

## IMMEDIATE COMMUNICATION

# Differences in neuroanatomical sites of apoD elevation discriminate between schizophrenia and bipolar disorder

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We previously demonstrated that apolipoprotein D (apoD) levels are elevated in the dorsolateral prefrontal cortex and caudate obtained postmortem from subjects with schizophrenia and bipolar disorder compared to controls, suggesting a focal compensatory response to neuropathology associated with psychiatric disorders. We have now extended those studies by measuring apoD protein levels in additional brain regions from post-mortem samples of schizophrenic and bipolar disorder subjects using an enzyme-linked immunosorbent assay. Increased apoD levels were observed in the lateral prefrontal cortex (Brodmann Area 46) in both schizophrenia (46%) and bipolar disorder (111%), and in the orbitofrontal cortex (Brodmann Area 11) (44.3 and 37.9% for schizophrenia and bipolar disorder, respectively). However, differences between the disease groups were observed in other brain regions. In subjects with schizophrenia, but not bipolar disorder, apoD levels were significantly elevated in the amygdala (42.8%) and thalamus (31.7%), while in bipolar disorder, but not schizophrenia, additional increases were detected in the parietal cortex (Brodmann Area 40; 123%) and the cingulate cortex (Brodmann Area 24; 57.7%). These data demonstrate that there is anatomical overlap in the pathophysiologies of schizophrenia and bipolar disorder, as well as areas of pathology that distinguish the two disorders.

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Apolipoprotein D (apoD), a 29 kDa glycoprotein, was initially identified as a component of human plasma high-density lipoproteins (HDLs),<sup>1</sup> and thus was designated to be an apolipoprotein. However, apoD does not show high sequence or structural similarity to other apolipoproteins, but rather is a member of the lipocalin superfamily of transporter proteins that bind small hydrophobic ligands.<sup>2</sup> *In vitro*, apoD has been shown to bind arachidonic acid, a human axillary odorant, heme-related molecules, and steroid hormones, such as progesterone and pregnenolone.<sup>3–7</sup> Despite the ability of apoD to bind these molecules, the physiological ligand(s) remain to be identified. It is possible that apoD has multiple, tissue-specific physiological ligands that may function differently under normal and pathological circumstances.

ApoD exhibits a wide range of expression both in peripheral tissues and in the central nervous system (CNS) of various species.<sup>2</sup> In the healthy CNS, apoD is expressed by oligodendrocytes, astrocytes and some neurons. Its expression is increased under pathologi-

cal conditions and, in many cases, these increases occur in distinct brain regions. For example, in the rat, increased apoD immunoreactivity and mRNA levels have been observed in the hippocampus after kainic acid or entorhinal cortex lesioning<sup>8,9</sup> and in the cortex after traumatic brain injury.<sup>10</sup> ApoD mRNA and protein levels were elevated in the cerebellum of a mouse strain considered to be a model of Niemann–Pick disease, a human condition characterized by abnormal lysosomal cholesterol storage and chronic progressive neurodegeneration.<sup>11,12</sup> Elevated apoD levels have also been observed in human neurological disorders. Increases in apoD immunoreactivity have been reported in both cerebrospinal fluid (CSF) and brains of Alzheimer's patients, and in the CSF of patients with cerebrovascular disease, motoneuron diseases and meningoencephalitis.<sup>13–15</sup> Additionally, increased intrathecal production of apoD has been observed in multiple sclerosis.<sup>16</sup> Together these data suggest that apoD is increased in response to neurological stress, because of either clinical or mechanical lesioning or active disease pathology. Our previous studies demonstrated an increase in apoD expression in the dorsolateral prefrontal cortex and caudate, obtained postmortem from subjects with schizophrenia and bipolar disorder, but not in other regions, such as cerebellum, substantia nigra, hippocampus and occipital cortex.<sup>17</sup> Since apoD has been

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shown to be elevated under diverse conditions, we suggest that apoD could represent a marker for the anatomical regions involved in particular pathological processes related to CNS disorders.

The aim of the present study was to measure apoD protein levels in additional brain regions from subjects with schizophrenia and bipolar disorder so as to determine if there were regionally localized, disease-specific changes in apoD expression in schizophrenia vs bipolar disorder.

