Table 5
Relationship of categorical variables with delayed recall and finger movement

Categories (n)	Delayed recall				Finger movement			
	Mean	S.D.	t^*	P	Mean	S.D.	t^*	P
Male (16)	70.3	18.8	0.4	0.719	13.5	6.2	0.3	0.735
Female (11)	67.6	18.8			12.5	8.7		
Outpatient (12)	70.0	17.3	0.2	0.849	17.0	6.9	2.6	0.014
Inpatient (15)	68.6	19.9			9.9	7.0		
Familial history + (7)	61.6	15.5	1.3	0.209	14.6	6.6	−0.6	0.559
Familial history − (20)	71.9	19.0			12.6	8.1		

* t-test for independent groups; $df = 2$.

of the originally grouped subjects (Wilks' $\lambda = 0.28$, $P = 4.9 \times 10^{-19}$). When the correlation between the delayed recall and finger movement tests was examined within the patient group, there was no correlation (Pearson's $r = -0.001$, $P = 1.0$).

3.5. Relationship of demographic and clinical variables with delayed recall and finger movement

The correlations of the demographic and clinical variables (continuous variables) with the delayed recall and finger movement tests are shown in Table 4. While the educational level was significantly correlated with the performance in the delayed recall test ($r = 0.51$, $P = 0.01$), there was no correlation between years of education and performance on the finger movement test. No significant correlation was observed for the remaining variables.

Differences in performance in the delayed recall and finger movement tests between the groups classified by categorical variables (sex, family history, and outpatient or inpatient status) are shown in Table 5. We found a significant difference in finger movement between outpatients and inpatients (t-test, $P = 0.014$). The scores of outpatients were significantly higher than those of inpatients in the finger movement test, although this difference was not observed for the delayed recall test. In regard to sex or family history, there was no significant difference in the scores in either test.

Table 6
Correlation of motor and cognitive functions with delayed recall and finger movement

	Delayed recall		Finger movement	
	r	P	r	P
Pegboard	−0.27	0.18	−0.51	0.01
Normal drawing	−0.44	0.02	−0.32	0.10
Finger movement	−0.00	1.00	–	–
General memory (WMS-R)	0.88	0.00	0.73	0.72
Attention and concentration (WMS-R)	0.45	0.02	0.20	0.32
Delayed recall (WMS-R)	–	–	−0.00	1.00
Full scale IQ (WAIS-R)	0.65	0.00	0.21	0.30
Completed categories (WCST)	0.34	0.12	0.34	0.11

r = Pearson's product-moment correlation coefficient.

3.6. Relationship of motor and cognitive functions with delayed recall and finger movement

The correlations of the motor and cognitive functions with the delayed recall and finger movement tests are shown in Table 6. Finger movement was correlated with pegboard only, while delayed recall was correlated with many of the variables: normal drawing, WMS-R indexes, and full scale IQ.

4. Discussion

By using a series of tests, we confirmed that both motor and cognitive functions are profoundly impaired in patients with chronic schizophrenia. The scores of all of the motor (pegboard, mirror drawing, normal drawing, and finger movement) and cognitive (WAIS-R and WMS-R) tests were significantly poorer in the schizophrenic patients than in the healthy control subjects even when sex, age, and years of education were controlled for. A discriminant analysis revealed that the functions that most successfully distinguished patients and controls were delayed recall and finger movement among the tests. Because the finger movement test that we developed is independent of motor speed, our results suggest that motor dexterity is intrinsically impaired in chronic schizophrenia. Furthermore, the score of the finger movement test did not correlate with that of the delayed recall, suggesting that these two functions are dimensionally different.

In accordance with our results, schizophrenia is characterized by a substantial deficit in memory functions (Saykin et al., 1991, 1994; Censits et al., 1997). Among the WMS-R subscales, the most impaired function in our patients was delayed recall (index score of 69.2),

followed by visual memory (73.4), verbal memory (76.6), and attention and concentration (83.7). This order is not consistent with previous studies (Wechsler, 1987; Randolph et al., 1994; Hawkins et al., 1997). For example, Gold et al. (1992) studied 45 schizophrenic patients and reported that the scores on the WMS-R subscales scores were similarly impaired (attention and concentration: 80.0; verbal memory: 81.8; visual memory: 81.5; delayed recall: 81.6). The inconsistency between previous studies and the present study may be attributable to the ethnic difference or relatively older age of our subjects (mean age: 44 years). With respect to the WCST, the difference between patients and controls just failed to reach statistical significance ($P=0.051$), although many earlier studies have shown that deficits on the WCST are prominent in schizophrenia (e.g. Franke et al., 1992; Scarone et al., 1993). The failure to reach statistical significance may be attributable to the small sample and relatively large inter-individual differences (i.e., a wide range of standard deviations) in the test scores.

In addition to memory functions, motor functions can be another factor to distinguish schizophrenic patients from healthy controls. The finger movement test we developed was found to have a highly discriminative power between patients and controls; this power might be even higher than that of the conventional motor tests, such as the pegboard test. Motor dysfunction in schizophrenia has been noted since the time of Kraepelin (Kraepelin, 1919; King, 1958; Weaver and Brooks, 1964; Heinrichs and Zakzanis, 1998). Our present results add further evidence for this. The majority of previous studies employed the finger tapping and pegboard tests (Heinrichs and Zakzanis, 1998). Scores on the latter test are, however, influenced by not only dexterity but also motor speed (Rosofsky et al., 1982). Therefore, we developed the finger movement test to evaluate motor dexterity alone. There was a highly significant correlation between the finger movement and pegboard tests, confirming the validity of the newly developed finger movement test as a tool to measure motor dexterity. Our observation that the finger movement test had a highly discriminative power between patients and controls suggests that impairment in motor dexterity *per se* might be a major characteristic of chronic schizophrenia.

However, it could be argued that impaired motor dexterity might have been due to undesirable side effects of the antipsychotic drugs (i.e., extrapyramidal symptoms). Finger rigidity, for example, may influence finger movement test results. Though we found no significant correlation between daily dose of antipsychotic drugs and performance in the finger movement test, there have been conflicting reports concerning the effect of antipsychotic medication. Some reports showed that motor deficit was independent of antipsychotics (Saykin et al., 1994; Tigges et al., 2000), but others showed that motor deficit in medicated patients was much worse than in drug-naïve patients (Putzhammer et al., 2004, 2005). One plausible explanation was that drug-naïve patients suffer from a primary motor deficit and antipsychotic medication (especially typical antipsychotic medication) worsens this primary deficit (Putzhammer and Klein, 2006). In sum, however, the, poor performance observed in the finger movement test is unlikely to be attributed solely to side effects of antipsychotic medication.

