



Published in final edited form as:

Neuroscience. 2010 May 5; 167(2): 384–395. doi:10.1016/j.neuroscience.2010.01.016.

A cross-laboratory comparison of expression profiling data from normal human postmortem brain

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Abstract

Expression profiling of post-mortem human brain tissue has been widely used to study molecular changes associated with neuropsychiatric diseases as well as normal processes such as aging. Changes in expression associated with factors such as age, gender or postmortem interval are often more pronounced than changes associated with disease. Therefore in addition to being of interest in their own right, careful consideration of these effects are important in the interpretation of disease studies. We performed a large meta-analysis of genome-wide expression studies of normal human cortex to more fully catalogue the effects of age, gender, postmortem interval and brain pH, yielding a “meta-signature” of gene expression changes for each factor. We validated our results by showing a significant overlap with independent gene lists extracted from the literature. Importantly, meta-analysis identifies genes which are not significant in any individual study. Finally, we show that many schizophrenia candidate genes appear in the meta-signatures, reinforcing the idea that studies must be carefully controlled for interactions between these factors and disease. In addition to the inherent value of the meta-signatures, our results provide critical information for future studies of disease effects in the human brain.

Introduction

Many studies have concerned genome-wide expression analysis of human postmortem brain tissue with an aim to identify changes in expression associated with neuropsychiatric disease (Mirmics et al., 2006). Human brain tissue presents a particular challenge for the analysis of gene expression due to variability between individuals and tissue heterogeneity, making the detection of small expression changes difficult. It is routine to match samples across conditions and check for confounding effects of gender, age and other factors. This is not always easy, as postmortem brain tissue is a limited resource and often sample sizes are small. Another common method of reducing the effects of these factors involves adjustment during data analysis. These methods include stratification of samples or implementation of statistical techniques based on observed covariate distributions in the compared populations. However, many studies are underpowered to detect genes so affected. This greatly complicates the

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detection of molecular changes associated with neuropsychiatric disorders such as schizophrenia and bipolar disorder (Tomita et al., 2004).

It is therefore important to understand the effects of factors such as age, gender, brain pH and postmortem interval (PMI) on gene expression, both to control for confounding sources of variability when seeking disease effects, and as a means of elucidating biologically interesting patterns due to the factors themselves. A number of studies have examined expression differences associated with age (Lu et al., 2004, Erraji-Benchekroun et al., 2005), sex (Galfalvy et al., 2003, Vawter et al., 2004, Reinius et al., 2008) and brain pH (Li et al., 2004, Mexal et al., 2006). Because of small samples sizes and the presence of noise, our knowledge of gene expression changes associated with these factors is likely to be incomplete.

One approach to detecting weak patterns is to use meta-analysis. In a meta-analysis, the results from multiple studies are statistically pooled to provide an overall estimate of significance of an effect. While meta-analysis has been increasingly used in the study of gene expression data (Rhodes et al., 2004, Cahan et al., 2007, Borozan et al., 2008), to our knowledge only a few studies have done so with post-mortem human brain data (Elashoff et al., 2007, Choi et al., 2008, de Magalhaes et al., 2009).

In this paper we present a large cross-laboratory meta-analysis of human postmortem brain data, integrating expression data from multiple studies, rather than a simple comparative analysis of published gene lists. Our primary focus in this study is to examine gene expression changes in normal brain with respect to four factors: age, gender, post-mortem interval (PMI) and brain pH, for which data sets from control subjects are publicly available. While many studies treat these factors as a nuisance and attempt to limit their range or control for them, we show that considerable variability in gene expression exists due to these factors. Our results provide new information on gene expression changes attributable to these factors.

Experimental Procedures

Data Collection

Genome-wide expression data sets were selected on the basis of public availability, inclusion of normal subjects, use of neocortical tissue, and the availability sample characteristic data. Details on each of the eleven datasets, including the source citation, can be found in Table 1. Sources include the Stanley Medical Research Institute (SMRI), the Harvard Brain Bank, and the Gene Expression Omnibus (GEO). GEO studies were identified by extensive manual and keyword searches. While the SMRI has additional data sets, these represent repeated runs of the samples from the same subjects, so we arbitrarily selected one data set to represent each of the two SMRI brain collections. Sample characteristics for the normal subjects within each dataset were collected (see Supplementary Table 1 for a summary). Datasets consisted of single-channel intensity data generated from various Affymetrix platforms and one dataset from the Illumina HumanRef-8 BeadArray. For the majority of the datasets, we obtained pre-processed data in which the expression levels were summarized, log transformed and normalized by using the 'rma' function in the R bioconductor 'affy' package (RDevelopmentCoreTeam, 2005). Where possible, we obtained the raw data (.cel files) for the remaining datasets and reprocessed it using the 'rma' function. For the studies in which the raw data was not available, we used the data in its given format.

Regression Analysis

Gene expression values for each probe, in each dataset, were modeled as a function of each of the factors (age, sex, pH, and PMI). P-values were computed using one-sided tests, performed independently for the two alternative null hypotheses (i.e. gene expression does not increase

with the confounding variable and gene expression does not decrease with the confounding variable). To make a fair comparison across studies, we re-annotated probe sequences for each array and mapped them to the corresponding GenBank gene using the Gemma database (<http://www.chibi.ubc.ca/Gemma/>). Probes which were annotated to more than one gene were removed from consideration. For cases in which multiple probes mapped to a single gene, we combined p-values by retaining only the minimum p-value. Analyses were conducted in R (RDevelopmentCoreTeam, 2005); code is available at <http://chibi.ubc.ca/~mmistry/>.

