Molecular Neurobiology

ISSN0893-7648/02/26(2–3): 369–388/$20.00

The Neurobiology of Apolipoproteins in Psychiatric Disorders

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Abstract

A genetic contribution to the transmission of psychiatric disorders has been established and it is now accepted that several genes confer susceptibility to schizophrenia, and similar disorders, giving rise to a complex polygenic mode of inheritance. With the high-throughput molecular profiling techniques available, apolipoproteins have emerged as being important factors in psychiatric disorders. This review will focus on three apolipoproteins that have recently been shown to be elevated in neuropsychiatric disorders: apoD, apoE, and apoL. Furthermore, the authors discuss the role of apoD in the pathology and pharmacotherapy of schizophrenia and bipolar disorder.

Index Entries: Apolipoprotein; aracidonic acid; schizophrenia; gene expression; pathology.

Introduction

Psychiatric disturbances, such as schizophrenia and bipolar disorder, bear similarities to one another in several epidemiologic aspects, including lifetime risk, age of onset, course of illness, and genetic susceptibility *(1)*. Symptoms of both disorders, which each affect about 1–2% of the world's population, typically surface during the late teens and 20s. The manifestations of schizophrenia include a diversity of both "positive" symptoms, which include delusions, hallucinations, disordered thinking and bizarre behavior, and "negative" symptoms, such as social withdrawal and affective blunting. Bipolar disorder is characterized by episodes of mania and depression and exhibits increasing chronicity and severity over time, requiring long-term prophylactic drug treatment.

A genetic contribution to the transmission of psychiatric disorders has been established and

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it is now widely accepted that several genes confer susceptibility to schizophrenia, and similar disorders, giving rise to a complex polygenic mode of inheritance *(2,3).* High-throughput molecular profiling techniques, including cDNA microarrays and differential display techniques, provide global approaches for the study of alterations in gene expression. From these types of studies, apolipoproteins have emerged as being important factors in psychiatric disorders. Three different apolipoproteins, apolipoprotein D (apoD), apolipoprotein E (apoE), and apolipoprotein L (apoL), have been shown to be regulated in the brains of subjects with schizophrenia and/or bipolar disorder. This review will focus on the role of apolipoproteins in neuropsychiatric disorders, with emphasis on apoD and its involvement in the pathology and pharmacotherapy of schizophrenia and bipolar disorder. The role of apoE in Alzheimer's disease has been extensively reviewed elsewhere *(4,5)* and will not be discussed here.

Differential Gene Expression in Neuropsychiatric Disorders

Alterations in gene expression play a role in several aspects of psychiatric disorders. Schizophrenia is known to be a multifactorial disorder with several genes contributing to the inheritance of the disease *(3).* Genetic defects include causative genes, which are rare, and susceptibility genes. In addition, the expression of any number of genes may be altered as a secondary response to primary genetic defects. Changes in gene expression may also be associated with environmental risk factors, generalized pathology, or specific symptoms that may be common to several neurological disorders. The expression of some genes may also be altered with disease progression. Genes that are expressed in response to antipsychotic drug treatment are also important in the understanding of mechanisms contributing to disease symptoms and of the clinical benefits of these drugs.

High-throughput molecular profiling studies have allowed the investigations of global changes in gene expression in various disease paradigms. These methods include the open system technologies, such as differential display *(6),* Serial Analysis of Gene Expression (SAGE) *(7),* TOtal Gene expression Analysis, (TOGA®) *(9),* and the closed system cDNA microarray methods (cf. *8*). Older differential screening methods, such as subtractive hybridization techniques, have also contributed to novel gene discovery. These approaches are not limited by the current state of knowledge, which can restrict the focus of pathological analysis, nor do they require *a priori* assumptions about the underlying basis of psychiatric disorders. This is in contrast to the candidate gene method, which is based on previous knowledge of suspected pathways or neurotransmitter receptors in schizophrenia. The application of systematic open- and closed-system analyses to animal models or postmortem tissue provides a powerful tool for the discovery of genes involved in psychiatric disorders.

Reports from cDNA microarray studies on postmortem samples from subjects with schizophrenia or bipolar disorder have revealed abnormalities in the expression of genes associated with a variety of biological processes, including synaptic plasticity, neuronal development, myelination, and signal transduction *(10–12).* Relevant to this review are results from cDNA array and other differential screening analyses that have revealed the upregulation of apolipoproteins in psychiatric disorders. Using the PCR-based mRNA display technique, TOGA®, apoD was identified as a gene regulated in mouse striatum by clozapine treatment *(13)* and subsequent studies demonstrated increases in apoD expression in the brains of subjects with schizophrenia and bipolar disorder *(14,15).* ApoE's involvement in neurological disorders was first discovered when it was identified in a subtractive hybridization screen of genes differentially expressed in scrapie and Alzheimer's disease (*16,17*). Subsequent studies examined apoE in schizophrenia and

other neurological disorders. Finally, in a cDNA microarray screen of genes located on known high-susceptibility chromosomal locations, Mimmack and collaborators recognized apoL as a molecule increased in the brains of schizophrenic subjects *(18).*

Apolipoprotein Expression in the Central Nervous System (CNS)

Lipoproteins are a large family of macromolecular complexes consisting of lipids, free cholesterol, cholesteryl esters and triglycerides, and proteins. The protein moieties of these complexes are referred to as apolipoproteins. Apolipoproteins make up a diverse class of proteins (14 members identified to date), whose common feature is that they were all identified as components of low-, intermediate- or high-density lipoprotein complexes, (LDL, IDL, and HDL respectively). Lipoprotein complexes play a role in plasma lipoprotein metabolism and cholesterol homeostasis in the periphery; however, some apolipoproteins are also expressed within the CNS. With the discoveries of apolipoprotein expression in the brain, new functions for these proteins are beginning to emerge.