With respect to the mirror drawing task, more than half of the patients did not complete it. So we excluded the results on this task from further analysis; however, the observation that so many patients could not complete the task may suggest a major difficulty with visuo-motor coordination in schizophrenia.

There is some evidence that deficits in motor function or dexterity have a developmental origin and manifest before the onset of schizophrenia. Cannon et al. (1999) examined various activities of school age in individuals who later developed schizophrenia, and found that pre-schizophrenic subjects performed significantly worse than control subjects only in nonacademic areas such as sports and handicrafts. Gschwandtner et al. (2005) reported that poorer fine motor functions as well as cognitive functions during childhood were crucial risk factors for later development of schizophrenia. Such deficits in motor functions may be related to genetic factors. Sautter et al. (1997) reported that familial patients were much worse than non-familial patients in the domains of motor and frontal lobe function. In the present study, however, we obtained no evidence supporting this possibility.

In the present study, the inpatients performed more poorly on the finger movement test than the outpatients. In line with this, Weaver and Brooks (1964) reported that scores in motor function tests were highly associated with the probability of discharge from the hospital. Lehoux et al. (2003) studied the relationship of several cognitive and motor tests with social functioning and revealed that the best fitting multivariate model to explain social functioning included fine motor dexterity and executive functioning. Sota and Heinrichs (2004) showed that motor dexterity was one of the crucial cognitive factors which predicted overall quality of life 3 years after initial measurement. These observations may suggest motor dexterity is related to functional outcome.

Several limitations are present in this study. First, the sample size was not very large. There is a possibility that some valid discriminators between patients and controls

have been missed due to the small sample size (type II error). Second, the mean IQ and memory indices of our controls were relatively high (around 110); therefore, it is possible that motor functions might also be relatively higher in our controls than in the general population. Thus, the differences in motor functions between patients and controls may have been overestimated in our study. Third, we did not administer a test for motor speed alone; the possibility remains that motor speed may be an important factor in discriminating patients and controls. Indeed, deficits in motor speed have been reported in both patients with schizophrenia and individuals at risk for the disorder (Niendam et al., 2006). Fourth, scoring of the finger movement test was performed by a single examiner without monitoring by a second examiner or objective instrument; thus some scoring errors might have occurred. Furthermore, strength of the fixed finger might have had some influence on the finger movement test score; controlling for such strength should have been done in the test.

In conclusion, we demonstrated profound impairment in motor dexterity as well as cognitive impairment in chronic schizophrenia using a newly developed motor test, the finger movement test. Impaired motor dexterity is a major characteristic of schizophrenia, which is relatively independent of cognitive functions.

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References

Bilder, R.M., Goldman, R.S., Robinson, D., Reiter, G., Bell, L., Bates, J.A., Pappadopulos, E., Willson, D.F., Alvir, J.M., Woerner, M.G., Geisler, S., Kane, J.M., Lieberman, J.A., 2000. Neuropsychology of first-episode schizophrenia: initial characterization and clinical correlates. American Journal of Psychiatry 157, 549–559.

Cannon, M., Jones, P., Huttunen, M.O., Tanskanen, A., Huttunen, T., Rabe-Hesketh, S., Murray, R.M., 1999. School performance in Finnish children and later development of schizophrenia: a population-based longitudinal study. Archives of General Psychiatry 56, 457–463.

Censits, D.M., Ragland, J.D., Gur, R.C., Gur, R.E., 1997. Neuropsychological evidence supporting a neurodevelopmental model of schizophrenia: a longitudinal study. Schizophrenia Research 24, 289–298.

Franke, P., Maier, W., Hain, C., Klingler, T., 1992. Wisconsin Card Sorting Test: an indicator of vulnerability to schizophrenia? Schizophrenia Research 6, 243–249.

Gold, J.M., Randolph, C., Carpenter, C.J., Goldberg, T.E., Weinberger, D.R., 1992. Forms of memory failure in schizophrenia. Journal of Abnormal Psychology 101, 487–494.

Goldberg, E., Seidman, L., 1991. Higher cortical functions in normals and in schizophrenia: a selective review. In: Steinhauer, S.R., Gruzelier, J.H., Zubin, J. (Eds.), Handbook of Schizophrenia: Neuropsychology, Psychophysiology and Information Processing. Elsevier, New York, pp. 553–597.

Gschwandtner, U., Pfluger, M., Aston, J., Borgwardt, S., Drewe, M., Stieglitz, R.D., Riecher-Rossler, A., 2005. Fine motor function and neuropsychological deficits in individuals at risk for schizophrenia. European Archives of Psychiatry and Clinical Neuroscience 256, 201–206.

Hawkins, K.A., Sullivan, T.E., Choi, E.J., 1997. Memory deficits in schizophrenia: inadequate assimilation or true amnesia? Findings from the Wechsler Memory Scale-Revised. Journal of Psychiatry & Neuroscience 22, 169–179.

Heinrichs, R.W., Zakzanis, K.K., 1998. Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. Neuropsychology 12, 426–445.

Jahn, T., Hubmann, W., Karr, M., Mohr, F., Schlenker, R., Heidenreich, T., Cohen, R., Schroder, J., 2006. Motoric neurological soft signs and psychopathological symptoms in schizophrenic psychoses. Psychiatry Research 142, 191–199.

Kashima, H., Handa, T., Kato, M., Sakuma, K., Yokoyama, N., Murakami, M., Shigemori, K., Muramatsu, T., Saito, H., Ooe, Y., Mimura, M., Asai, M., Hosaki, H., 1987. Neuropsychological investigation on chronic schizophrenia-aspects of its frontal functions. In: Takahashi, R., Flor-Henry, P., Gruzelier, J., Niwa, S. (Eds.), Cerebral Dynamics, Laterality and Psychopathology. Elsevier, Amsterdam, pp. 337–345.

King, H.E., 1958. Relation between fine and gross psychomotor movement in schizophrenia. A.M.A. Archives of Neurology and Psychiatry 79, 427–433.

Kraepelin, E., 1919. Dementia Praecox. Livingstone, Edinburgh.

Kurita, M., 1995. Effects of bilateral finger rotation exercise on flexibility and the efficiency of intellectual performance. In: Kikuchi, T., Sakuma, H., Saito, I., Tsuboi, K. (Eds.), Biobehavioral Self-Regulation: Eastern and Western Perspectives. Springer-Verlag, Tokyo, pp. 14–19.

Lehoux, C., Everett, J., Laplante, L., Emond, C., Trepanier, J., Brassard, A., Rene, L., Cayer, M., Merette, C., Maziade, M., Roy, M.A., 2003. Fine motor dexterity is correlated to social functioning in schizophrenia. Schizophrenia Research 62, 269–273.