Meta-analysis of Differential Expression

The following meta-analysis was carried out for each of the four confounding factors, and each hypothesis independently. We computed a summary statistic S across n studies for each gene t using Fisher's method (Fisher, 1948), which has been used previously in other microarray meta-analyses (Rhodes et al., 2002, Hess and Iyer, 2007)

$$S(t) = -2 \times \sum_{i=1}^n \log(p_i),$$

where p_i is the regression p-value in the i^{th} experiment. A given gene was included in the analysis given if it was measured in at least three datasets and the particular sample characteristic was reported. A p-value for $S(t)$ is computed by observing that, under the null hypothesis of uniform p-values within each study, $S(t)$ has a χ^2 distribution with $2n$ degrees of freedom. The meta-analysis p-values for each signature were processed with the R 'qvalue' package to control the false discovery rate, yielding a q-value measure for each gene (Storey and Tibshirani, 2003).

Validation analysis

We extracted genes lists from the expression analysis literature for age (Erraji-Benchekroun et al., 2005), gender (Galfalvy et al., 2003) and brain pH (Mexal et al., 2006). Each set consisted of a list of probes (Affymetrix probe sets) differentially expressed in human postmortem brain as reported in their respective studies, which were then split based on direction of change. Each probe was mapped to its corresponding gene using Gemma. Genes were removed if they were not included in our meta-analysis. Details on each of the validation sets can be found in Supplementary Table 1. Agreement of the meta-signature ranking with the validation sets was performed using receiver operating characteristic (ROC) curve analysis.

Each meta-signature was further analyzed for functional enrichment of Gene Ontology (GO) terms (Ashburner et al., 2000), using the 'over-representation analysis' (ORA) method in ErmineJ (Lee et al., 2005). ORA evaluates the genes that meet a specified selection criterion (meta-q < 0.001) and determines if there are gene sets which are statistically over-represented. Probabilities are computed using the binomial approximation to the hypergeometric distribution then corrected for multiple testing using the Benjamini-Hochberg procedure.

Results

Meta-analysis of differential expression

We assessed the global levels of gene expression across datasets by assigning rank values to each gene based on its mean expression value within a dataset. While we observe variation between studies, there still emerges a clear pattern of genes which are consistently strongly or weakly expressed in the brain (data not shown) supporting the feasibility of comparing studies to one another.

We used linear regression to evaluate gene expression changes with respect to four factors (age, sex, brain pH, and PMI), within each dataset. We considered both directions of change (up- and down-regulation) for each factor, creating up to eight different scenarios for each dataset. Although the focus of this paper is to report on the results from the meta-analysis across datasets, we briefly summarized here the results from individual studies. The numbers of genes that show evidence change with each of the factors ($q < 0.01$) is given in Table 2. Not surprisingly, the datasets with smaller sample size showed fewer statistically significant changes associated with the factors. Overall the factors associated with the most robust differential expression were age and brain pH. Genes found to change in individual datasets but not from meta-analysis can be found in Supplementary Table 7 at <http://chibi.ubc.ca/~mmistry/>.

To examine changes in gene expression that were consistent across all datasets, or supported by evidence from multiple data sets, we implemented a cross-study meta-analysis approach (see Methods). The output of this analysis is eight meta-signatures (up or down for each of the four factors). The top ten genes from each meta-signature can be found in Table 7. To examine the results, we first extracted the corresponding p-values from each individual dataset and visualized them as (smoothed) plots in the order determined by the meta-signature order (Figure 1). We observe that genes that have good meta-q-values tended to have good p-values in multiple, but not necessarily all studies. We also demonstrate this using an alternative heat map visualization (Supplementary Figure 1). More detailed results are plotted for some example genes in Figure 2. These plots show that p-values for a given gene vary across individual datasets, and the meta-analysis can identify genes which show only weak or non-significant effects in some data sets. For example, in Figure 2, for age genes *Gfap* and *Rgs4*, we see weak changes in expression level (up and down, respectively), that are not significant after multiple test correction in those studies. On the other hand, we also find genes that show significant effects in most if not all studies (e.g., *Xist*, Figure 2). In Figure 3 we assembled the top 50 genes down-regulated with age, and plotted expression levels within each dataset with samples ordered by increasing age. For most of the studies, we observe a gradient across the dataset as expression decreases from high to low levels, illustrating that the meta-analysis recovers many genes which show fairly consistent trends across data sets.

While we have presented results from an analysis which treated each factor independently (linear regression), we also performed a meta-analysis which models the factors simultaneously in an analysis of covariance, yielding very similar results (available at <http://chibi.ubc.ca/~mmistry/>). We also attempted to model interactions among factors, but this was difficult due to lack of power.

Meta-profile evaluation

We tested the robustness of our meta-profiles by using a jackknife procedure. This involved sequentially removing a dataset, performing the meta-analysis on the remaining datasets, and then selecting genes at a meta- $q < 0.01$. This procedure was repeated for each data set in turn, and genes found in all rounds retained as a “core” signature. Each of the core signatures encompass more than half of the genes found in the corresponding meta-profiles, with the pH meta-profiles as an exception. The “core” signatures can be found in Supplementary Table 6 at <http://chibi.ubc.ca/~mmistry/>.

The studies we selected for meta-analysis were, in general, not designed to test the effects of age, sex, pH or PMI; in fact attempts may have been made to limit the range of these factors (especially in the case of PMI and pH). However, because human brain samples are difficult to obtain, enough variability is allowed into studies to allow us to perform a meaningful meta-analysis. We still questioned the extent to which our results would agree with more targeted studies. Therefore, for the purpose of validation of our approach, we identified independent

gene lists from the literature for age, sex and brain pH (we could not find a comprehensive validation set for PMI). Details on validation sets can be found in Supplementary Table 2. Each validation gene list was then separated into two groups based on direction of change, to correspond with meta-signatures obtained from our meta-analysis. Obviously none of these validation gene lists can be considered true gold standards, but help put our results in the context of previous findings.