Apolipoprotein L

Apolipoprotein L (apoL) is a newly discovered 42 kDa plasma apolipoprotein. In the plasma, apoL is found almost exclusively associated with apoA-I forming high density lipoproteins (HDLs) *(19).* There are four forms of apoL, apoL I–IV, which are encoded by separate genes, closely arrayed on chromosome 22. Alternative splicing of these genes results in up to 12 different transcripts *(19).* Variations in the signal peptides of the different apoL isoforms predict both intracellular and extracellular locations for this protein *(20).* ApoL exhibits abundant expression in the lung, pancreas, prostate, spleen, liver, and placenta. ApoL1 is ubiquitously expressed in human CNS, but at lower levels than in peripheral tissues *(20). In situ* hybridization studies have demonstrated pan-neuronal expression of apoL1 and apoL2 mRNA in the frontal cortex of humans *(18).*

Little is known about the functional role of apoL in the brain or peripheral tissues and, to date, few studies are represented in the literature. However, speculated roles arise from its nucleotide and amino acid sequences and the nature of the protein itself. The presence of sterol response elements in the promoter region of the apoL gene suggests roles relaed to lipid synthesis and/or transport *(20).* With regards to its protein structure, all isoforms of apoL maintain a classical amphipathic helix formation *(20).* This suggests an interaction with lipid molecules or a catalyst for lipid transfer. ApoL, the most recently described apolipoprotein, was first identified in a study investigating novel constituents of pancreatic HDLs, which are large lipoprotein complexes that function in reverse cholesterol transport in the periphery *(21).* However, HDLs may have other functions in the CNS. Hence, as a component of HDLs, apoL may function in the cholesterol homeostasis or other CNS-related HDL functions.

Apolipoprotein E

In the plasma, apolipoprotein E (apoE) is associated with very low density, intermediate density, high density lipoproteins and chylomicrons, and plays an important role in the metabolism of cholesterol and triglyceride-rich lipoproteins. ApoE is a 37 kDa protein that exists in three major isoforms (E2, E3, and E4), which can be distinguished by cysteine-arginine interchanges at residues 112 and 158 *(22).* These proteins are encoded by three alleles (ε2, ε3, and ε4), which combine into six genotypes *(23).* The brain is a major site of apoE expression in many species, although apoE is also widely expressed in other tissues *(24,25).* ApoE mRNA transcripts are distributed throughout the brain and are localized primarily to astrocytes and microglia *(26,27).* ApoE protein is synthesized and secreted by

astrocytes throughout both gray and white matter regions *(26–28).*

Numerous in vitro studies have identified molecular interactions of apoE with other molecules. Many of these interactions are isoform-specific and are possibly related to its role in neurological disorders. Isoform-specific interactions of apoE with lipoprotein particles, Aβ, APP, cytoskeletal proteins, laminin, and growth factors have been identified and proposed as important interactions in this disease (for review, *see* refs. *4,5*). Several of these molecular interactions alter the metabolism or function of these proteins in cultured cells. ApoE mediates cellular uptake of cholesterol and lipid complexes via interaction with a family of low-density lipoprotein (LDL) receptors *(29),* which are widely expressed throughout the body and are present on both neurons and glial cells in the brain *(30).* A more recently described family member, the apoE receptor2, is expressed only in brain and testis *(31).*

A central role for apoE in the brain appears to focus around cholesterol metabolism and homeostasis. The CNS contains approx 25% of the unesterified cholesterol in the body *(32).* Cholesterol is a major component of the lipid membranes of glial cells and neurons, and is essential to the formation of axonal myelin sheaths *(32).* ApoE and its LDL family of receptors play a pivotal role in the mobilization and redistribution of cholesterol in repair, growth, and maintenance of myelin and neuronal membranes during development or after injury in the PNS, as well as during membrane remodeling associated with synaptic plasticity *(26,29,33).* As neurons undergo dendritic remodeling and synaptogenesis, cholesterol is internalized through the apoE/LDL-receptor pathway, with a subsequent negative feedback inhibition of cholesterol synthesis *(4).* These observations suggest that cholesterol delivery and synthesis in the brain are tightly regulated through an apoE-dependent mechanism. The absence of other classical plasma apolipoproteins, such as apo A1–IV, C1, and apo B *(34)* from the brain emphasizes the critical role of apoE in the CNS.

Apolipoprotein D

Apolipoprotein D (apoD), a 29 kDa glycoprotein, was initially identified as a component of human plasma high-density lipoproteins (HDLs) *(35),* and was thus was designated as an apolipoprotein. However, apoD does not show high sequence or structural similarity to other apolipoproteins, which possess an amphipathic helical domain as a characteristic feature. Rather, it is a member of the lipocalin superfamily of transporter proteins, which are characterized by an eight-stranded beta-barrel conformation and function in the transport of small hydrophobic molecules in a wide variety of biological contexts *(36).* Similar to other apolipoproteins, apoD is abundantly expressed in the plasma, where it interacts with apolipoprotein A–I and lecithin-cholesterol acyltransferase forming, along with other components, the HDLs. Unlike most other apolipoproteins, whose expression is limited to the liver and/or intestine, apoD is also widely expressed within the CNS where its physiological role is distinct from its peripheral function, yet remains unclear. Clues to the functional role of apoD in the CNS, however, may also be provided by its putative binding partners.

Biochemical studies have shown that apoD can interact with several different types of molecules, although none has definitively been identified as its physiological ligand. Separate from its association with HDLs, apoD was independently isolated as an abundant protein from mammary gross-cystic disease fluid, and was found to bind with high affinity to progesterone and pregnenolone, but not to other steroid derivatives *(37–39).* Others found that apoD binds to bilirubin, as predicted by structural analysis of the binding pocket in apoD *(40),* and an abundant axillary-secreted odorant, E-3-methyl-2-hexenoic acid *(41).* Most interestingly, two groups have demonstrated that apoD interacts with arachidonic acid (AA). Morais-Cabral and collaborators (1995) found that apoD, purified from gross-cystic disease fluid, binds specifically to AA, but not its metabolites. They observed a 20-fold higher

affinity for this interaction than apoD binding to progesterone, its originally identified binding ligand *(42).* Another study, using bacterially produced recombinant apoD protein, demonstrated that apoD binds both AA and progesterone with approximately the same affinity, but did not recognize the other presumed ligands, pregnenolone, bilirubin, and the odorant component *(43)*. Proposed explanations to account for these discrepancies include differences in protein purity and the pH-dependence of the binding studies *(43).* Since AA is a major component of phospholipids, and is the precursor for eicosanoid and prostaglandin synthesis, these studies implicate apoD in pathways associated with membrane phospholipids, signal transduction, and lipid metabolism.