Manschreck, T.C., 1986. Motor abnormalities in schizophrenia. In: Handbook of Schizophrenia. The Neurology of Schizophrenia, vol. 1. Elsevier Science Publishers B.V., Amsterdam.

Matthews, C.G., Klove, H., 1964. Instruction Manual for the Adult Neuropsychological Test Battery. University of Wisconsin Medical School, Madison, WI.

Milner, B., Corkin, S., Teuber, H.-L., 1968. Further analysis of the hippocampal amnesic syndrome: 14-year follow-up study of H.M. Neuropsychologia 6, 215–234.

Niendam, T.A., Bearden, C.E., Johnson, J.K., McKinley, M., Loewy, R., O'Brien, M., Nuechterlein, K.H., Green, M.F., Cannon, T.D., 2006. Neurocognitive performance and functional disability in the psychosis prodrome. Schizophrenia Research 84, 100–111.

Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97–113.

Putzhammer, A., Klein, H.E., 2006. Quantitative analysis of motor disturbances in schizophrenic patients. Dialogues in Clinical Neuroscience 8, 123–130.

Putzhammer, A., Heindle, B., Broll, K., Pfeiff, L., Perfahl, M., Hajak, G., 2004. Spatial and temporal parameters of gait disturbances in schizophrenic patients. Schizophrenia Research 69, 159–166.

Putzhammer, A., Perfahl, M., Pfeiff, L., Ibach, B., Johann, M., Zitzelsberger, U., Hajak, G., 2005. Performance of diadochokinetic movements in schizophrenic patients. Schizophrenia Research 79, 271–280.

Randolph, C., Gold, J.M., Kozora, E., Cullum, C.M., Hermann, B.P., Wyler, A.R., 1994. Estimating memory function: disparity of Wechsler Memory Scale-Revised and California Verbal Learning Test indices in clinical and normal samples. The Clinical Neuropsychologist 8, 99–108.

Rogowska, J., Gruber, S.A., Yurgelun-Todd, D.A., 2004. Functional magnetic resonance imaging in schizophrenia: cortical response to motor stimulation. Psychiatry Research. Neuroimaging 130, 227–243.

Rosofsky, I., Levin, S., Holzman, P.S., 1982. Psychomotility in the functional psychoses. Journal of Abnormal Psychology 91, 71–74.

Sautter, F.J., McDermott, B.E., Cornwell, J.M., Borges, A., Johnson, J., Vasterling, J.J., Marcontell, D.K., 1997. A comparison of neuropsychological deficits in familial schizophrenics, nonfamilial schizophrenics, and normal controls. Journal of Nervous and Mental Disease 185, 641–644.

Saykin, A.J., Gur, R.C., Gur, R.E., Mozley, P.D., Mozley, L.H., Resnick, S.M., Kester, D.B., Stafiniak, P., 1991. Neuropsychological function in schizophrenia. Selective impairment in memory and learning. Archives of General Psychiatry 48, 618–624.

Saykin, A.J., Shtasel, D.L., Gur, R.E., Kester, D.B., Mozley, L.H., Stafiniak, P., Gur, R.C., 1994. Neuropsychological deficits in neuroleptic naive patients with first-episode schizophrenia. Archives of General Psychiatry 51, 124–131.

Scarone, S., Abbruzzese, M., Gambini, O., 1993. The Wisconsin Card Sorting Test discriminates schizophrenic patients and their siblings. Schizophrenia Research 10, 103–107.

Scherer, H., Stip, E., Paquet, F., Bedard, M.A., 2003. Mild procedural learning disturbances in neuroleptic-naive patients with schizophrenia. Journal of Neuropsychiatry and Clinical Neurosciences 15, 58–63.

Shinagawa, F., Kobayashi, S., Fujita, K., Maekawa, H., 1990. Japanese Wechsler Adult Intelligence Scale-Revised. Nihon Bunka Kagakusya, Tokyo.

Sota, T.L., Heinrichs, R.W., 2004. Demographic, clinical, and neurocognitive predictors of quality of life in schizophrenia patients receiving conventional neuroleptics. Comprehensive Psychiatry 45, 415–421.

Sugishita, M., 2001. The Japanese Version of the Wechsler Memory Scale-Revised. Nihon Bunka Kagakusha, Tokyo.

Tigges, P., Mergl, R., Frodl, T., Meisenzahl, E.M., Gallinat, J., Schroter, A., Riedel, M., Muller, N., Moller, H.J., Hegerl, U., 2000. Digitized analysis of abnormal hand-motor performance in schizophrenic patients. Schizophrenia Research 45, 133–143.

Weaver, L.A., Brooks, G.W., 1964. The use of psychomotor tests in predicting the potential of chronic schizophrenia. Journal of Neuropsychiatry 170–180.

Wechsler, D., 1981. WAIS-R Manual. The Psychological Corporation, New York.

Wechsler, D., 1987. Wechsler Memory Scale-Revised Manual. The Psychological Corporation, San Antonio, TX.

p75NTR as a Therapeutic Target for Neuropsychiatric Diseases

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Abstract: The p75 neurotrophin receptor (p75NTR) was originally identified as a low-affinity receptor for neurotrophins. Recent studies have revealed that p75NTR can promote cell death or survival and modulate neurite outgrowth depending on the operative ligands and co-receptors. Up-regulation and ligand activation of p75NTR have been shown to be involved in neuronal cell death in cultured cells and animal models of neurodegenerative diseases. The levels of proneurotrophins, which bind to p75NTR to promote neuronal death, have been found to be increased in postmortem brains of patients with Alzheimer's disease. Furthermore, there is some evidence for the involvement of this molecule in psychiatric diseases, such as depression and schizophrenia. Mice lacking *p75NTR* have been shown to have several alterations in central nervous system and cognitive function. Notably, recent progress in genome-based drug discovery has enabled the identification of peptides and non-peptide small molecules targeting p75NTR, which may be potentially beneficial in the treatment of neuropsychiatric diseases. In this review, we focus on recent findings on p75NTR as a therapeutic target for neuropsychiatric diseases.

Keywords: p75NTR, neurotrophin, proneurotrophin, depression, schizophrenia, Alzheimer's disease, drug discovery, knockout (KO) mouse.

INTRODUCTION

p75NTR was identified as a receptor for neurotrophins, namely, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), and neurotrophin 4/5 (NT4/5), and cloned as a type I transmembrane protein, with its molecular weight of 75 kDa being glycosylated through both N- and O-linkages in the extracellular domain [1-5]. Neurotrophins influence numerous cellular activities such as proliferation, growth, differentiation, and regeneration [6]. The most extensively studied neurotrophin, BDNF, for example, has been implicated in Alzheimer's disease [7-9] and psychiatric diseases such as depression [10, 11] and schizophrenia [12, 13]. It is therefore feasible to speculate that the pan-neurotrophin receptor, p75NTR, might play critical roles in the pathogenesis of neuropsychiatric diseases. It might be a possible target molecule for the treatment of such diseases, although little attention has thus far been paid to p75NTR.

