onset decreases from 84 to 68 years with an increasing number of ApoE-4 alleles, confirming the dosage effect of the ApoE-4 allele, which in ApoE-4/4 homozygotes anticipates the age at onset to their 60s. The combination of low head circumference and ApoE-4 is also a strong predictor of early-onset AD.[52]

ApoE is found in amyloid plaques and neurofibrillary tangles (NFT) in AD brains. The accumulation of potentially pathogenic C-terminally truncated fragments of ApoE depends on both the isoform and the cellular source of ApoE. Neuron-specific proteolytic cleavage of ApoE-4 is associated with increased phosphorylation of tau and may play a key role in the development of AD-related neuronal deficits.[53] Hippocampal ApoE levels correlate with NFT formation, especially in ApoE-3/3 autopsy samples, but not in ApoE-4 carriers.[54] Monocyte-derived macrophages exhibit a significantly greater increase in nitric oxide production during immune activation in AD patients with the ApoE-4 allele. Enhanced macrophage responsiveness and increased production of nitric oxide in ApoE-4 may predispose the CNS to an increased potential for nitration and nitrosation, consistent with the reduction-oxidation imbalance and neuroinflammatory state observed in AD.[55]

ApoE may affect NFT and β-amyloid peptide (BAP) deposition in AD.[56] ApoE-4-related proteins may interfere with binding of tau to microtubules, altering tau glycation and phosphorylation.[57] The presence of ApoE-4 increases the odds ratio for cerebral amyloid angiopathy; and ApoE-4 is strongly associated with increased BAP deposition in AD.[58–60] The oxidized form of purified ApoE-4 shows a higher affinity binding to synthetic BAP and MAP2 than the ApoE-3 isoform, and probably ApoE may affect microtubule function and BAP accumulation in AD.[56,61] Carriers of ApoE-2 and ApoE-4 alleles are also more prone to recurrent cerebral amyloid angiopathy than ApoE-3/3 carriers.[62] AD ApoE-4 carriers show reduced glucose metabolism in selected brain regions.[63] There is also an ApoE-related cognitive decline in AD patients, which is more accelerated in subjects with the ApoE-4/4 genotypes. ApoE-related differences in serum ApoE levels,[64,65] blood pressure values[66] and lymphocyte apoptosis[67,68] have been demonstrated in AD. ApoE-4/4 patients are also the worst responders to different treatments.[26] ApoE-4 carriers also show a poorer brain metabolism.[63,69,70]

The ApoE-4 genotype is accompanied by lower metabolic activity in the nucleus basalis of Meynert neurons in AD and controls.[71] Dubelaar *et al.* used the size of the Golgi apparatus as an indicator of metabolic activity to show that control subjects harboring the ApoE-4 allele have reduced neuronal metabolism and show more neurons with smaller Golgi apparatus size compared with ApoE-4 non-carriers. As the disease progresses into later stages of AD (Braak V-VI stages) neuronal metabolism strongly diminishes, resulting in neurons with extremely small Golgi apparatus size, irrespective of ApoE genotype.[71]

ApoE-4 may influence AD pathology interacting with APP metabolism and BAP accumulation, enhancing hyperphosphorylation of tau protein and NFT formation, reducing choline acetyltransferase activity, increasing oxidative processes, modifying inflammation-related neuroimmunotrophic activity and glial activation, altering lipid metabolism, lipid transport and membrane biosynthesis in sprouting and synaptic remodeling, and inducing neuronal apoptosis.

A critical review in the literature provides convincing support to the hypothesis of ApoE as a major player in AD pathogenesis and risk of dementia. The major facts demonstrating that ApoE is associated with AD can be summarized as follows: (i) increased frequency of the ApoE-4 allele in AD and protective effect of ApoE-2; (ii) association of ApoE-4 with an anticipation of the age-at-onset; (iii) negative influence of ApoE on cognitive performance; (iv) deleterious associations of ApoE-4 with other genes as potential risk factors for AD; (v) ApoE and sex differences in AD; (vi) association of ApoE with BAP and tau in AD pathology; (vii) ApoE and alterations in lipid metabolism; (viii) ApoE and neuroendocrine function in AD; (ix) ApoE and behavior; (x) ApoE and brain atrophy; (xi) ApoE and survival; and (xii) ApoE in other CNS disorders.[29,72]

Association of ApoE with BAP and tau in AD pathology

The ApoE-4 isoform binds to BAP more rapidly than the ApoE-3 isoform;[73] and the ApoE-4 allele is strongly associated with increased senile plaques but not NFT in AD and in the AD Lewy body variant.[58] However, isoform-specific differences have been identified in the binding of ApoE to microtubule-associated protein tau, which forms NFT, and to BAP, a major component of senile plaques.[56] Other studies have reported that the presence of ApoE-4 is significantly associated with both BAP and NFT in autopsy

brains,[74] but the effect is differentially modified by age and gender. For instance, the effect of ApoE-4 on NFT is noted at ages 80 and above, but not between ages 60 and 79, in both genders, whereas the association between the ApoE-4 allele and senile plaques for women is found only from ages 60 to 79, but not above 80 years, with no age difference in men.[75] The amount of deposited BAP40 is significantly increased in AD brain samples with ApoE-4 allele and also in cases with the -491 AA genotype independent of ApoE-4 status, suggesting that the association between increased BAP load and alleles of the ApoE promoter polymorphism is independent of ApoE genotype.[76]

In animal models, overexpression of human ApoE-4 in transgenic mice led to an increase in plaque formation, with the association of the ApoE-4 isoform with APP and BAP in the plaques, a decrease in presynaptic terminals, and an increase in tau phosphorylation and in surrounding gliosis, all these events corresponding with major neuropathological hallmarks of AD. ApoE reduces BAP levels by 20–80% in cell cultures. ApoE may function independently of BAP, and conformational changes in its molecular structure might contribute to neurodegeneration. Characterization of the 3-D structure of ApoE shows four helix bundles in between the amino and carboxy terminal of the molecule. ApoE-4 is the most unstable isoform in terms of protein folding; ApoE-2 folds in the most stable conformation; and ApoE-3 shows an intermediate stability. The molten globule conformation linked to greater stability is acquired most often by ApoE-4 than ApoE-3 or ApoE-2, and ApoE-4 exhibits the highest tendency among these three proteins to form molten globules whose conformational features may lead to increased degradation, alterations in cell signaling, increased binding to lipids, modifications in protein–protein interactions, increased membrane binding, changes in transport through membranes, and modified interactivity with cellular receptors.[77] The structural changes in ApoE-4 seem to be related to an interaction between Arg112 and Arg61 with Glu225 that does not occur in ApoE-3 owing to the presence of a Cys residue at position 112.[74] Wild-type ApoE-4 seems to be associated with higher BAP production, more extensive disruption of the cytoskeleton, and increased lysosomal cleavage.[78–80] Astrocytes appear to play a critical role in the clearance of BAP in the brain following migration to areas of the brain rich in neurotoxic deposits. A receptor-specific uptake seems to mediate internalization and degradation, but defects in these steps associated with ApoE may impair clearance, thus favoring further accumulation of BAP and the appearance of neurodegenerative events. Expression of ApoE-3 in a transgenic model decreased the BAP load in a dose-dependent manner in PDAPP mice at 12–15 months of age, and expression of ApoE-4 led to increased deposition of BAP in these PDAPP/ApoE-knockout mice. ApoE-2 induced a marked decrease in BAP accumulation.[78] So, it appears that ApoE polymorphic variants affect the amount of BAP deposited in the brain, and ApoE is able to reduce γ-secretase cleavage of APP, lowering BAP levels. In neuronal and non-neuronal cell lines, ApoE treatment reduced BAP40 by 60–80% and BAP42 to a lesser extent (20–30%) in the conditioned media. ApoE treatment resulted in an accumulation of APP-C-terminal fragments in cell extracts and a marked reduction of APP intracellular domain-mediated signaling, consistent with diminished γ-secretase processing of APP. All three isoforms of ApoE had similar effects on BAP and APP-C-terminal fragments, and the effects were independent of the LDL receptor family.

