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IMMEDIATE COMMUNICATION

Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress

S Prabakaran*[,1,2], JE Swatton*[,1,2], MM Ryan*[,1,2], SJ Huffaker*[,1,2], JT-J Huang[1,2], JL Griffin[3], M Wayland[4], T Freeman[4], F Dudbridge[4], KS Lilley[5], NA Karp[5], S Hester[5], D Tkachev[1,2], ML Mimmack[1,2], RH Yolken[6], MJ Webster[7], EF Torrey[7] and S Bahn[1,2]

[1]*Department of Neurobiology, Babraham Institute, Cambridge, UK;* [2]*Department of Psychiatry, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK;* [3]*Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Sanger Building, Cambridge, UK;* [4]*MRC Rosalind Franklin Centre for Genomics Research (formerly MRC Human Genome Mapping Project Resource Centre), Hinxton, Cambridge, UK;* [5]*Department of Biochemistry, Cambridge Centre for Proteomics, University of Cambridge, Cambridge, UK;* [6]*Stanley Division of Developmental Neurovirology, Johns Hopkins School of Medicine, Baltimore, MD, USA;* [7]*Stanley Laboratory of Brain Research, Department of Psychiatry, Uniformed Services University for the Health Sciences, Bethesda, MD, USA*

The etiology and pathophysiology of schizophrenia remain unknown. A parallel transcriptomics, proteomics and metabolomics approach was employed on human brain tissue to explore the molecular disease signatures. Almost half the altered proteins identified by proteomics were associated with mitochondrial function and oxidative stress responses. This was mirrored by transcriptional and metabolite perturbations. Cluster analysis of transcriptional alterations showed that genes related to energy metabolism and oxidative stress differentiated almost 90% of schizophrenia patients from controls, while confounding drug effects could be ruled out. We propose that oxidative stress and the ensuing cellular adaptations are linked to the schizophrenia disease process and hope that this new disease concept may advance the approach to treatment, diagnosis and disease prevention of schizophrenia and related syndromes.
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The onset and etiology of schizophrenia has been associated with a wide range of genetic and epigenetic factors with little agreement as to the true causality. Despite the abundance of hypotheses, the list of reproducible findings is limited. Schizophrenia is clearly a genetically complex, if not a diverse, disorder and it is possible that multiple etiologies could converge to the same disease phenotype and pathophysiology.

Microarray analysis is a powerful tool for identifying gene expression alterations in disease tissue and has been successfully employed to study a variety of disorders, including complex neuropsychiatric disorders. For example, two recent microarray studies on schizophrenia prefrontal cortex found evidence for metabolic alterations and myelin-related gene changes in schizophrenia.[1,2] However, a prominent problem with gene expression profiling studies in neuropsychiatric disorders is that individual expression changes are usually modest and that interindividual variability is high, which makes it difficult to distinguish true physiological differences from normal human variation. Thus, we employed an integrative approach on a large sample set to define disease-specific abnormalities within schizophrenia post-mortem brain tissue by analyses at the level of mRNA (transcriptome), protein (proteome) and low-molecular-weight intermediates (metabolome). The investigation of gene and protein expression combined with measurements of metabolites provides insights into disease-specific regulatory mechanisms and metabolic networks, generating a more comprehensive and converging picture of the diseased brain. Using this approach, we identified several significantly altered metabolic pathways in schizophrenia brain tissue. In view of these findings, we propose that mitochondrial dysfunction and oxidative stress are strongly involved in, and may underlie, the pathophysiology of schizophrenia. These abnormalities may pertain to abnormal oxygen or glucose supply and/or growth factor signaling resulting in

Correspondence: S Bahn, Department of Psychiatry, University of Cambridge, Cambridge CB2 2QQ, UK.
E-mail: sabine.bahn@bbsrc.ac.uk
*These authors contributed equally to this work
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global metabolic disturbances and precipitate the clinical syndrome of schizophrenia.

Materials and methods

Proteomics

Tissue collection and sample preparation Fresh-frozen prefrontal cortex tissue (Brodmann area 9) from white and gray matter of 10 schizophrenia and 10 well-matched control individuals was obtained from the Neuropathology Consortium of the Stanley brain collection (Stanley Medical Research Institute, USA).[3] Samples were homogenized in amido sulfobetaine 14 (ASB14) buffer (8 M urea, 2% ASB14, 5 mM Mg acetate, 20 mM Tris base, pH 8) containing complete protease inhibitor cocktail (Roche) and phosphatase inhibitors (1 mM Na pyrophosphate, 1 mM Na orthovanadate, 10 mM β-glycerophosphate, 50 mM NaF and 1% Triton). Proteins were extracted by precipitation using 100 mM ammonium acetate in methanol and resuspended in ASB14 buffer. The concentration of protein was determined using a detergent-compatible protein assay kit (BioRad).

2D fluorescence difference gel electrophoresis (2D-DIGE) 2D-DIGE was performed as described previously.[4] In short, individual protein samples (100 μg) were minimally labeled with Cy3 or Cy5 (200 pmol, Amersham Biosciences). A protein pool consisting of all protein samples included in the study was generated for use as an internal standard, and was minimally labeled with Cy2. All CyDyes™ were supplied as charge-balanced NHS esters. Proteins labeled with Cy2 (pool), Cy3 and Cy5 were mixed and separated by isoelectric focusing using 24 cm nonlinear IPG DryStrips, pH 3–10 (Amersham Biosciences) according to the manufacturer's instructions. Proteins were further separated according to molecular weight using SDS-polyacrylamide gels (12%, Ettan DALT Twelve apparatus, Amersham Biosciences). Following electrophoresis, gels were scanned at appropriate wavelengths for Cy2, Cy3 and Cy5 fluorescence using Typhoon™ 9400 (Amersham Biosciences). Gel images were cropped using ImageQuant™ V5.2 (Amersham Biosciences), and protein expression was quantified using DeCyder Batch Processor and Biological Variation Analysis (BVA) software V5.02, (Amersham Biosciences). The Cy2 internal standard included in all experiments for normalization purposes allowed both intra- and intergel analyses. Protein spots showing significant changes ($P \leq 0.05$) in schizophrenia with respect to controls were picked from a colloidal Coomassie-stained gel, digested with trypsin (MassPrep Station, Micromass) and the resulting peptides were analyzed using LC-MS/MS (QTof2, Micromass). The data obtained were used to identify proteins from primary sequence databases using Mascot search engine (www.matrixscience.com).

Processing of proteomics data Following gel-to-gel matching of spots, statistical analysis (Student's *t*-test) of normalized protein abundance changes between samples was performed using BVA software module as described.[4]

Metabolomics

Acquisition of metabolomics data For NMR-based metabolomics analysis, high-resolution magic angle spinning (HRMAS) ^{1}H NMR spectroscopy was performed on white and gray matter brain tissue as described previously.[5] HRMAS ^{1}H NMR spectra were acquired using a Bruker 700 MHz (Bruker Avance, Bruker GmBH, Rheinstetten, Germany) at $+4^\circ$C using a conventional solvent suppressed pulse/acquire sequence (RD $-\pi/2-t_1-\pi/2-t_m-\pi/2$ TR = 3 s, SW = 10 kHz, 32k data points, solvent suppression applied during the preparation time (t_1) of 4 μs and mixing time (t_m) of 150 ms, number of acquisitions = 128, 7 kHz spinning rate). Spectra were also acquired with a Carr Purcell Meiboom and Gill (CPMG) pulse sequence to attenuate selectively lipid resonances and bound metabolites relative to aqueous metabolites. CPMG spectra were obtained using a 40 ms total spin echo time (spin echo delay = 500 μs, total number of spin echoes = 40, with other parameters identical to those described for the above NOESY pulse sequence).

Processing of metabolomics data Following Fourier transformation, spectra were integrated across 0.04 ppm spectral regions between 0.4 and 9.4 ppm using a routine with Matlab (Mathworks, CA, USA). The output vector representing each spectrum was normalized across the integral regions, excluding the water resonance, and this formed the input to the pattern recognition routine.