MOLECULAR OUTLINE

Human p75NTR is a 427 amino acid protein containing a 28 amino acid signal peptide, four extracellular cysteine-rich domains (CRD1 to CRD4), an extracellular stalk domain, a single transmembrane domain, and a 155 amino acid cytoplasmic domain. There is a short splicing variant of p75NTR, which will be described in more detail later (Fig. 1). p75NTR binds neurotrophins through interactions with the CRDs, each with six cysteine residues at conserved positions [14, 15]. The extracellular stalk domain is serine/threonine-rich and contains *O*-linked glycosylation sites [16]. The cytoplasmic juxtamembrane region, called the chopper domain, has been found to be necessary and sufficient to initiate neural cell death [17]. The second half of the intracellular domain is the death domain whose activation induces apoptosis [18, 19]. Signaling mediators, which are activated subsequent to ligand binding to p75NTR, include ceramide [20], nuclear factor κB (NF-κB) [21], Akt (also known as protein kinase B) [22], c-Jun N-terminal kinase (JNK) [23], and caspases [17].

p75NTR is a receptor for all mature neurotrophins (NGF, BDNF, NT3, and NT4/5) and immature proneurotrophin forms [24] (Fig. 2). Neither tropomyosin-related kinase A (TRKA) nor p75NTR forms a high-affinity binding site when expressed alone, whereas coexpression of the two receptors results in formation of high-affinity mature neurotrophin binding sites [25]. In forming a complex with TRK receptors (TRKA, TRKB, and TRKC) for the mature neurotrophins, p75NTR modulates the affinity and activity of these kinases that promote neuronal survival [25-30]. The high-affinity binding of proneurotrophins to p75NTR is mediated by interaction of the receptor with a co-receptor, sortilin, which is thought to promote apoptosis [31, 32]. p75NTR can also bind ligands other than neurotrophins, for example, amyloid beta [33], prion peptides (PrPs) [34], and rabies virus glycoprotein (RVG) [35, 36]. Moreover, it interacts with co-receptors other than TRKs and sortilin, for example, the NOGO receptor (NOGOR) and leucine rich repeat and Ig domain containing 1 (LINGO-1) [37, 38]. Further details are described in recent reviews [24, 39].

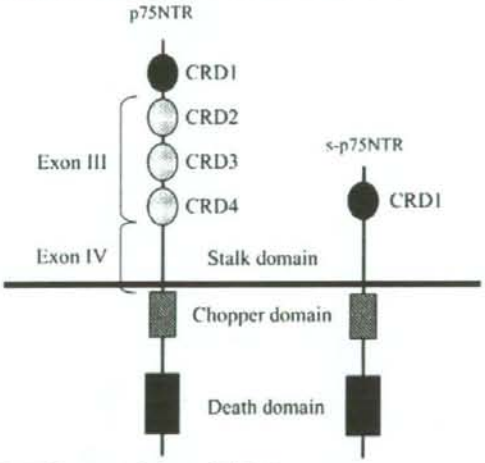

Fig. (1). The structural schema of p75NTR.
Schematic representation of full-length p75NTR and its short splice variant (s-p75NTR)
CRD: cysteine-rich domain

SPLICING VARIANT

Although p75NTR is mainly expressed as a 75 kDa transmembrane glycoprotein, there is a protein isoform of p75NTR that arises from alternative splicing of exon III (Fig. 1). This isoform is left intact in a *p75NTR* mutant mouse line generated by Lee *et al.* [40,

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41]. In its extracellular domain, the short p75NTR variant (s-p75NTR) differs from the full-length p75NTR protein by the absence of three (CRD2, CRD3, and CRD4) of the four CRDs [42]. Neurotrophins bind to CRD2, CDR3 and CRD4 of p75NTR [15, 43]. Indeed, in HEK293 cells expressing recombinant s-p75NTR, the receptor did not bind to any neurotrophin [40]. Reverse transcription-polymerase chain reaction (RT-PCR) analysis performed on mouse, rat and human cells revealed that *s-p75NTR* is evolutionarily conserved and coexpressed with full-length *p75NTR* transcript at different embryonic stages, generally at substantially lower levels. Although the functions of s-p75NTR are largely unknown, some studies suggest that it is a functional molecule *in vivo*. s-p75NTR binds to RVG through CRD1, in contrast to requiring the other three CRDs for neurotrophin binding [36, 44]. Recently, a mammalian homologue of p75NTR, neurotrophin receptor homologue 2 (NRH2), was identified [45-47]. NRH2 contains transmembrane and cytoplasmic domains homologous to those of s-p75NTR; however, it lacks all of the CRDs [45]. NRH2 can interact with p75NTR and Trks, and mediate death or promote survival signals [46, 48].

KNOCKOUT MICE

Lee *et al.* [41] generated mice lacking functional *p75NTR* by targeted disruption of exon III, which encodes CRD2, CRD3 and CRD4 (designated henceforth $p75NTR^{exonIII-/-}$). $p75NTR^{exonIII-/-}$ mice were reported to be viable and fertile, and to develop deficits in heat sensitivity and skin defects in all extremities [41]. Immunohistochemistry revealed a lack of peripheral sensory nerve fibers expressing calcitonin-related peptide alpha (CALCA) and substance P [41]. Neonatal sympathetic and embryonic sensory neurons derived from $p75NTR^{exonIII-/-}$ mice showed reduced sensitivity to NGF and displayed deficits in developmental and injury-induced apoptosis [49, 50].

Because both the *s-p75NTR* transcript and its encoded protein are expressed in $p75NTR^{exonIII-/-}$ mice, von Schack *et al.* [40] targeted exon IV to generate a null mutation (designated henceforth $p75NTR^{exonIV-/-}$). In both $p75NTR^{exonII-/-}$ and $p75NTR^{exonIV-/-}$ mutants, alterations in cholinergic neurons in the basal forebrain, hippocampal neurons, and neurogenic precursor cells in the subventricular zone (SVZ) have been observed (Table 1) [40, 41, 49-74]. $p75NTR^{exonIV-/-}$ mice display more severe phenotypes than $p75NTR^{exonIII-/-}$ mice, particularly in the nervous systems. $p75NTR^{exonIV-/-}$ mice displayed a larger reduction in the number of dorsal root ganglia (DRG) neurons and Schwann cells, partial perinatal lethality, and defects in the vascular system that have not been observed in $p75NTR^{exonIII-/-}$ mice [40].

