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Transcription and Pathway Analysis of the Superior Temporal Cortex and Anterior Prefrontal Cortex in Schizophrenia

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The molecular basis of schizophrenia is poorly understood; however, different brain regions are believed to play distinct roles in disease symptomology. We have studied gene expression in the superior temporal cortex (Brodmann area 22; BA22), which may play a role in positive pathophysiology, and compared our results with data from the anterior prefrontal cortex (BA10), which shows evidence for a role in negative symptoms. Genome-wide mRNA expression was determined in the BA22 region in 23 schizophrenics and 19 controls and compared with a BA10 data set from the same subjects. After adjustments for confounding sources of variation, we carried out GeneGO pathway enrichment analysis in each region. Significant differences were seen in age-related transcriptional changes between the BA22 and the BA10 regions, 21.8% and 41.4% of disease-associated transcripts showing age association, respectively. After removing age associated changes from our data, we saw the highest enrichment in processes mediating cell adhesion, synaptic contact, cytoskeletal remodelling, and apoptosis in the BA22 region. For the BA10 region, we observed the strongest changes in reproductive signalling, tissue remodelling, and cell differentiation. Further exploratory analysis also identified potentially disease-relevant processes that were undetected in our more stringent primary analysis, including autophagy in the BA22 region and the amyloid process in the BA10 region. Collectively, our analysis suggests disruption of many common pathways and processes underpinning synaptic plasticity in both regions in schizophrenia, whereas individual regions emphasize changes in certain pathways that may help to highlight pathway-specific therapeutic opportunities to treat negative or positive symptoms of the disease.

Key words: BA22; BA10; amyloid; autophagy; synaptic plasticity

Schizophrenia is a severe psychiatric illness with enormous societal impact. Accurate diagnosis requires two or more characteristic symptoms to be present, including positive symptoms such as hallucinations and delusions, and negative symptoms such as blunted emotional responding and substantial impairment of social function (Gottesman, 1989). Recent genome-wide association studies (GWAS), demonstrate that schizophreniic pathology may be underpinned by a moderate number of common alleles with individually weak effects, which are potentiated by environment, and a large number of rare alleles that act independently or with environment. Three recent, well-powered GWAS replicated genetic

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associations of common schizophrenia alleles, including the major histocompatibility complex region (Purcell et al., 2009; Shi et al., 2009; Stefansson et al., 2009). Deletion analysis has also identified rare alleles that may predispose to schizophrenia, including ERBB4, NRXN1, CHRNA7 (Stefansson et al., 2008; Walsh et al., 2008), and most notably DISC1, which allowed the elucidation of a novel disease pathway in both schizophrenia and bipolar disorder (Hennah and Porteous, 2009). Transcriptomic analysis in high-quality post-mortem brain tissue can complement genetic studies by identifying disease-associated changes in mRNA expression, some of which may be mediated by disease alleles. In the present study, we assessed transcriptomic changes in a carefully curated schizophrenia cohort, the Charing Cross Hospital Prospective Collection (CCHPC). We analyzed gene expression in the superior temporal cortex (Brodmann area 22; BA22) from 23 schizophrenics and 19 controls. We previously reported a transcriptomic analysis of the anterior prefrontal cortex (BA10) collected from the same subjects (Maycox et al., 2009), using the same array platform. This gave us an opportunity to compare expression directly between the BA22 and BA10 regions. There is some evidence that these regions are involved in the mediation of distinct aspects of schizophrenia pathology. Imaging studies of the prefrontal cortex point to a role in higher cognitive dysfunction in the disease, which may manifest in some of the negative symptoms (Barbalat et al., 2009), whereas structural imaging studies have emphasized left-sided temporal cortex abnormalities in schizophrenia, with low volumes of gray matter, which have been consistently linked to auditory hallucinations, one of the main positive symptoms (Woodruff et al., 1997). The progressive BA22 volume loss appears to occur early in the illness (Rapoport et al., 1999), with some evidence of a proapoptotic mechanism (Jarskog et al., 2004). However, a recent meta-analysis of over 25 imaging studies (Goghari et al., 2010) suggests that regional involvement may be more complex than previously expected, particularly in the superior temporal cortex, which showed no consistent relationship with specific disease symptoms.