Genomics

Tissue collection and RNA extraction The procedure has been described previously.[6] In brief, fresh-frozen prefrontal cortex tissue from 54 schizophrenia and 50 well-matched control individuals were obtained from the Neuropathology Consortium of the Stanley brain collection (Stanley Medical Research Institute, USA). Key demographic characteristics are summarized in Table 3. Total RNA was extracted from post-mortem prefrontal cortices (Brodmann region 9) of schizophrenia and control brains using Tri-reagent (Sigma, UK) or Trizol (Gibco-Brl). RNA quality was assessed using a high-resolution electrophoresis system (Agilent Technologies, Palo Alto, CA, USA).

Microarray analysis Isolated total RNA was processed through the Affymetrix preparation protocol (www.affymetrix.com), hybridized to HG-U133A GeneChips (Affymetrix CA, USA) and

subjected to stringent sample and data quality control steps as described previously.[6]

Data preprocessing Hybridized arrays were subjected to a rigorous quality control process as described previously,[6] resulting in the exclusion (blind to diagnosis) of 12 experimentally outlying samples (six schizophrenia and six control samples). Affymetrix Microarray Suite (MAS 5.0) was used for image processing and data acquisition. The robust multiarray average (RMA) method was used to compute expression values and normalization of the remaining 92 GeneChips.[7] The normalized expression data were imported into GeneSpring 5.1 (Silicon Genetics, CA, USA) for visualization and expression analysis.

Data filtering In order to exclude inaccurate data while including reliable low expression values, probes were filtered based on coefficient of variation (CV) across all chips. Rosner's test for multivariate outliers[8] across all 92 GeneChip data sets was applied before analyzing the distribution of CVs and was used to eliminate up to four extremely spurious points that consequently would bias the entire CV for that probe (for further discussion with regard to the theory and application of this technique, please see supplementary notes). An 80 : 20 mixture of gamma distributions was fit to the distribution of CVs across all chips and was found to fit the data best both visually (see below) and in terms of Akaike's information criterion.[9] From the fitted mixture of gamma distributions, this would ensure that about 47% of the unreliable readings and about 6% of the reliable readings would be removed. The estimated mixture was significantly distinguished from a single gamma distribution for all the probes by the likelihood-ratio test for nested models. Using this model, probe sets with a coefficient of variance (across all 92 microarrays) greater than 0.22 were removed from further analysis. Rosner's test for multivariate outlier detection[8] was then applied to filtered RMA normalized control and schizophrenia probe data sets, respectively, using a *P*-value cutoff of $P = 0.05$.[8] Of a total of 22 282 probe sets on the HG-U133A chip, 17 622 probe sets met all expression level filtering criteria and 3406 were found to be significant.

Detection of differentially expressed genes Differential gene expression between controls and schizophrenia samples was analyzed by applying a *t*-test (for equal variances). Significance was assigned to *P*-values less than $P = 0.05$. Benjamini and Hochberg false discovery rate controlling procedure ($q = 0.05$) was also performed on the filtered data set. FDR found 152 genes to be significantly altered above the false-positive rate (see supplementary notes for details). Transcripts that passed FDR are highlighted in the EASE pathways in the supplementary information. Lists of differentially expressed genes selected by the above methods were subjected to functional profiling using EASE (http://david.niaid.nih.gov/david/ease.htm) and GO Surfer (http://biosun1.harvard.edu/complab/gosurfer/).

Statistical analysis of demographic variables Available demographic variables were subjected to an extensive analysis of correlation with transcript levels of significant genes. The variables investigated included: age, gender, race, psychosis, age of onset, duration of illness, suicide status, agonal state (rate of death: quick death <1 h and slow death >1 h), fluphenazine equivalent, alcohol, time in hospital, recreational drug use, post-mortem interval (PMI), brain weight, refrigerator interval, body height, body weight, side of brain, problem in pregnancy or birth, problem in child development, exacerbation, global severity, smoking, family history, insight, mood stabilizers at time of death and brain pH. Correlation with expression levels was assessed by Spearman's rank-correlation test or one-way ANOVA, as appropriate, with a Bonferroni correction applied to the tests of multiple genes. Correlation with disease status was assessed by one-way ANOVA or Fisher's exact test, as appropriate. Variables showing correlation with both disease status and expression levels were then included in an analysis of covariance model (ANCOVA) to determine the primary sources of variation, using the 'aov' function in R[10] (see supplementary information for further discussion). As gene expression levels are correlated, we expected the Bonferroni correction to be conservative and therefore relaxed our significance threshold to include 'alcohol' ($P = 0.18$) and 'recreational drug use' ($P = 0.054$) in the ANCOVA model. 'Global severity' was excluded from the analysis of covariance since it is assumed to be secondary to disease status.

Gene expression clustering Genes for hierarchical clustering were derived from those probe sets passing the 0.22 coefficient of variance filter and found to be significant after applying Benjamini and Hochberg false discovery rate controlling procedure ($q = 0.05$). Probe sets demonstrating greater than three outliers in either schizophrenia or control groups were removed. The remaining probe list was subjected to Bonferroni correction with a 0.01 cutoff. Hierarchical clustering based on Pearson's product moment coefficient was performed using GeneSpring (Silicon Genetics). In all, 59 probe sets were found to separate 89.6% of schizophrenia samples from controls (Figure 1c). Ontological classification of these 59 probe sets using EASE and GO Surfer showed significant changes in the same and similar pathways as those found to be among the most significantly affected within the entire significant probe set list (data not shown).

Results

2D-DIGE and BVA (Amersham) were used to assess the relative protein expression of prefrontal cortex

tissue, white and gray matter separately, from 10 schizophrenia and 10 control patients. Out of an average of 2045 protein spots identified by BVA, 215 were found to be significantly altered in schizophrenia *vs* control brains. Of these 215 spots, 170 were identified by LC-MS/MS (see supplementary notes) with 114 corresponding to a single known protein (comigrating protein mixtures were eliminated from further analysis). Several proteins were identified at multiple positions on the gel suggestive of post-translational modifications and/or different isoforms. Thus, the 114 single protein spots identified a total of 50 individual proteins (Table 1). Of these, 19 are associated with mitochondrial function,[11,12] 16 with oxidative stress[11,13] and three with peroxisomal function[14–16] (Table 1). Other prominently altered classes of proteins were cytoskeletal proteins and proteins associated with protein trafficking/turnover (Table 1). Principal component analysis (PCA) showed that white and gray matter proteomics profiles from schizophrenia brains differed significantly from control brains and could be used to classify the disease status (see supplementary notes).

The same samples used for the proteomics investigation were analyzed by HRMAS ^{1}H NMR spectroscopy and PCA to characterize metabolic profiles in intact brain tissue. We found 10 (out of $\sim$60 identifiable metabolites) significantly altered ($P<0.05$) between schizophrenia and control tissues in white matter (Table 2). In gray matter, a similar pattern was observed using PCA, but did not reach significance using univariate statistical analysis (see supplementary notes).

Protein and metabolite investigations were further coupled with a microarray study using Affymetrix U133A chips comprising $\sim$23 000 annotated transcripts to screen 104 post-mortem prefrontal cortex samples (54 schizophrenia and 50 controls; six schizophrenia and six control samples were identified as experimental outliers (blind to disease status) and removed from the analysis. The 20 samples used in the proteomics and metabolomics studies are included in the microarray study; (see Table 3a and b for respective patient demographics). Following data normalization and statistical analysis (see supplementary notes), 3406 transcripts were found to be significantly altered in schizophrenia when compared to control samples. Significant genes were classified according to their cellular localization (Figure 1a; GO Surfer http://biosun1.harvard.edu/complab/gosurfer/) and biological function (Figure 1b; EASE http://david.niaid.nih.gov/david/ease.htm). By far, the largest number of significantly altered genes was found to encode mitochondrial and mitochondria-related proteins (Figure 1a; Table 1).