In the healthy CNS, apoD is expressed by oligodendrocytes, astrocytes, and some neurons. However, expression in these cell types is increased under pathological conditions, which has also given clues to the functional role for apoD in the CNS. For example, increased apoD immunoreactivity and mRNA levels have been observed in the rat hippocampus after kainic acid and entorhinal cortex lesioning *(44,45),* and in the cortex after traumatic brain injury *(46).* ApoD mRNA and protein levels are elevated in the cerebellum of a mouse strain considered to be a model of Niemann-Pick disease, a human condition that is characterized by abnormal lysosomal cholesterol storage and chronic progressive neurodegeneration *(47,48).* 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.

Apolipoproteins in Psychiatric Disorders

Most of the studies examining gene expression changes in schizophrenia have focused on one particular brain region, the prefrontal cortex. This is a region widely implicated in the pathophysiology of schizophrenia (for review *see* ref. *49*). Apolipoproteins have been shown to be elevated in the prefrontal cortex of subjects with schizophrenia and bipolar disorder (*see* Table 1) and these studies are reviewed below. Additionally, studies from the authors' group have examined apoD protein levels in an extended panel of brain regions from subjects with schizophrenia and bipolar disorder and have found differential increases in apoD expression throughout the brain. These findings suggest that apoD may discriminate between different psychiatric disorders.

Apolipoprotein L in Schizophrenia and Bipolar Disorder

Recently, the elevated expression of apoL in the prefrontal cortex of schizophrenic subjects was shown. A cDNA microarray was designed to screen genes located on known high-susceptibility chromosomal locations in schizophrenia. Pooled samples from prefrontal cortex of schizophrenic subjects (n = 10) and controls (n = 10) were investigated for gene expression differences *(18).* Of approx 300 candidate cDNAs, one whose expression increased 2.6-fold was identified as apoL1 *(18).* Experiments on additional brain samples from a different cohort of subjects with schizophrenia, depression, or bipolar disorder revealed a consistent 1.8-fold increase in apoL1 expression in the schizophrenic subjects. No significant changes in apoL1 were observed in subjects with depression, or bipolar disorder. Real-time PCR analysis using primers specific to the other isoforms, apoL2-L6, was subsequently performed. Specific increases in the apoL2 and apoL4 isoforms were detected in the schizophrenic samples, but not in subjects with depression, while only the apoL2 form was elevated in bipolar disorder. No changes in apoL3, L5 or L6 were apparent in the schizophrenic samples and were not tested in the other diseases *(18).*

Few clues are available regarding the potential function role for apoL in schizophrenia and

Table 1
Fold-Increases in Apolipoprotein Levels in Prefrontal Cortex and Other Brain Regions of Schizophrenic and Bipolar Subjects

Apo	BA	Schz	*p* value	BD	*p* value	Ref.
Prefrontal Cortex:						
ApoD	9	1.92***	0.0002	2.02**	0.02	*(13)*
ApoD	46	1.46**	0.004	2.11***	0.0001	*(15)*
ApoE	10	1.2**	0.008	nd	nd	Unpublished[†]
ApoL1	ns	1.85*	0.013	1.07	0.69	*(18)*
ApoL2	ns	2.37****	0.00004	1.6*	0.05	*(18)*
ApoL4	ns	2.7**	0.009	1.4	0.77	*(18)*
ApoD in Other Brain Regions:						
Caudate	–	1.69*	0.04	1.89*	0.02	*(13)*
Thalamus	–	1.32**	0.008	1.15	0.37	*(15)*
Amygdala	–	1.42*	0.036	1.38	0.15	*(15)*
Orbfrontal	11	1.44*	0.014	1.38[#]	0.051	*(15)*
Cingulate	24	1.26	0.099	1.57[#]	0.057	*(15)*
Parietal	40	1.16	0.273	2.23**	0.008	*(15)*

ApoD protein concentrations were measured by ELISA using purified apoD as a standard. ApoE protein levels were determined by Western blot analysis. ApoL mRNA levels were measured by real-time PCR analysis. BA = Brodmann Area; Schz = schizophrenia; [#]BD = bipolar disorder; ns = not specified; nd = not determined. Asterisks indicate significant differences as determined in the given reference. [†]Unpublished studies: Dean, B., Laws, S. M., Hone, E., Taddei, K., Scarr, E., Thomas, E. A., Harper, C., McClean, C., Masters, C., Lautenschlager, N., Gandy, S. E., and Martins, R. N.

bipolar disorder. However, one study has identified apoL in a differential display analysis as a molecule induced by tumor necrosis factor-α (TNFα) in endothelium cells *(50)*. TNFα is a pro-inflammatory cytokine that is implicated in neuronally-mediated responses to disease and injury. These findings suggest that apoL may play a role in the inflammatory process. This is consistent with suggestions that the pathophysiology of schizophrenia involves activation of the inflammatory response system *(51,52)*.

ApoE and Schizophrenia

ApoE is most widely known for its association with Alzheimer's disease. Many studies have confirmed that the inheritance of the apoε4 allele is associated with an increased susceptibility to familial and sporadic late-onset Alzheimer's disease (for reviews *see* refs. *4,5,34*). ApoEs association with neurological disorders was first discovered when it was identified as a differentially expressed gene in a subtractive hybridization screen examining genes associated with the pathology of scrapie. Due to the similarities between scrapie and Alzheimer's disease, apoE was subsequently examined in brains from Alzheimer's subjects and also found to be upregulated *(14,17)*. Several genotyping studies have also investigated apoE polymorphisms in schizophrenia, which make up the bulk of the research investigating apoE in schizophrenia. An early study using DNA from postmortem brain tissue reported an increased frequency of the ε4 allele in schiz-

ophrenia *(23).* This was the first suggestion of a possible role for apoE in the pathology of schizophrenia. The study was motivated by the possibility that decline in the cognitive function observed in both schizophrenia and Alzheimer's diseases may have a similar pathological basis. Additional findings suggest that the ε4 allele could be associated with either an increased susceptibility for schizophrenia or specific symptoms associated with the illness. For example, the ε4 allele has been associated with less severe psychotic symptoms *(53):* a blunted ketamine-induced exacerbation of psychosis *(54),* an increase in positive symptoms *(55),* a worse prognosis in females *(56),* and an increased frequency of the early-onset form of the illness *(57).* A decreased ε2 allelic frequency has been observed in early-onset schizophrenia *(58),* which may suggest that the ε2 allele is protective against the illness developing early in life. Additionally, it has been suggested that the ε3 allele confers an increased risk of schizophrenia *(59).* Other studies, however, have failed to show any changes in allelic frequency of the *APOE* gene in schizophrenia (unpublished studies: Dean, B., Laws, S. M., Hone, E., Taddei, K., Scarr, E., Thomas, E. A., Harper, C., McClean, C., Masters, C., Lautenschlager, N., Gandy, S. E., and Martins, R. N.; *60*), or in another common polymorphism (–491A/T) present in the transcriptional regulatory region of the gene *(61,62).*

