Supplemental Table: Primer pairs and efficiency of amplified gene products

Gene	Accession	Size (position)	Forward primer	Reverse primer	Ex
Beta actin (ACTB)	NM_001101	73 bp (23-95)	AGCCTCGCCTTTGCCGA	GCGCGGCGATATATCATCATC	0.97
Cyclophilin A (CYC)	NM_021130	126 bp (159-284)	GCAGACAAGGTCCCAAAG	GAAGTCACCACCCTGACAAC	0.98
glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	NM_002046	87 bp (556-642)	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG	0.97
beta-2-microglobulin (B2M)	NM_004048	106 bp (330-435)	CACCCCCACTGAAAAAGATG	CCTCCATGATGCTGCTTACA	0.98
mitochondrial ATP synthase 6 (ATP6)	NC_001807	89 bp (8848-8936)	CTTATGAGCGGGCACAGTGA	GGGGTGTAGGTGTGCCTTGT	1.08
18S ribosomal RNA (18S)	X03205	143 bp (1247-1389)	CTCAACACGGGAAACCTC	GTCTCGTTCGTTATCGGAAT	1.00
67 kDa glutamate decarboxylase (GAD_{67})	NM_000817	86 bp (2495-2580)	GTTTCCCGCTCCAAGAGAAT	TGGAGTTGTTGGACAAGCTG	1.09
65 kDa glutamate decarboxylase (GAD_{65})	NM_000818	105 bp (177-281)	GTCGGAAGATGGCTCTGG	AGGGCGCACAGTTTGTTT	0.98
Parvalbumin (PV)	NM_002854	140 bp (111-250)	GCTACCGACTCCTTCGAC	ATGAATCCCAGCTCATCC	1.00
Somatostatin (SST)	NM_001048	93 bp (319-411)	ATGCCCTGGAACCTGAAGAT	CCATAGCCGGGTTTGAGTTA	1.07
Calretinin (CR)	NM_001740	86 bp (485-570)	CTGCTGAAGAAGGCGAAC	CCGTTCAAGTCAAACATCC	0.97
$GABA_A$ receptor, α1	NM_000806	87 bp (1202-1288)	TGAGCATCAGTGCCAGAAAC	ACACAAAGGCATAGCACACG	0.98
$GABA_A$ receptor, δ	NM_000815	108 bp (683-791)	ACCACGGAGCTGATGAACTT	AGGGCATGTAGGATTGGATG	0.97
GABA transporter 1 (GAT1)	NM_003042	130 bp (1081-1210)	CTGGACTGGAAAGGTGGTCTA	GCGGAAGTTGGGTGTGAT	1.00

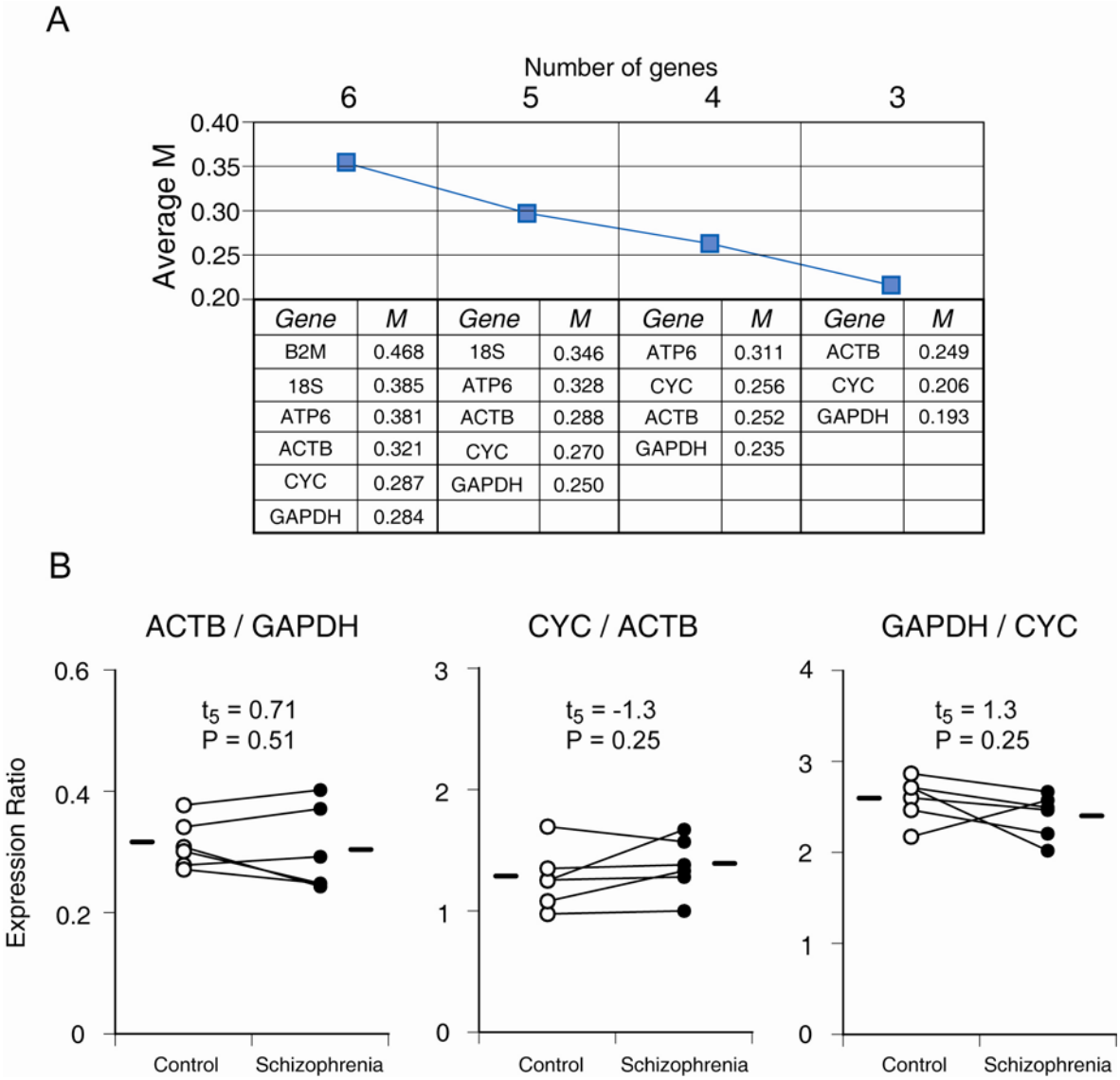
Ex (primer efficiency): Ex is defined by $Rn = Ro*(1+Ex)^n$. Rn = amplification signal of target molecules at cycle n, Ro = initial signal, n = number of cycles. For all primer sets, PCR thermal cycling was 10 min polymerase activation at 95°C followed by 40 cycles at 95°C for 15 sec and 62°C for 1 min.