## Materials and methods

### Tissue collection

Following approval from the North-Western Health Care Human Ethics Committee of the Victorian Institute of Forensic Medicine, tissue samples were obtained from the left brain hemisphere. In all cases, the cadavers were refrigerated within 5 h of being found, tissue was rapidly frozen to  $-70^{\circ}\text{C}$  within 30 min of autopsy and stored until used. The mean post-mortem interval (PMI) and pH for the tissue from each group was not significantly different (Tables 1 and 2). The groups consisted of 19 subjects with a diagnosis of schizophrenia (of which 16 were the same as those in Thomas *et al*<sup>17</sup>, 20 subjects (controls) with no known history of psychiatric illness (of

which 17 were as in Thomas *et al*<sup>17</sup> (Table 1), eight subjects with a diagnosis of bipolar disorder (see Thomas *et al*<sup>17</sup>) and eight controls (six of which were controls used in Thomas *et al*<sup>17</sup>) (Table 2). The exclusion of previously used subjects and the inclusion of new ones were made without reference to apoD status in the previous study and was due to tissue availability.

All psychiatric diagnoses of the subjects from whom tissue was collected were made using DSM-IV criteria<sup>18</sup> by a senior psychiatrist and psychologist following an extensive case-history review using a structured diagnostic instrument (DIBS).<sup>19</sup> All schizophrenic subjects and five of the eight bipolar subjects had a history of treatment with typical antipsychotic drugs. The drug doses listed in Tables 1 and 2 reflect doses at death, not cumulative lifetime doses. The sex distribution and mean age of the schizophrenic and bipolar disorder groups in this study were not significantly different from their respective controls (Table 1).

### Membrane preparation

Membrane homogenates were prepared from the following brain regions: lateral prefrontal cortex (Brodmann Area (BA) 46), parietal cortex (BA40), cingulate cortex (BA24), orbitofrontal cortex (BA11),

**Table 1** Demographic data for the schizophrenic and control subjects

Schizophrenia							Control				
	Sex	Age years	Tissue pH	PMI (hours)	DOI	Drug dose (mg) <sup>a</sup>		Sex	Age (years)	Tissue pH	PMI (hours)
1	F	36	6.28	45	4	160	1	M	23	6.13	36
2	M	23	6.40	42	6	1750	2	M	50	6.43	69
3	M	22	6.29	49	20	2920	3	M	22	6.58	51
4	M	44	6.28	32	23	600	4	M	25	6.15	35
5	F	27	5.85	41	10	N/A	5	F	21	6.03	58
6	M	55	6.10	25	33	400	6	M	43	6.25	45
7	M	38	5.52	40	N/A	160	7	F	32	6.16	56
8	M	42	6.26	47	22	N/A	8	M	38	6.42	38
9	M	25	6.38	49	2	200	9	M	35	6.40	27
10	M	22	6.07	37	3	450	10	M	42	6.32	26
11	M	42	6.26	35	15	610	11	M	34	6.40	16
12	M	22	6.17	37	3	200	12	M	43	6.43	51
13	M	38	6.02	50	4	50	13	M	29	6.46	15
14	F	35	6.26	15	7	300	14	M	25	6.48	50
15	M	42	6.44	47	8	128	15	F	33	6.41	42
16	M	41	6.20	31	11	500	16	M	26	6.42	24
17	M	19	6.22	43	3	750	17	M	30	5.86	27
18	F	38	6.43	20	17	N/A	18	M	42	6.61	43
19	M	45	6.48	68	N/A	300	19	M	38	6.19	44
							20	F	38	6.26	52
Mean $\pm$ SEM		34.15 $\pm 2.3$	6.22 $\pm 0.05$	39.6 $\pm 2.7$			Mean $\pm$ SEM		33.4 $\pm 1.8$	6.32 $\pm 0.04$	40.3 $\pm 3.2$

PMI, post-mortem interval; DOI, duration of illness; N/A, not available.

<sup>a</sup>Drug doses are given as chlorpromazine equivalents.