There has been increasing interest in a potential role for fatty acids in adversely affecting organismal substrate utilization and contributing to the cardiovascular complications in insulin resistance. Fatty acids have already been implicated in regulating the expression of a number of genes in resident cells of the vessel wall. In this regard, it has been demonstrated that oleic acid increases ApoE secretion from macrophages at a locus involving post-translational glycosylation.[81]

ApoE in other forms of dementia and CNS Disorders

The distribution of ApoE genotypes clearly differs among different CNS disorders, with an accumulation of the ApoE-4 allele in dementia, especially in AD and mixed-type dementia (MXD). In early-onset AD (EOAD), the ApoE-3/4 and ApoE-4/4 genotypes account for 54.35% of the cases, and the presence of the ApoE-4 allele is more frequent in women (52.42%) than in men (35.47%). In late-onset AD (LOAD), the ApoE-4 allele is present in 55.88% of the cases (55.87% in women and 55.92% in men). According to these results, the frequency of the ApoE-4 allele is similar in women with EOAD and LOAD, but significantly higher in men with LOAD as

compared with EOAD men; however, women and men show an identical distribution in LOAD. Integrating both types of age-related AD phenotypes (EOAD+LOAD), the presence of the ApoE-4 alleles accounts for 51.38% of AD cases (54.38% in women and 45.43% in men).[29]

In pure vascular dementia (VD), secondary to severe cardiovascular and cerebrovascular disorders (e.g. stroke, atrial fibrillation, hypertension), the ApoE-4 allele is present in 37.60% of the cases, with a relative distribution similar in women (39.08%) and men (35.57%), but significantly different from the distribution pattern seen in AD. The highest accumulation of ApoE-4 carriers is observed in MXD (53.01%), with a distribution in women (58.76%) and men (45.10%) similar to that detected in AD; however, the ApoE-4/4 genotype is over-represented in both women (12.55%) and men (13.41%) with MXD. In this regard, patients with MXD exhibit the highest frequency of the ApoE-4/4 as compared with any other cluster or pathological group in the Spanish population.[29,37,39]

In AD patients with history of cerebrovascular disorders (excluding stroke), such as chronic cerebrovascular insufficiency, migraine, hypotension or dizziness, a high frequency of ApoE-4 was also found (44.71%), with identical distribution of the ApoE-4/4 genotype in women and men. Nevertheless, in patients with cerebrovascular disorders without cognitive impairment, the frequency of the ApoE-4 allele (24.65%) was similar to that of controls (24.76%), suggesting that the risk of developing dementia in patients with chronic cerebrovascular disorders may be associated with the presence of the ApoE-4 allele. In patients with different CNS disorders, including Parkinson's disease, schizophrenia, depression, anxiety and epilepsy, an increased frequency of the ApoE-4 allele was detected (41.13%), with a similar distribution in women and men, probably indicating that the ApoE-4 allele might represent a factor of brain vulnerability in different medical conditions. Finally, we found a low frequency of ApoE-4 (24.99%) in patients with stroke, practically the same as in controls and in patients with cerebrovascular disorders without cognitive deterioration. Surprisingly, a high frequency of ApoE-4 was also observed in patients with anxiety (39%), diabetes (40%) and hypertension (36%). The highest frequencies of the ApoE-4/4 genotype in decreasing order were identified in MXD, diabetes, VD, headache, and AD. The fact that patients with stroke and/or cerebrovascular disorders without cognitive impairment show a frequency of ApoE-4 similar to controls (20–30%) together with the evidence that patients with MXD and AD represent the population with the highest frequency of ApoE-4 (50–60%) suggests that the inheritance of ApoE-4 is an important risk factor in dementia in general, and that the presence of ApoE-4 in patients with cerebrovascular disorders and/or stroke may be determinant for these patients to develop dementia as a secondary event following cerebrovascular damage.[29,37,39]

Vascular dementia and cerebrovascular disorders

The frequency of the ApoE-4 allele has been found to be increased in vascular dementia (VD).[82–84] In early reports it was suggested that the increased plasma cholesterol concentrations and resulting atherosclerosis associated with ApoE-4 might contribute to VD.[82] Wieringa *et al.*[85] found a higher frequency of ApoE-4 in multi-infarct dementia, but the increased prevalence of the ApoE-4 allele was not related to serum lipid levels, and they concluded that the hypothesis that the onset of multi-infarct dementia may be precipitated by ApoE-4's mediation of higher serum cholesterol levels was not supported. Some authors did not find a great difference in ApoE-4 allele frequency between AD and VD.[86,87]

A high frequency of the ApoE-2 allele was observed in patients with cerebral amyloid angiopathy-related hemorrhage, suggesting that patients with ApoE-2 may be protected from parenchymal AD but may be susceptible to rupture of amyloid-laden vessels.[88,89] Lin *et al.* reported that ApoE-4 plays no significant role in the development of ischemic cerebrovascular disease and VD, but that ApoE-2 has a protective effect with regard to the development of ischemic cerebrovascular disorders and VD for Taiwanese–Chinese subjects younger than 65 years.[90] Greenberg *et al.* also found association between ApoE-2 and vasculopathy in cerebral amyloid angiopathy, postulating that ApoE-2 and ApoE-4 might promote hemorrhage through separate mechanisms: ApoE-4 by enhancing amyloid deposition, and ApoE-2 by promoting rupture. ApoE-2 is also a risk factor for early recurrence of cerebral amyloid angiopathy.[91] Others have reported that possession of ApoE-4 does not by itself confer an increased risk of cerebral amyloid angiopathy but may be associated with reduced longevity even in the absence of AD or

cerebral hemorrhage.[92] The ApoE-2 allele may influence the therapeutic response in some cases. For instance, there is evidence that the efficacy of i.v. tissue plasminogen activator in patients with acute ischemic stroke may be enhanced in those carrying the ApoE-2 allele.[93]