To quantify the predictive power of our analysis meta-signatures with respect to the corresponding validation sets, we first performed a standard receiver operating characteristic (ROC) analysis. The score reported for each profile is the area under the ROC curve (AUC), a value between 0 and 1, where 1.0 is perfect agreement with the external list and 0.5 would reflect a random order. The AUC values for each of the profiles are reported in Table 3. Individual ROC plots can be found in Supplementary Figure 2. We also tested the effect of using a specific statistical threshold for selecting genes from the meta-profiles, by collecting genes at two significance levels (meta- $q < 0.01$, and meta- $q < 0.001$). The overlap with the validation set was significant ($p < 0.001$, Fisher's exact test; Table 3) for all signatures. We also found a comparable overlap between each of the core signatures and the validation sets.

The brain pH profiles gave high AUC scores of 0.91 and 0.86 (up- and down-regulated genes, respectively), when validated against DLPFC pH-sensitive genes taken from Vawter et al. (2006). Additionally, we obtained reasonably high scores using a smaller independent pH gene list obtained from Mexal et al. (2006), despite a difference in the brain region used between the validation study and the meta-analysis (data not shown). Because pH itself probably covaries across brain regions (Mexal et al., 2006), our results are consistent with the hypothesis that pH-related changes in gene expression are similar across brain regions. The age profiles on the other hand, exhibited slightly lower scores than those obtained for brain pH. Erraji-Benchekroun et al. used samples from Brodmann areas 9 (dorsolateral PFC, BA9) and 47 (orbitofrontal PFC, BA47) from each subject, to evaluate age expression differences, showing comparable changes in both brain regions (Erraji-Benchekroun et al., 2005). As such, our validation set consisted of genes showing age expression changes collectively within both neocortical brain regions BA9 and BA47. While many of these genes appear at the top of our ranked lists, some are also dispersed throughout our ranking. The gender meta-signature from our meta-analysis scored high when validated with a set of genes from Galfalvy et al. (2003), with most validation genes sitting at the top of the ranking.

Overlap with schizophrenia candidate genes

We compared significant genes (meta- $q < 0.001$) from each of our meta-signatures with genes known to be associated with schizophrenia. We extracted a list of 34 schizophrenia candidate genes provided in a comprehensive literature review (Colantuoni et al., 2008), and searched this list of genes within each of our meta-signatures. We found that 12 of these genes identified with at least one of our meta-signatures, though the majority of overlap was observed amongst the age and pH meta-profiles (Table 4). Overlap of genes with each of the age meta-signatures was significant at $p < 0.01$.

Affected Biological Pathways

To derive a high-level biological interpretation of our meta-signatures we performed a Gene Ontology (GO) (Ashburner et al., 2000) enrichment analysis using ErmineJ (Lee et al., 2005). We extracted the 'top' GO categories for each of the profiles and a comparison amongst them revealed various 'biological processes' unique to each meta-signature, in addition to a number of shared categories. In Figure 4, we have displayed the top ten categories for each age meta-signature depicting the number of genes represented in each GO category and the

associated p-value (corrected for multiple testing). The ORA data generated for all eight meta-signatures can be found in Supplementary Table 5 at <http://chibi.ubc.ca/~mmistry/>.

For genes increasing in expression with the progression of age, top GO categories included those involved in cell growth and proliferation, and cell-cell interaction, consistent with previous studies (Erraji-Benchekroun et al., 2005, Hong et al., 2008). Other processes included the insulin receptor signaling pathway, encompassing a number of genes involved in longevity and aging (Bartke, 2008). The age down-regulated genes presented an enrichment in synaptic and/or receptor activity with GO categories such as neuron recognition, neurotransmitter transport and secretion, and regulation of neurotransmitter levels. This finding is concordant with existing aging studies in mouse and human (Erraji-Benchekroun et al., 2005, Oh et al., 2009). Similarly, we found an enrichment of genes involved in neurotransmitter secretion in the pH up-regulated meta-signature, in addition to genes implicated in metabolism and a different array of pathways (i.e. G-protein signaling). The female and male meta-signatures identified a similar enrichment of terms including relevant processes such as sex determination. However, the genes associated with these shared terms were quite different between the two meta-signatures. Functional enrichment analysis of our meta-signatures, although far from providing hard cellular evidence, still provided a useful indication of the biological processes altered by each factor and greater insight at the molecular level.

Correlation between factors and meta-profiles

Because we analyzed each factor independently, we wished to check whether factors were correlated with each other across the 415 samples (Table 5). Age and PMI displayed the highest correlation of 0.35, consistent with a positive correlation reported in (Galfalvy et al., 2003). Age and brain pH displayed a slight negative correlation of -0.2 . Further investigation of these two factors revealed that categorizing the values of age into ‘young’ (< 50 years of age) and ‘old’ (≥ 50 years of age) groups resulted in a lower mean pH in the ‘old’ group versus the ‘young’. This was the general trend within each dataset (Supplementary Figure 3).

Due to these correlations, we expected that individual genes in some meta-profiles might overlap with other meta-profiles (Table 6). Accordingly, the two factors displaying the highest number of overlapping genes were those [up or down] in age and those [down or up] with brain pH, respectively. However, these effects were weak and were even weaker in the ‘core’ signatures (Supplementary Table 4). We also found that a number of genes up-regulated with PMI were also identified amongst the profiles for brain pH and age in both directions, but were unable to extract any definite patterns.