**Table 2** Demographic data for the bipolar and control subjects

	Sex	Age (years)	Tissue pH	PMI (h)	DOI	Neuroleptic Drugs
<i>Bipolar</i>						
1	F	74	6.26	45	12	Fluphenazine
2	F	58	5.68	41	40	None
3	M	59	6.46	34	24	None
4	M	38	6.42	24	10	Chlorpromazine
5	M	66	6.41	17	3	Fluphenazine
6	F	55	6.46	52	14	Trifluorperazine
7	F	60	6.08	50	23	Flupenthixol
8	M	61	6.44	58	35	Melleril
Mean ± SEM		58.8 ± 3.6	6.27 ± 0.09	40.1 ± 5.0	20.1 ± 4.5	
<i>Control</i>						
1	F	73	6.37	28		
2	F	38	6.26	52		
3	M	42	6.61	43		
4	M	42	6.32	26		
5	M	50	6.43	69		
6	F	62	6.45	40		
7	M	43	6.25	45		
8	M	43	6.43	51		
Mean ± SEM		49.1 ± 4.3	6.39 ± 0.04	44.3 ± 4.8		

amygdala and thalamus. Dissection of the cortical regions was carried out with reference to a Brodmann map. All tissues excised from the cortex excluded the underlying white matter. Tissues from these regions were homogenized in Tris buffer (20 mM Tris-HCl, 0.2 mM EGTA, 0.1 mM EDTA, pH 7.4) including 3 × 'complete<sup>TM</sup>' protein inhibitor tablets (Boehringer Mannheim).

#### Enzyme-linked immunosorbent assay (ELISA)

ApoD was quantified in membrane homogenates using a modified ELISA. Two monoclonal antibodies to apoD from Signet Laboratories, Inc (Dedham, MA, USA) were used in a sandwich assay: a coating antibody and an HRP-conjugated apoD antibody. Microtiter high-capacity binding plates (Costar) were coated with 50 µl of a 4.7 µg/ml of apoD antibody for 1 h at room temperature. The wells were washed 4 × with T-TBS (Tris-buffered saline + 0.1% Tween-20), blocked with 5% bovine serum albumin in T-TBS for 1 h at room temperature and then washed again 4 × with T-TBS. An aliquot (50 µl) of the tissue homogenates (50 µg total protein) was added and incubated for 1–3 h at room temperature. The wells were washed 4 × with T-TBS, and then 50 µl of a second, HRP-conjugated, apoD antibody was added to each well and incubated 1 h at room temperature. After extensive washing with T-TBS, 50 µl of 3,3',5,5'-tetramethyl-benzidine (TMB) substrate system (Sigma Chemical Co.) was added to allow color formation. The reaction was quenched with 0.2 N HCl (50 µl) and absorbance was read at 450 nm. Purified apoD (kindly provided by Dr DA Haagenen, Sacramento, CA) was used as a standard in all assays. An internal control

was included on each assay plate to assess assay-to-assay variation.

#### Statistical analysis

Analysis of the ELISA data using two-way ANOVA showed that there was a significant variance with diagnosis ( $P < 0.0001$ ), a significant variance with region ( $P < 0.0001$ ), but no significant interaction between the variables ( $P = 0.775$ ). Further analysis within diagnosis using one-way ANOVA (with Bonferroni's Multiple Comparison *post hoc* test) revealed significant differences in various brain regions in both schizophrenic and bipolar cohorts. Student's *t*-test (two-tailed; unpaired) was used to determine exact *P* values. A Pearson product moment correlation analysis of experimental data vs demographic data (age and duration of illness (DOI)), treatment-related (final recorded drug dose) and tissue-related data (pH) was carried out using an assumed straight-line curve fit. With the exception of two-way ANOVA, which were carried out using Minitab<sup>®</sup> (Minitab Inc. State College, PA, USA) all statistical analyses were carried out using GraphPad Prism computer software (GraphPad software, Inc. San Diego, CA, USA).