Other dementias

A high frequency of ApoE-4 has been found in Lewy body dementia[94,95] where ApoE-4 carriers also showed a greater neuritic degeneration in hippocampal CA2-3 regions. The ApoE-2/3 genotype has been associated with significantly earlier age of onset of Huntington's disease.[95] ApoE-2/2 has been associated with frontotemporal dementia, but the rarity of this genotype recommends being cautious in the interpretation of results.[96] In Chamorros with amyotrophic lateral sclerosis/parkinsonism dementia complex, the ApoE-4 allele was not found to be associated with this form of dementia and the presence of the ApoE-3 allele did not reveal any protective effect against NFT formation in this population.[97] Itabashi *et al.* compared the distribution of ApoE genotypes in a necropsy series of AD and other dementias (Parkinson's disease with dementia, progressive supranuclear palsy, Lewy body dementia, polyglucosan-body disease, Pick's disease, dementia+ hydrocephalus, Wernicke–Korsakoff syndrome)[98] and found no major differences in the distribution of the ApoE-4 allele in AD and the other dementias, suggesting that the ApoE-4 allele is not predictive of AD. An increased frequency of the ApoE-4 allele was reported in bulbar-onset motor neuron disease,[99] but this could not be replicated in another study.[100] No association has been found between ApoE-4 and the incidence or the age of onset of sporadic or autosomal dominant amyotrophic lateral sclerosis.[101]

Down syndrome

Senile plaques in Down syndrome are particularly large in ApoE-4 carriers and less abundant than in AD, suggesting that pathology in Down syndrome is due to increased amyloid production and deposition with ApoE-4 probably increasing senile plaque initiation.[102] In patients with Down syndrome, ApoE-2 was associated with increased longevity and decreased frequency of dementia.[103] No ApoE-4/4 was seen in Down syndrome cases in some studies;[103] in contrast, others could not find significant differences in the distribution of ApoE genotypes between AD and Down syndrome.[104] In general terms, it appears that the frequency of the ApoE-4 allele in Down syndrome does not differ from that of the general population and that ApoE-2 may exert a protective effect.[105]

Schizophrenia

Several studies attempted to associate ApoE-4 with schizophrenia. Harrington *et al.* reported an increased frequency of ApoE-4 in schizophrenia,[106] but subsequent studies in different populations failed to replicate this finding.[107–113] However, ApoE-4 was associated with an early onset of schizophrenia,[109,114] with a reduced outcome of positive symptoms,[115,116] and with a worse prognosis in women,[112] but these results could not be replicated by others.[110,117] Some authors found that ApoE-3 might increase the risk of schizophrenia,[118] but this finding could not be confirmed.[119] Both early-onset schizophrenia[120] and a poor response to neuroleptics were associated with ApoE-2.[121] In a recent study, no differences in ApoE allele or genotype frequencies were observed in schizophrenia, although a possible association between schizophrenia in men and the ApoE-2/3 genotype was postulated.[122] In patients with paraphrenia or late-onset schizophrenia, Howard *et al.* found comparable frequencies of the ApoE-4 allele to that found in centenarians.[123]

The ApoE genotype was related to the incidence of psychiatric symptomatology.[124] The presence of one ApoE-4 allele conferred a 2.5-fold risk and the presence of two ApoE-4 alleles conferred a 5.6-fold risk for development of delusions; however, no association was found for depressive symptoms or behavioral disturbances in some studies;[121] in contrast, others have found a small increment of psychiatric symptoms and aberrant behaviors in AD patients with ApoE-4.[125]

Some authors suggest that increased levels of ApoE in the frontal cortex of schizophrenics may be associated with the pathology of schizophrenia and that antipsychotic drugs decrease ApoE levels as part of their therapeutic action.[126]

Multiple sclerosis

The ApoE-4 allele was associated with significantly faster progression of disability and more extensive axonal damage in patients with multiple sclerosis,[127]

but some studies found that ApoE-4 and/or the -491 A/T ApoE promoter polymorphism were not associated with a more rapid course of multiple sclerosis.[128] ApoE-4 was also associated with slightly earlier disease onset, but it does not constitute a risk factor for multiple sclerosis.[129–132] Niino *et al.* found no relation between ApoE and multiple sclerosis in Japan.[133]

N-acetylaspartate (NAA) is exclusively present in mature neurons, and it appears decreased in multiple sclerosis, reflecting neuronal loss, axonal loss, and generalized neuronal dysfunction. Multiple sclerosis patients with ApoE-4(+) exhibit a higher degree of disability and a lower NAA : creatine ratio than patients with ApoE-4(−)(244). ApoE-4(+) carriers have more relapses and have a 5-fold higher rate of annual brain volume loss compared to ApoE-4(−) carriers. ApoE-4(+) carriers also show an increase in individual lesions on magnetic resonance imaging. In contrast, ApoE-2 carriers show the lowest annual volume loss of brain volume.[127] These results by Enzinger *et al.* clearly demonstrate the negative influence that ApoE-4 exerts on brain volume, contributing to increasing brain atrophy in multiple sclerosis.[127]

Head injury

It has been reported that ApoE-4 may negatively influence recovery in patients with head injury. Teasdale *et al.* found that ApoE-4(+) carriers were more likely to have an unfavorable outcome 6 months after injury than ApoE-4(−) carriers.[134] ApoE-4(+) patients also have more difficulties with memory than matched patients without ApoE-4. The performance of ApoE-4(+) carriers is poor regardless of severity of injury, whereas performance in ApoE-4(−) carriers worsens in parallel with more severe injury.[135] In patients with mild to moderate traumatic brain injury the ApoE-4 allele also affects short-term recovery.[136] The frequency of the ApoE-4 allele was also found to be increased in patients with prolonged post-traumatic unawareness who did not recover consciousness. In addition, ApoE-4 was associated with BAP deposition following head injury. CSF ApoE and BAP levels decrease after traumatic brain injury, whereas CSF S100B levels increase. There is also a correlation between injury severity and the decrease in BAP after brain injury.[137]

Parkinson's disease

The ApoE-4 allele does not function as a risk factor that influences the development of AD lesions in Parkinson's disease.[138] The ApoE-4 allele frequency in Parkinson's disease patients with dementia (0.068) and in those without dementia (0.13) does not greatly differ from controls (0.102), indicating that the biological basis of dementia in Parkinson's disease may differ from that of AD (254). In general, ApoE-4 was not associated with Parkinson's disease in the Caucasian population.[139] However, the age at onset of Parkinson's disease appears to be significantly earlier in ApoE-3/4 and ApoE-4/4 carriers than in patients with the ApoE-3/3 genotype.[140]