Aside from the genotyping studies, apoE protein levels have also been measured in the brains of subjects with schizophrenia using Western blot analysis. A significant increase in the levels of apoE was detected in the prefrontal cortex of schizophrenic subjects (n = 23) compared to the control subjects (n = 47) (Dean et al., 2002, unpublished observations).

What might be the role of apoE in schizophrenia? ApoE is the primary regulator of CNS cholesterol, which has multiple important functions in the brain. Alterations in the levels of apoE, therefore, may have critical consequences for normal brain function. Abnormalities in cholesterol metabolism have also been previously implicated in schizophrenia (as reviewed by ref. *63*). It will be interesting to see the extent of apoE expression differences in the CNS of subjects with schizophrenia and other psychiatric disorders, and if isoform specific effects exist, as observed with the apoL family of proteins. Like apoL, apoE has also been implicated in the inflammation process. ApoE knock-out mice show an increase in pro-inflammatory markers suggesting that apoE may act to inhibit the inflammatory response *(64).* These observations provide additional support for inflammatory mechanisms in schizophrenia *(51,52).*

ApoD in Psychiatric Disorders

Clozapine and ApoD Regulation

Typical antipsychotic drugs, including haloperidol and chlorpromazine, are dopamine D_2-receptor antagonists. Clozapine is an atypical antipsychotic drug that represents a significant advancement in the treatment of mental illness. Clozapine displays improved clinical efficacy over typical antipsychotics and also has been proven to be effective in patients who have not responded to conventional pharmacotherapies *(65,66).* In contrast to typical antipsychotics, clozapine exhibits affinity for neurotransmitter receptors, in addition to the dopamine family, including serotonin, histamine, muscarinic, and adrenergic receptors *(66).* However, it is possible that the clinical efficacy of clozapine may not be attributed exclusively to its drug-blocking profile. Additional factors may be involved, perhaps those related to lipid-signaling and metabolism. The efficacy of medications, such as clozapine, in schizophrenia and other mental disorders has been well established, in that antipsychotic drugs reduce symptomatology and prevent relapse in a large percentage of patients. Therefore, molecular and biochemical changes resulting from administration of antipsychotic drugs may be associated with antagonism of the pathology of psychoses.

Considerable evidence suggests that the ameliorative effects of clozapine and other antipsychotic drugs are the result of changes in

gene expression. Imaging studies have shown that dopamine receptors are blocked after only a few hours of antipsychotic administration *(66a)*, whereas relief of psychotic symptoms may take several weeks of drug administration. This temporal discrepancy between receptor occupancy and clinical benefit suggests that molecular changes are occurring downstream from receptor blockade attributing to the delayed actions. The unwanted motor side effects that often accompany antipsychotic drug regimens also exhibit a similar delayed onset *(67).* In rodents, antipsychotic drug treatment has been shown to induce expression of immediate early genes, many of which are transcriptional regulatory factors (for review *see* ref. *67a*). Thus, the latency in antipsychotic benefits might result from the progression of a cascade of gene expression changes that cause gradual changes in the expression of critical proteins. These modifications in gene expression may function to overcome the underlying neurochemical deficits of the disorder, thereby restoring a state of normal mental activity, and can additionally cause detrimental consequences in some cases.

In order to gain insight into the molecular mechanisms of clozapine action, we used a PCR-based, high-throughput genomics method, TOGA® (Total Gene Expression Analysis) *(9)* to identify genes whose expression is altered by clozapine administration to rodents. Groups of mice received daily subcutaneous injections of clozapine (7.5 mg/kg) for up to 2 wk. Poly A^+-selected mRNA samples were isolated from the striatum and subjected to TOGA® in duplicate *(13).* Nearly 11,000 mRNAs were detected and their expression levels in the samples were automatically compiled in an electronic database. The database was queried for species whose concentration gradually increased or decreased to a threshold of 1.6-fold over the control level as a result of clozapine exposure. Between 30–40 mRNAs were identified, one of which encoded apoD. The PCR panel from the TOGA® analysis (one of 256 panels generated for each sample) is shown in Figure 1A. The same RNA preparations were quantified by Northern blot analysis (Figure 1B) and the two methods were compared (Figure 1C). These studies indicate that apoD mRNA concentrations are gradually increased by chronic clozapine administration ultimately rising 2.5- to 3-fold *(13).*

The authors investigated the neuroanatomical expression of apoD using *in situ* hybridization and immunohistochemical analyses, and found that apoD mRNA and protein were expressed in several different cell types in clozapine-treated mice. Increases in apoD mRNA were observed after 5 d and even more so after 2 wk of clozapine treatment in gray matter regions, such as the striatum, globus pallidus, and thalamus. Increases in expression were also detected in white matter tracts, predominantly in the corpus callosum, anterior commissure, internal capsule, and optic tract *(13). In situ* hybridization experiments performed on brains from haloperidol-treated (4 mg/kg) mice revealed small, but inconsistent increases in apoD expression in gray or white matter regions. The increases in apoD mRNA expression were also correlated with increases in apoD protein, as indicated by immunohistochemical experiments using antisera specific for apoD. In the striatum, both neurons and astrocytes displayed increased apoD protein expression, while in white matter regions, the cell types giving rise to the elevated apoD levels were astrocytes and oligodendrocytes *(13).*