Susceptibility to schizophrenia is likely to depend on the cumulative modest effects of multiple genes interacting across multiple overlapping functional pathways (Balu and Coyle, 2011). We use the term ''pathway'' with the caveat that knowledge of pathways is incomplete and biased toward a particular disease processes. However, at the simplest level, it might be useful to consider pathways as modules of interacting genes that alter the function or expression of other genes, leading to a biological phenotypic endpoint. Similar approaches have been successful in other complex diseases (Baranzini et al., 2009; Holmans et al., 2009; Beech et al., 2010). Here we show that the application of a pathway approach to transcriptomic analysis can yield interesting insights into the biology of schizophrenia by implicating novel pathways. Although the involvement of specific brain regions in different aspects of disease pathology is somewhat contentious, pathway analysis can improve the molecular understanding of each brain region, which in turn should improve overall understanding of schizophrenia. Ultimately, we believe that a better molecular understanding of the role of specific brain regions in the disease may improve the overall understanding of schizophrenia pathology, progression, and response to treatment, leading to novel and improved therapeutic opportunities.

TABLE I. Summary of Patient Demographics for Samples That Were Included in the Statistical Analysis

	Schizophrenic	Control
No. of samples	23	19
Gender (male/females)	13/10	11/8
Age (years)		
Mean	72.2	67.7
SD	16.9	22.2
Median	77.0	72.0
Range	28–97	25–94
PMD (hr)		
Mean	7.1	9.1
SD	5.7	4.3
Median	5.0	8
Range	3–30	4–17
Brain pH		
Mean	6.2	6.5
SD	0.2	0.3
Median	6.2	6.6
Range	5.7–6.5	5.7–6.9

MATERIALS AND METHODS

Tissue Collection

Samples were collected as part of a prospective collection program coordinated through Imperial College London. The group was elderly patients whose schizophrenic illnesses had started before the introduction of antipsychotic medication and who had progressed to long-stay psychiatric nursing facilities. Demographic, diagnostic, and clinical data were ascertained from personal knowledge and scrutiny of all case notes available (Table I). Patients were diagnosed prospectively according to DSM-III criteria by A.M.M., supplemented by scrutiny of all case notes. All of the patients met diagnostic criteria for residual schizophrenia with pronounced negative symptoms alongside attenuated positive symptoms and intellectual dysfunction. The mean age of onset was 26 years, and duration between onset and death was almost 5 decades, with a mean of 48 years. Two patients had been ill for 73 years. Thirteen patients had never been discharged after their first admission, whereas only five had more than four periods of discharge between their first admission and their death in hospital. Eleven patients had had electroconvulsive therapy in the past. Most patients had been treated with neuroleptic drugs when they became available. The mean duration of treatment was 33 years. One patient was neuroleptic naive at death. Doses were relatively low, and only four patients took doses as expressed in chlorpromazine equivalents of more than 750 mg per day. The ethnicity of patients was Caucasian. All

patients, with the agreement of their nearest relative or authorized representative, have given written informed consent for use of tissue obtained post-mortem for research.

Control brain samples were obtained from mentally normal Caucasian tissue donors from the community, Charing Cross Hospital, and local nursing homes. The causes of death were similar in the control and schizophrenia cohorts, bronchopneumonia being the most common cause, followed by carcinoma (lung, bowel, prostate, bladder, esophagus) and, to a lesser extent, ischemic heart disease and coronary artery occlusion. At autopsy, gross examination of brains revealed no major atrophy. As for the patient group, the right hemisphere was sampled. Cases of widespread damage from stroke were excluded. Histological screening was carried out, and cases with evidence of Alzheimer's disease, Parkinson's disease, or multiple sclerosis were excluded. The control and schizophrenia cohorts were collected over the same period, and the storage conditions are identical. This study has been approved by the West London Mental Health Ethical Research Committee and complies with the conditions of the Research Governance Office of the Imperial College of Science, Technology and Medicine Clinical Research Office.

Array Processing of CCHPC Samples

Total RNA was extracted from frozen BA22 samples obtained from 69 donors as described previously (Maycox et al., 2009). Although pH was analyzed in brain lysates with a pH meter, this was not considered to be rigorous enough to exclude or include samples, and instead the RNA integrity number (RIN) was used to assess the quality of the RNA as the primary inclusion criterion. The quantity of extracted RNA was determined by spectrophotometry, and quality was assessed using an Agilent 2100 Bioanalyzer (Agilent, South Plainfield, NJ) to determine the RIN. Based on the RIN, samples were classified into three quality groups, pass (RIN $>$ 7.0), borderline (RIN 6.0–7.0), and fail (RIN $<$ 6.0). After classification, there were 31 pass (RIN range of samples 7.0–9.0, average 7.7), 30 borderline (RIN range of samples 6.0–6.9; average 6.4), and six fail samples. Two donors were withdrawn. Samples in the fail category were excluded from the study, and the remaining samples were randomized into four batches, containing an equal number of schizophrenic/control and male/female samples, for target generation and hybridization. For each batch, 10 μg total RNA was processed to biotin-labeled cRNA and hybridized to HG-U133_Plus_2.0 GeneChips in accordance with the Affymetrix protocol (Affymetrix, Santa Clara, CA). Arrays were scanned on a GeneChip Scanner 3000, and fluorescence intensity was obtained by using GeneChip Operating Software. In total 60 samples were successfully hybridized. Cel files were submitted to the NCBI GEO database (http://www.ncbi.nlm.nih.gov/geo/) and are available under accession No. GSE21935.