Pathway analysis using EASE showed pathways associated with glutathione biosynthesis/metabolism, oxygen and reactive oxygen species metabolism, protein kinase cascade, receptor protein signaling, proteolysis and peptidolysis, protein catabolism, cell communication and signal transduction to be significantly upregulated, while oxidative phosphorylation, energy pathways, RNA metabolism, vesicle transport, protein transport, carbohydrate biosynthesis, lipid biosynthesis and glycolysis were among the most significantly downregulated pathways (Figure 1b and supplementary notes). Based on these pathways, hierarchical clustering (see supplementary notes) identified 59 significantly altered genes, which allowed an 89.6% separation of schizophrenia from control samples across the 92 GeneChips (48 schizophrenia and 44 control brains; Figure 1c). These genes related mainly to mitochondria and energy metabolism according to GO classifications, as determined by EASE (Figure 1c and supplementary notes).

Taken together, the three functional genomics tiers provide converging evidence for perturbations in a number of key metabolic pathways and evidence for increased oxidative stress in the schizophrenia brain.

Alterations in glucose, fatty acid and oxidative phosphorylation pathways

EASE analysis found the 'glycolysis' pathway to be significantly downregulated at the transcript level (Figure 1b), with four glycolytic genes significantly decreased, and our proteomics analysis demonstrated a significant downregulation of seven out of the 10 key glycolytic enzymes (Table 1). Pyruvate dehydrogenase (PDH) was significantly decreased at the protein level and showed a significant reduction at the transcript level for two subunits (DLAT, PDHα1) and an E3 binding protein (PDX1) (Table 1; Figure 2a). Deficiencies of PDH have been shown to result in increased lactate concentrations and cellular acidosis,[17] correlating with the detection of significantly raised lactate levels in schizophrenia brains by NMR-based metabolomics (Table 2). An increase in lactate and a decreased flux through the PDH complex implies a shift towards anaerobic respiration and could be in the context of hypoxia and/or mitochondrial dysfunction within the schizophrenia prefrontal cortex.

Increased cellular lactate levels result in decreased pH and are closely linked to brain energy deficits.[18] The pH in schizophrenia brains was slightly but significantly lowered compared to controls (Table 3a), with about 30% of the significantly altered genes showing some correlation between pH and expression levels (however, correction for pH did not eliminate the statistical significance of the implicated genes; see supplementary notes). There was no correlation between brain pH, agonal state or cause of death and the significantly altered genes within the control group (see supplementary notes). In fact, lactate levels *per se* have been found to be stable for up to 195 h post-mortem[19] further supporting that increased lactate levels and decreased pH in the schizophrenia prefrontal cortex are not post-mortem artifacts and may relate to the underlying disease process.

We also found evidence suggestive of increased utilization/depletion of glycogen stores in schizophrenia, supported by the upregulation of two iso-

Table 1 Protein expression is significantly altered in the prefrontal cortex of schizophrenia

Peroxisome	Oxidative Stress	Mitochondria-associated	Protein	White matter		Gray matter		Accession number (SwissProt/ TrEMBL)	Mascot score	Sequence coverage (%)
				Fold change	P-value	Fold change	P-value			
		√	**Pyruvate kinase, muscle (PKM1)**	−1.29 to −1.58 (4)	0.00045 − 0.0074	−1.3	0.039	P14618	27 8-807	14-48
		√	**Pyruvate kinase, muscle (PKM2)**					P14786	278-807	14-48
		√	**Aconitase 2, mitochondrial (ACO2)**	−1.25 to −1.29 (2)	0.035 − 0.043	−1.37 to −1.41 (3)	0.000011 − 0.0012	Q99798	65-723	5-33
		√	Phosphoglycerate dehydrogenase (PHGDH)			−1.3	0.004	O43175	75	4
		√	**EH-domain containing protein 2 (EHD2)**	1.29	0.048			Q9NZN4	69-141	4
		√	**EH-domain containing protein 3 (EHD3)**					Q9NZN3	69-141	4
		√	Triosephosphate isomerase 1 (TPI1)			−1.47	0.0036	P00938	72	8
		√	**Hexokinase 1 (HK1)**	−1.31	0.035	1.71	0.01	P19367	165	8
		√	Tu translation elongation factor, mitochondrial (TUFM)			−1.4	0.0043	P49411	430	23
		√	**Ubiquinol-cytochrome *c* reductase core protein 1 (UQCRC1)**	−1.44	0.016	−1.35	0.0023	P31930	143	11
		√	Glucose-regulated protein, 58kDa (GRP58)			−1.27	0.0068	P30101	247	20
		√	**Moesin (MSN)**			1.19	0.029	P26038	115	4
		√	Gelsolin (amyloidosis, Finnish type) (GSN)	−1.42	0.0028	−1.36	0.025	P06396	332-459	19-20
	√	√	**Malate dehydrogenase 1, NAD (soluble) (MDH1)**	−1.29	0.00094	−1.55	0.00027	P40925	79	6
	√	√	Peroxiredoxin 1 (PRDX1)			−1.6	0.024	Q06830	89	14
	√	√	**Peroxiredoxin 2 (PRDX2)**	−1.28	0.02	−1.38	0.000032	P32119	124	22
		√	**Heat-shock 70 kDa protein 1 (HSPA1A)**					P08107	520	24
		√	**Heat-shock-related 70 kDa protein 2 (HSPA2)**					P54652	465-564	23-28
		√	**Heat-shock 70 kDa protein-like 1 (HSPA1L)**	−1.31 to −1.56 (3)	0.012 - 0.039	−1.23 to−1.41 (4)	0.00086 - 0.032	P34931	520	24
		√	**Heat-shock 70 kDa protein 8 (HSPA8)**					P11142	507	28
		√	**Heat-shock 70 kDa protein 5 (HSPA5)**					P11021	59	6
		√	**Aldolase A, fructose-bisphosphate (ALDOA)**	−1.25	0.027	−1.24 to −1.47 (5)	0.000086 - 0.035	P04075	367-611	39-56
		√	**Aldolase C, fructose-bisphosphate (ALDOC)**					P09972	119-509	15-48
		√	**Glyceraldehyde-3-phosphate dehydrogenase (GAPD)**			−1.32 to −1.5 (5)	0.000086 - 0.016	P00354	162-279	23-29
		√						P04406	162-279	23-29
		√	Pyruvate dehydrogenase E1 component, alpha 1 (PDHA1)			−1.48	0.0051	P08559	387	22
		√	**NADH dehydrogenase (ubiquinone) Fe-S protein 1 (NDUFS1)**	−1.38 to −1.39 (2)	0.0064 - 0.024	−1.36 to −1.52 (2)	0.0013 - 0.0022	Q8N1C4	54-75	2
		√	**Tubulin, alpha 2 (TUBA2)**					Q13748	90	13
		√	**Tubulin, alpha 6 (TUBA6)**	−1.24 to −1.61 (2)	0.034 - 0.0035	−1.29 to −1.53 (5)	0.0031 - 0.043	Q9BQE3	90-283	9-20
		√	**Tubulin, alpha 1 (testis specific) (TUBA1)**					P05209	109-283	11-20
		√	**Tubulin, beta 5 (TUBB5)**					P05218	248-294	18-25
		√	Glutathione-S-transferase M3 (brain) (GSTM3)			−1.19	0.044	P21266	143	14
		√	Glutathione transferase omega (GSTTLp28)					P78417	94	14
		√	**Enolase 2, (gamma, neuronal) (ENO2)**	−1.29	0.042	−1.18 to−1.41 (6)	0.00085-.033	P09104	441	26
		√	**Enolase 1, (alpha) (ENO1)**					P06733	167-610	11-37
		√	Leucine aminopeptidase 3 (LAP3)			−1.25	0.026	P28838	201	11
		√	**Aldehyde dehydrogenase 1, family member A1 (ALDH1A1)**	−1.28	0.014			P00352	294	14
		√	Fascin homolog 1, actin binding protein (FSCN1)	−1.26	0.039			Q16658	389	20
		√	*N*-ethylmaleimide-sensitive factor (NSF)			1.62	0.011	P46459	141-153	8
		√	**CDC10 cell division cycle 10 homolog *(S. cerevisiae)* (CDC10)**	−1.22 to−1.36 (2)	0.028 - 0.038			Q16181	128-214	11-18
		√	**Glutamate-ammonia ligase (glutamine synthase) (GLUL)**			−1.52	0.0017	P15104	153	10
		√	**Actin, alpha 2, smooth muscle, aorta (ACTA2)**					P03996	222-369	16-29
		√	**Actin, beta (ACTB)**					P02570	193-369	15-26
		√	**Actin, alpha cardiac muscle (ACTC)**	−1.15 to−1.55 (5)	0.011 − 0.048	−1.18	0.026	P04270	222-316	16-29
		√	**Actin, gamma 1 (ACTG1)**					P02571	259-518	18-42
		√	**Actin, alpha 1, skeletal muscle (ACTA1)**					P02568	222-316	16-29
		√	**Spectrin, alpha, noneryrythrocytic 1 (alpha-fodrin) (SPTAN1)**	−1.41	0.012	−1.29 (2)	0.037 - 0.048	Q13813	104-512	2-9
		√	**Creatine kinase, brain (CKB)**			−1.24 to −1.3 (3)	0.0014 - 0.0023	P12277	96-193	9-12
		√	**Actinin, alpha 4 (ACTN4)**	−1.3 to−1.61 (2)	0.0055 - 0.0074	1.58	0.026	O43707	155-234	4-8
		√	**Carbonyl reductase 3 (CBR3)**			−1.23 to−1.41 (2)	0.0029 - 0.03	O75828	103	11
		√	**Carbonyl reductase 1 (CBR1)**					P16152	151-387	26-39
		√	**Quinoid dihydropteridine reductase (QDPR)**	−1.36	0.021			P09417	154	12
		√	Phosphoglycerate mutase 1 (brain) (PGAM1)			−1.18	0.026	P18669	95	15
		√	Phosphoglycerate mutase 2 (muscle) (PGAM2)					P15259	95	15
		√	**Phospholysine phosphohistidine inorganic (LHPP) pyrophosphate phosphatase**			−1.25	0.0067	Q9H008	102	12
		√	Ubiquitin carboxyl-terminal esterase L1 (UCHL1)			−1.41	0.0022	P09936	141	18
		√	**Esterase D/formylglutathione hydrolase (ESD)**	−1.35	0.02			P10768	245	32
		√	Tyrosyl-tRNA synthetase (YARS)	−1.3	0.021			P54577	340	17
		√	**Glycolipid transfer protein (GLTP)**			−1.34	0.0042	Q9NZD2	159	8-14
		√	**Actin-related protein 2/3 complex, subunit 1A, 41 kDa ARPC1A)**			−1.62	0.0017	Q92747	60	4
		√	**Actin-related protein 2/3 complex, subunit 1B, 41 kDa ARPC1B)**					O15143	60	4
		√	**Dynamin 1 (DNM1)**	−1.17	0.033	1.48 to 1.62 (2)	0.01 - 0.034	Q05193	150-186	5-6
		√	**Dynamin 2 (DNM2)**					P50570	150-186	5-6
		√	**Transferrin (TF)**	1.39	0.007	−1.17 to−1.22 (2)	0.0094 - 0.02	P02787	63-126	7-9
		√	ATPase, H+ transporting, (vacuolar proton pump) (ATP6V1E1)			−1.33	0.0059	P36543	336	33
		√	**2',3'-cyclic nucleotide 3' phosphodiesterase (CNP)**	−1.28 to−1.63 (7)	0.0024 - 0.032	−1.41	0.0069	P09543	54-853	2-51
		√	**Brain abundant, membrane attached signal protein 1 (BASP1)**	2.03 to 2.4 (2)	0.0089 - 0.035			P80723	177-204	33-47
		√	**Dihydropyrimidinase-like 2 (DPYSL2)**					Q16555	100-486	10-33
		√	**Dihydropyrimidinase-like 5 (DPYSL5)**	−1.33 to -1.53 (4)	0.0001 - 0.034	−1.33 to -1.46 (3)	0.0031 - 0.041	Q9BPU6	85	5
		√	**Dihydropyrimidinase-like 4 (DPYSL4)**					O14531	52	2
		√	**Collapsin response mediator protein 1 (CRMP1)**					Q14194	321	16
		√	**Septin 3 (SEPT3)**	−1.2	0.022	−1.19	0.0063	Q9UH03	53	6
		√	SH3-domain GRB2-like 2 (Endophilin 1) (SH3GL2)			−1.32	0.014	Q99962	68	6
		√	**Albumin (ALB)**	−1.69	0.0063			P02768	240-261	14