The finding that clozapine elevates apoD expression in rodent brains suggests that apoD may be associated with the mechanisms of clozapine action. It is possible that elevated levels of apoD, such as those caused by clozapine treatment, are beneficial to patients with schizophrenia by compensating for deficiencies in other aspects of lipid metabolism. For example, several researchers have demonstrated decreases in the content and incorporation of AA into membrane phospholipids in schizophrenics *(68–72).* ApoD, by binding AA, may help counteract these deficits. This view is consistent with previous studies that have investigated the effects of clozapine treatment

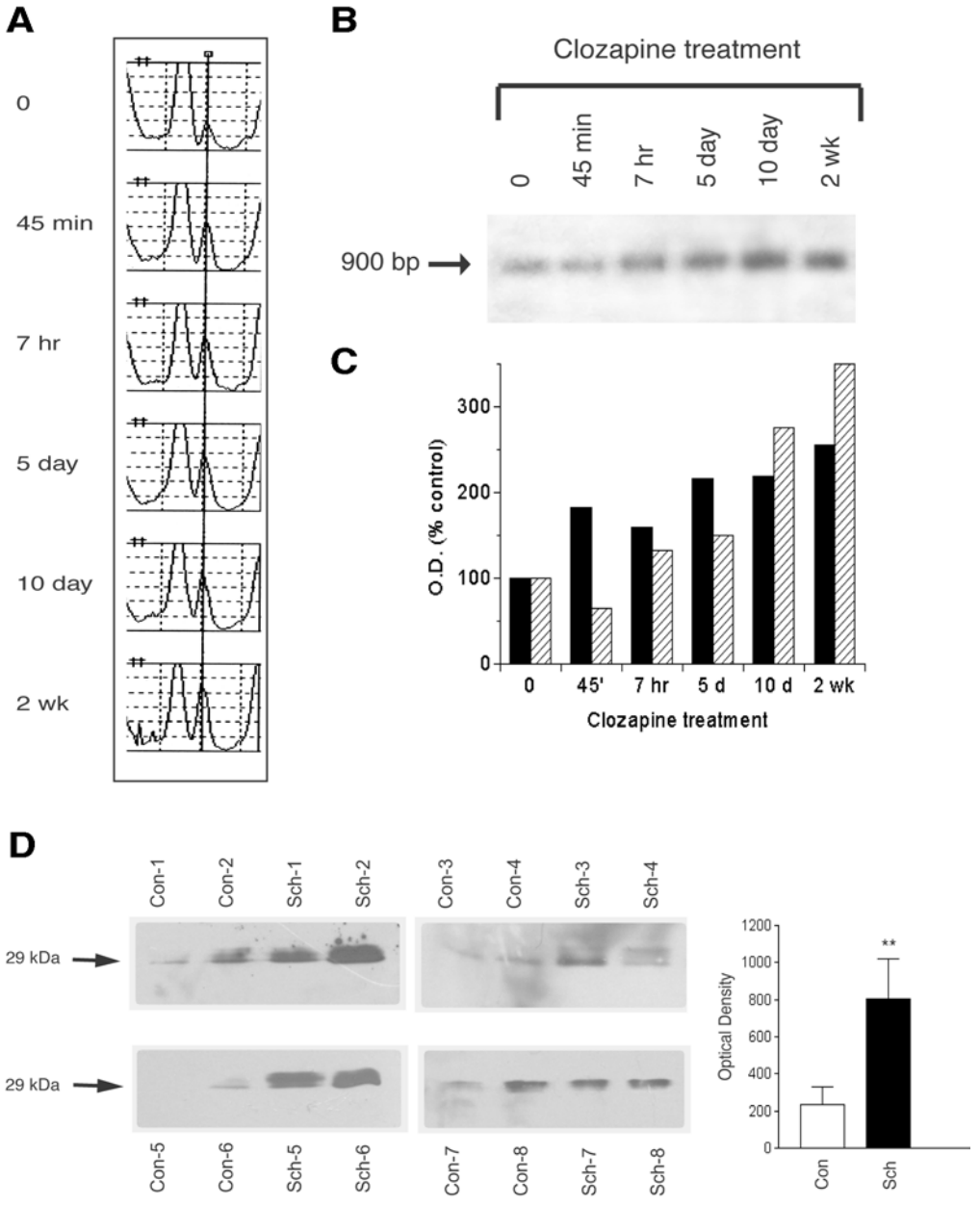

Fig. 1. **(A)** TOGA profile for product corresponding to apoD. Vertical line is drawn through the peak corresponding to apoD. **(B)** Northern blot showing an increase in apoD expression with clozapine treatment. Northern blots with 2 µg polyA$^+$-selected RNA from the striatum (including nucleus accumbens) of mice treated with clozapine (7.5 mg/kg) for the indicated time periods were probed with a ^{32}P-labeled apoD cDNA clone. **(C)** Bar graph demonstrating the quantitative correlation between TOGA and Northern blot analysis. Solid and stippled bars reflect TOGA and Northern values, respectively. (For more details *see* ref. *13*.) **(D)** Western blot analysis demonstrating apoD expression in dorsolateral prefrontal cortex of control (Con) and schizophrenic (Sch) subjects. Western blots containing 50 µg total protein/lane were probed with a monoclonal antibody directed against human apoD. Eight schizophrenic subjects (Sch1–Sch8) are shown with their age- and sex-matched controls (Con1–Con8). Only the regions of the blots corresponding to the 29 kDa apoD immunoreactive species are shown.

on fatty acid levels in schizophrenic patients. Horrobin and collaborators showed that 8–12 wk of clozapine administration resulted in a significant increase in AA and docosahexaenoic (DHA) levels in red-cell membranes of schizophrenic patients *(73).* Suggested mechanisms for this effect of clozapine include the inhibition of PLA_2 or PLC enzymes and/or antioxidant/anti-free radical effects of clozapine. However, pathways related to apoD provide an alternative explanation. In other studies dating back to 1985, clozapine was shown to inhibit AA-induced aggregation of rabbit platelets, suggesting an inhibition of the generation of prostaglandin endoperoxides or thromboxanes *(74).* Hence, it is possible that clozapine, and other antipsychotic drugs, exert some of their effects via lipid/fatty-acid modulation. In a further note of interest, apoD has been shown to interact with the leptin receptor, which may explain the weight gain associated with antipsychotic drug treatment *(75).*

Apolipoprotein D in Schizophrenia and Bipolar Disorder

We hypothesized that apoD levels might be low in patients with schizophrenia and that long-term treatment with clozapine would be beneficial for patients by elevating apoD. Therefore, we quantified apoD concentrations in serum samples from normal subjects and patients with schizophrenia using an enzyme-linked immunosorbent Assay (ELISA) with two different antibodies to human apoD. This schizophrenic group consisted of a mixture of patients that had received typical antipsychotic drugs, clozapine, or were deemed antipsychotic-free (those that had not received antipsychotic drugs orally for 1 mo or depot injection for 3 mo before blood collection). A significant decrease in the concentration of apoD was observed in schizophrenic patients relative to normal subjects (256 μg/mL ± 11 vs 303 μg/mL ± 12; $P = 0.0084$) *(14)*.