Alzheimer's disease, which causes deficits in learning and memory processes, is accompanied by a loss of cholinergic function [75-77]. Some studies have indicated an increase in the number of cholinergic forebrain neurons in $p75NTR^{exonIII-/-}$ mice [53, 54, 78], while others reported a decrease [67] or no significant change in the numbers of such neurons [52]. To resolve these conflicting results, Naumann *et al.* analyzed the numbers of cholinergic neurons in the medial septal nucleus of $p75NTR^{exonIII-/-}$ and $p75NTR^{exonIV-/-}$ mice on a Sv129/BALB/c genetic background and a back-crossed congenic strain (C57BL/6). The $p75NTR^{exonIII-/-}$ mutation led to a moderate increase (+13%) in the number of cholinergic neurons only after back-crossing onto a C57BL/6 background. Interestingly, s-p75NTR was present at substantially higher levels in mice with the Sv129 background compared with C57BL/6 mice, which might help to explain this result. In contrast to the $p75NTR^{exonIII-/-}$ mutation, the $p75NTR^{exonIV-/-}$ mutation resulted in an over 20% increase in the number of cholinergic neurons, independent of genetic background. They concluded that $p75NTR^{exonIV-/-}$ mutation increases the number of cholinergic neurons in the medial septum [54].

The $p75NTR^{exonIV-/-}$ mutation results in severe ataxia in mice and precludes detailed behavioral testing [40]. When spatial learning was examined in $p75NTR^{exonIII-/-}$ mice, conflicting results were observed, depending on test paradigms. Peterson *et al.*, who found a markedly reduced number of cholinergic septal neurons, reported deficits in acquisition of the Morris water maze, inhibitory avoidance, and habituation tasks in adult $p75NTR^{exonIII-/-}$ mice [64]. Such deficits in $p75NTR^{exonIII-/-}$ mice in the Morris water maze were subsequently supported by Wright *et al.* [66]. Performance in the Barnes maze, by contrast, was superior in $p75NTR^{exonIII-/-}$ mice than in control mice [52]. The Barnes maze is a dry version of the circu-

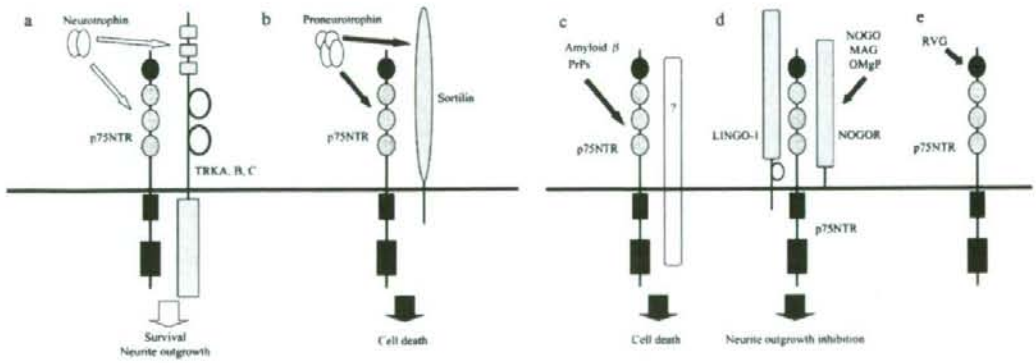

Fig. (2). p75NTR is involved in different biological activities, depending on its different ligands and co-receptors.

a). p75NTR physically interacts with TRK receptors (TRKA, TRKB, and TRKC) and enhances their abilities to respond to neurotrophins.

b). Interaction of p75NTR with sortilin mediates proapoptotic signals in response to proneurotrophin binding.

c). Amyloid beta binds to p75NTR and promotes cell death.

d). p75NTR forms a complex with NOGOR that results in growth inhibitory signals to be transduced in response to NOGO, myelin-associated glycoprotein (MAG), or oligodendrocyte myelin-glycoprotein (OMgP).

e). RGV binding occurs on CRD1, although this binding is not essential for RV infection.

Table 1. Neuronal and Behavioral Phenotypes of *p75NTR* Knockout Mice

Year	Authors	Region	KO	Phenotypes
1992	Lee, KF et al.[41]	SN	III	Marked decrease in sensory innervation by calcitonin gene-related peptide- and substance P-immunoreactive fibers and loss of heat sensitivity
1993	Davies, AM et al.[49]	TSN	III	Altered response to NGF in trigeminal sensory neurons
1994	Lee, KF et al. [50]	DRG, SCG	III	Excessive loss of DRG and SCG neurons *in vivo*
1995	Curtis, R. et al. [70]	DRG	III	Retrograde axonal transport of NT-4 and BDNF, but not NGF, was dramatically reduced
1997	Yeo, TT et al. [53]	BFCN	III	Increase in the number, size, activity, and target innervation of BFCNs
1997	Peterson, DA et al. [67]	BFCN	III	Decrease in the number of BFCNs
1997	Bergmann, I. et al.[72]	Cutaneous SN	III	Reduction of the epidermal innervation density
1998	Bamji, SX et al. [55]	SCG	III	Sympathetic neuron death was developmentally delayed
1998	Fern, CC et al. [57]	Facial MN	III	Significant improvement of survival in the adult KO mice, compared to WT, following injury
1999	Brennan, C. et al. [65]	SCG	III	In *p75NTR*$^{-/-}$ *NGF*$^{-/-}$ mice, SCG neuron number was restored to WT levels. (*NGF*$^{-/-}$ mice had 50% fewer SCG neurons.)
1999	Peterson, DA et al. [64]	BFCN	III	Impairments in several learning and memory tasks, such as Morris water maze, inhibitory avoidance, motor activity, and habituation tasks
1999	Yamashita, T. et al. [71]	SN, MN	III	Reduced outgrowth of sensory and motor axons
1999	Frade, JM et al. [56]	Retina, Spinal cord	III	In *p75NTR*$^{-/-}$ embryos, cell death was reduced in the retina and in the spinal cord
2000	Bentley, CA et al. [51]	DRG	III	Abnormalities of axon growth, arborization, and Schwann cell migration during development
2000	Greferath, U. et al. [52]	BFCN	III	Increase in the number of BFCNs, marked increase of the size of BFCNs, and better spatial learning performance
2000	Coulson, EJ et al. [69]	DRG	III	Resistance for cell death after withdrawal from cultured medium
2001	von Schack et al. [40]	DRG	IV	Larger reduction in the number of DRG neurons and Schwann cells
2002	Naumann, T. et al. [54]	BFCN	III, IV	Increase in the number of BFCNs (medial septum), compared with controls
2002	Troy, CM et al. [58]	HN	III	Marked reduction in the number of dying neurons after induced seizures
2004	Wright, JW et al. [66]	(Behavior)	III	Impairments in Morris water maze task
2005	Zagrebelsky, M. et al. [59]	HN	III, IV	Postnatal hippocampal pyramidal cells in both mutant lines had a higher spine density and greater dendritic complexity than WT mice
2005	Rosch, H. et al. [60]	HN	III, IV	Hippocampal LTD was impaired in both *p75NTR*-deficient strains
2005	Woo, NH et al. [61]	HN	III	Impairment of the NMDA receptor-dependent LTD and a decrease in the expression of *NR2B*
2005	Scott, ALM et al.[73]	Spinal cord	III	In rhizotomy-treated *p75NTR*$^{-/-}$ mice, intraspinal sprouting was significantly augmented
2006	Sato, T et al. [62]	SGN, HCs	III	Progressive hearing loss, degeneration of SGNs, and severe loss of HCs
2006	Volosin, M. et al. [68]	BFCN	III	65% decrease in the number of dying neurons in the medial septum and 80% decrease in the diagonal band compared with the WT mice 1day after kainic acid treatment
2007	Young, KM et al. [63]	NPC in SVZ	III	70% reduction in neurogenic potential *in vitro*, significant reductions of numbers of PSA-NCAM positive SVZ neuroblasts *in vivo*, and a lower OB weight
2007	Jansen, P. et al.[74]	SCG	III	Significant increase in the number of sympathetic neurons and protection against age-associated cell death