Discussion

We have conducted a meta-analysis of gene expression in human cortex, examining gender, age, postmortem interval and brain pH, as well as expression level. This meta-analysis was made possible by the fact that many gene expression analyses have useful data for each of these factors, even though they were usually considered potential “confounds” to be controlled. Our results have at least two potential uses for future studies. First, the results of our meta-analysis provide new information on the effects of each of the factors on gene expression and can be studied further independently or used to bolster support for other studies. Second, the identification of robust signatures associated with these factors will provide a ‘watch list’ of genes which might be viewed cautiously if they are found to be implicated in neuropsychiatric disease by expression studies. To facilitate the use of these lists in future studies, they are provided in Supplementary Table 6 at <http://chibi.ubc.ca/~mmistry/>, with the top ten from each list displayed in Table 7.

There are some limitations of our study. We used a relatively simple meta-analysis method, and other techniques may have even higher sensitivity. We combined datasets generated using differing platforms, which may contribute noise and potentially reduce the power of our meta-analysis. The MAQC (MicroArray Quality Control) project recently initiated a number of studies to specifically address these concerns, and in general, reported a high agreement between platforms (Canales et al., 2006, Shi et al., 2006, Shippy et al., 2006). A number of other studies have been conducted to this end, showing agreement between platforms (Dobbin et al., 2005, Irizarry et al., 2005, Petersen et al., 2005), and a high concordance between the top functions identified by each platform (Pedotti et al., 2008, Li et al., 2009). While we acknowledge that there still remain small differences between studies, we are only focussed on the consistencies and combining them to extract more robust expression changes than can be derived from single dataset studies. To maximize total sample size in our study, we used a coarsely defined neocortical brain region. The majority of our datasets utilized samples from the frontal cortex, but we also included a number of samples from the temporal and parietal cortices. Various groups have studied regional patterns of gene expression in the postmortem human brain, revealing cortical regions to generally cluster together indicating a shared global expression profile (Khaltovich et al., 2004, Roth et al., 2006, Ernst et al., 2007). Finally, we only considered linear model fits to age, pH and PMI. Future studies can address some of these issues, and also include more data as studies become available.

Comparison of our results to the validation sets strongly supported the relevance of the meta-signatures. However, the overlap in 'top genes' between meta-profiles and validation sets, while statistically significant, was only a subset of the genes in the validation lists. There are several possible explanations for this effect. One is that most of the studies we used treated these factors as nuisances to be eliminated, which may have reduced our power to find real changes. For example, the majority of the datasets have no subjects under the age of 30 at the time of death, and most are over 40. In particular the Kato and Chen data sets, which use samples from the Stanley Foundation, have a particularly well-controlled (narrow) age range. In contrast the age validation set used a broader age range (13 to 79 years of age) (Erraji-Benchekroun et al., 2005). Additionally, we expect biological variation among sample groups (and therefore studies). Strong signals in any given data set, including the validation sets, may be specific to that study. That is, none of the validation sets are truly gold standards. Further examination of genes on the validation lists within each individual dataset supports this notion. The agreement between the meta-analysis and the validation lists is better than the agreement between the validation and any single dataset, with only a few to none of the validation genes being identified in each dataset. In summary, the limited overlap of the meta-signatures with the validation sets may simply be contingent on the data we used, and does not call into question the validation sets or the meta-analysis.

Using a jackknife analysis, we obtained core signatures for each of the factors. We found a large proportion of meta-profile genes overlap with the 'core' signatures, illustrating the ability of our meta-analysis to extract gene profiles robust to influences from individual datasets. The exception was brain pH, for which the 'core' signatures consisted of very few genes (Supplementary Table 4). This was due to a large pH effect in the Kato dataset. However, examination of the other data sets revealed that many genes showing large effects in the Kato data set also show trends with pH (Supplementary Figure 4). Thus, even though the pH meta-profiles are arguably biased by strong effects from the Kato dataset, these genes appear to show weaker changes in many other data sets.

The inverse relationship we observed between our age and brain pH meta-profiles is in agreement with a previous study (Harrison et al., 1995). A review of the literature also reveals that results from independent gene expression studies examining changes with age are strikingly similar to results derived from brain pH profiling studies, (Li et al., 2004, Erraji-

Bencheikroun et al., 2005, Berchtold et al., 2008, Hong et al., 2008, de Magalhaes et al., 2009), further supporting this relationship. It has been suggested that the relationship between age and pH is likely a result of slower modes of death experienced by elderly subjects (Harrison et al., 1995), but this has not yet been fully explored. Previous studies however, have found brain pH to be a proxy for agonal stress (Li et al., 2007). Subjects experiencing a longer terminal phase of death results in lower brain pH levels than would be observed in subjects experiencing a sudden death. We were unable to obtain cause of death information for the majority of our datasets, and thus were unable to incorporate it into the meta-analysis. This raises the question of whether the reasoning behind the inverse relationship is as hypothesized by Harrison et al. (1995), or if the process of aging actually results in a general decline of brain pH.