Since psychiatric illnesses are brain disorders, we investigated whether apoD was differentially expressed in brain tissue obtained postmortem from control and schizophrenic subjects. For the initial studies we focused on the dorsolateral prefrontal cortex (Brodmann Area [BA] 9), a region widely implicated in the pathophysiology of schizophrenia (for review *see* ref. *49*). Using Western blot analysis, we detected an increase in apoD levels in the dorsolateral prefrontal cortex schizophrenic subjects compared to their age-and sex-matched controls (Figure 1D) *(14).* This was opposite to the direction of change observed in the serum samples, suggesting a potentially complex regulation of apoD. In additional studies, we used an ELISA to measure apoD levels in tissue homogenates from 12 different brain regions of control subjects and those diagnosed with either schizophrenia or bipolar disorder. It was found that both disorders are accompanied by apoD increases in several areas, many of which are shared by the disorders (*see* Table 1; Figure 2). Nevertheless, there are areas of apoD increases that discriminate between schizophrenia and bipolar disorder *(15)*. These findings are summarized in Figure 2. Brain regions that exhibited elevated apoD levels in both disorders were the dorsolateral (BA9) and lateral (BA46) prefrontal cortex, orbitofrontal cortex (BA11), and caudate. Additionally, in schizophrenic subjects, elevated apoD proteins levels were observed in the thalamus and amygdala. In subjects with bipolar disorder, additional regions of increased apoD expression were the parietal (BA40) and cingulate (BA24) cortices. No significant differences were detected in the occipital cortex, hippocampus, substantia nigra or cerebellum of either disease group.

Neuroanatomical Sites Implicated in Schizophrenia

Schizophrenia is a heterogeneous disorder and no single anatomical region has been proven to be focal to pathology. Numerous experimental and clinical studies have provided evidence of pathophysiological changes in the prefrontal cortex of patients with schizophrenia *(76–81).* Hence, the prefrontal cortex was the focus of studies examining changes in apoE and apoL, as well as the authors' own studies on apoD. In addition to the prefrontal

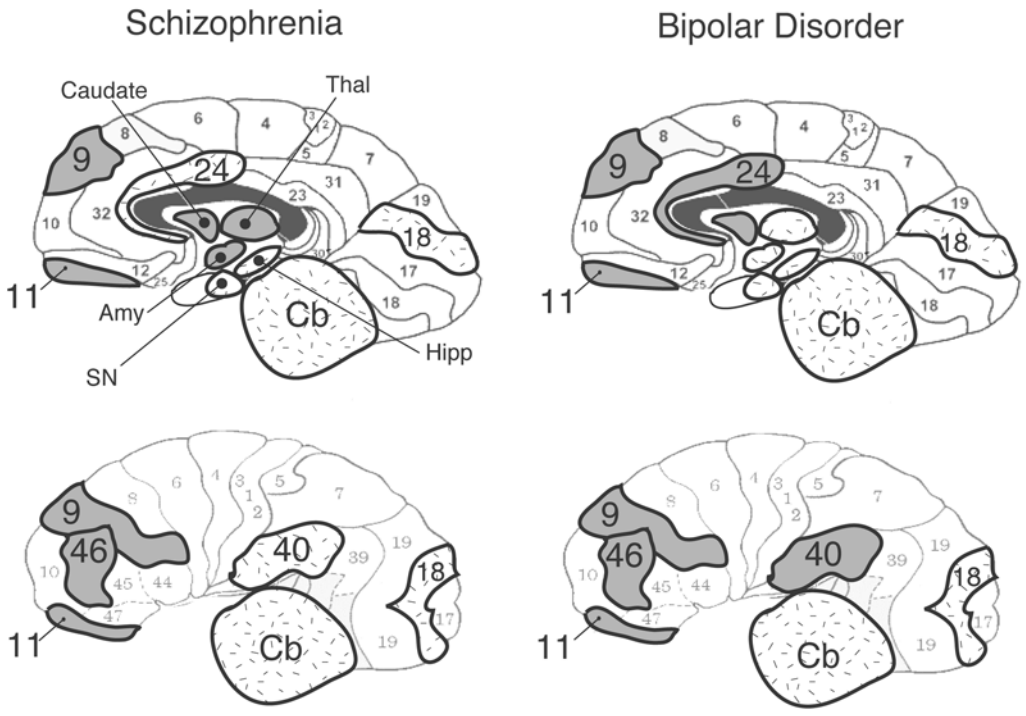

Fig. 2. Schematic depiction of brain regions exhibiting elevated apoD expression in schizophrenia and bipolar disorder. Shaded areas indicate specific regions of elevated apoD expression, which are different between the two disorders. Speckled regions denote brain regions that were examined for apoD expression, but did not show increased apoD expression. Numbered areas on brain surface are according to Brodmann.

cortex, the brain regions displaying elevated protein levels of apoD have been previously implicated in the pathophysiology of schizophrenia and/or bipolar disorder. Components of bipolar disorder have also been associated with abnormal functioning of the prefrontal cortex *(82–84).* Emerging studies have also recognized the orbital frontal cortex as an important region in both schizophrenia and bipolar disorder *(85–87).*

The elevation of apoD levels in the caudate, thalamus and amygdala, is also consistent with previous reports, as these regions have also been implicated in schizophrenia. The cortical-striatal-thalamic circuit modulates cognitive processing; hence, in addition to the prefrontal cortex, the caudate and thalamus are implicated in cognitive disturbances as well as other behavioral abnormalities observed in schizophrenia *(88).* PET-scans have shown decreased metabolic activity in the thalamus of schizophrenic patients *(89),* while MRI-scans have also shown regional abnormalities in the thalamus *(90).* 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 *(91).* The amygdala is a structure reponsible for emotional and social behavior. Hence, it is thought that amygdala dysfunction may contribute to behavioral changes that accompany schizophrenia.