Prion disease

Initial studies did not find association between ApoE-4 and other amyloid-forming diseases, including Creutzfeldt–Jakob disease, familial amyloidotic polyneuropathy, and Down syndrome. Subsequent studies concluded that ApoE-4 might be a major susceptibility factor for Creutzfeldt–Jakob disease.[141]

Other diseases

Increased frequency of ApoE-4 has been found in patients with inclusion body myositis.[142] The probability of moderate to severe sleep-disordered breathing (apnea/hypopnea) was reported to be significantly higher in ApoE-4(+) carriers, independent of age, sex, body mass index, and ethnicity.[142] Patients with primary dystonia harboring the ApoE-4 genotype tend to have an earlier age at onset than ApoE-4(−) carriers.[143]

Copin *et al.* have reported that two ApoE-promoter SNP previously associated with AD also modified the primary open-angle glaucoma (POAG) genotype. ApoE(-219G) is associated with increased optic nerve damage,[144] and ApoE(-491T), interacting at a highly significant level with a SNP in the myoclin gene (MYOC) promoter (MYOC-1000G), is associated with increased intra-ocular pressure and with limited effectiveness of intra-ocular pressure-lowering treatments in patients with POAG. Some studies have speculated with an increased frequency of glaucoma in AD patients; however, the studies of Copin *et al.*[145] have been criticized by Bunce *et al.*,[146] and Ressiniotis *et al.*[147] reported that ApoE is not a risk factor for developing POAG, even in patients with normal

tension glaucoma. Other studies also indicate that the ApoE genotype does not constitute a risk factor for developing POAG.[147]

Although there is no apparent association of particular ApoE genotypes with macular degeneration,[148] the inheritance of specific ApoE alleles has been linked to the incidence of age-related macular degeneration (ARMD). The ApoE-4 allele appears to be protective, or at least, to delay the age at diagnosis of the disease, whereas the ApoE-2 allele appears to have a modifier effect by bringing forward the mean age of disease diagnosis.[149,150] ApoE is an intrinsic component of drusen, the hallmark of ARMD. Age-related alteration of lipoprotein biosynthesis and processing at the levels of the retinal pigment epithelium, where ApoE can be locally synthesized, and/or Bruch membrane might be a significant contributing factor in drusen formation and ARMD pathogenesis.[151] ApoE has also been implicated in pupil dilation, and a hypersensitive pupil dilation response to tropicamide was reported in cognitively normal individuals with the ApoE-4 allele.[152] Hypersensitivity responses of the pupil to the cholinergic agonist pilocarpine and the antagonist tropicamide have also been reported in AD,[153,154] but these findings could not always be replicated.[155]

Estrogen use was associated with less cognitive decline among 2716 women (>65 years) who did not have the ApoE-4 allele, but not among women who had at least one ApoE-4 allele,[156] probably indicating that ApoE-4(+) carriers under estrogen regimens may have a higher risk of cognitive deterioration.

The ApoE-4 allele frequency is not increased in familial non-insulin-dependent diabetes mellitus (NIDDM), despite the presence of ApoE in the pancreatic islet amyloid in NIDDM.[157] In China, Liu *et al.*[158] found that: (i) the heparan sulfate proteoglycan (HSPG) T allele is a risk factor for the development of severe diabetic nephropathy in type 2 diabetic patients; (ii) the ApoE-2 allele is a risk factor for the occurrence of type 2 diabetes mellitus in the Chinese general population; and (iii) the co-inheritance of HSPG-T/ApoE-2 confers a higher risk of type 2 diabetes mellitus progression to diabetic nephropathy in Chinese.[158] In the Japanese population, the ApoE-2 is a prognostic risk factor for both the onset and progression of diabetic nephropathy in type 2 diabetes.[159]

Herpes simplex virus type 1 (HSV1) is present in certain regions of the brain in a high proportion of elderly subjects and patients with AD. It has been reported by Itzhaki and co-workers that the combination of HSV1 in the brain, and carriage of the ApoE-4 allele, was a strong risk factor for AD.[160–163] Corder *et al.* also showed that HIV-infected subjects with the ApoE-4 allele have excess dementia and peripheral neuropathy, postulating that long-term survivors of HIV infection with ApoE-4 may be at high risk for dementia and that gene–viral interaction may speed AD pathogenesis.[164] Tursen *et al.* recently reported that the presence of ApoE-2/3, high-density lipoprotein (HDL)-cholesterol levels and the absence of the ApoE-3/3 genotype can be regarded as risk factors for superficial fungal disease, especially dermatophytosis.[165]

Cardiopulmonary bypass induces a rise in cytokine release by activated monocytes. ApoE-4 and TNFB polymorphisms (TNFB-A329G) are risk factors for atherosclerosis. The presence of TNFB*A329G and ApoE-4 is associated with significantly higher releases of IL8 and TNFA, prolonged intubation, and increased transfusion in patients undergoing coronary artery bypass grafting, relative to patients without genetic variants.[166]

The ApoE-2 allele seems to be associated with the lowest reproductive efficiency and the ApoE-3 with the highest. The different total cholesterol levels associated with ApoE genotypes could have an effect on steroidogenesis and as a consequence determine the observed differential fertility.[167]

Exercise

Physical activity improves lipid levels by altering triglyceride metabolism, and ApoE facilitates triglyceride clearance by mediating lipoprotein binding to hepatic receptors. Thompson *et al.* studied the influence of ApoE variants on lipid and physiological response to exercise training in the USA.[168] This prospective study demonstrates that the serum lipid response to exercise training differs by ApoE genotype in a pattern consistent with known metabolic differences among the variants. TG were slightly higher in ApoE-2/3, whereas LDL-cholesterol was lower. TG decreased by 11% with training for the entire cohort, and 7%, 12%, and 14% for ApoE-2/3, ApoE-3/3 and ApoE-3/4, respectively. LDL-cholesterol did not change in the cohort, but decreased slightly in ApoE-2/3 and ApoE-3/3 subjects, and increased 4% in the ApoE-3/4 group. Total cholesterol/HDL and LDL/HDL decreased with training in ApoE-2/3 and ApoE-3/3, but increased in

ApoE-3/4. The ApoE genotype also affected the increase in aerobic capacity produced by exercise training possibly via undefined effects on nerve and skeletal muscle function.[168] In another study, no association was found between ApoE and maximal oxygen uptake levels either in the sedentary state or in response to exercise training.[169] In summary, all these studies globally indicate that ApoE-related polymorphic variants, especially the ApoE-4 allele, represent a biological disadvantage for brain function and lipid metabolism.