Selection of internal control transcripts

In real-time qPCR, the use of stable internal control transcripts is crucial for the accurate quantification of genes of interest(1). Thus, we first evaluated the expression stability of six candidate internal controls, including beta-2-microglobulin (B2M), beta actin (ACTB), mitochondrial ATP synthase 6 (ATP6), cyclophilin A (CYC), glyceraldehyde-3-phosphate dehydrogenese (GAPDH), and 18S ribosomal protein (18S), in A9 across the 12 subjects in pairs 1-6 (Table 1). These transcripts were selected based on their frequent usage in real-time qPCR studies in general, our previous microarray findings of their stable expression in the DLPFC between normal comparison and schizophrenia subjects (for ATP6)(2), and on the membership of these transcripts in different functional classes, which reduces the likelihood that their expression is co-regulated. Because the expression ratio of two ideal internal control transcripts should be

identical across all samples, the variation of the expression ratios of two potential control transcripts reflects differences, or instability, in the expression of one (or both) of these transcripts across samples. Thus, for every transcript, the standard deviations of the $\log_2$-transformed expression ratios to other transcripts were determined across the 12 subjects, and the stability (M) of that particular transcript was expressed by the average of these standard deviations (SDs) determined against each of the other transcripts(1). The rank-order of the stability of the six candidates was determined by stepwise exclusion of the transcript with the highest M value, resulting in three transcripts (ACTB, GAPDH, and CYC) with the most stable expression across samples (Supplemental Figure 1A). Due to the inherent variation in expression of internal control transcripts, it is recommended to use the geometric mean of at least 3 stable control transcripts as the normalization factor (NF)(1). To determine whether three control transcripts was sufficient, we compared the NF for ACTB, CYC and GAPDH (NF3) with that of these three transcripts plus ATP6 (NF4) across the 12 subjects. The highly significant correlation between NF3 and NF4 ($r > 0.97$, $P < 0.01$) indicated that the three selected internal control transcripts (ACTB, CYC and GAPDH) provided a stable baseline for comparisons across subject groups. Indeed, none of the expression ratios among these three transcripts in A9 (Supplemental Figure 1B) differed between normal comparison and schizophrenia subjects ($t_5 \leq 1.3$, $P \geq 0.25$).