The Influence of Long-Term Antipsychotic Drug Treatment

One of the universal drawbacks of using postmortem tissue to study pathological features of psychiatric disease is the potential

influence of long-term antipsychotic drug treatment. Accordingly, all of the schizophrenic subjects and most of the bipolar subjects examined for apoD expression had been treated with typical antipsychotic drugs prior to death. The elevated apoD CNS levels detected might have resulted from long-term drug treatment. However, several arguments suggest that these changes are not simply due to drug treatment before death. First, the authors did not observe a correlation between apoD levels and antipsychotic drug dose (chlorpromazine equivalents) in these subjects, nor a correlation between apoD levels and duration of illness (DOI) *(14)*. DOI may be considered an indication of how long subjects have been exposed to antipsychotic drugs. Secondly, in the rodent studies, the authors demonstrated an increase in apoD in response to clozapine, while all the schizophrenic and bipolar subjects had been treated with typical antipsychotics (haloperidol, chlorpromazine, fluphenazine). It is possible, though, that typical antipsychotic drugs may regulate apoD levels to some extent. 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 were not exposed to antipsychotic drugs *(92–94)*. Some behaviorally disturbed Alzheimer's patients may have received antipsychotic drug treatment, however, the authors also observed increases in apoD expression in a mouse model of Alzheimer's disease and these mice were not treated with antipsychotic drugs *(95)*. Finally, the authors observed disease-specific changes in apoD expression in schizophrenia and bipolar disorder, despite the subjects in both groups being similarly treated with antipsychotic drugs prior to death. Together these findings would argue against the changes in apoD observed in schizophrenia and bipolar disorder being simply an effect of antipsychotic drugs.

ApoD as a Region-Specific Marker for Pathology

Overall, these results have demonstrated that apoD levels are increased in rodents by clozapine administration, as well as being elevated in the brains of subjects with schizophrenia and bipolar disorder. The finding that clozapine-induced apoD accumulation in rodent brains had suggested the simple hypothesis that increases in apoD may be beneficial to patients with neuropsychiatric disorders. The results from the author's human brain analysis suggest that apoD accumulation in certain brain regions might be a natural response to regional neuropathology. Accordingly, one reason clozapine is an effective antipsychotic drug may be because of its ability to elevate apoD levels and/or augment increases in apoD already present in the brain.

Considering the upregulation of apoD levels observed in rat brain in response to mechanical insult, the authors suggest that apoD may be elevated in human brains in response to local pathophysiology. The low levels of apoD detected in the serum may reflect systemic lipid metabolic insufficiencies. This is consistent with the hypothesis that deficiencies in various aspects of phospholipid metabolism are a pathogenic feature of schizophrenia. While the apoD gene itself may not be site for a causative mutation, its association with potentially multiple lipid-related pathways provides a plausible link. Alternatively, the brain, with its complex regulatory pathways, may be attempting to compensate for intrinsic lipid abnormalities by increasing apoD expression. This compensation would occur only in regions manifesting dysfunction in the diseased CNS. Therefore, apoD elevation may represent a compensatory, regional response to neuropathological processes that occur in brain areas particularly affected by schizophrenia and bipolar disorder.

Possible Functions for ApoD in Psychiatric Disorders

The physiological role for apoD in psychiatric disorders remains unclear. Increases in apoD expression have been observed in response to diverse neuropathologies, hence, apoD expression 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 patterns of apoD induction observed in human diseases *(44–46,48,92–94)*, the authors hypothesize that apoD represents a specific pathological response. What might be the consequences of increased levels of apoD in the brain? While apoD might have a similar function to apoE in the CNS, it does not share a similar protein structure as apoE or the classical apolipoproteins, and does not bind cholesterol with high affinity. Hence, apoD likely exerts its effect via binding of different ligands. By binding to AA, a major component of the lipid bilayer, apoD may cause alterations in membrane structure and composition or may affect dendritic/synaptic remodeling and reorganization in response to neuropathology. Alternatively, 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, and may therefore act to prevent oxidative damage to membranes.

Arachidonic Acid and ApoD

Membrane Effects

Among the hydrophobic compounds that apoD binds, AA is of particular interest. AA is one of the most important fatty acids. AA, together with DHA, makes up ~90% of the polyunsaturated fatty acid content in the CNS *(96)*. In its esterified form, it is a major component of the lipid bilayer of cellular membranes and is released by a number of phospholipases upon extracellular signals. Variation in composition and hydrocarbon chain saturation state determine membrane order and fluidity, and these properties affect the binding and function of extrinsic membrane proteins and second messenger signaling. Hence, changes in the levels of apoD can potentially affect membrane phospholipid composition, by modulating the transport and uptake of AA or of other membrane constituents. Phospholipids play a critical role in almost every function of the cell membrane, from the formation and remodeling of dendrites and synapses to the release of neurotransmitters and cell-to-cell communication. Plasma membrane phospholipids act as precursors in numerous signaling systems, e.g., inositol phosphate, platelet activating factors, lysophosphatidic acid and eicosanoids, and comprise the membrane microenvironment for neurotransmitter receptors, ion channel, and enzymes. In the prefrontal cortex and other sites of increased apoD expression, numerous reports have demonstrated increases and/or decreases in neurotransmitter receptors, ion channels and membrane-bound proteins, all of which can be affected by membrane alterations in subjects with schizophrenia (for review *see* ref. *88*). Synaptic organization is also dependent upon the integrity of the membrane structure. Recent studies have demonstrated increases in various presynaptic proteins *(97)*, such as synapsin and synaptophysin, two synaptic vesicle-associated proteins *(98,99)* in the cerebral cortex of schizophrenic subjects. The increase in apoD observed after sciatic nerve injury suggests it may play a role in synaptic reorganization and reinnervation after injury or in response to pathology.