*Key findings (see text).

Accession numbers, fold changes and *P*-values are listed for single-hit proteins that showed significant expression changes ($P \leq 0.05$) in the prefrontal cortex, white and gray matter, of 10 schizophrenia and 10 matched controls. Fold changes represent the mean of 10 independent data points. Proteins detected in more than one spot (shown in bold) may be indicative of either different isoforms and/or post-translational modifications. The number of detected spots is noted within parentheses, while the fold differences and *P*-values are given as a range. Multiple accession numbers are given for proteins where it was not possible to identify a single isoform. Mascot scores >50 indicate significant identity or extensive homology to sequenced proteins ($P<0.05$). The percent coverage for each polypeptide is also shown. Associations of each protein with mitochondrial, oxidative stress and peroxisomal ontologies are indicated by ticks on the left-hand side, and have been determined by gene ontology database and literature searches (see supplementary notes for additional information).

forms of the initial glycogen catabolism enzyme, 1,4-debranching enzyme glycogen phosphorylase, as well as the glucose-6-phosphate transporter-1. In addition, transcripts for the main glycogen synthesis enzymes were downregulated, including glycogenin and the glycogen branching enzyme (Figures 1b and 2a). Likewise, uridine and its derivatives (ie UDP), which act as high-energy adjuncts in glycogenesis, were significantly decreased at the metabolite level (Table 2). These findings suggest a highly altered energy metabolism profile within the schizophrenia brain, particularly implying an increased demand for glucose, resulting in increased glycogen catabolism. The expression of glycolytic and other energy pathway enzymes is tightly regulated by growth factors/hormones such as insulin (INS) and insulin-like growth factors (IGFs).[20] We found a significant upregulation of INS, insulin-like growth factor (IGF2) and insulin binding proteins 4 and 5 (IGFBP4, IGFBP5). IGF and IGFBP gene expression can be induced in response to hypoxia/ischemia and play a role in neuroprotection.[21] Thus, the corresponding evidence of decreased glycolytic enzymes, electron transport chain transcripts/proteins (see below) and increased INS signaling implies abnormal brain glucose utilization/regulation, and is in keeping with increased oxidative stress and perhaps hypoxic conditions within the prefrontal cortex of schizophrenia (Figures 2a and b). Biological systems limited in their supply of glucose as an energy source are forced to alter their gene expression in order to utilize alternate energy sources. We found significant increases at the transcript level of several fatty acid β-oxidation enzymes, including peroxisomal acyl-CoA oxidase, short- and long-chain dehydrogenases (ACADS, ACADL) and DCI that encodes an isomerase required for the breakdown of saturated fatty acids. Conversely, the 'lipid biosynthesis' pathway was significantly downregulated (Figure 1b), which includes several transcripts for key enzymes of fatty acid and lipid biosynthesis, such as long-chain fatty-acid-coenzyme A ligase, acetyl-coenzyme A acyltransferase 2 (ACAT2), ATP citrate lyase and alkylglycerone phosphate synthase (AGPS). Finally, our metabolomics results also showed a decrease in long-chain fatty acids (data not shown). AGPS is also involved in phospholipid synthesis and is important in the generation and maintenance of myelin within the central nervous system.[22] Previous expression profiling studies by others and our own group,[2,23] have found evidence for abnormalities in myelin-associated genes in schizophrenia. Within this study, we found the early growth response protein genes (EGR1 & 2) to be significantly downregulated, a transcription factor that has been shown to regulate late myelination genes, including MAG, MPZ, PMP22, MBP,[24] all of which have previously been found to be decreased in the schizophrenia brain.[2,23] A large number of genes involved in cholesterol biosynthesis were also significantly downregulated (ACAT, HMGCS, SQLE, FDFT1, GGPS1, IDI1 and CYP51). Cholesterol and long-chain fatty acids are essential components of myelin. Many genes involved in lipid metabolism and particularly cholesterol biosynthesis are strictly coregulated with myelin-related genes.[25] In keeping with this, our metabolomics findings suggest greater perturbations in white matter compared to gray matter in schizophrenia patients (Table 2; supplementary notes). Thus, decreased myelin-related transcription factors, cholesterol synthesis genes and fatty-acid synthesis genes support findings of myelin-related changes in the schizophrenia brain.