In the KO column, III and IV represent *p75NTR*^{exonIII} and *p75NTR*^{exonIV}, respectively.
BFCN: Basal forebrain cholinergic neuron, CNS: Central nervous system, DRG: Dorsal root ganglia, HC: Ear hair cell, HN: Hippocampal neurons, LTD: Long-term depression, MN: Motor neuron, NMDA: N-methyl-d-aspartate, NPC: Neurogenic precursor cells, NR2B: N-methyl-D-aspartate receptor 2B, OB: Olfactory bulb, PSA-NCAM: Polysialic acid neural cell adhesion molecule, SCG: Superior cervical ganglion, SGN: Spiral ganglion neuron, SN: Sensory neuron, SVZ: Subventricular zone, TSN: Trigeminal sensory neuron, WT: Wild-type.

lar water maze that provides a spatial cue to permit navigation to a safe location. Unlike the study of Peterson *et al.* [64, 67], Greferath *et al.* [52] did not find a markedly reduced number of cholinergic septal neurons, and reported that cholinergic cells in *p75NTR*$^{exonIII-/-}$ mice were significantly larger than those in control mice in the medial septal area and in the diagonal band of Broca. They discussed that the improved performance of *p75NTR*$^{exonIII-/-}$ mice in the Barnes maze correlates with their hypertrophied cholinergic neurons. Taken together, these findings suggest that *p75NTR* may act as a negative regulator of cholinergic neurons in the forebrain.

p75NTR and Neurodegenerative Disease

Alzheimer's disease is pathologically characterized by extensive neuronal cell death, synaptic loss, intracellular neurofibrillary tangles, and extracellular senile plaques and, as described above, is associated with a loss of cholinergic neurons resulting in profound memory disturbances and irreversible impairment of cognitive function [75-77, 79-81]. In the postmortem brains of patients with Alzheimer's disease, the levels of proneurotrophins, which bind to p75NTR and promote neuronal death, have been found to be increased in several studies [82-86]. In cellular models of neurodegenerative diseases, up-regulation and ligand activation of p75NTR have been shown to mediate neuronal cell death [31, 32, 69]. Sortilin, a member of the vacuolar protein sorting 10 protein (Vps10p) domain receptor family, was shown to form a receptor complex with p75NTR as an essential component for transmitting proneurotrophin-dependent cell death signals [31, 32]. Sortilin KO (*Sort1*$^{-/-}$) mice were shown to be resistant to age-dependent degeneration of sympathetic neurons [74]. In *p75NTR*$^{exonIII-/-}$ mice, protection against age-associated cell death was also observed, supporting the possibility of a functional interaction between p75NTR and

sortilin in this process [74]. Of note, amyloid beta peptides, the major constituents of senile plaques, have been characterized as ligands for p75NTR [33, 87, 88]. Although this signaling *in vivo* is still unclear, a number of reports have suggested that p75NTR is involved in the promotion of cell death signaling by amyloid beta peptides *in vitro* [89-95]. A recent study provided evidence of a direct link between p75NTR signaling and amyloid beta-induced toxicity in hippocampal neurons *in vitro* and in cholinergic basal forebrain neurons *in vivo* [88]. Both proneurotrophin- and amyloid beta-regulated signaling pathways involving p75NTR *in vivo* seem promising in order to understand the etiology of Alzheimer's disease and to develop novel therapeutic drugs.

p75NTR in Neuropsychiatric Diseases

A number of studies indicate that neurotrophins also play an important role in neuropsychiatric diseases such as depression and schizophrenia (reviewed in [7-13, 96]). Therefore, p75NTR, as a pan neurotrophin receptor, might play a key role in neuropsychiatric diseases. As the first study examining a possible association between *p75NTR* and psychiatric diseases, we reported that a missense polymorphism (S205L) in *p75NTR* was associated with depressive disorder and attempted suicide in a Japanese population [97]. The frequency of mutant-type (L205) was significantly decreased in patients compared with controls ($P < 0.05$, odds ratio 0.54, 95% CI 0.31-0.94), suggesting that this variant may have a protective effect against the development of major depression. Furthermore, this association was more strongly observed in patients with a history of attempted suicide than in those without such a history. A recent study in a North American population, however, failed to obtain evidence for an association between *p75NTR* polymorphisms, including S205L, and a risk of childhood-onset mood disorder (COMD) or suicide attempt in COMD [98]. In order to clarify the relationship between *p75NTR* and depressive disorder and suicidal behavior, further studies in large samples are required.

Many lines of evidence indicate that early neurodevelopmental abnormalities contribute to the pathogenesis of schizophrenia [99-101]. Schizophrenia is also characterized by adult-onset subcortical dopaminergic hyperactivity (see, for example, [102, 103]) and disrupted prepulse inhibition (PPI) of acoustic startle (see, for example, [104-107]). *p75NTR* is widely expressed in the developing central and peripheral nervous systems during the period of synaptogenesis and developmental cell death [108]. Rats treated with neonatal injections of p75NTR antibody conjugated to saporin into the developing prefrontal cortex showed impaired PPI and behavioral changes characteristic of adult-onset dopaminergic hyperresponsivity [109]. It has been suggested that prenatal vitamin D3 depletion can lead to changes in many features of brain development, including morphology, cellular proliferation and neurotrophin systems, which suggests a potential risk-modifying factor for schizophrenia. Interestingly, this change induced by vitamin D3 depletion includes a marked decrease in the expression of *p75NTR*, and vitamin D3-responsive elements are present in the promoter region of *p75NTR* [110]. Recently, the early growth response (Egr) transcriptional regulators, Egr1 and Egr3, were identified as direct modulators of *p75NTR* expression [111]. Egr1 and Egr3 bind to and transactivate the *p75NTR* promoter *in vitro* and *in vivo* [111]. *EGR3* was identified as a potential susceptibility gene in schizophrenia by a recent genetic association study and postmortem brain analysis [112].