Microarray Quality Control of CCHPC Samples

After application of standard MAS5.0 Affymetrix quality control criteria, all samples had background levels (42.3–68.3) and scale factors (1.30–3.59) within the acceptable range, with an average percentage present of 39.8%. To ensure that only the highest quality microarray data were used in the analysis, β-actin 3′/5′ ratios were assessed as a surrogate for quality. Sixteen samples with a β-actin 3′/5′ ratio $>$3.8 failed this measure of quality and were excluded from further analysis. A gender check was performed as an additional quality control to ensure that all samples had been annotated correctly by assessing the gene expression levels of the male-specific transcript DDX3Y (205000_at) and the female-specific XIST (224589_at). Two samples were excluded on the basis of gender checking.

TABLE II. PCA Model Used To Analyze the Final 42 CCHPC BA22 Samples

Scores from PC1 of QC metrics PCA	Covariate
Age	Covariate
Gender	Factor
Disease	Factor
Disease × gender	Interaction
Gender × age	Interaction

CCHPC Data Analysis

After application of quality control measures, 19 control and 23 schizophrenia samples were subjected to statistical analysis. The raw signal intensities (Cel files) for each scan were imported into Resolver version 4.0 (Rosetta Biosoftware, Seattle, WA). Signal extraction was performed within Resolver, and the normalized data were then exported for further analysis (Weng et al., 2006). An initial principal components analysis (PCA) was performed on detected probe sets (28,065 probe sets, defined as detected in Resolver as probe sets with $P < 0.01$ for detection in $\geq$20 samples) and indicated that the major source of variability (first principal component, PC1) was sample quality, measured using β-actin 3′/5′ ratios. There was no obvious structure resulting from disease (control vs. schizophrenic) or gender. To account for the variability seen by the expression PCA, a PCA was performed on the QC metrics (average signal, background, standard deviation of the background, number present, raw q, scale factor, GAPDH 3′/5′ ratio, β-actin 3′/5′ ratio), and the scores from the first principal component (PC1) were taken to be used as a covariate in all subsequent analysis. This approach allows us to combine all of the information from the QC metrics into a single value that can be used in our analysis model to account for variability in data quality resulting from sample degradation and other factors. Table II describes the model used to analyze the final 42 CCHPC BA22 samples. All probe sets (54,613) were analyzed; negative intensity values were ignored, and data were analyzed on a $\log_{10}$ scale in SAS 9.1.

Real-Time PCR for CCHPC

Real-time (RT) PCR results were generated using either the 5′ nuclease assay (TaqMan) and the ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) or SYBR Green I using the Prism 7700 sequence detection system (Applied Biosystems) as described in our earlier study of the BA10 region (Maycox et al., 2009). Assay sequence information is indicated in Supporting Information

TABLE III. Global Comparison of Transcriptomic Changes in the BA22 and BA10 Regions

	BA22	BA10
Probe sets assayed	54,613	54,613
Probes passing QC	24,715	33,558
Disease effect ($P < 0.05/P < 0.1$)	1,998/3,685	2,077/4,089
Gender effect ($P < 0.05$)	2,069	2,013
Age effect ($P < 0.05$)	4,597	9,431

Table I. TaqMan normalization was also performed as described previously (Bond et al., 2002).

Pathway Analysis

We compared differentially regulated genes in the BA22 and BA10 brain regions using GeneGO Metacore pathway analysis (db version 6.2, build 24095; GeneGO, St. Joseph, MI; Nikolsky et al., 2005). This method identified transcripts that are overrepresented in defined ontologies. A false discovery rate (FDR) filter was applied to preliminary *P* values, using a q-value calculation. After the enrichment, *P* values are calculated for all the terms within the given ontology, each term is tested as a separate hypothesis, and the resulting q-values represent corrected *P* values with account of total terms in the given ontology and the rank order of the particular term. Our pathway analysis was undirected and detected only enrichment against a background of the genes tested in the experiment (i.e., the genes assayed on the HG_U133_plus_2 chip). To evaluate age effects, we performed pathway analysis on results obtained before and after the removal of strongly age-associated ($P < 0.01$) changes. Because schizophrenia is a highly complex and heterogeneous disease, mediated by modest effects across many genes, we explored different pathway analysis approaches. In our primary analysis, we considered all transcripts showing disease $P < 0.05$, and results from this analysis are the focus of this article. We also took an exploratory approach emphasizing power over type 1 error, using a generous differential disease expression threshold of $P < 0.1$; these results are also discussed.