To this point we have focused on evaluating the meta-analysis in light of other data sets, but clearly one of the reasons to do a meta-analysis is to integrate information on weak patterns. Indeed, our meta-analysis has confirmed previous finding and also added to them. By assembling the significant genes ($q < 0.01$) from individual datasets, we were able to generate a 'union' signature, for each of the factors (Table 2). A comparison of the union signatures against the corresponding meta-signatures revealed greater than 50% of the genes in each of our meta-signatures to be novel (not found in any of the individual studies, and only revealed by the meta-analysis). These novel genes span a broad range of cellular functions, implicating various gene families with each of the different factors. An example is alterations of the gamma-aminobutyric acid (GABA)-related transcriptome in age. We found two GABA receptors (Gabbr and Gabrg2) and two glutamic acid decarboxylases (Gad67 and Gad65) to be down-regulated with age. Animal studies have demonstrated that GABA receptors are markedly decreased with age, and there is evidence to suggest this plays a role in age-related cognitive changes (Jiang et al., 2001, El Idrissi, 2008). Evidence of reduced inhibitory neurotransmission in the human brain is supported by evidence from a recent study (Loerch et al., 2008) using the Lu et al. (2004) dataset, and is also observed in the results of our meta-analysis. Also consistently altered in our age meta-signature are members of the regulator of G-protein signalling (RGS) family. RGS family members, expressed in the brain and periphery, plays a critical role in signal transduction by negatively regulating G-protein-coupled receptors (GPCR) by means of their GTPase accelerating activity. These proteins have been implicated in normal behavioural processes and various brain disorders. In the brain, they function to modulate neurotransmission resulting from the activation of metabotropic GPCRs. Rgs4, a member of this family has been previously shown to be down-regulated with age (Colantuoni et al., 2008). In our study, we confirm this finding and additionally report four other members of the family, (Rgs6, Rgs7, Rgs12 and Rgs17) that display an age-related decline in expression. Alterations in expression of RGS genes presents a possible molecular mechanism that could affect neuronal functioning during aging.

One motivation of our study was to identify gene expression changes which need to be accounted for when studying potential expression changes in neuropsychiatric disorders such as schizophrenia. This is important because changes in expression due to the factors we studied can be large, compared to the reported effects of psychiatric disease (Mirnics et al., 2006). Thus even a mild bias in age might cause a change in expression which is as large, or larger than, the effect of disease. Therefore a gene which is known to change expression with age (for example) must be analyzed very carefully if it is to be considered a candidate marker for disease, because it is difficult to control perfectly for age. We searched our meta-signatures for a list of schizophrenia associated genes and found that 12 of these genes identified with at least one of our meta-signatures (Table 4). One such example is Rgs4, a gene that has been extensively characterized in schizophrenia studies (Levitt et al., 2006) and which we find to be down-regulated with age. Our results confirm previous work showing that Rgs4 is down-regulated with age (Colantuoni et al., 2008). We also identified the receptor-ligand pair ErbB3 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 3) and Nrg1 (neuregulin 1) in our

age up- and down-regulated meta-signatures, respectively. Nrg1 and its receptor ErbB3 are implicated in key neurodevelopmental processes in the nervous system (Falls, 2003), and have also implicated in schizophrenia (Corfas et al., 2004). Evidence of a role for Nrg1-ErbB in schizophrenia includes reduction in the level of ErbB3 expression in human postmortem prefrontal cortex samples and genetic association evidence linking Nrg1 to schizophrenia (Corfas et al., 2004). Another notable candidate is Gad67, a gene that is down-regulated across our meta-signatures for age (consistent with some reports in the literature (Cashion et al., 2004, Siegmund et al., 2007)), and up-regulated with pH. The reduction of Gad67 expression in schizophrenia is arguably one of the best established changes for this disorder (Mirnics et al., 2006). Together these findings of expression alterations of genes implicated in schizophrenia with respect to these factors contribute an added complexity to their pre-existing relationships with the disorder.

Looking specifically at findings from microarray studies of schizophrenia (Middleton et al., 2002, Vawter et al., 2002, Iwamoto et al., 2005, Arion et al., 2007), we find additional overlap with the results from our meta-analysis. Synaptic machinery transcripts such as Syn2 and Synj1, reported as down-regulated in subjects with schizophrenia, are also down-regulated with age in our meta-signatures. We see similar patterns between our data and other genes reported to be down-regulated in schizophrenia such as Mapk1, Kcnk1, and Crym. Careful analysis of such genes will allow us to explore the potential of interrelationships between these factors and schizophrenia, and reveal the underlying factors driving the changes in gene expression.

In summary, our results show that meta-analysis of human brain gene expression data is both feasible and informative. We hope our study will encourage future meta-analyses on the effects of neuropsychiatric disease on human brain gene expression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

MM was partly supported by the Canadian Institute for Health Research (CIHR) bioinformatics training program. PP is supported by a career award from the Michael Smith Foundation for Health Research, a CIHR New Investigator award, the Canadian Foundation for Innovation, and a Human Brain Project grant from the National Institutes of Health (GM076990). We thank Kelsey Hamer for technical support. We thank Clare Beasley for critical reading of the manuscript. We also thank the groups and institutions who made their data publicly available, including the SMRI and the Harvard Brain bank. We thank Nicole Berchtold (University of California, Irvine), Mehmet Somel (Max Planck Institute for Evolutionary Anthropology), Alice Chen-Plotkin (Center for Neurodegenerative Disease Research) for providing additional information on their data sets.

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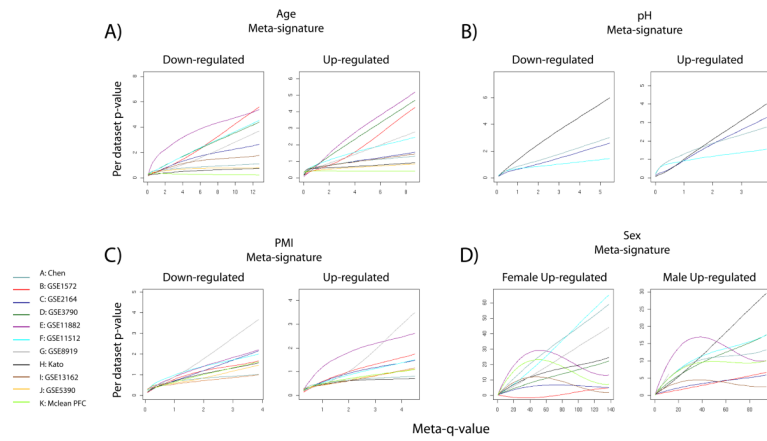


Figure 1. Distribution of dataset p-values across meta-signature q-values

For each dataset used, gene p-values were plotted against the corresponding meta-q value and a loess fit was computed to generate a smooth curve between points. The fact that most data sets show a rise in p-values correlated with the meta-q-values indicates the contribution of signals of varying strengths to the meta-signatures. An alternative view of the data using heat maps is available in the supplement. The distorted curves for gender are due to the strong effects of a small number of genes with very small meta-q-values (note the difference in scale of D compared to A–C).