GENOTYPE-PHENOTYPE CORRELATIONS

Different ApoE genotypes confer specific phenotypic profiles to AD patients. Some of these profiles may add risk or benefit when the patients are treated with conventional drugs, and in many instances the clinical phenotype demands the administration of additional drugs, which increase the complexity of therapeutic protocols. From studies designed to define ApoE-related AD phenotypes,[29,37,72,170–175] several confirmed conclusions can be drawn: (i) the age-at-onset is 5–10 years earlier in approximately 80% of AD cases harboring the ApoE-4/4 genotype; (ii) the serum levels of ApoE are lowest in ApoE-4/4, intermediate in ApoE-3/3 and ApoE-3/4, and highest in ApoE-2/3 and ApoE-2/4; (iii) serum cholesterol levels are higher in ApoE-4/4 than in the other genotypes; (iv) HDL-cholesterol levels tend to be lower in ApoE-3 homozygotes than in ApoE-4 allele carriers; (v) LDL-cholesterol levels are systematically higher in ApoE-4/4 than in any other genotype; (vi) triglyceride levels are significantly lower in ApoE-4/4; (vii) nitric oxide levels are slightly lower in ApoE-4/4; (viii) serum Aβ levels do not differ between ApoE-4/4 and the other most frequent genotypes (ApoE-3/3, ApoE-3/4); (ix) blood histamine levels are dramatically reduced in ApoE-4/4 as compared with the other genotypes; (x) brain atrophy is markedly increased in ApoE-4/4>ApoE-3/4>ApoE-3/3; (xi) brain mapping activity shows a significant increase in slow wave activity in ApoE-4/4 from early stages of the disease; (xii) brain hemodynamics, as reflected by reduced brain blood flow velocity and increased pulsatility and resistance indices, is significantly worse in ApoE-4/4 (and in ApoE-4 carriers, in general, as compared with ApoE-3 carriers); (xiii) lymphocyte apoptosis is markedly enhanced in ApoE-4 carriers; (xiv) cognitive deterioration is faster in ApoE-4/4 patients than in carriers of any other ApoE genotype; (xv) occasionally, in approximately 3–8% of the AD cases, the presence of some dementia-related metabolic dysfunctions (e.g. iron, folic acid, vitamin B_{12} deficiencies) accumulate more in ApoE-4 carriers than in ApoE-3 carriers; (xvi) some behavioral disturbances (bizarre behaviors, psychotic symptoms), alterations in circadian rhythm patterns (e.g. sleep disorders), and mood disorders (anxiety, depression) are slightly more frequent in ApoE-4 carriers; (xvii) aortic and systemic atherosclerosis is also more frequent in ApoE-4 carriers; (xviii) liver metabolism and transaminase activity also differ in ApoE-4/4 with respect to other genotypes; (xix) blood pressure (hypertension) and other cardiovascular risk factors also accumulate in ApoE-4; and (xx) ApoE-4/4 carriers are the poorest responders to conventional drugs. These 20 major phenotypic features clearly illustrate the biological disadvantage of ApoE-4 homozygotes and the potential consequences that these patients may experience when they receive pharmacological treatment.[170–176]

CONCLUSION

AD is a multifactorial and complex disorder in which over 150 different genes distributed across the human genome may be involved. Among AD-causing genes, APP, PS1, and PS2 mutations in part explain AD pathogenesis, however Mendelian mutations in those three genes only account for less than 10% of AD cases, indicating that many other networking mechanisms must be involved in neurodegeneration and premature neuronal death in AD. ApoE-related polymorphic variants (ApoE-4 allele) represent the most significant susceptibility genetic defect in AD, contributing to neuronal dysfunction in approximately 30–40% of AD cases. The precise mechanism by which ApoE affects neurodegeneration is still unclear. ApoE-4 is a genetic risk factor of cognitive impairment in many neurodegenerative disorders, including AD and other types of dementia.

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RESEARCH ARTICLE

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The Impact of a Genome-Wide Supported Psychosis Variant in the *ZNF804A* Gene on Memory Function in Schizophrenia

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A recent genome-wide association study showed that a variant (rs1344706) in the *ZNF804A* gene was associated with schizophrenia and bipolar disorder. Replication studies supported the evidence for association between this variant in the *ZNF804A* gene and schizophrenia and that this variant is the most likely susceptibility variant. Subsequent functional magnetic resonance imaging studies in healthy subjects demonstrated the association of the high-risk *ZNF804A* variant with neural activation during a memory task and a theory of mind task. As these cognitive performances are disturbed in patients with schizophrenia, this gene may play a role in cognitive dysfunction in schizophrenia. The aim of the current study was to investigate the potential relationship between this *ZNF804A* polymorphism and memory function. The effects of the high-risk *ZNF804A* genotype, diagnosis, and genotype–diagnosis interaction on verbal memory, visual memory (VisM), attention/concentration, and delayed recall (measured by the Wechsler Memory Scale-Revised) were analyzed by two-way analysis of covariance in 113 patients with schizophrenia and 184 healthy subjects. Consistent with previous studies, patients with schizophrenia exhibited poorer performance on all indices as compared to healthy control subjects ($P < 0.001$). A significant *ZNF804A* genotype–diagnosis interaction was found for VisM performance ($P = 0.0012$). Patients with the high-risk T/T genotype scored significantly lower on VisM than G carriers did ($P = 0.018$). In contrast, there was no genotype effect for any index in the healthy control subjects ($P > 0.05$). Our data suggest that rs1344706 may be related to memory dysfunction in schizophrenia. © 2010 Wiley-Liss, Inc.

Key words: ZNF804A; memory; schizophrenia; polymorphism; rs1344706

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NTRODUCTION

Schizophrenia (OMIM: 181500) is a common complex psychiatric lisease with a lifetime risk of approximately 1%. There are strong genetic components of this disease, with an estimated heritability of pproximately 80% [Cardno and Gottesman, 2000; Tsuang, 2000]. n a genome-wide association study and follow-up studies, a single ucleotide polymorphism (SNP) in the *ZNF804A* gene (rs1344706) vas found to be associated with schizophrenia and bipolar disorder O'Donovan et al., 2008]. Subsequent replication studies demon-trated the association between schizophrenia and the *ZNF804A* ene and that rs1344706 remained the most strongly associated narker in the gene after fine mapping of *ZNF804* locus [Riley et al., 010; Steinberg et al., 2010; Williams et al., 2010; Zhang et al., 2010].