RESULTS

Review of Transcriptional Changes in the BA22 Region in Schizophrenia Subjects

After analysis of all 54,613 probe sets in our BA22 sample using the linear model described, the differentially expressed transcript list was filtered to remove low-intensity probes and probes mapping to introns or the antisense strand. A total of 24,715 probe sets remained after filtering, of which 1,998 showed a significant ($P < 0.05$) disease effect (Table III). We investigated the biological rationale of the 73 transcripts showing the strongest evidence of differential disease expression (disease $P < 0.001$; Supp. Info. Table II). Several are plausible schizophrenia candidates, including the DISC1 interacting protein FEZ1 (–1.26, $P = 0.0009$; Matsuzaki and Tohyama, 2007); GSTM3 (+1.41, $P = 0.0006$), a brain-specific glutathione S-transferase that colocalizes with amyloid-β plaques, previously associated with Alzheimer's disease and cognition (Maes et al., 2010); and finally AGA (–1.50, $P = 0.0002$), an aspartylglucosaminidase that has previously been linked to schizophrenia in the prefrontal cortex (Vawter et al., 2006). We confirmed a selection of the most significant ($P < 0.001$) transcriptional changes observed in the BA22 region (C1QB, GSTM3, YWHAQ) and a broader range of marginally associated ($P < 0.1$) schizophrenia candidate genes within the CCHPC cohort by RT-PCR (Supp. Info. Table I). Many of the genes altered in the arrays wetre not confirmed by RT-PCR and so should be considered to some extent as provisional observations.

Comparison of Expression Changes in the CCHPC BA22 and BA10 Brain Regions

We compared our BA22 results with our previously published analysis of the BA10 region from the same CCHPC subjects, the laboratory analysis of which was performed in parallel to the current study, using the same platform, QC and linear model (Maycox et al., 2009). This allowed us to compare results directly across both regions (Table III). Two hundred twenty probe sets showed a significant ($P = 0.05$) change in both regions. Among these 220 probe sets, 212 (representing 184 genes) showed the same direction of disease associated regulation (94 down-regulated vs. 118 up-regulated). We observed similar numbers of disease and gender effects in both regions, but notably we saw substantial differences in age-related changes; 18.6% of probes that passed QC showed an age effect in BA22 compared with 28.1% in BA10. A summary of analysis results for all probe sets from the BA22 brain region and previous results recorded in the BA10 region (Maycox et al., 2009) is provided in Supporting Information Table III.

Pathway Analysis of Transcriptional Changes in the BA22 and BA10 Regions

We investigated data from both regions for enrichment at the level of specific biological processes and at the higher level of GeneGO maps, which aggregate related processes and pathways to highlight enriched biological systems. We took different approaches to maximize the informativity of our analysis, summarized in Supporting Information Figure 1. Our primary analysis sought to enhance the specificity for disease effects over age effects by excluding disease-regulated (disease $P < 0.05$) transcripts that also showed a strong age-related effect (age $P < 0.01$). In our exploratory analysis, we maximized the sensitivity of our pathway analysis by including suggestive but nonsignificant transcriptional changes (disease $P < 0.1$) while continuing to exclude strong age effects ($P < 0.01$).

Primary Analysis: Process and Map Enrichment After Correction for Age Effects

Several processes and maps were significantly overrepresented (FDR $P < 0.05$) in the BA22 and BA10 brain regions (Figs. 1, 2). Process and map enrichment in