#### Results

In previous studies, we demonstrated an increase in apoD expression in the dorsolateral prefrontal cortex and caudate of schizophrenic and bipolar subjects, but not other brain regions.<sup>17</sup> Here, we have quantified apoD levels in six additional brain regions from schizophrenic and normal subjects using an ELISA. Significant increases were detected in the lateral

prefrontal cortex (BA46) (46% increase), orbitofrontal cortex (BA11) (23.9%), amygdala (42.8%) and thalamus (31.7%) of the schizophrenic subjects (Figure 1; Table 3). No significant differences in apoD expression were found in parietal or cingulate cortices (BA40 and BA24, respectively).

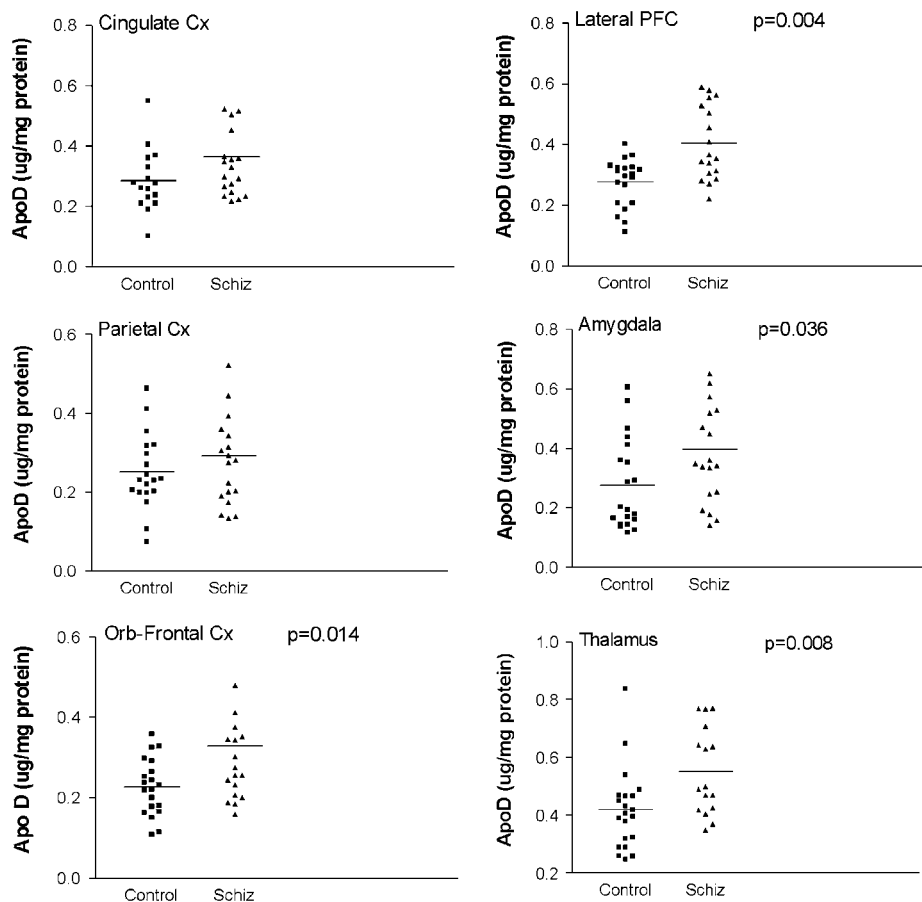
Subjects with bipolar disorder exhibited a different pattern of altered apoD expression. Significantly elevated levels of apoD protein expression were detected in the lateral prefrontal cortex (111%) and the parietal cortex (123%) (Figure 2; Table 3). A trend towards an increase in apoD expression was also observed in the orbitofrontal cortex (37.9%) and cingulate cortex (57.7%), but these did not reach significance ( $P=0.051$  and  $0.057$  for orbitofrontal and cingulate cortices, respectively) (Figure 2; Table 3). No appreciable differences were detected in the amygdala or thalamus.

To attempt to address the issues of the effects of antipsychotic drug exposure of the subjects, we determined the correlations between the apoD levels in each brain region with the drug dose and DOI of each subject. The highest correlation between demographic and generalized brain parameters and mea-

surements of apoD was between DOI and apoD in the cingulate cortex ( $r^2=0.39$ ).