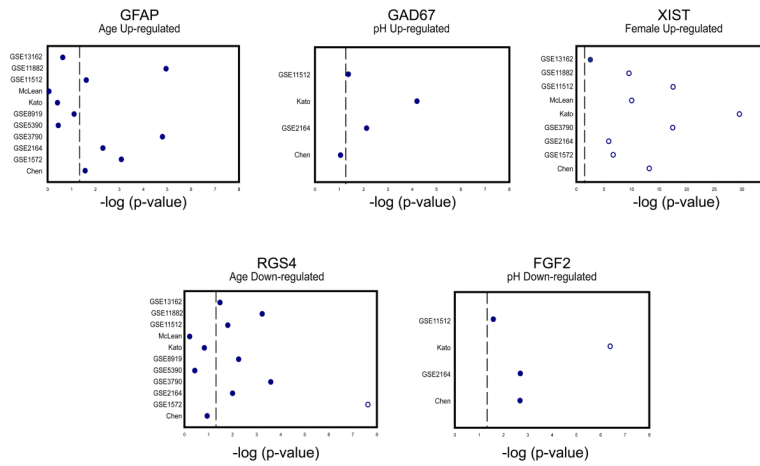


Figure 2. Distribution of dataset p-values for individual genes: a magnified view

For selected genes from each of the meta-signatures we have plotted the log regression p-values from each dataset. Open circles represent the datasets for which the gene was found to be significant after multiple test correction ($q < 0.01$). Dashed line indicates a per-study p-value significance level of 0.05 for reference.

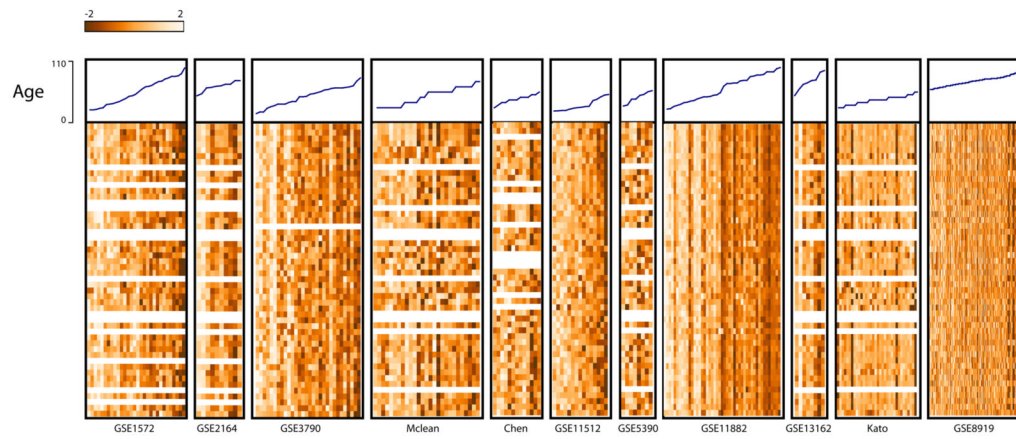


Figure 3. Top genes down-regulated with age

The top 50 age down-regulated genes were selected based on meta-analysis q-value ranking. For each gene, the corresponding data from each study was extracted and converted to a heat map. Expression values were normalized across samples within each dataset, and ordered by age. Age is plotted at the top of each heat map. Light values in heat map indicate higher expression. Grey bars indicate missing values. All data sets are at approximately the same horizontal scale except the last, which is compressed to fit on the page.