In addition to changes observed in β-oxidation enzymes, we found significant increases in many of the elements of the carnitine transport system. This process first requires the attachment of fatty acids to carnitine, facilitated by carnitine palmitoyltransferases 1 and 2 (CPT1, CPT2) on the outer and inner mitochondrial membranes (respectively), followed by the movement of acylcarnitine across the mitochondrial membranes. CPT1 and CPT2 were significantly increased at the transcript level in the schizophrenia brain. Initiation and regulation of β-oxidation within the peroxisome and mitochondria also occurs through the activity of carnitine acetyltransferase (CRAT),[26] which was significantly upregulated at the transcript

Table 2 Metabolite changes in white matter of the schizophrenia prefrontal cortex

Metabolite	*White matter*	
	Fold change	*P-value*
Adenosine (C8, C6 ring) (8.22 ppm)	0.51	0.0011
Uridine (C6 ring) (8.00 ppm)	0.5	0.013
Lipid (CH = CH) (5.28 ppm)	1.23	0.017
Myoinositol (H4/H6, H1/H3, H2) (3.63 ppm)	0.87	0.0002
Phosphocholine ($N(CH_3)_3$)(3.22 ppm)	0.88	0.0018
Taurine (SO_3-CH_2) (3.26 ppm)	1.19	0.03
Glutamate/glutamine (C4) (2.46 ppm)	1.35[a]	0.04[a]
Acetate (C2) (1.95 ppm)	0.71[a]	0.0004[a]
GABA	0.6[a]	0.015[a]
Lactate (C2, C3) (1.34 ppm)	1.51[a]	0.014[a]

[a]Values determined by CPMG analysis.
Major metabolic changes were identified by HRMAS ^{1}H NMR spectroscopy-based metabolomics analysis of the brain tissue. Significant white matter changes were detected using both conventional and CPMG spectra (see Materials and methods). All metabolites listed were found to be significant according to the multivariate partial least-squares discriminate analysis (PLS-DA) separation of schizophrenia samples compared to controls. Identified resonances and corresponding chemical shifts are shown within parentheses, with the resonance used for ratio calculation and statistical analysis underlined. Gray matter from schizophrenia patients and controls could not be distinguished using the CPMG pulse sequence [see supplementary notes for additional information].

Table 3 Summary of patient demographics

Patient (n)	*Schizophrenia (48)*	*Control (44)*	*t-test P-value*
(a) Microarray samples			
Age (years)	42.4 (±9.8)	44.5 (±8.5)	0.27
Gender (M/F)	23/23	21/23	N/A
Age onset (years)	21.1 (±5.7)	N/A	N/A
Duration of illness (years)	21.3 (±10.9)	N/A	N/A
Fluphenazine mg. Equivalents	69482 (±91981)	N/A	N/A
PMI (hours)	32.4 (±15.7)	28.0 (±12.6)	0.15
Side of brain (L/R)	24/24	21/23	N/A
Brain pH	6.4 (±0.27)	6.54 (±0.29)	**0.02**
(b) Proteomics/Metabolomics samples			
Patient (n)	*Schizophrenia (10)*	*Control (10)*	*t-test P-value*
Age (years)	39.6 (±11.8)	45.6(±8.3)	0.21
Gender (M/F)	8/2	7/3	N/A
Age onset (years)	22.9 (±6.0)	N/A	N/A
Duration of illness (years)	16.7 (±9.58)	N/A	N/A
Fluphenazine mg. Equivalents	58950 (±60161)	N/A	N/A
PMI (hours)	28.4 (±13.28)	31.5 (±13.98)	0.62
Side of brain (L/R)	4/6	3/7	N/A
Brain pH	6.43 (±0.25)	6.49 (±0.28)	0.60

level (Figure 2a). Increased carnitine transfer is one of the key counter-regulation mechanisms in brain 'famine' conditions (the other being a reduction in PDH activity in response to increased fatty acid oxidation).[27]

Among the significantly downregulated pathways, oxidative phosphorylation and ATP synthesis were most prominently affected. We found significant downregulation of 11 nuclear encoded subunits of electron transport chain (ETC) complex I, four of ETC complex III and 11 of ETC complex IV within the prefrontal cortex of schizophrenia patients (Figure 2a). Proteomics results also demonstrated significant decreases in subunits of ETC complexes I and III (Table 1). In addition to these alterations, significantly lowered transcript levels were observed for cytochrome-*c*, holocytochrome-*c* synthetase, and 11 nuclear encoded transcripts of the ATP synthase complex (Figure 2a). Expression and activity of mitochondrial ETC complexes has been shown to be highly associated with the generation of reactive oxygen species (ROS), with inhibition of ETC activity resulting in increased ROS generation[28] and decreased ETC-related gene expression.[29]

Oxidative stress

A large number of the significantly upregulated gene pathways in the schizophrenia brain are known to deal with the response to stressors, signal transduction and transcriptional regulation (Figure 1b; see supplementary notes). In addition, oxygen and ROS metabolism pathways were found to be significantly increased (Figure 1a), suggesting that there is an increased level of ROS and oxidative stress generation within the prefrontal cortex of the schizophrenia brain. The full effects of ROS within cells, let alone complex tissues, is not fully understood, yet the list of putative markers for ROS continues to grow. 'Marker proteins' that are particularly prone to ROS damage include aconitase (ACO), enolase (ENO), pyruvate dehydrogenase (PDH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH)[30] and complex I of the ETC.[31] The relative level of ROS present in mitochondria also directly correlates with ACO activity as this enzyme is sensitively inhibited by ROS reactions.[32] We found ACO2 (mitochondria specific) as well as ENO, PDHA1 and GAPDH to be significantly reduced at the protein level, further suggesting an increase in ROS (Table 1).

In addition to enzymes known to be ROS targets, other markers for hypoxia and oxidative stress include a large network of cellular machinery involved in the quenching of ROS such as taurine, glutathione, thioredoxin, superoxide dismutases and metallothioneins. Taurine, found to be increased in the schizophrenia brain by NMR-based metabolomics (Table 2), is neuroprotective in dealing with oxidative stress, hypoxia/ischemia and other metabolic poisons.[33] Surges in taurine have also been shown to follow increases in cellular ROS and hypoxia most markedly in neurons.[33] Glutathione pathways (synthesis and metabolism), which are important in free radical scavenging,[34] were also significantly upregulated at the transcript level (Figure 1b).