## Discussion

In this study, we detected increases in apoD expression in several CNS regions from subjects with schizophrenia and bipolar disorder. In tissue from the schizophrenic subjects, elevated apoD levels were detected in the lateral prefrontal cortex, orbitofrontal cortex, thalamus and amygdala, whereas increased levels of apoD were only observed in cortical regions from subjects with bipolar disorder. In our previous study investigating apoD expression in psychiatric disorders, we detected increases in apoD expression in the dorsolateral prefrontal and caudate of subjects with schizophrenia and bipolar disorder, but not occipital cortex, cerebellum, substantia nigra or hippocampus.<sup>17</sup> Together, these studies have examined 12 different brain regions in two disease cohorts; the results from both studies are summarized in Table 4. These data leave little doubt that apoD levels are increased in both psychiatric diseases. Furthermore, these increases



**Figure 1** ApoD levels in the indicated brain regions of control and schizophrenic subjects. ApoD concentrations were measured by ELISA using purified apoD as a standard. Each data point represents one subject. Significant differences were determined by one-way ANOVA and exact  $P$  values by Student's  $t$ -test (two-tailed), as indicated.

**Table 3** ApoD protein levels and correlation coefficients for DOI and drug dose in various brain regions from control, schizophrenic and bipolar subjects

Region	ApoD level ( $\mu\text{g}/\text{mg}$ protein)			Correlation	
	Control	Schizophrenic	P value	$r^2$ (dose)	$r^2$ (DOI)
Cortex					
Lateral prefrontal	0.277 $\pm$ 0.018	0.405 $\pm$ 0.029***	0.004	0.013	0.021
Orbitofrontal	0.228 $\pm$ 0.016	0.329 $\pm$ 0.037*	0.014	0.097	0.035
Parietal	0.251 $\pm$ 0.002	0.292 $\pm$ 0.030	0.273	0.001	0.287
Cingulate	0.287 $\pm$ 0.026	0.364 $\pm$ 0.036	0.099	0.007	0.393
Amygdala	0.278 $\pm$ 0.034	0.397 $\pm$ 0.043*	0.036	0.034	0.026
Thalamus	0.419 $\pm$ 0.030	0.552 $\pm$ 0.037*	0.008	0.062	0.090
Region	ApoD level ( $\mu\text{g}/\text{mg}$ protein)			Correlation	
	Control	Bipolar	P value	$r^2$ (dose)	$r^2$ (DOI)
Cortex					
Lateral prefrontal	0.303 $\pm$ .051	0.641 $\pm$ 0.043***	0.0001	0.177	0.019
Orbitofrontal	0.224 $\pm$ .025	0.309 $\pm$ 0.031#	0.051	0.138	0.220
Parietal	0.263 $\pm$ .059	0.589 $\pm$ 0.084**	0.008	0.208	0.001
Cingulate	0.317 $\pm$ .049	0.500 $\pm$ 0.073#	0.057	0.188	0.198
Amygdala	0.385 $\pm$ .071	0.532 $\pm$ 0.066	0.150	0.458	0.016
Thalamus	0.407 $\pm$ .051	0.472 $\pm$ 0.050	0.377	0.209	0.076