Numerous studies have found subregional abnormalities of the brain in patients with schizophrenia, including smaller hippocampal volume, larger ventricles, smaller cerebral volume, reversed asymmetry in the superior temporal gyrus, and smaller volume of the medial temporal lobes (reviewed in [113-115]), and in those with major depression, including reduced volumes of hippocampus, amygdala and anterior cingulate (reviewed in [113, 116, 117]). There are several lines of evidence suggesting alterations of oligodendrocytes in schizophrenia, for example, lowered density of oligodendroglia [118, 119]. Possibly pertinent to this, proneurotrophins induce death of oligodendrocytes expressing p75NTR [31]. Taken together, these findings suggest that p75NTR might play an important role as a key molecule in such volume changes of the brain in patients with neuropsychiatric diseases.

DRUG CANDIDATES

Agonists or antagonists for p75NTR would contain structural determinants of one or more neurotrophin active sites that interact with p75NTR. Longo *et al.* [120] revealed that a peptide corresponding to the region between amino acid residues 28 and 38 of NGF inhibits its neurotrophic effects on DRG neurons. Subsequently, short synthetic peptides corresponding to the beta-hairpin loop of NGF were designed, blocking neuronal death in culture [121]. This NGF-inhibitory activity was p75NTR dependent, requiring both peptide cyclization and dimerization [121]. Turner *et al.* [122] showed that application of a cyclic decapeptide p75NTR antagonist, containing amyloid beta residues 28–30 (Lys-Gly-Ala), protects against NGF-induced death signaling in cultured NSC-34 cells.

Recently, Massa *et al.* [123] identified several small molecules as novel ligands of p75NTR, including a derivative of caffeine, LM11A-24. These compounds are non-peptidyl mimetics of the neurotrophin loop 1 domain identified by tandem *in silico* and *in vitro* screening. LM11A-24 bound to p75NTR, exerted potent neuroprotective effects through one or more p75NTR-dependent mechanisms, and stimulated survival pathways in hippocampal neurons. It also prevented p75NTR-dependent apoptosis induced by proNGF in oligodendrocytes [123]. Subsequently, Pehar *et al.* [124] showed that LM11A-24 was able to inhibit p75NTR-dependent motor neuron death induced by NGF. Intriguingly, the apparent potency of LM11A-24 was considerably higher than that of the above-mentioned peptide-based antagonist containing amyloid beta residues 28–30 (Lys-Gly-Ala) in motor neuron-like NSC-34 cells [123, 122]. LM11A-24 and its related derivatives capable of crossing the blood–brain barrier are expected to become leading candidates in the development of therapeutic strategies targeting p75NTR.

CONCLUSION

We focused on recent findings concerning p75NTR in relation to neuronal function, its possible relevance to neuropsychiatric diseases, and progress in genome-based drug discovery targeting p75NTR. p75NTR associates with many kinds of co-receptors and ligands, and transduces various signals, which complicate the understanding of the role of p75NTR *in vivo*. However, in *p75NTR* KO mice, an increase in the number and size of cholinergic neurons in the medial septum and protection against age-associated neuronal cell death were observed, which raises the possibility that *p75NTR* might regulate cholinergic neurons negatively in the forebrain. p75NTR, as a mediator of death signaling in both neurons and oligodendrocytes, might contribute to the morphological changes in the brain and subsequent development of neuropsychiatric diseases. Thus, suppression of p75NTR might be a possible therapeutic strategy. Recently, virtual screening *in silico* has been put to practical use in drug discovery and some small molecule ligands for p75NTR have been identified (for example, LM11A-24). Studies on such small ligands for p75NTR with respect to their therapeutic and protective effects in neuropsychiatric diseases are warranted.

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REFERENCES

[1] Chao, M. V.; Bothwell, M. A.; Ross, A. H.; Koprowski, H.; Lanahan, A. A.; Buck, C. R.; Sehgal, A. Gene transfer and molecular cloning of the human NGF receptor. *Science* **1986**, *232*, 518-521.

[2] Grob, P. M.; Ross, A. H.; Koprowski, H.; Bothwell, M. Characterization of the human melanoma nerve growth factor receptor. *J. Biol. Chem.* **1985**, *260*, 8044-8049.

[3] Herrup, K.; Shooter, E. M. Properties of the beta nerve growth factor receptor of avian dorsal root ganglia. *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 3884-3888.

[4] Johnson, D.; Lanahan, A.; Buck, C. R.; Sehgal, A.; Morgan, C.; Mercer, E.; Bothwell, M.; Chao, M. Expression and structure of the human NGF receptor. *Cell* **1986**, *47*, 545-554.

[5] Radeke, M. J.; Misko, T. P.; Hsu, C.; Herzenberg, L. A.; Shooter, E. M. Gene transfer and molecular cloning of the rat nerve growth factor receptor. *Nature* **1987**, *325*, 593-597.

[6] Reichardt, L. F. Neurotrophin-regulated signalling pathways. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **2006**, *361*, 1545-1564.

[7] Sohrabji, F.; Lewis, D. K. Estrogen-BDNF interactions: implications for neurodegenerative diseases. *Front. Neuroendocrinol.* **2006**, *27*, 404-414.

[8] Hubka, P. Neural network plasticity, BDNF and behavioral interventions in Alzheimer's disease. *Bratisl. Lek. Listy* **2006**, *107*, 395-401.

[9] Schulte-Herbruggen, O.; Braun, A.; Rochlitzer, S.; Jockers-Scherubl, M. C.; Hellweg, R. Neurotrophic factors--a tool for therapeutic strategies in neurological, neuropsychiatric and neuroimmunological diseases? *Curr. Med. Chem.* **2007**, *14*, 2318-2329.

[10] Martinowich, K.; Manji, H.; Lu, B. New insights into BDNF function in depression and anxiety. *Nat. Neurosci.* **2007**, *10*, 1089-1093.

[11] Post, R. M. Role of BDNF in bipolar and unipolar disorder: clinical and theoretical implications. *J. Psychiatr. Res.* **2007**, *41*, 979-990.

[12] Durany, N.; Thome, J. Neurotrophic factors and the pathophysiology of schizophrenic psychoses. *Eur. Psychiatry* **2004**, *19*, 326-337.