The thioredoxin pathway is activated under numerous ROS-generating conditions including hypoxia,[35] and is thought to be a regulator of hypoxic-induced gene expression and a crucial component of hypoxia-induced microvasculature change.[36] We found an isoform of thioredoxin 2, thioredoxin domain-con-

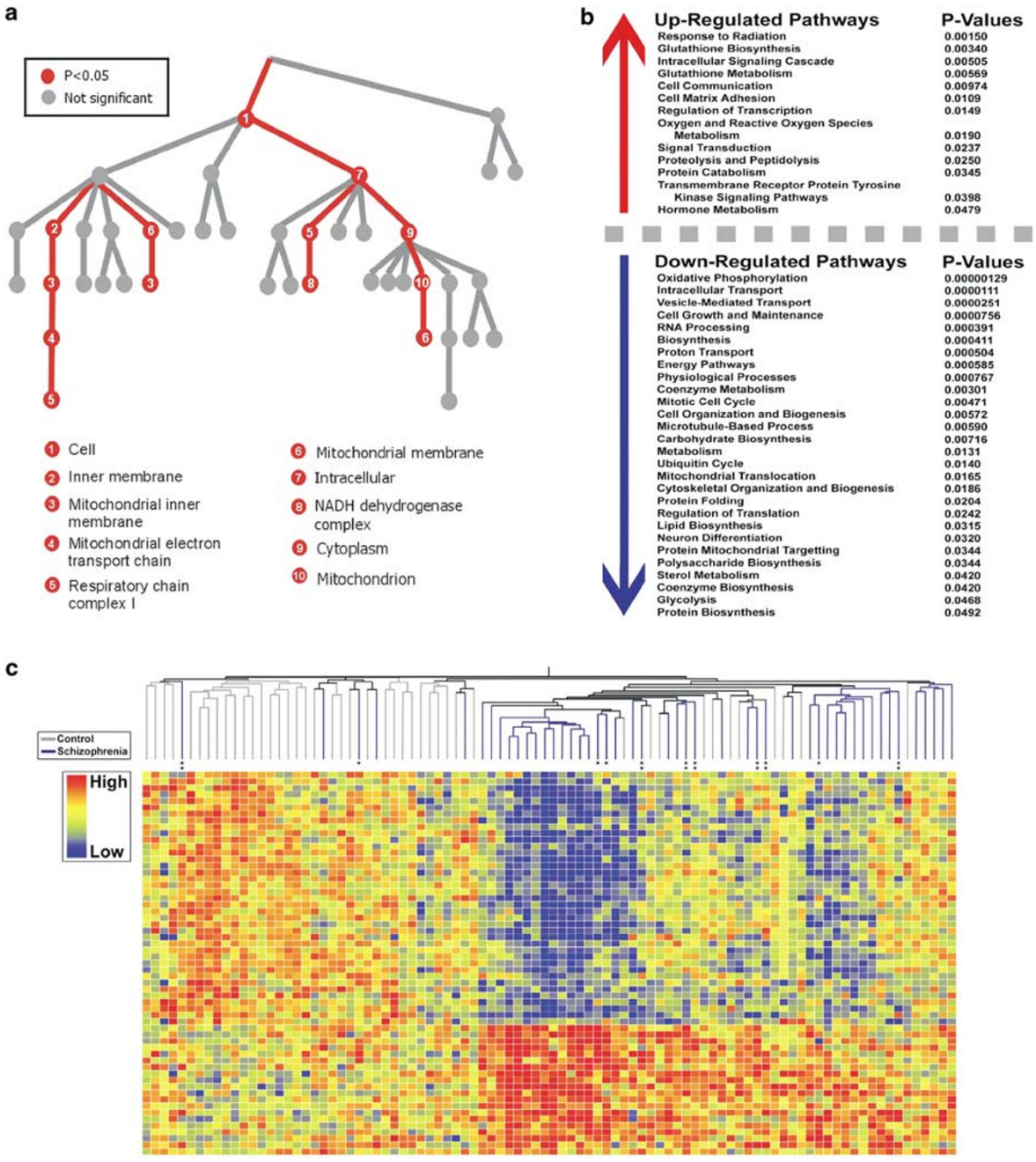


Figure 1 Transcript changes and altered pathways in schizophrenia prefrontal cortex. **(a)** Mitochondria are the most affected cellular components at the transcript level in schizophrenia. Cellular localization of the significantly altered genes (both up- and downregulated) that passed RMA and filtering procedures (see Materials and Methods) were analyzed and visualized using GO Surfer (http://biosun1.harvard.edu/complab/gosurfer/). Branches and nodes represent pathways containing greater than five genes. Significantly altered 'cellular components' ($P<0.05$) are highlighted in red and their categories are indicated. Details of nonsignificant pathways are listed in the supplementary notes. **(b)** Metabolic categories found to be most significantly altered at the transcript level. EASE (http://david.niaid.nih.gov/david/ease.htm) was used for pathway analysis of microarray results and to determine significantly up- and/or downregulated GO biological processes and KEGG metabolic pathways. Categories shown are among the most significantly altered pathways (see supplementary notes for full gene and pathway list). **(c)** Hierarchical clustering tree of schizophrenia (vertical green lines) and controls (vertical gray lines) microarray chips on the basis of 59 significantly altered genes related to energy metabolism and oxidative stress. Drug-näive schizophrenia patients are denoted by **($n=7$), while minimally treated patients are marked by *(<6000 lifetime fluphenazine units; $n=4$). Note that the schizophrenia group appears to fall into two subclusters with respect to lowered transcript expression as indicated by the prominent blue shading.

taining 3 (TXNDC3) and thioredoxin reductase 2 (TXNRD2) were significantly increased at the transcript level in schizophrenia brains. Interestingly, peroxiredoxins 1 and 2 (PRDX1, PRDX2) were significantly decreased at the protein level and several forms at the transcript level (although they did not pass our expression level filters). TXNRDs act to reduce disulfide bonds and are an effective means of removing 'protein casualties' of oxidative damage, while PRDXs reduce H_2O_2 to molecular oxygen and water through the use of thioredoxin electrons.[37] TXNRD2 has also been shown to be significantly increased in cells that were exposed to hypoxic conditions and can act as a preconditioning response to ameliorate future hypoxic events.[35]

Superoxide dismutases are a family of enzymes that are responsible for mopping up ROS molecules and are markers for oxidative stress and are induced in hypoxic conditions.[38] We found a significant upregulation of SOD3 transcripts in schizophrenia brains. The extracellular isoform, SOD3, is not only important in quenching ROS reactivity but is also strongly involved in regulating vasodialation via its role in NO signaling.[39] We also found a significant increase in nitric oxide synthase 3 (NOS3), which is directly involved in ROS-related microvascular changes.[39]

SODs and metallothioneins (MT) are transcriptionally activated in response to a variety of cellular stressors, especially hypoxia and oxidative stress, and have been found to protect against future insults.[40,41] MT-1G and MT-like 5 (MTL5) genes were significantly increased in schizophrenia brains. MT transcripts are most significantly upregulated in cells neighboring sites following ischemic damage with increased expression lasting for extended periods following trauma.[41] MT1 and hemeoxygenase 1 (HO-1) genes, which were also significantly upregulated, have been shown to be increased following hypoxic preconditioning.[42] Thus, taken together, there is evidence not only for oxidative stress responses within the schizophrenia prefrontal cortex but also support that this stress may be partially or specifically due to hypoxic conditions.