The *ZNF804A* gene (OMIM: 612282) is located on chromosome q32.1 and consists of four exons and three introns spanning 41 kb. Although little is known about the encoded protein and ts function, the sequence contains predicted zinc ion and DNA-inding domains, suggesting a role in the regulation of gene xpression. Two imaging genetics studies using functional nagnetic resonance imaging (fMRI) have demonstrated associa-ions between the high-risk *ZNF804A* variant and neural activation luring a memory task and a theory of mind task in healthy subjects Esslinger et al., 2009; Walter et al., 2010]. The high-risk *ZNF804A* ariant had impact on brain functional dysconnectivity between lorsolateral prefrontal cortex (DLPFC) and hippocampal forma-ion during an N-back memory task in healthy subjects [Esslinger t al., 2009]. This altered connectivity between DLPFC and hippo-ampal formation might be a basis of human memory function.

Patients with schizophrenia have pronounced deficits in aspects f neurocognitive function such as speed of processing, attention/ igilance, working memory, verbal learning and memory, visual earning and memory, reasoning and problem solving, and social ognition [Nuechterlein et al., 2004]. Cognitive impairments are trongly related to functioning in areas such as work, social relation-hips, and independent living in schizophrenia. The lack of marked ognitive benefit of present antipsychotics has led to the investiga-ion of alternative drugs and mechanisms for the treatment of these mpairments [Buchanan et al., 2007]. Intermediate phenotypes/ ndophenotypes represent simpler clues to genetic underpinnings han the disease syndrome itself, promoting the view that psychi-tric diagnoses can be decomposed or deconstructed, which can esult in more straightforward and successful genetic analysis Gottesman and Gould, 2003; Preston and Weinberger, 2005]. Memory deficits are prominent trait markers of schizophrenia, vith impairments also observed in first-degree relatives [Snitz et al., 006]. Genetic risk for schizophrenia could affect functional activi-y in the brain; such changes have been shown to mediate disturbed nemory function [Meyer-Lindenberg and Weinberger, 2006]. In he present study, we examined the effect of the genome-wide upported variant in the *ZNF804A* gene on memory functions in atients with schizophrenia.

MATERIALS AND METHODS

Sample Description

The subjects of this study consisted of 113 patients with chizophrenia [53.1% males, mean age ± standard deviation: 38.3 ± 12.1 years] and 184 healthy control subjects [47.8% males, 36.2 ± 11.5 years]. The sex ratio and mean age did not differ significantly between patients and control subjects ($P > 0.05$), whereas the years of education were significantly lower among patients with schizophrenia (14.2 ± 2.4) than among control subjects (15.4 ± 2.4) [$z = -4.20$, $P < 0.001$]. All subjects were biologically unrelated Japanese individuals. Subjects were excluded from this study if they had neurological or medical conditions that could affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, cancer in an active stage, cerebrovascular disease, epilepsy, seizures, substance-related disorders, or mental retardation. Cases were both outpatients and inpatients at Osaka University Hospital. Each patient with schizophrenia had been diagnosed by a trained psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria based on the Structured Clinical Interview for DSM-IV (SCID) for schizophrenia. Healthy control subjects were recruited through local advertisements at Osaka University. Psychiatrically, medically, and neurologically healthy control subjects were evaluated using the DSM-IV-Non-Patient version of the Structured Clinical Interview to exclude individuals who had current or past contact with psychiatric services or had received psychiatric medication [Ohi et al., 2009]. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University.

Genotyping

We selected rs1344706 in the *ZNF804A* gene because this SNP has been found to be associated with schizophrenia and bipolar disorder in genome-wide association and follow-up studies [O'Donovan et al., 2008] and the four replication studies confirmed the association [Riley et al., 2010; Steinberg et al., 2010; Williams et al., 2010; Zhang et al., 2010]. Furthermore, this SNP was related to functional brain activity in healthy subjects [Esslinger et al., 2009; Walter et al., 2010]. Venous blood was collected from the subjects, and genomic DNA was extracted from whole blood according to standard procedures. The SNP was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, CA) as described previously [Hashimoto et al., 2006, 2007]. Detailed information on the PCR conditions is available upon request.

Phenotype Measures

A full version of the Wechsler Memory Scale-Revised (WMS-R) [Sugishita, 2001], which is generally used to measure memory functions, was administered to the subjects. The four indices of the WMS-R, that is, verbal memory (VerM), visual memory (VisM), attention/concentration (AC), and delayed recall (DR), were used for the analysis. Psychiatric symptoms in patients with schizophrenia were evaluated using the positive and negative syndrome scale (PANSS) [Kay et al., 1987].

TABLE I. Demographic and Clinical Characteristics of Patients with Schizophrenia and Controls

	Schizophrenia (n = 113)						Control (n = 184)					
	T/T (n = 21)		G carrier (n = 92)				T/T (n = 44)		G carrier (n = 140)			
Variables	**Mean**	**SD**	**Mean**	**SD**	***P*-value**	**z**	**Mean**	**SD**	**Mean**	**SD**	***P*-value**	**z**
Age (years)	38.1	11.2	38.4	12.4	0.99	0.01	36.5	10.8	36.1	11.8	0.68	0.42
Sex (male/female)[a]	10/11		49/43		0.94	0.01	24/20		64/76		0.31	1.05
Education (years)	14.2	2.2	14.2	2.4	0.80	0.25	14.7	1.9	15.6	2.5	**0.05**	1.99
CPZeq (mg/day)	586.2	518.6	535.9	443.1	0.95	0.06	—	—	—	—	—	—
Age at onset (years)	23.7	10.2	24.2	8.6	0.76	0.31	—	—	—	—	—	—
Duration of illness (years)	14.4	9.7	14.2	11.1	0.74	0.33	—	—	—	—	—	—
Positive symptoms[b,c]	16.0	7.9	18.2	5.5	0.10	1.62	—	—	—	—	—	—
Negative symptoms[b,c]	18.6	7.5	18.6	7.0	0.89	0.14	—	—	—	—	—	—

CPZeq, chlorpromazine equivalents of total antipsychotics; [b]PANSS, positive and negative syndrome scale; SD, standard deviation. T/T: individuals with T/T genotype of rs1344706. G carriers: individuals with G/G and G/T genotypes of rs1344706. Differences in clinical characteristics between genotype groups were analyzed using the Mann–Whitney *U*-test, except for [a]χ^2 test. [c]T/T: n = 18; G carrier: n = 84. A significant *P*-value is shown as bold face and underlined.

Statistical Analyses

Statistical analyses were performed using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan) and PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and control subjects as well as between genotype groups were analyzed using χ^2 tests for categorical variables and the Mann–Whitney *U*-test for continuous variables. The presence of Hardy–Weinberg equilibrium was examined using the χ^2 test for goodness of fit. No deviation from Hardy–Weinberg equilibrium was detected in cases or in controls ($P > 0.05$). To examine the effect of ZNF804A rs1344706 genotype on memory function, the effects of *ZNF804A* genotype, diagnosis, and genotype–diagnosis interactions on four memory domains were analyzed by a two-way analysis of variance (ANOVA). In further analysis to control for confounding factors, the genotype effects, diagnosis effects, and genotype–diagnosis interactions on the memory functions were adjusted by a two-way analysis of covariance (ANCOVA) with sex and years of education as covariates (the scores of indices were previously corrected by age). When genotype–diagnosis interaction was found, cases and controls were separately analyzed by ANOVA and ANCOVA. The Bonferroni correction was applied for multiple testing on four indices of the WMS-R to avoid type I error. Standardized effect sizes were calculated using Cohen's *d* method (http:www.uccs.edu/faculty/lbecker). The significance level for statistical tests was set at two-tailed $P < 0.05$.