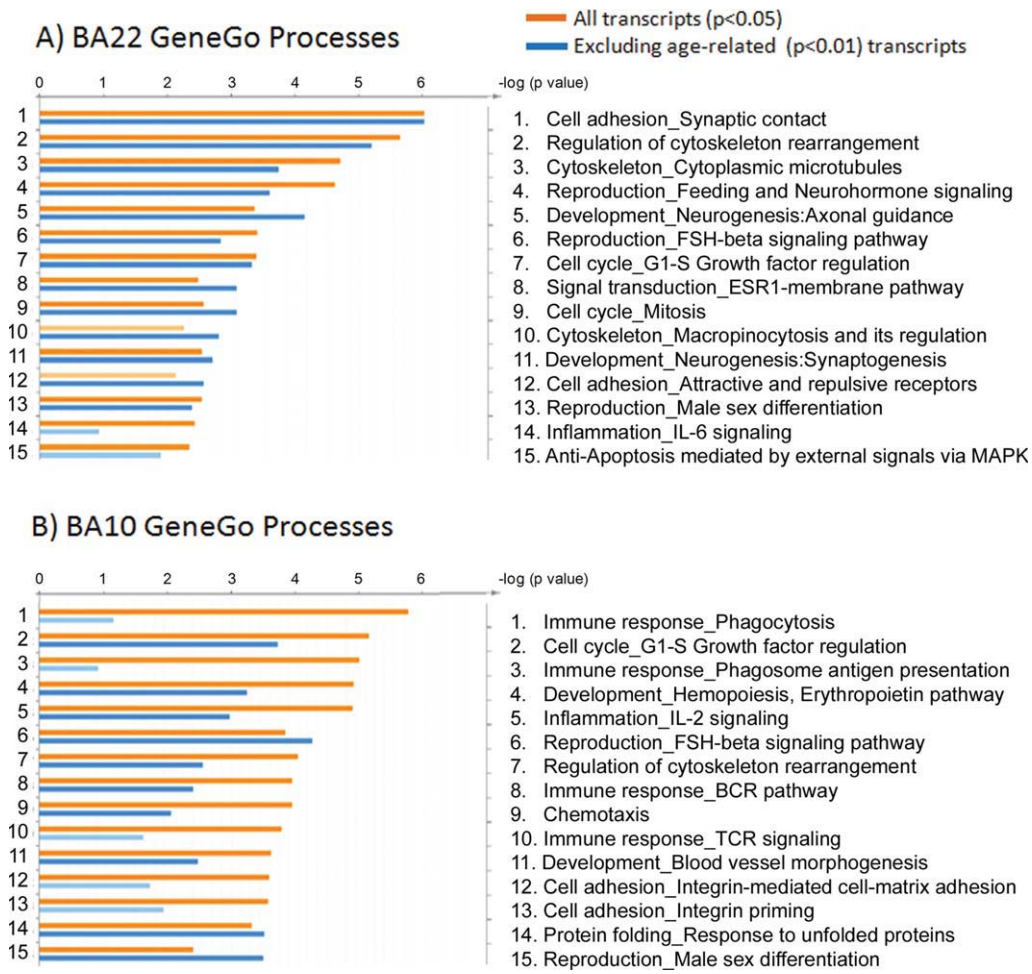

Fig. 1. **A,B:** GeneGO BA22 and BA10 process enrichment before and after removal of strongly age-related transcript data. Translucent colored bars indicate an association that falls below the pathway enrichment false discovery rate threshold. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the BA22 region was not altered greatly after removal of age-related transcripts, with the exception of the immune system response map, which was reduced dramatically (Fig. 2A). The most highly enriched processes in the BA22 region included cell adhesion and synaptic contact ($P = 9.51 \times 10^{-7}$) and regulation of cytoskeletal rearrangement ($P = 6.37 \times 10^{-6}$). At the map level, tissue remodelling and wound repair ($P = 2.35 \times 10^{-10}$) and apoptosis ($P = 1.28 \times 10^{-9}$) showed the strongest enrichment, supporting previous observations that the progressive atrophy of the BA22 region observed in schizophrenia may proceed by a proapoptotic mechanism (Woodruff et al., 1997; Jarskog et al., 2004).

In sharp contrast to the BA22 region, adjustment for age-associated transcriptional changes in the BA10 region substantially reduced overrepresentation of many GeneGO processes and maps (Figs. 1B, 2B), reflecting the higher number of age-associated changes in the region. After exclusion of strongly age-associated transcripts, process enrichment was seen in reproductive hormone signalling processes, including follicle-stimulating hormone (FSH)-beta signalling ($P = 5.56 \times 10^{-5}$), male sex differentiation ($P = 3.19 \times 10^{-4}$), and gonadotropin regulation ($P = 4.14 \times 10^{-4}$). These changes may reflect disease-related processes in the prefrontal cortex, where negative symptom severity correlates with reproductive hormone imbalance (Akhondzadeh et al., 2006), and may also reflect the influence of long-term medication, which also impacts reproductive hormone signalling (Konarzewska et al., 2009). At the higher map level, we observed enrichments in cell differentiation ($P = 1.07 \times 10^{-11}$) and tissue remodelling and wound repair ($P = 4.44 \times 10^{-11}$), also suggesting some degree of cellular plasticity and remodelling that could include synaptic remodelling. We present the FDR-corrected results of our pathway analysis in Supporting Information Table IV.