Hypoxia

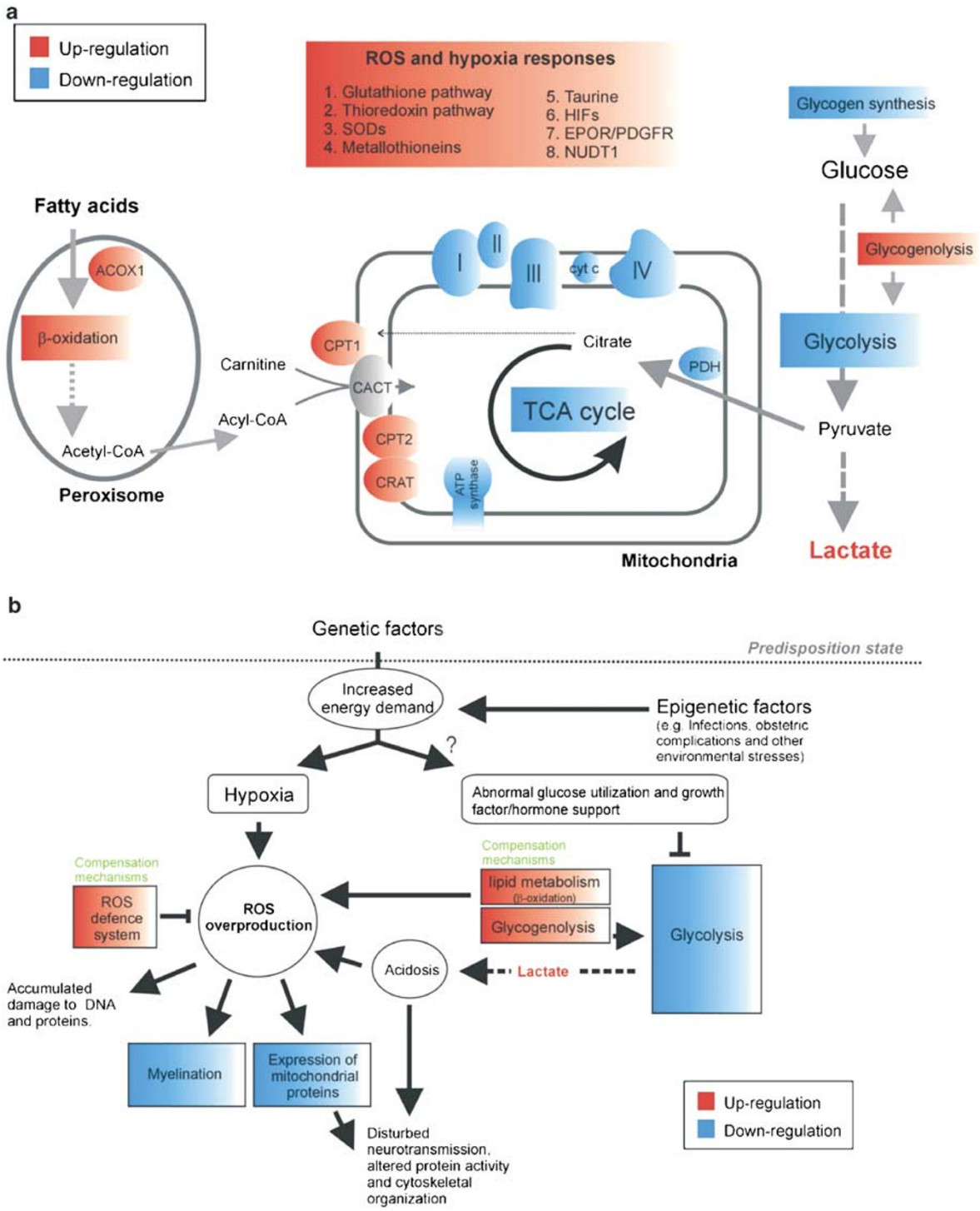
Under hypoxic conditions, cells are forced to alter their energy metabolism and, as a result of low oxygen and increased anaerobic respiration, high levels of ROS are produced.[43] The expression of a number of hypoxia 'marker genes' is reliably increased or decreased in conditions leading to oxidative damage: hypoxia has been shown to trigger the phosphorylation of constitutively expressed hypoxia-inducible factors (HIFs) directly leading to an increased antioxidant response (to mop-up ROS) and altered expression of glycolytic and oxidative phosphorylation proteins.[44] Two hypoxia-inducible factors (HIF-3α and HIF-1β) were significantly increased at the transcript level in the schizophrenia prefrontal cortex. HIF-3α and HIF-1β mRNA are both upregulated in response to hypoxia and in hypoxic preconditioning.[45,46] HIF activation has also been linked to increased lactate concentration and hypoxia itself is a known cause of oxidative stress.[44]

Known transcriptional targets of HIFs include antioxidants, oxygen-dependent energy pathways (ie TCA and oxidative phosphorylation) and glycolytic enzymes as well as many other genes that are controlled through hypoxia response elements.[47] Erythropoietin expression is sensitively regulated by HIF activity and acts on several cell types.[47] Erythropoietin receptors (EPOR) are expressed in CNS neurons and have been shown to protect primary neuronal cultures against hypoxia-induced oxidative stress.[48] Transient hypoxia is also linked to increased platelet-derived growth factor (PDGF) signaling,[49] which has also been shown to reduce oxidative stress-related effects.[50] Both EPOR and PDGF receptor-β (PDGFRβ) transcripts were significantly increased in schizophrenia brains. EPOR and PDGFRβ transcription is increased following acute hypoxic stress and transcripts remain significantly upregulated for prolonged periods following ischemic

Figure 2 Schematic summary of prominent findings and hypothesis. (a) Summary of functional genomics findings from the prefrontal cortex of schizophrenia brains. Significantly upregulated transcripts, proteins, metabolites and pathways are depicted in red, while downregulations are shown in blue. Functional genomics results showed decreased glycolytic proteins/transcripts along with increases in transcripts encoding glycogenolytic enzymes and decreases in transcripts associated with glycogen synthesis. Decreases in PDH complex proteins/transcripts were accompanied by increased lactate concentrations. Transcripts encoding β-oxidation enzymes and carnitine transport subunits were significantly upregulated, while those encoding fatty acid synthesis enzymes were significantly decreased. Aerobic energy pathways were also significantly decreased with lowered protein and transcript expression of TCA and oxidative phosphorylation enzymes. Antioxidants and ROS defense enzymes were significantly increased at the protein and transcript level. **(**b**)** Schematic representation of hypothesis. Genetic factors predispose patients to the adverse effects of epigenetic factors. Diverse epigenetic triggers increase energy demands within the prefrontal cortex, which in turn outrun glucose and oxygen and result in the depletion of glycogen stores, increased lipid metabolism and hypoxia. Hypoxia decreases the expression and activity of aerobic energy pathways including TCA and oxidative phosphorylation while inducing the expression of ROS defense enzymes. Hypoxic conditions also increase lactate concentrations (due to increased anaerobic respiration), which can disrupt neurotransmission, alter protein activity and cytoskeletal organization and amplify ROS production, the latter causing damage to proteins, DNA and white matter. The noted decreases in glycolytic and oxidative phosphorylation pathways are accompanied by alteration in growth factor signaling such as INS/IGF. Thus, global metabolic changes as a result of hypoxia and/or altered growth factor signaling might explain disturbed neurotransmitter systems in schizophrenia.

trauma, suggesting a neuroprotective function in response to hypoxia.[48,49,51]

Finally, if hypoxia and ETC-born ROS are prominent within cells, then oxidative damage to proteins, metabolites and DNA could result in inactive proteins, metabolites and potential DNA damage. We found protein catabolism and peptidolysis pathways to be significantly upregulated, conceivably as a result of increased protein damage (Figure 2b). In addition, 8-oxo-G, 2-hydroxy-A, 8-oxo-A, 2-hydroxy-A are some of the most common nucleotide mutations that occur as a result of oxidative stress.[52] These oxidized purines are converted to monophosphates by NUDT1 (nucleoside diphosphate-linked moiety X motif 1, also MTH1) to prevent the inclusion of the base during transcription and DNA synthesis.[52] NUDT1 was significantly increased at the transcript level in schizophrenia brains compared to controls.