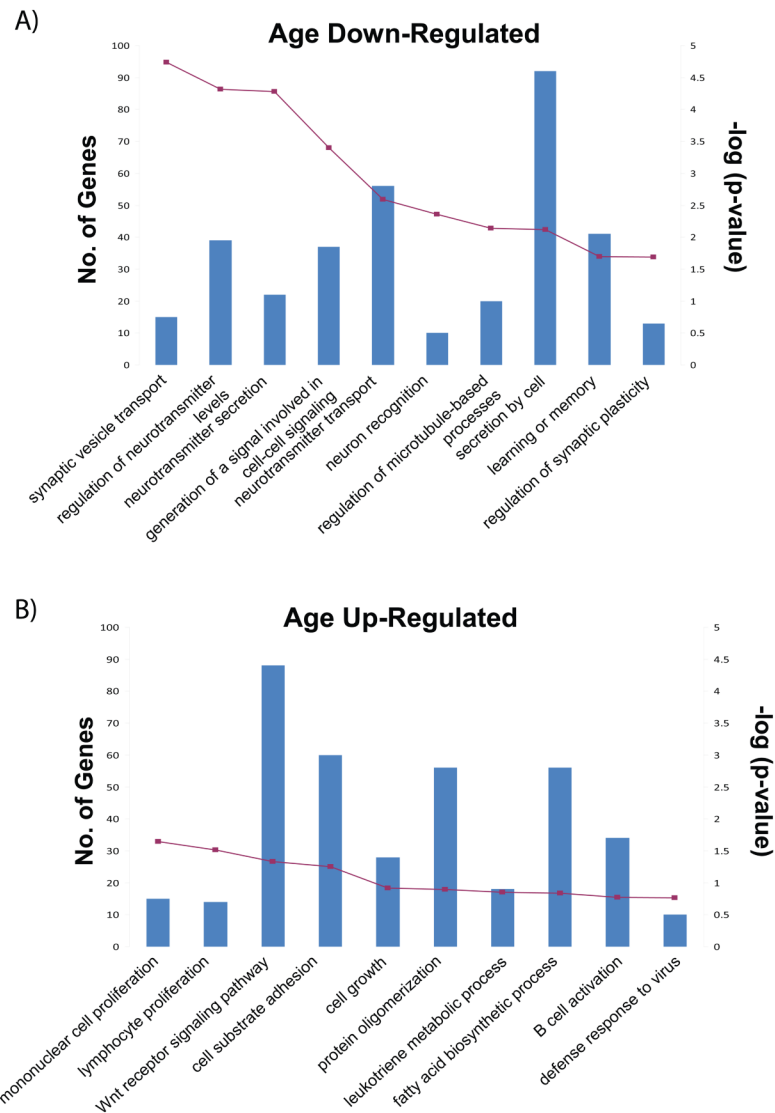


Figure 4. Gene Ontology Enrichment Analysis

For the age meta-signatures, we have displayed the top 10 GO terms identified using a GO over-representation analysis. The primary y-axis displays the number of meta-signature genes that fall in the given 'biological process' category, while the secondary axis displays the associated p-value. GO terms were collapsed to parent term if parent and child both appeared in the top ten.

Table 1

Human postmortem brain datasets included in meta-analysis

Dataset	Reference	Description	Microarray Platform	Brain region(s)	No. of normal Subjects
A	Stanley Chen n/a	Schizophrenia, Bipolar, depression	HG-U133A/B (RMA)	DLPFC	13
B	GSE1572 Lu et al. (2004)	Aging study	HG-U95vA (RMA)	Frontal lobe	30
C	GSE2164 Vawter et al. (2004)	Gender differences in expression	HG-U95vA (RMA)	DLPFC	10
D	GSE3790 Hodges et al. (2006)	Huntington's	HG-U133A/B (MAS 5.0)	Frontal cortex	36
E	GSE11882 Berchtold et al. (2008)	Gender and aging	HG-U133 Plus 2.0 (GC-RMA)	Superior Frontal Gyrus	47
F	GSE11512 Somel et al. (2009)	Transcriptional neoteny	HG-U133 Plus2.0 (RMA)	Frontal cortex	15
G	GSE8919 Myers et al. (2007)	Cortical gene expression	Illumina Sentrix BeadChip (Illumina Software)	Cerebral cortex, temporal lobe, frontal lobe, parietal lobe	193
H	Stanley Kato Iwamoto et al. (2005)	Schizophrenia, Bipolar	HG-U133A (RMA)	DLPFC	34
I	GSE13162 Chen-Plotkin et al. (2008)	Frontotemporal lobar degeneration	HG-U133A (RMA)	Frontal cortex	8
J	GSE5390 Lockstone et al. (2007)	Down Syndrome	HG-U133A (RMA)	DLPFC	8
K	McLean PFC n/a	Schizophrenia, Bipolar	HG-U133A (RMA)	PFC	27

Table 2

Significant genes ($q < 0.01$) identified within each individual dataset

Dataset	Age		Sex		pH		PMI	
	Down	Up	Female	Male	Down	Up	Down	Up
Stanley Chen	0	0	2	7	0	0	0	0
Stanley Kato	0	0	1	10	703	0	0	0
GSE1572	162	64	1	6	n/a	n/a	0	0
GSE2164	0	0	1	2	0	0	0	0
GSE8919	153	133	6	19	n/a	n/a	2	278
GSE11512	0	0	3	13	1	0	0	0
GSE11882	428	0	2	14	n/a	n/a	1	0
GSE13162	0	0	0	0	n/a	n/a	0	0
GSE5390	1	1	n/a	n/a	n/a	n/a	0	0
McLeanPFC	0	0	1	5	0	0	n/a	n/a
GSE3790	0	0	1	13	n/a	n/a	n/a	n/a
'Union' Signature	689	198	10	33	704	0	2	278
Meta-signature Overlap	415	102	6	19	191	0	1	46

Q-values were calculated from regression p-values for each factor within each dataset. The number of genes reported here are significant at $q < 0.01$. The 'union' signature represents the union of unique genes identified for each factor across all datasets. The meta-signature overlap indicates the number of union signature genes overlapping with the corresponding meta-signature.

Table 3

Comparison of meta-signature profiles against validation gene sets

	No. of profile genes ($q < 0.001$)	Overlap with validation set ($q < 0.001$)	No. of profile genes ($q < 0.01$)	Overlap with validation set ($q < 0.01$)	AUC score
Age Genes					
Up-regulated	404	40	1241	69	0.74
Down-regulated	1134	113	2247	136	0.76
pH Genes					
Up-regulated	25	11	368	131	0.91
Down-regulated	215	55	1018	122	0.86
Sex Genes					
Male	14	7	38	7	0.90
Female	19	1	128	1	n/a
PMI Genes					
Up-regulated	75	n/a	691	n/a	n/a
Down- regulated	4	n/a	49	n/a	n/a

Table 4

Schizophrenia candidate gene analysis

Schizophrenia genes identified in meta-profiles		
Age	Down	Opcml, Pldn, Nrg1, Rgs4, Bdnf, Dlg4, Gad67
	Up	Ntrk2, Ppp1r1b, Erbb3
pH	Down	Ntrk2, Slc1a2
	Up	Rgs4, Gad67
PMI	Up	Nrg1

Table 5

Rank correlations between factors

	Age	Sex	pH	PMI
Age				
Sex	-0.12 **			
pH	-0.2 *	0.17		
PMI	0.35 ***	-0.2 ***	-0.06	

Spearman rank correlations were computed using sample characteristic information for individual subjects.