RESULTS

The Effect of the High-Risk *ZNF804A* Polymorphism on Memory Functions

We examined potential associations between the *ZNF804A* genotype and memory functions in patients with schizophrenia and healthy controls. There was no difference in age, sex, chlorpromazine equivalents of total antipsychotics, age at onset, duration of illness, or PANSS scores between genotype groups. The only difference in demographic variables was a significantly greater number of years of education in the control groups ($z = 1.99$, $P = 0.05$; Table I). The *ZNF804A* genotype effects, diagnosis effects, and genotype–diagnosis interactions on memory functions are shown in Table II. We found significant effects of diagnosis (VerM: $F_{1,293} = 146.91$, $P < 0.001$; adjusted $F_{1,291} = 133.70$, $P < 0.001$, VisM: $F_{1,293} = 114.30$, $P < 0.001$; adjusted $F_{1,291} = 103.87$, $P < 0.001$, AC: $F_{1,293} = 53.46$, $P < 0.001$; adjusted $F_{1,291} = 48.59$; $P < 0.001$, DR: $F_{1,293} = 200.36$, $P < 0.001$; adjusted $F_{1,291} = 186.09$, $P < 0.001$) and genotype–diagnosis interaction (VisM: $F_{1,293} = 8.21$, $P = 0.0045$, adjusted $F_{1,291} = 10.76$, $P = 0.0012$). Significant genotype effects were only found for VisM ($F_{1,293} = 4.46$, $P = 0.036$, adjusted $F_{1,291} = 3.40$, $P = 0.066$). The effect of diagnosis and the diagnosis–genotype interaction remained positive after correction for multiple tests (corrected *P*-values, VerM: $P < 0.001$, VisM: $P < 0.001$, AC: $P < 0.001$, DR: $P < 0.001$, interaction in VisM: $P = 0.0048$), whereas the genotype effect on VisM did not remain after correction for multiple tests ($P > 0.14$). Patients with schizophrenia displayed lower scores on all memory indices than did controls, and the effect sizes of VerM, VisM, AC, and DR were −1.72, −1.21, −1.17, and −1.89, respectively. As a genotype– diagnosis interaction was found for VisM, we separately analyzed the effects of genotype on VisM in patients and controls (Fig. 1). There was a significant genotype effect in patients with schizophrenia ($F_{1,111} = 5.05$, $P = 0.027$; adjusted $F_{1,109} = 5.82$, $P = 0.018$), whereas there was no genotype effect in controls ($F_{1,182} = 0.88$, $P = 0.35$; adjusted $F_{1,180} = 1.43$, $P = 0.23$). The patients with the high-risk T/T genotype scored significantly lower on VisM than did those who carry a G genotype (effect size: −0.56).

When the two genotypes were divided into three genotype groups (patients with T/T genotype, T/G genotype, and G/G genotype), the patients with the high-risk T/T genotype scored significantly lower on VisM than patients with the T/G genotype (adjusted $F_{1,68} = 8.59$, $P = 0.0046$) and marginally lower than patients with the G/G genotype (adjusted $F_{1,58} = 2.89$, $P = 0.09$;

TABLE II. Effects of the *ZNF804A* Genotype on Memory Function Determined Using WMS-R

	Schizophrenia (n = 113)				Control (n = 184)				ANOVA						ANCOVA (adjusted)					
	T/T (n = 21)		G carrier (n = 92)		T/T (n = 44)		G carrier (n = 140)		Diagnosis effect		Genotype effect		Interaction		Diagnosis effect		Genotype effect		Interaction	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*-value	$F_{1,293}$	*P*-value	$F_{1,293}$	*P*-value	$F_{1,293}$	*P*-value	$F_{1,291}$	*P*-value	$F_{1,291}$	*P*-value	$F_{1,291}$
VerM	82.6	14.6	84.6	18.3	110.2	14.1	111.3	13.0	**$<10^{-3}$**	**146.9**	0.50	0.45	0.83	0.04	**$<10^{-3}$**	**133.7**	0.78	0.08	0.47	0.52
VisM	81.7	18.2	92.3	19.7	110.5	8.3	108.9	10.3	**$<10^{-3}$**	**114.3**	**0.036**	**4.46**	**0.0045**	**8.21**	**$<10^{-3}$**	**103.9**	0.066	3.40	**0.0012**	**10.8**
AC	92.0	16.5	90.9	15.2	105.1	13.6	109.2	13.9	**$<10^{-3}$**	**53.5**	0.48	0.50	0.23	1.44	**$<10^{-3}$**	**48.6**	0.54	0.37	0.28	1.17
DR	77.1	18.4	82.6	19.5	111.7	12.7	112.0	11.8	**$<10^{-3}$**	**200.4**	0.20	1.65	0.25	1.31	**$<10^{-3}$**	**186.1**	0.35	0.88	0.10	2.71

WMS-R, Wechsler Memory Scale-Revised; VerM, verbal memory; VisM, visual memory; AC, attention/concentration; DR, delayed recall; SD, standard deviation. *T/T*: individuals with T/T genotype of rs1344706. G carriers: individuals with G/G or G/T genotype of rs1344706. The effects of the ZNF804A genotype and the effects of diagnosis on the memory function were analyzed by a two-way analysis of variance (ANOVA). Adjusted effects of genotype were analyzed by a two-way analysis of covariance (ANCOVA) with sex and years of education as covariates. Significant *P*-values are shown as bold face and underlined.

FIG. 1. The association between the high-risk *ZNF804A* genotype and visual memory in patients with schizophrenia. X-axis: gray bars, individuals with T/T genotype of rs1344706; white bars, individuals with a G allele (G/T and G/G genotypes) of rs1344706. Y-axis: scores of visual memory from the WMS-R. Error bars represent standard errors of the mean. *$P < 0.05$, compared with patients with a G allele.

Table III). However, there was no significant difference in scores between patients with the T/G genotype and G/G genotype ($F_{1,88} = 1.39$, $P = 0.24$).