Exploratory Analysis: Improving Analysis Sensitivity at a Pathway Level

We emphasized the power and sensitivity of our analysis by including suggestive ($P < 0.1$) transcriptomic

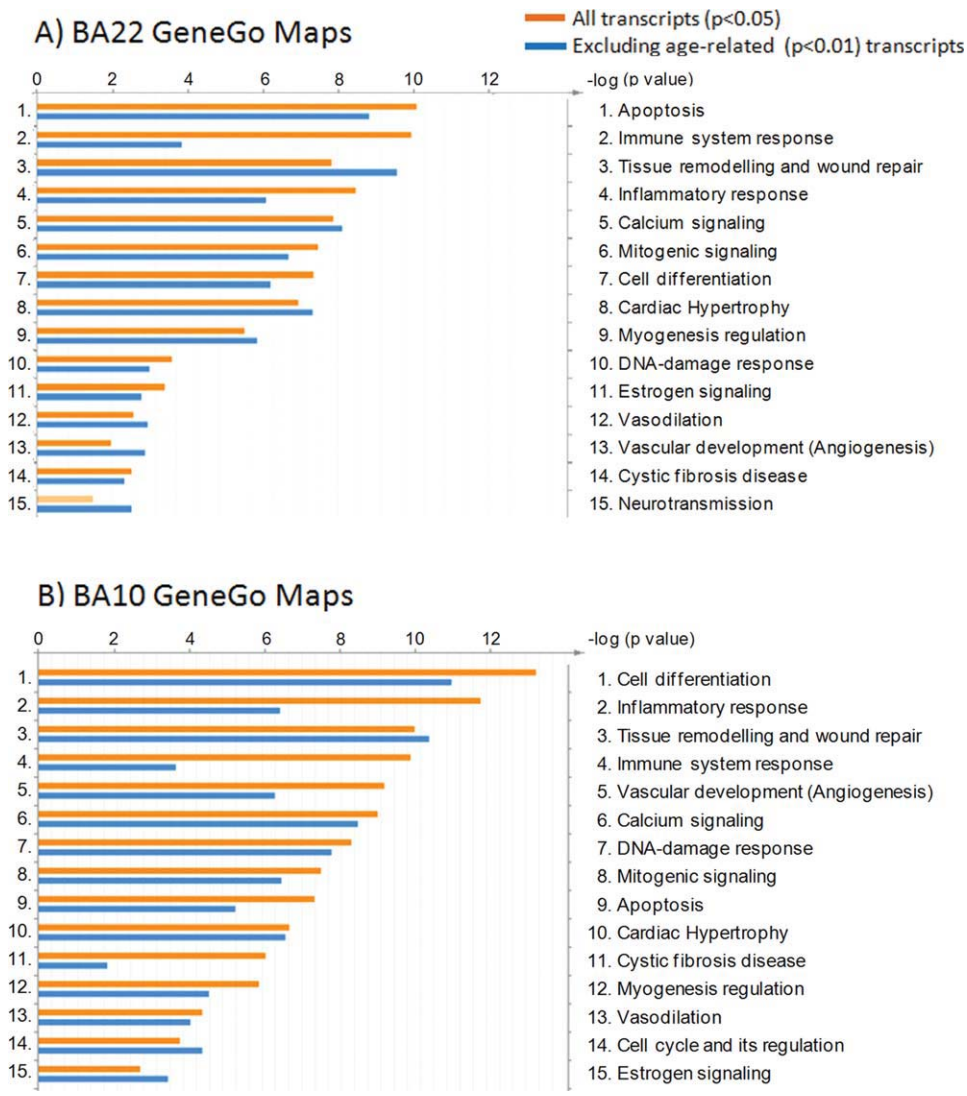

Fig. 2. **A,B:** GeneGO BA22 and BA10 map enrichment before and after removal of strongly age-related transcript data. Translucent colored bars indicate an association that falls below the pathway enrichment false discovery rate threshold. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

changes, accepting a probable increase in type I error. The results are presented in Supporting Information Figures 2 and 3. At the process level, BA22 enrichment showed a striking increase in two processes, cell adhesion: synaptic contact ($P = 4.04 \times 10^{-12}$) and development neurogenesis: synaptogenesis ($P = 5.42 \times 10^{-7}$). Other processes were not altered substantially over our primary analysis, although two additional processes of therapeutic interest did emerge, transmission of nerve impulse ($P = 1.621^{-4}$) and autophagy ($P = 1.203^{-3}$); both show unique BA22 enrichment, making them potentially relevant to positive pathophysiology. The BA10 region showed a substantial enrichment in three processes that were nonsignificant in our primary analysis. The most striking and strongest overall BA10 process enrichment was seen in cell adhesion: amyloid proteins ($P = 4.34 \times 10^{-8}$), which we discuss further below. Enrichment among GeneGO maps remained largely unchanged from our primary analysis in both the BA22 and the BA10 regions (Supp. Info. Fig. 3).