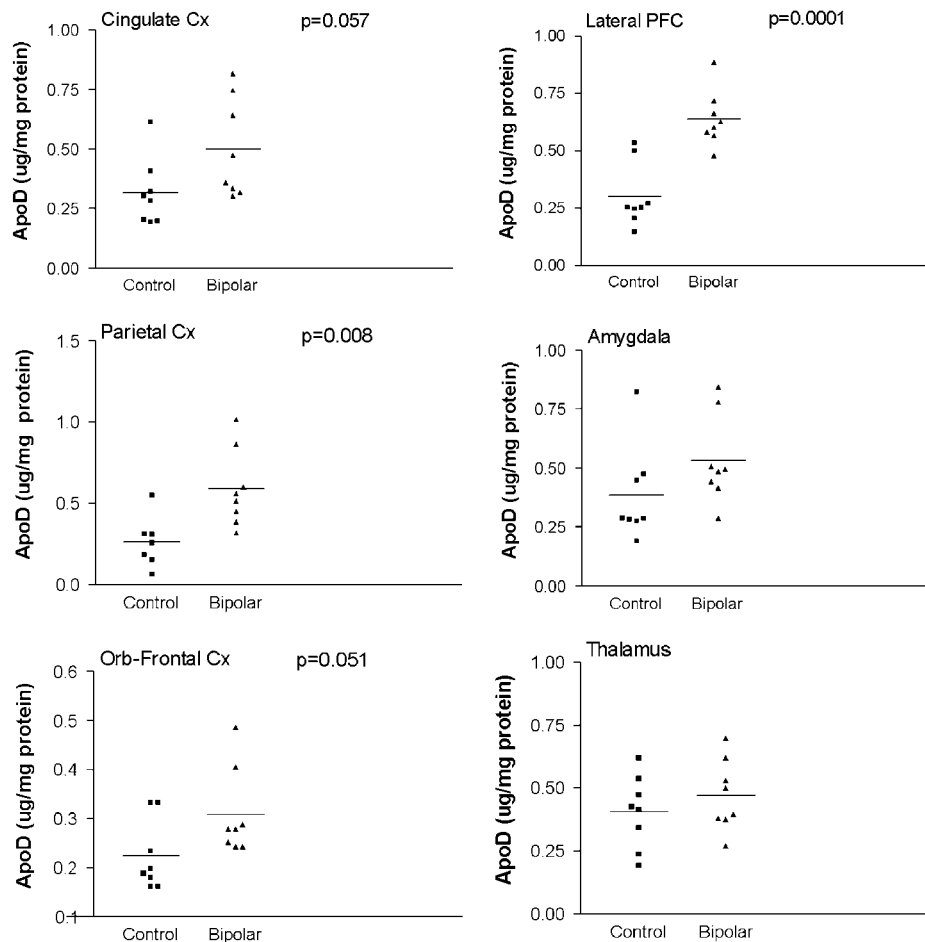
ApoD concentrations were measured by ELISA using purified apoD as a standard. Values are mean concentration  $\pm$  s.e.m. Significant differences were determined by one-way ANOVA and exact *P* values determined by Student's *t*-test (two-tailed). # Represents trend towards significance.

occur in brain regions that have previously been implicated in psychiatric disorders. Our results also reveal anatomically selective similarities and differences, from which one could infer that the pathologies of the two disorders may be similar in the cortical regions of the CNS but differ distinctly with regard to the subcortical structures that are affected.

Schizophrenia and bipolar disorder share some common features;<sup>20</sup> hence it may not be entirely surprising that elevated apoD expression in the two disorders was observed in overlapping regions of the CNS. These include the dorsolateral prefrontal cortex and caudate<sup>17</sup> and the lateral prefrontal cortex and orbitofrontal cortex (this study). The prefrontal cortex is a key structure for cognitive function, such as planning, judgment, decision making, and the mediation of working memory, all of which are affected in schizophrenia and bipolar disorder.<sup>21–28</sup> Emerging studies have also recognized the orbital frontal cortex as an important region in schizophrenia and bipolar disorder.<sup>29–32</sup> The orbitofrontal cortex is associated with social conduct, cognitive functions and prospective memory.<sup>33</sup> Dysfunction in these behavioral correlates are characteristic of both schizophrenia and bipolar disorder, although symptoms are not always clear-cut since patients with the same diagnosis may present with different symptoms, which could possibly be due to additional abnormalities in other brain regions.

The potential for dysfunction in multiple cortical regions and the interconnectivity of the frontal regions may account for the waxing and waning of symptoms that are often related to bipolar disorder. Accordingly, additional cortical regions were found to be elevated in their apoD expression in the subjects with bipolar disorder: the parietal and cingulate cortices. The parietal cortex is one region that has not been widely studied in psychiatric disease; however, parietal lobe dysfunction can result in perceptual impairment, recognition defect and neglect. To our surprise, this region exhibited the greatest elevation in apoD in the subjects with bipolar disorder. The anterior cingulate cortex appears to play a crucial role in motivation, goal-directed behavior and attention.<sup>34</sup> Interestingly, attention-deficit disorders are noted to be comorbid with a variety of psychiatric disorders, including bipolar disorder.<sup>35,36</sup> The elevations in apoD detected in this region are consistent with previous studies demonstrating synaptic pathology and decreased neuronal density in the anterior cingulate cortex of subjects with bipolar disorder.<sup>37,38</sup>