AA and PLA_2 Signaling

The phospholipase A_2 enzymes are responsible for the incorporation of AA into membrane phospholipids, as well as the intracellular release of AA, which acts as a precursor for prostaglandin and leukotriene biosynthesis. In schizophrenic and schizoaffective patients, there is substantial evidence for AA and PLA_2 dysfunction. Studies have also demonstrated a marked depletion of AA in membranes of red blood cells, fibroblasts, and brain tissue and an

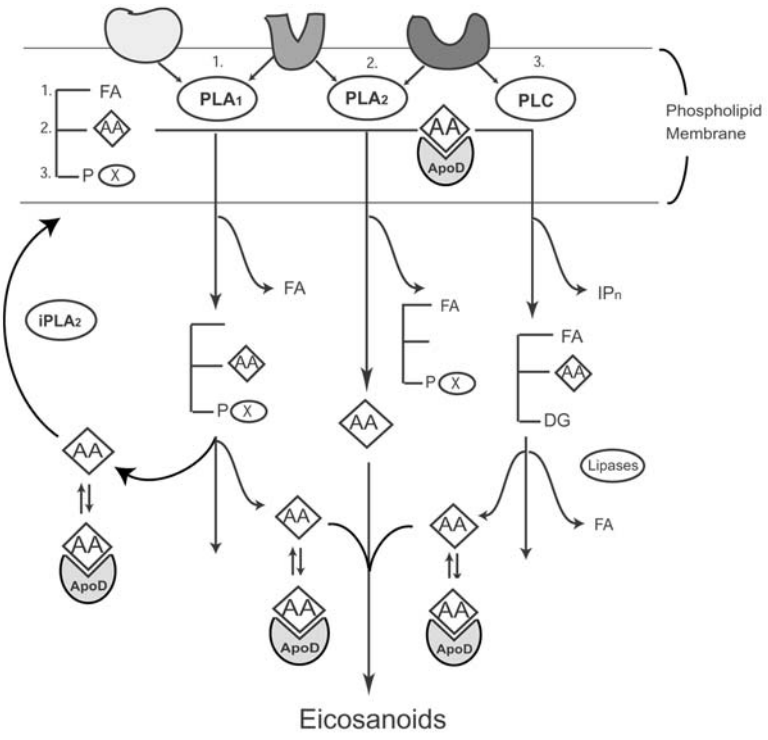

Fig. 3. Schematic view of arachidonic acid (AA) signaling and potential role for apoD intervention. Upon activation of extracellular receptors, phospholipase A_1 (PLA_1), A_2 (PLA_2), and C (PLC) are responsible for the release of phospholipids from the membrane at the given positions 1, 2, or 3 respectively. The calcium-independent PLA_2 ($iPLA_2$) regulates incorporation of AA back into phospholipids. FA, fatty acid; P, phosphate; X, choline, ethanolamine, inositol or serine; IPn, inositol phosphates; DG, diacylglycerol. ApoD may act to stabilize membrane AA or bind free AA, thereby modulating re-incorporation of AA into the membrane and/or synthesis of eicosanoids.

abnormal incorporation/esterification of AA into platelet membranes of patients with schizophrenia *(68–71,100–102).* In addition, an impairment of AA-related signal transduction is observed in schizophrenic patients, as revealed by reduced niacin-initiated skin flushing *(103–105).* These effects may be related to alterations in the activity of the enzyme phospholipase A_2 (PLA_2), which is responsible for AA incorporation into, and release from phospholipid membranes. The activity of PLA_2 (the calcium-independent isoform) has been shown to be elevated in the serum and cortex of schizophrenic patients *(106–108).* The ability of apoD to bind AA may provide a link for apoD to these phospholipid pathways. By binding AA, apoD may facilitate or inhibit the incorporation of AA into membrane phospholipids, and may affect substrate availability for PLA_2-induced release of AA and the subsequent eicosanoid signaling cascade (*see* Figure 3). Many eicosanoids (prostaglandins, leukotrienes, etc.) are mediators of inflammation, which again implicates inflammatory mechanisms in schizophrenia.

Oxidative Stress and ApoD

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 *(109).* A similar role may be played by apoD in psychiatric disorders when considering the possibility that oxidative stress or damage contributes to the pathophysiology of schizophrenia, as has been suggested *(102).* Free radicals are reactive chemical species generated during normal metabolic processes and can damage lipids, proteins, and DNA. Neuronal membranes are exceedingly vulnerable to free radical-mediated damage. Several investigators have demonstrated decreased levels of polyunsaturated fatty acids in both peripheral and central membranes of patients with schizophrenia (for review *see* ref. *110*). Oxidative stress may provide an explanation for the specific membrane abnormalities that have been previously observed in schizophrenia and could represent the pathological stimulus for apoD upregulation in psychiatric disorders.

Concluding Thoughts

Neuropsychiatric disorders are accompanied by abnormalities in various aspects of lipid metabolism and signal transduction, which has led to the hypothesis that schizophrenia, and related illnesses, are disorders of lipid metabolism *(110–112).* Numerous studies have reported alterations in the content and distribution of membrane lipids and their fatty acids, as well as fatty-acid (primarily AA) metabolism and signaling in red blood cells, fibroblasts, and brain tissue of schizophrenic subjects compared to controls *(110–112).* This hypothesis provides an explanation for the diversity of symptoms associated with psychiatric disorders. Furthermore, it suggests that polymorphisms in any of a large number of genes, whose products coordinate lipid metabolism, could perturb neuronal function and result in behavioral abnormalities, thereby providing an explanation of the multigenetic nature of these disorders. This hypothesis is supported by the recent discoveries that three different apolipoproteins are elevated in the brain of subjects with psychiatric disorders. All of the brain-expressed apolipoproteins, which include apoJ and apoH (unpublished studies), which have not been studied in psychiatric disorders, may act in concert to regulate CNS lipid metabolism. However, due to differences in activities, they may have distinct roles relating to different aspects of lipid function. Future studies on these proteins might provide insights into the mechanisms that produce the variety of partially overlapping symptoms of the affective disorders and might possibly lead to molecular criteria that would discriminate subtypes of these disorders.

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