DISCUSSION

In our analysis of post-mortem tissue from the BA22 region, we have attempted to gain a system-level overview of transcriptional changes that are perturbed in schizophrenia. We have compared our BA22 data with BA10 region data from the same subjects (Maycox et al., 2009). In addition to evaluating individual genes, we evaluated process-level changes involving ~50–200 genes and map-level changes aggregating related processes to gain a system-level view, including ~200–1,000

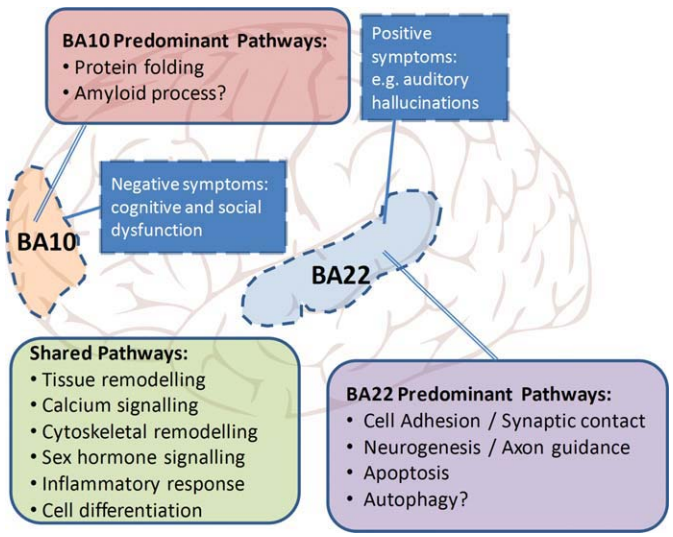

Fig. 3. Gaining insight into schizophrenia disease pathology by pathway analysis. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

genes. We tried to maximize the informativity of our analysis, enhancing the specificity for disease effects over age effects, and maximizing sensitivity by including suggestive but nonsignificant transcriptional changes. This allowed us to evaluate ''group behavior'' among the marginal results in our analysis. We believe that this has been an effective strategy for increasing the sensitivity of our analysis to identify disease-relevant processes that evaded detection in our more stringent primary analysis, e.g., autophagy in the BA22 region and the amyloid process in the BA10 region.

Potential Confounding Factors in Our Analysis

In common with almost all post-mortem studies of schizophrenia are a number of potential confounding factors that might influence the results of our study. Perhaps the most difficult to correct for is exposure to medication. Almost all our subjects had a history of neuroleptic treatment, so we cannot exclude the possibility that some changes correlate with exposure to medication.

The influence of aging on the brain is also a potential confounder, which we have been able to accommodate in our statistical analysis. We observed striking differences in the age-associated behavior of transcripts in the BA22 and BA10 regions, with, respectively, 21.8% and 41.4% of disease-associated transcripts also showing age association. This may reflect brain region-specific rates in structural decline that have been seen in imaging studies of normal aging brains (Yankner et al., 2008). However, there is also evidence of region-specific differences in schizophrenia. One study reported a lack of normal age-associated structural changes in the BA22 region in schizophrenia (Chance et al., 2008). When we excluded strongly age-associated transcripts from our BA22 analysis, few processes or maps were altered greatly, with the exception of the immune system response map (Fig. 2A). In marked contrast, the BA10 region showed strikingly reduced enrichment of multiple processes and maps after exclusion of age-associated transcripts (Figs. 1B, 2B). This presented a conundrum for our analysis strategy. On one hand, the emergence of schizophrenia disease symptoms are strongly age related, particularly in early life, so age is by definition a factor in the disease. However, there is evidence of similarity between expression profiles seen in normal aging and in schizophrenia, particularly in the prefrontal cortex. In an independent study of the BA10 region in healthy subjects spanning a range of ages, Colantuoni et al. (2008) noted changes in a number of known schizophrenia susceptibility genes. In another study of the prefrontal cortex (Tang et al., 2009), significant overlap (29–34%) was seen between genes whose expression was correlated with aging in normal subjects and early-stage schizophrenia, leading to the hypothesis that schizophrenia onset might anticipate the normal aging process and, furthermore, that some symptoms of aging, such as dementia and psychosis, might be explained by these common molecular profiles, a view supported by a recent report of shared molecular expression profiles between Alzhiemer's disease and schizophrenia (Horesh et al., 2010). Although changes in gene expression associated with both disease and age may be disease relevant, we chose to exclude the strongest age-related changes ($P < 0.01$) from our pathway analysis. Our observations in the BA22 region (the main focus of this study) suggest that very little power is lost after exclusion of these transcripts. For consistency, we treated our analysis of the BA10 region in an identical manner.