In the schizophrenic subjects, we also observed increases in apoD in the thalamus and amygdala, two regions increasingly implicated in schizophrenia. The cortical–striatal–thalamic circuit modulates cognitive processing; hence, in addition to the prefrontal cortex, the thalamus has also been implicated in



**Figure 2** ApoD levels in various brain regions of controls and subjects with bipolar disorder. ApoD concentrations were measured by ELISA using purified apoD as a standard. Each data point represents one subject. Significant differences were determined by one-way ANOVA and exact *P* values by Student's *t*-test (two-tailed), as indicated.

cognitive disturbances observed in schizophrenia. PET-scans have shown decreased metabolic activity in the thalamus of schizophrenic patients,<sup>39</sup> while MRI-scans have also shown regional abnormalities in the thalamus.<sup>40</sup> In addition, several researchers have demonstrated a significant reduction in total neuron number in the mediodorsal thalamic nucleus, which projects to the dorsolateral prefrontal cortex.<sup>41</sup> The amygdala is a structure responsible for emotional and social behavior.<sup>42</sup> Hence, it is thought that amygdalar dysfunction may contribute to behavioral changes that accompany schizophrenia. Due to its complexity, the amygdala has been less studied however, studies implicating this structure have begun to emerge,<sup>43</sup> including recent imaging studies that have reported a decrease in gray matter density in the amygdala of schizophrenic patients.<sup>44</sup>

While many studies have suggested that abnormalities in the cingulate cortex contribute to the pathophysiology of schizophrenia (for review see Tamminga *et al*<sup>45</sup>), this has not been consistently supported in the literature<sup>37,38,46</sup> Given the hetero-

geneity of schizophrenia, it is possible that abnormalities in the cingulate cortex may accompany certain subtypes of the disease (that have yet to be identified). Carter *et al*<sup>47</sup> have reported that in vivo functional imaging abnormalities in anterior cingulate are associated with neuropsychologic deficits, including attention. Hence, perhaps cingulate abnormalities are associated primarily with those patients presenting attention deficits as part of their symptomatology.

One apparent inconsistency in our findings is the lack of apoD induction in the hippocampus.<sup>17</sup> The hippocampus has been the subject of numerous studies on schizophrenia and abnormalities in different subregions of the hippocampus have been widely demonstrated in the brains of subjects with schizophrenia (for a review see Harrison<sup>48</sup>). In our studies, we measured apoD levels in hippocampal homogenates, which consisted of multiple subregions of the hippocampus. ApoD is expressed in different subregions of the hippocampus (CA1, CA2/3, dentate, subiculum and parahippocampal gyrus).<sup>17</sup> It is possible that the expression of apoD in these subregions is

**Table 4** Summary of neuroanatomical regions exhibiting elevated apoD levels in psychiatric disorders

<i>Schizophrenia</i>	<i>Bipolar disorder</i>
<i>Cortical regions</i>	
<b>Dorsolateral prefrontal</b>	<b>Dorsolateral prefrontal</b>
<b>Lateral prefrontal</b>	<b>Lateral prefrontal</b>
<b>Orbito-frontal</b>	<b>Orbitofrontal<sup>a</sup></b>
Parietal	<b>Parietal</b>
Cingulate	<b>Cingulate<sup>a</sup></b>
Occipital	Occipital
<i>Other regions</i>	
<b>Caudate</b>	<b>Caudate</b>
Substantia nigra	Substantia nigra
Cerebellum	Cerebellum
Hippocampus	Hippocampus
<b>Amygdala</b>	Amygdala
<b>Thalamus</b>	Thalamus

Brain regions listed in bold lettering represent those with elevated apoD expression in either schizophrenia or bipolar disorder.

<sup>a</sup>Denotes regions demonstrating trends of elevated apoD levels that did not reach significance (see Table 3). The following brain regions were taken from Thomas *et al.*<sup>17</sup> the dorsolateral prefrontal cortex, occipital cortex, caudate, substantia nigra, cerebellum and hippocampus.

differentially altered in schizophrenia; however, such putative differences would not be discernable in homogenate preparations.

