTG CAGAATA | CDC42EP4-R4 | GGGAGGGAGGGAA TAACTCA | 100 |
| *PAK1* | PAK1-Hu-F2 | TTGGGGATGTTTG CTACCTC | PAK1-Hu-R2 | GACATGACAAGCC ACAATGC | 98 |
| *PAK2* | PAK2-Hu-F3 | CTCACTGACTATGT G CCAACG | PAK2-Hu-R3 | TCGCAACTACAAAT CCCA AA | 100 |
| *PAK3* | PAK3-Hu-F2 | TGGGGGTTCTTTAC CTTTCA | PAK3-Hu-R2 | CTGCGATTCAGGC TTACAAA | 95 |
| *LIMK1* | LIMK1-Hu-F2 | GAAGAGGCCATCC TTTGTGA | LIMK1-Hu-R2 | CTCCCAGAAACCT CTGTCCA | 95 |
| *LIMK2* | LIMK2-Hu-F1 | GCAGGGGGA ATTGATAAAGG | LIMK2-Hu-R1 | ACATCTGGTCCCA CA ACTCA | 103 |

**Supplemental References**

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