Evidence for Dysregulation of Synaptic Plasticity in the BA22 Region

When all the enriched processes in the BA22 region are considered, an overall theme emerges that may point to a widespread disruption in synaptic plasticity. This is seen directly in the processes of cell adhesion and synaptic contact, but also in cytoskeletal remodelling, an essential process underpinning plasticity, and in apoptosis and autophagy, two processes that are essential in the maintenance of synaptic structure (Bateup and Sabatini, 2010; Shen and Ganetzky, 2010). Changes in these pathways are robust to adjustment for age and are strengthened substantially when an exploratory disease threshold of $P < 0.1$ is used. This suggests that aberrant BA22 synaptic plasticity may contribute to some of the positive disease symptomology. Other studies also support a deficit in plasticity; in a recent meta-analysis of 15 imaging studies of schizophrenia (Honea et al., 2005), the most consistent gray and white matter deficits were seen in the superior temporal gyrus, which includes the BA22 region. Others have also pointed to multiple lines of molecular evidence implicating a complex set of alterations in cortical circuitry of the BA22 region in the dis-

ease (Lewis and Sweet, 2009). If these observations hold true, it also follows that therapies acting on cortical plasticity may help to ameliorate positive disease symptoms. In this context, the preliminary evidence we found implicating changes in autophagy may be important, insofar as autophagy is key to synaptic plasticity (Shen and Ganetzky, 2010) and is aberrant in neurodegenerative diseases, including Alzheimer's, Huntington's, Parkinson's, motor neuron, and prion diseases (Cherra et al., 2010). Recently, Horesh et al. (2010) also reported BA22-specific expression changes in the autophagy pathway in both Alzheimer's disease and schizophrenia. In our study, autophagy-related genes are widely down-regulated in the BA22 region [ATG7, −1.31 ($P = 0.019$); ATG3, −1.18 ($P = 0.001$, also down-regulated in the study of Horesh et al. (2010); BCL-XL (BCL2L1), −1.21 ($P = 0.026$)]. Antipsychotic drugs may exhibit efficacy in part by induction of autophagy. Sarkar et al. (2005) showed that the mood-stabilizing drug lithium induces autophagy, whereas Zhang et al. (2007) identified eight small-molecule inducers of autophagy in a high-throughput screen, including three FDA-approved antipsychotic drugs, fluspirilene, trifluoperazine, and pimozide. The tentative evidence that we find for down-regulation of the autophagy pathway in our subjects is a potentially important finding that should be explored further by independent confirmation of the expression changes observed. If these changes prove to be robust, this would argue for repositioning small-molecule autophagy activators that are currently in clinical development for neurodegenerative diseases (Sarkar and Rubinsztein, 2008; Renna et al., 2010).

Novel Disease Insight From the BA10 Region

Focusing on our primary analysis of the BA10 region, the strongest theme emerging was dysregulation of reproductive hormone signalling. It may be pertinent, that schizophrenia symptoms generally develop during the onset of the reproductive period; this has led some to suggest a link between the disease and the flood of reproductive hormones to the brain that disturbs the balance between glutamatergic and cholinergic excitation and dopaminergic, serotonergic, and GABA-ergic inhibition (Stevens, 2002). These are likely to trigger compensatory remodelling of synapses in specific brain areas, which may be aberrant in schizophrenia. Our results argue for more consideration of therapies that target reproductive hormone signalling in schizophrenia, a view that seems to be gaining wider support (Hughes et al., 2009).

Our exploratory BA10 analysis also highlighted the process of cell adhesion mediated by amyloid proteins as highly significantly enriched. This process was nonsignificant in our primary analysis and highlights a large number of marginal ($P < 0.1$) changes in amyloid proteins in the region, including APOC1, APOC2, APOM, APP, APPBP1, and APPBP2. Contrary to expectations, none of these changes was age-related, suggesting a distinct pathology for Alzheimer's disease. Although these findings are tentative at best, considering the exploratory nature of our analysis, they are intriguing nevertheless and potentially relevant considering recent links identified between schizophrenia and amyloid pathology via DISC1 (Seshadri et al., 2010). This argues for more investigation of the role of amyloid proteins in schizophrenia.

Therapeutic Insight From Schizophrenia Pathway Analysis

Our ultimate study objective has been to identify new therapeutic targets that might show improved clinical efficacy, fewer side effects, or better treatment of positive or negative symptoms of schizophrenia. Current antipsychotic medicines show high levels of polypharmacology (Mestres and Gregori-Puigjane, 2009), which appears to be necessary for therapeutic efficacy (Hopkins, 2008) but may also be the primary source of extrapyramidal side effects (Dayalu and Chou, 2008). We have identified multiple overlapping pathways containing many potential targets that might be worth further investigation (Fig. 3). We believe that this study might help to highlight some key pathways underpinning schizophrenia, which might provide a better opportunity to identify new medicines, or to find new indications for existing medicines, that more effectively target schizophrenia symptoms while avoiding unnecessary side effects.

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