Alternatively, the lack of apoD elevation in the hippocampus, as well as in the cingulate cortex, may indicate that these regions are not sites of active pathology. ApoD may only be elevated in neuroanatomical sites of primary pathology, while secondary responses to pathology may be occurring in several other areas. The hippocampus and cingulate cortex are two highly innervated areas and it is possible that abnormalities reported in these regions reflect secondary responses to other innervating areas.

As samples from never-medicated patients are difficult to acquire, all of the schizophrenic subjects and most of the bipolar subjects in this study had been treated with typical antipsychotic drugs prior to death. The elevated apoD CNS levels detected in this study are, therefore, seemingly consistent with our previous studies, which demonstrated that apoD levels were elevated in the rodent brain after clozapine administration.<sup>49</sup> However, several arguments suggest that these changes are not simply because of drug treatment before death. Most importantly, we detected disease-specific changes in apoD that would not be expected if such a change was simply a function of antipsychotic drugs as both cohorts had similar treatments for their psychoses. Secondly, we did not observe a correlation between apoD levels and antipsychotic drug dose (chlorpromazine equivalents) in these subjects, nor a correla-

tion between apoD levels and DOI. DOI may be an indication of how long subjects have been exposed to antipsychotic drugs. Thirdly, there has recently been a large body of literature describing apoD induction under various other neuropathological conditions. For example, apoD levels have been shown to be elevated in brains of patients with other neurological disorders, such as Alzheimer's disease, cerebrovascular disease, motoneuron diseases and meningoencephalitis, and presumably these patients had not been exposed to antipsychotic drugs.<sup>13–15</sup> Some behaviorally disturbed Alzheimer's patients may have received antipsychotic drug treatment; however, we also observed increases in apoD expression in a mouse model of Alzheimer's disease and these mice were not treated with antipsychotic drugs.<sup>50</sup> Together, these findings would argue against the changes in apoD reported in this study being simply an effect of antipsychotic drugs.

The physiological role for apoD in psychiatric disorders remains unclear. The increase of apoD expression that has been observed in response to diverse neuropathologies may represent a nonspecific response to cellular injury. However, given the distinct sites of apoD upregulation observed after CNS insult in the rodent studies and the regional specificity of apoD induction observed in human disease,<sup>8–11,13–15</sup> we hypothesize that apoD represents a response to a pathological process in affected brain regions. Given its role as a lipid-binding protein and member of the lipocalin family of transport proteins, apoD may be involved in the binding of steroids or fatty acids released upon CNS insult, or the transport of lipid molecules necessary for dendritic or synaptic remodeling in response to neuropathology. Moreover, recent studies have demonstrated that another lipocalin, human tear lipocalin, acts as a scavenger for harmful lipophilic molecules and, hence, may work as a defense against the deleterious effects of oxidative stress.<sup>51</sup> A similar role may be provided by apoD in psychiatric disorders as it has been suggested that oxidative stress or damage contributes to the pathophysiology of schizophrenia.<sup>52</sup>

Free radicals are reactive chemical species generated during normal metabolic processes and can damage lipids, proteins and DNA. Neuronal membranes are uniquely vulnerable to radical-mediated damage. Several investigators have demonstrated decreased levels of polyunsaturated fatty acids in both peripheral and central membranes of patients with schizophrenia (for a review see Horrobin and Bennet<sup>53</sup>). Oxidative stress may provide an explanation for the specific membrane abnormalities that have been observed in schizophrenia and may represent the pathological stimulus for apoD upregulation in these disorders.

In summary, we have shown that apoD concentrations are elevated in brain regions of schizophrenic and bipolar subjects. Some areas of elevation are common between the disorders, but others are

distinct, indicating that there are at least some anatomical disturbances that differ between schizophrenia and bipolar disorder. In schizophrenic subjects, the regions displaying increased apoD expression have previously been implicated in the pathology of the disease. The areas of selective elevation in bipolar disorder may give clues as to areas of selective neuropathology in that disease. These differences may have value in discriminating between, and, perhaps, even within the two psychiatric conditions.

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