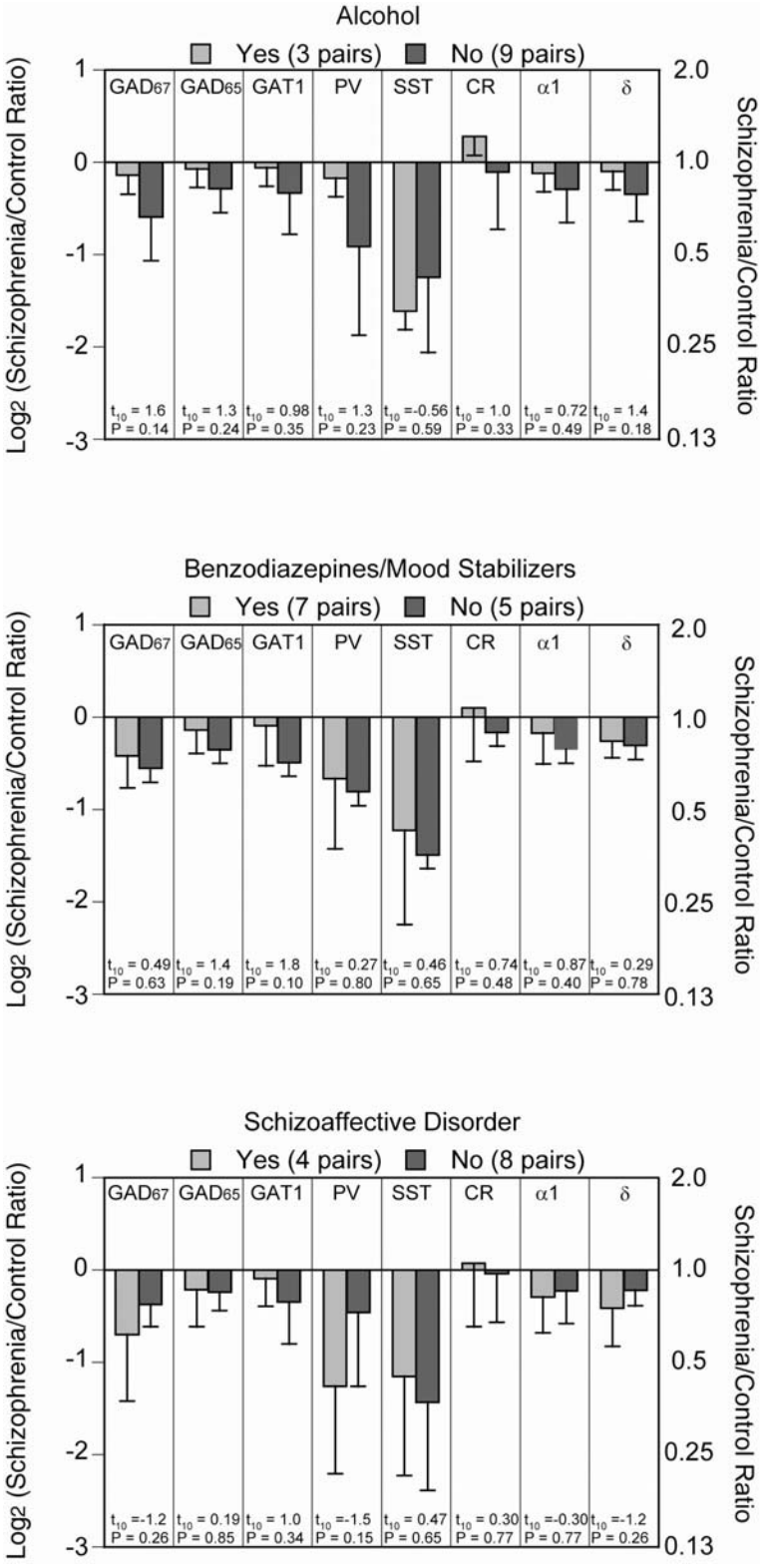
Supplemental Figure 1. Assessment of the expression stability of control transcripts



Gene	*M*	*Gene*	*M*	*Gene*	*M*	*Gene*	*M*
B2M	0.468	18S	0.346	ATP6	0.311	ACTB	0.249
18S	0.385	ATP6	0.328	CYC	0.256	CYC	0.206
ATP6	0.381	ACTB	0.288	ACTB	0.252	GAPDH	0.193
ACTB	0.321	CYC	0.270	GAPDH	0.235		
CYC	0.287	GAPDH	0.250				
GAPDH	0.284						

(A) The average expression stability (M) of internal control transcripts was plotted as a function of the stepwise exclusion of the least stable control transcripts. The stability of each remaining transcript is shown for each step in the table. The transcripts are ordered by M from the largest (least stable) to the smallest (most stable). (B) The expression ratios among the three most stable control transcripts in the DLPFC do not differ between normal comparison and schizophrenia subjects in pairs 1-6 (Table 1).

Supplemental Figure 2. The effects of confounding factors on expression changes of the GABA-related transcripts



Mean (SD) log_2-transformed schizophrenia to normal comparison expression ratios for each of the eight GABA-related transcripts in A9 between subject pairs with or without the indicated potential confounding factors in the subject with schizophrenia at the time of death. The corresponding non-transformed schizophrenia to normal comparison expression ratios are shown on the right axis. Neither presence of alcohol abuse/dependence, usage of benzodiazepines/mood stabilizers history, nor diagnosis of schizoaffective disorder affected the expression changes in any of these transcripts.

References

1. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F: Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 2002; 3:34.1-34.11

2. Hashimoto, T, Arion, D, Volk DW, Mirnics K, and Lewis, DA: Alterations in GABA-related transcriptome in the dorsolateral prefrontal cortex of subjects with schizophrenia. Molecular Psychiatry 2007; Epub ahead of print