Several antipsychotics (mainly atypical antipsychotics) have been found to have antioxidant activity,[53] suggesting that their pharmacokinetics may reside in their ability to scavenge ROS as well as their effects on neurotransmission. Middleton *et al*,[1] in a microarray study, also observed metabolic alterations in amino-acid metabolism, TCA cycle and ubiquitin metabolism within the schizophrenia prefrontal cortex and, using a primate model, found evidence to suggest that antipsychotic medications may normalize metabolic disturbances associated with schizophrenia.[1] To ensure that the antioxidant activities and the metabolic dysfunctions that we observed were not a drug effect, this study included seven drug-naïve and four minimally antipsychotic-treated schizophrenia patients (2500–6000 lifetime fluphenazine units). The drug-naïve patients showed similar metabolic and antioxidant disturbances when compared to the antipsychotic-treated group (Figure 1c) and no correlation was found of the mentioned differentially expressed genes with fluphenazine equivalent drug exposure (see supplementary notes).

Discussion

Quantitatively, glucose is the major substrate utilized for brain oxidative metabolism. [18] Therefore, the cerebral metabolic rate for glucose and for oxygen mirror each other.[31] A depletion of oxygen results in an increase in anaerobic activity, an increase in lactate levels and a decrease in the efficiency of glucose conversion to ATP,[54] thus resulting in increased glucose demand in order to meet brain energy needs. Glycogen is a brain energy source that can be quickly mobilized in response to abnormally high glucose demand or insufficient glucose supply (such as under hypoxic conditions).[18] Abnormal serum glucose profiles in first-onset schizophrenia patients have previously been reported[55] and the prevalence of diabetes type II is significantly increased in schizophrenia patients (15.8% as compared to 2–3% in the general population).[56] Taken together with the findings of this study of altered glycolysis enzyme expression, increases in glycogenolysis-related transcripts and the decrease in glycogenesis-related genes and metabolites, there is substantial evidence for an increased glucose demand and/or cellular hypoxia within the schizophrenia prefrontal cortex.

Both anaerobic respiration and glycogenolysis result in an increase of lactate levels[18] and a decrease in cellular pH.[18] Lowered cellular pH has been associated with oxidative stress,[57] dopamine reuptake inhibition,[58] synaptic glutamate release,[59] and cognitive impairment[60] (all features of the schizophrenia syndrome). Thus, a significantly decreased pH observed in schizophrenia *vs* control brains further supports the notion of increased anaerobic respiration and hypoxic conditions in schizophrenia.

Studies into the effects of glucose depletion and increased β-oxidation (as suggested by the findings of this study) have shown significant increases in ROS and oxidative stress.[16] Increased β-oxidation and decreased fatty acid/lipid synthesis may be associated with the observed downregulation of myelination- and oligodendrocyte-related genes in schizophrenia.[23,61] Although lipids are not a major energy source in the brain, β-oxidation has been shown to occur in astrocytes, which in turn may sustain neurons and oligodendrocytes when energy levels are scarce.[18] The observed increase in fatty acid metabolic enzymes in this study may also be indicative of increased lipid turnover, possibly to replace peroxide damaged cell membrane and myelin lipids. Studies into white and gray matter abnormalities of patients with mitochondrial encephalomyopathy implied that white matter is particularly vulnerable to damage by oxidative stress,[62] and white matter abnormalities are also common in diabetes patients (69% of long-duration diabetes type I).[63] Thus, there is converging support that abnormalities in white matter within the frontal cortex of schizophrenia patients[2,23] may, in fact, be correlated with an excess of ROS.

Electron flow through the ETC is a highly regulated process and is directly linked to the availability of glucose, oxygen and the generation of ROS. ETC complex expression can also be controlled by growth factors such as INS.[64] Most known disorders associated with impaired ETC function and/or oxidative stress present with lactic acidosis and, in turn, with complex neuropsychiatric and psychomotor manifestations including frank psychosis.[31] Furthermore, alterations in complexes I–IV of the ETC have been found in multiple brain regions and in blood cells of schizophrenia patients in numerous independent investigations.[31,65] Thus, the significant downregulation of ETC subunits found in this study at both the transcript and protein levels provides convincing evidence for anaerobic respiration, increased levels of ROS and mitochondrial dysfunction within the prefrontal cortex of schizophrenia patients (Figure 2b).

In addition and beyond the 'more general' metabolic disturbances, we provide evidence for specific alterations (oxidative stress markers, ROS scavenging pathways and hypoxia coping mechanisms), which may precede the 'metabolic injury'. Almost 50% of the significantly changed proteins identified through our proteomics study are associated with mitochondrial function or oxidative stress (Table 1), a finding that is mirrored at the transcript level with mitochondria being one of the most significantly affected organelles (Figure 1a). These findings are further complemented by nearly 90% separation of schizophrenia from control samples based on transcripts from these gene categories (Figure 1c). Alterations in metabolites such as lactate and taurine accompanied by increases in transcripts encoding hypoxia-protecting genes confer further support for oxygen depletion and altered energy metabolism within the schizophrenia prefrontal cortex. These findings are summarized in Figure 2a. Many of the oxidative stress markers found to be significantly increased in

this study have been shown to play protective roles against intermittent hypoxia/ischemia. The upregulation of MTs is a noted consequence of ischemic insult and MT1 knockin mice show a significantly higher protection against focal ischemia.[66] In addition, NOS3 has been shown to be the most significantly increased NOS-related component in cells following chronic hypoxic insult and plays an important role in protection against future ischemic insults.[67] PDGF[49] and EPO[48] signaling have each been found to share similar protective properties against chronic hypoxia and ischemia. Thus, the increased expression of these markers may act in a neuroprotective manner and serve as an adaptation to a chronic/intermittent imbalance between oxygen supply and demand in schizophrenia.

Oxidative stress can occur as a result of several different insults; however, the findings of increased HIF transcripts, corresponding changes in some of their targets (such as decreased ETC complexes and increased EPO and PDGFB pathways) and the increase in lactate concentrations strongly suggests a state of hypoxia within the schizophrenia prefrontal cortex. The energy metabolism alterations at the protein, metabolite and transcript levels, the upregulation of alternate energy pathways and the increases in transcripts encoding insulin-signaling proteins suggest that there may be an accompanying state of glucose/energy starvation. Taken together, the strong evidence for hypoxia and energy depletion both point towards the possibility of microvasculature abnormalities that result either in inadequate supply of oxygen and nutrients during stress or are, perhaps, even constitutive. These issues are furthered by the observation that several of the noted findings have consequences on vasculature control, including INS signaling, EPOR, NOS and SOD3. Finally, our findings at the transcript, protein and metabolite level suggest major alterations in the enzyme complexes of mitochondria perhaps as a result of a cellular reaction to hypoxia or a sign of primary mitochondrial dysfunction within the schizophrenia brain.

It is important to note that this study investigates the end state of a chronic disorder and that many findings may be secondary to one (or more likely several) disease factor(s) that predispose and precipitate the syndrome. However, the consistent changes in entire pathways involved in oxidative stress, energy metabolism and mitochondrial function at the transcript, protein and metabolite level imply that these pathways are linked to the pathology in schizophrenia. This is further supported by the ability to separate schizophrenia patients (including some patients with a duration of illness of less than 1 year as well as drug-naïve patients) from controls based on a set of genes encoding mitochondrial complexes and redox-sensing proteins. We have not identified a single unifying cause of the illness that could fully explain the disorder; however, the majority of our results support a state of intermittent or chronic low-grade hypoxic stress or, perhaps, local ischemia within the schizophrenia prefrontal cortex (possibly due to abnormal cerebral blood flow). Periodic or ongoing deficiencies of oxygen (or glucose) result in adaptive changes in order to prevent the deleterious effects of future low oxygen situations. We hypothesize that diverse genetic and/or epigenetic factors predispose and precipitate hypoxic events in a constitutively vulnerable prefrontal cortex (ie as a result of altered microcirculation or glucose/oxygen utilization) and in turn lead to the acute and chronic deficits characteristic of schizophrenia (Figure 2b).

There is no doubt that more research is needed to disentangle the genetic and epigenetic components associated with schizophrenia to distinguish primary from secondary disease phenomena. We hope that our findings will enhance the understanding of the disorder and encourage new directions in schizophrenia research, which in turn should impact on treatment approach, diagnosis and disease prevention of schizophrenia and related syndromes.

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