DISCUSSION

In the present study, we first demonstrated an association between the high-risk *ZNF804A* SNP and memory performance in patients with schizophrenia. We provided evidence that patients with the high-risk T/T genotype had lower performance on VisM than patients who carry a G allele. The effect size of the difference in VisM scores between patients with the T/T genotype and G carriers was −0.56; this effect is typically considered a medium-sized effect. We do not know why we found the genotype effect on only VisM. A possible explanation is that a previous study reported suggestive linkage evidence for the VisM on 2q36 near the locus of the *ZNF804A* gene [Paunio et al., 2004]. Another possibility is that this SNP is associated with connectivity during N-back memory task, which is an fMRI task using visual cue [Esslinger et al., 2009]. This study showed no effect of genotype on a memory task in healthy subjects, which is consistent with our data [Esslinger et al., 2009].

A linear genotype effect on connectivity in DLPFC and hippocampal formation during a memory task was found in healthy control subjects in an fMRI study [Esslinger et al., 2009]. These data

TABLE III. Effects of the *ZNF804A* Genotype on Memory Performance

	Schizophrenia (n = 113)						Control (n = 184)						ANCOVA (adjusted)					
	T/T (n = 21)		T/G (n = 51)		G/G (n = 41)		T/T (n = 44)		T/G (n = 85)		G/G (n = 55)		Diagnosis effect		Genotype effect		Interaction	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*-value	$F_{2,289}$	*P*-value	$F_{2,289}$	*P*-value	$F_{2,289}$
VerM	82.6	14.6	83.9	19.1	85.5	17.3	110.2	14.1	112.5	12.8	109.4	13.2	$<\underline{10^{-3}}$	**168.5**	0.61	0.49	0.52	0.66
VisM	81.7	18.2	93.2	20.2	91.1	19.3	110.5	8.3	108.4	10.0	109.7	10.9	$<\underline{10^{-3}}$	**111.6**	0.15	1.94	**0.0028**	**5.99**
AC	92.0	16.5	89.9	14.6	92.1	15.9	105.1	13.6	109.8	14.8	108.2	12.4	$<\underline{10^{-3}}$	**70.7**	0.84	0.18	0.45	0.81
DR	77.1	18.4	83.1	20.8	82.0	18.1	111.7	12.7	113.2	11.6	110.2	12.0	$<\underline{10^{-3}}$	**227.5**	0.18	1.71	0.23	1.47

WMS-R, Wechsler Memory Scale-Revised; VerM, verbal memory; VisM, visual memory; AC, attention/concentration: DR, delayed recall; SD, standard deviation. T/T, T/G, G/G: individuals with three genotypes of rs1344706. Adjusted effects of three genotypes were analyzed by a two-way analysis of covariance (ANCOVA) with sex and years of education as covariates. Significant *P*-values are shown as bold face and underlined.

might indicate that quantitative traits (i.e., brain physiological activity measured by fMRI) are closer to the genetic substrate than behavioral traits, such as neuropsychological functions and psychiatric disorders, and should be observable in genetically at-risk but behaviorally unaffected individuals [Meyer-Lindenberg and Weinberger, 2006]. Such physiological quantitative traits are likely to influence a neuropsychological trait, memory performance, in patients with schizophrenia, however, they might not affect memory performance in healthy subjects. This phenomena suggests that the high-risk SNP in the *ZNF804A* gene might be related to the neuropsychological disturbance in schizophrenia.

There were several limitations to this study. Although the sample was moderate in size, it might not be representative of the schizophrenic population. A false-positive association cannot be excluded as a possibility in our study, despite the precautions of ethnic matching and correction for multiple testing. The effects of the ZNF804A gene on VisM could be an epiphenomenon of the severity of the disease and/or medication. In conclusion, we found an effect of the high-risk *ZNF804A* SNP on VisM in schizophrenia. Further research will be required to clarify the role of the high-risk *ZNF804A* SNP in the pathophysiology of schizophrenia.

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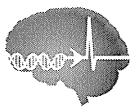

Molecular Brain

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NMDAR2B tyrosine phosphorylation regulates anxiety-like behavior and CRF expression in the amygdala

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Abstract

Background: Anxiety disorders are a highly prevalent and disabling class of psychiatric disorders. There is growing evidence implicating the glutamate system in the pathophysiology and treatment of anxiety disorders, though the molecular mechanism by which the glutamate system regulates anxiety-like behavior remains unclear.

Results: In this study, we provide evidence suggesting that tyrosine phosphorylation of the NMDA receptor, an ionotropic glutamate receptor, contributes to anxiety-like behavior. The GluN2B subunit of the NMDA receptor is tyrosine-phosphorylated: Tyr-1472 is the major phosphorylation site. Homozygous knock-in mice that express a Tyr-1472-Phe mutant of GluN2B, which prevents phosphorylation of this site, show enhanced anxiety-like behavior in the elevated plus-maze test. Expression of corticotropin-releasing factor (CRF), which is important for the regulation of anxiety-like behavior, is increased in the amygdala of the knock-in mice. Furthermore, injection of CRF receptor antagonist attenuated the enhanced anxiety-like behavior of the knock-in mice. We also show that elevated plus-maze exposure simultaneously induced de-phosphorylation of Tyr-1472 and increased CRF expression.

Conclusions: These data suggest that Tyr-1472 phosphorylation on GluN2B is important for anxiety-like behavior by negative regulation of CRF expression in the amygdala.

Background

Anxiety is commonly experienced and typically adaptive; however, excessive and dysfunctional anxiety leads to serious disorders. Anxiety disorders are the most prevalent class of psychiatric disorders in many countries [1]. Compounds that target of γ-aminobutyric acid and the serotonergic systems have received great attention within the development of treatments for anxiety disorders [2]. As some forms of anxiety are relatively resistant to treatment with these compounds, which include benzodiazepines and selective serotonin reuptake inhibitors, it has become increasingly apparent that alternative treatment strategies are needed. Recently, the glutamatergic system, the major mediator of excitatory synaptic transmission in the mammalian brain, has been the focus of pathophysiological studies of human anxiety disorders [3]. In rodents, *N*-methyl-D-aspartate (NMDA) receptor antagonists show anxiolytic effects in several test scenarios including the elevated plus-maze test [4,5]. While these reports point to the involvement of NMDA receptor-mediated signaling in the regulation of anxiety-like behaviors, molecular dissection of the role of NMDA receptor-mediated signaling is difficult because glutamate exerts its effects on various neural functions in a highly complex manner [6].

The NMDA receptor is crucial for neural development, synaptic plasticity, neuronal excitotoxicity, and behavior [6-9]. The NMDA receptor is composed of the GluN1 and GluN2 subunits: the GluN1 subunit is essential for the function of NMDAR channels, whereas the GluN2 subunits (GluN2A, GluN2B, GluN2C, and GluN2D) determine the characteristics of NMDAR channels by forming different heteromeric configurations with the GluN1 subunit [6]. The function of NMDA receptor-mediated signaling is in part regulated by Src tyrosine kinase-mediated phosphorylation of the

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