* indicates a p-value of ≤ 0.05

** indicates a p-value of ≤ 0.01

*** indicates a p-value of $\ll 0.001$

Table 6

Evaluating gene overlap between meta-signatures

	No. of Profile genes		Age		pH		PMI		Sex	
	Up	Down	Up	Down	Up	Down	Up	Down	Female	Male
Age	404									
Up			2							
Down	1134									
pH	25	18	0							
Up				18						
Down	215	1	75	1	0					
PMI	75	32	2		0	2				
Up				32	0					
Down	4	2	1	2	0	0	0			
Sex	19	1	4		0	1	0	0	0	0
Female				1						
Male	14	1	0	1	0	0	0	0	0	0

Using genes for each meta-profile at $q < 0.001$, we compared them against one another to evaluate the overlap and potential relationships between the factors. The observation of two genes in the age meta-signature that change in both directions is a consequence of multiple probes of different specificity for the same gene, which show different directions of change.

Table 7

Top meta-signature genes

Age Down-regulated	Age Up-regulated
OLFMI	NEBL
KCNFI	MED12
RGS4	BCL2
PPP3CB	GMPR
ADCY2	GFAP
SVOP	ADH1B
EFNB3	WWOX
ATP2B2	PLEC1
HPCA	VCAN
CALB1	AHCYL1
pH Down-regulated	pH Up-regulated
FGF2	SLC1A1
AHCYL1	LARGE
DTNA	C17orf81
MAPKAPK2	HISPPD2A
TJP1	PRKCD
S100A13	DLG3
RBBP6	KCNAB1
GNNG12	SLC8A1
ANP32E	GABRA5
BAALC	RIT2
Female Up-regulated	Male Up-regulated
XIST	JARID1D
HDHD1A	USP9Y

nebullette
mediator complex subunit 12
B-cell CLL/lymphoma 2
guanosine monophosphate reductase
glial fibrillary acidic protein
alcohol dehydrogenase 1B (class I), beta polypeptide
WW domain containing oxidoreductase
plectin 1, intermediate filament binding protein 500kDa
versican
S-adenosylhomocysteine hydrolase-like 1

solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system X_{ag}), member 1
like-glycosyltransferase
chromosome 17 open reading frame 81
histidine acid phosphatase domain containing 2A
protein kinase C, delta
discs, large homolog 3 (Drosophila)
potassium voltage-gated channel, shaker-related subfamily, beta member 1
solute carrier family 8 (sodium/calcium exchanger), member 1
gamma-aminobutyric acid (GABA) A receptor, alpha 5
Ras-like without CAAX 2

junonji, AT rich interactive domain 1D
ubiquitin specific peptidase 9, Y-linked (fat facets-like, Drosophila)

Age Down-regulated	Age Up-regulated
UTX	EIF1AY
JARID1C	CYorf15B
TSIX	DDX3Y
USP9X	UTY
LOC554203	RPS4Y1
ST5	TTTTY15
ZFX	CYorf15A
PNPLA4	TMSB4Y
PMI Down-regulated	PMI Up-regulated
BRD8	GOSR2
RBM5	CYBSB
PUM2	SNF8
ARHGEF7	GRLF1
NCOA3	C6orf1
DAPK1	MGMT
SORL1	MAX
KIAA0841	ST3GAL2
CAMK2G	PTGER3
DIP2C	EXT1
	ubiquitously transcribed tetratricopeptide repeat, X chromosome
	ubiquitously transcribed tetratricopeptide repeat, X chromosome
	jumonji, AT rich interactive domain 1C
	XIST antisense RNA (non-protein coding)
	ubiquitin specific peptidase 9, X-linked
	alanyl-tRNA synthetase domain containing 1 pseudogene
	steroid sulfatase (microsomal), isozyme S
	zinc finger protein, X-linked
	patatin-like phospholipase domain containing 4
	bromodomain containing 8
	RNA binding motif protein 5
	pumilio homolog 2 (Drosophila)
	Rho guanine nucleotide exchange factor (GEF) 7
	nuclear receptor coactivator 3
	death-associated protein kinase 1
	sortilin-related receptor, L(DLR class) A repeats-containing
	KIAA0841
	calcium/calmodulin-dependent protein kinase II gamma
	DIP2 disco-interacting protein 2 homolog C (Drosophila)
	eukaryotic translation initiation factor 1A, Y-linked
	chromosome Y open reading frame 15B
	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked
	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked
	ribosomal protein S4, Y-linked 1
	testis-specific transcript, Y-linked 15
	chromosome Y open reading frame 15A
	thymosin beta 4, Y-linked
	golgi SNAP receptor complex member 2
	cytochrome b5 type B (outer mitochondrial membrane)
	SNF8, ESCRT-II complex subunit, homolog (S. cerevisiae)
	glucocorticoid receptor DNA binding factor 1
	chromosome 6 open reading frame 1
	O-6-methylguanine-DNA methyltransferase
	MYC associated factor X
	ST3 beta-galactoside alpha-2,3-sialyltransferase 2
	prostaglandin E receptor 3 (subtype EP3)
	exostosins (multiple) 1

For each meta-signature we have listed the top ten genes, as ranked by meta-q-value (all at $q < 0.001$). For each gene we have listed the gene symbol an gene name. Complete meta-signature lists for each factor can be found in Supplementary Table 6 <http://chibi.ubc.ca/~nmistry/>.