[13] Buckley, P. F.; Mahadik, S.; Pillai, A.; Terry, A., Jr. Neurotrophins and schizophrenia. *Schizophr. Res.* **2007**, *94*, 1-11.

[14] Welcher, A. A.; Bitler, C. M.; Radeke, M. J.; Shooter, E. M. Nerve growth factor binding domain of the nerve growth factor receptor. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 159-163.

[15] He, X. L.; Garcia, K. C. Structure of nerve growth factor complexed with the shared neurotrophin receptor p75. *Science* **2004**, *304*, 870-875.

[16] Chapman, B. S.; Eckart, M. R.; Kaufman, S. E.; Lapointe, G. R. O-linked oligosaccharide on the 75-kDa neurotrophin receptor. *J. Neurochem.* **1996**, *66*, 1707-1716.

[17] Coulson, E. J.; Reid, K.; Baca, M.; Shipham, K. A.; Hulett, S. M.; Kilpatrick, T. J.; Bartlett, P. F. Chopper, a new death domain of the p75 neurotrophin receptor that mediates rapid neuronal cell death. *J. Biol. Chem.* **2000**, *275*, 30537-30545.

[18] Liepinsh, E.; Ilag, L. L.; Otting, G.; Ibanez, C. F. NMR structure of the death domain of the p75 neurotrophin receptor. *EMBO J.* **1997**, *16*, 4999-5005.

[19] Coulson, E. J.; Reid, K.; Shipham, K. M.; Morley, S.; Kilpatrick, T. J.; Bartlett, P. F. The role of neurotransmission and the Chopper domain in p75 neurotrophin receptor death signaling. *Prog. Brain Res.* **2004**, *146*, 41-62.

[20] Dobrowsky, R. T.; Werner, M. H.; Castellino, A. M.; Chao, M. V.; Hannun, Y. A. Activation of the sphingomyelin cycle through the low-affinity neurotrophin receptor. *Science* **1994**, *265*, 1596-1599.

[21] Carter, B. D.; Kaltschmidt, C.; Kaltschmidt, B.; Offenhauser, N.; Bohm-Matthaei, R.; Baeuerle, P. A.; Barde, Y. A. Selective activation of NF-kappa B by nerve growth factor through the neurotrophin receptor p75. *Science* **1996**, *272*, 542-545.

[22] Roux, P. P.; Bhakar, A. L.; Kennedy, T. E.; Barker, P. A. The p75 neurotrophin receptor activates Akt (protein kinase B) through a phosphatidylinositol 3-kinase-dependent pathway. *J. Biol. Chem.* **2001**, *276*, 23097-23104.

[23] Friedman, W. J. Neurotrophins induce death of hippocampal neurons *via* the p75 receptor. *J. Neurosci.* **2000**, *20*, 6340-6346.

[24] Barker, P. A. p75NTR is positively promiscuous: novel partners and new insights. *Neuron* **2004**, *42*, 529-533.

[25] Hempstead, B. L.; Martin-Zanca, D.; Kaplan, D. R.; Parada, L. F.; Chao, M. V. High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. *Nature* **1991**, *350*, 678-683.

[26] Battleman, D. S.; Geller, A. I.; Chao, M. V. HSV-1 vector-mediated gene transfer of the human nerve growth factor receptor p75hNGFR defines high-affinity NGF binding. *J. Neurosci.* **1993**, *13*, 941-951.

[27] Benedetti, M.; Levi, A.; Chao, M. V. Differential expression of nerve growth factor receptors leads to altered binding affinity and neurotrophin responsiveness. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 7859-7863.

[28] Mahadeo, D.; Kaplan, L.; Chao, M. V.; Hempstead, B. L. High affinity nerve growth factor binding displays a faster rate of association than p140trk binding. Implications for multi-subunit polypeptide receptors. *J. Biol. Chem.* **1994**, *269*, 6884-6891.

[29] Hantzopoulos, P. A.; Suri, C.; Glass, D. J.; Goldfarb, M. P.; Yancopoulos, G. D. The low affinity NGF receptor, p75, can collaborate with each of the Trks to potentiate functional responses to the neurotrophins. *Neuron* **1994**, *13*, 187-201.

[30] Canossa, M.; Twiss, J. L.; Verity, A. N.; Shooter, E. M. p75(NGFR) and TrkA receptors collaborate to rapidly activate a p75(NGFR)-associated protein kinase. *EMBO J.* **1996**, *15*, 3369-3376.

[31] Nykjaer, A.; Lee, R.; Teng, K. K.; Jansen, P.; Madsen, P.; Nielsen, M. S.; Jacobsen, C.; Kliemannel, M.; Schwarz, E.; Willnow, T. E.; Hempstead, B. L.; Petersen, C. M. Sortilin is essential for proNGF-induced neuronal cell death. *Nature* **2004**, *427*, 843-848.

[32] Teng, H. K.; Teng, K. K.; Lee, R.; Wright, S.; Tevar, S.; Almeida, R. D.; Kermani, P.; Torkin, R.; Chen, Z. Y.; Lee, F. S.; Kraemer, R. T.; Nykjaer, A.; Hempstead, B. L. ProBDNF induces neuronal apoptosis *via* activation of a receptor complex of p75NTR and sortilin. *J. Neurosci.* **2005**, *25*, 5455-5463.

[33] Yaar, M.; Zhai, S.; Pilch, P. F.; Doyle, S. M.; Eisenhauer, P. B.; Fine, R. E.; Gilchrest, B. A. Binding of beta-amyloid to the p75 neurotrophin receptor induces apoptosis. A possible mechanism for Alzheimer's disease. *J. Clin. Invest.* **1997**, *100*, 2333-2340.

[34] Della-Bianca, V.; Rossi, F.; Armato, U.; Dal-Pra, I.; Costantini, C.; Perini, G.; Politi, V.; Della Valle, G. Neurotrophin p75 receptor is involved in neuronal damage by prion peptide-(106-126). *J. Biol. Chem.* **2001**, *276*, 38929-38933.

[35] Tuffereau, C.; Benejean, J.; Blondel, D.; Kieffer, B.; Flamand, A. Low-affinity nerve-growth factor receptor (P75NTR) can serve as a receptor for rabies virus. *EMBO J.* **1998**, *17*, 7250-729.

[36] Tuffereau, C.; Schmidt, K.; Langevin, C.; Lafay, F.; Dechant, G.; Koltzenburg, M. The rabies virus glycoprotein receptor p75NTR is not essential for rabies virus infection. *J. Virol.* **2007**, *81*, 13622-30.

[37] Wang, K. C.; Kim, J. A.; Sivasankaran, R.; Segal, R.; He, Z. P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature* **2002**, *420*, 74-78.

[38] Mi, S.; Lee, X.; Shao, Z.; Thill, G.; Ji, B.; Relton, J.; Levesque, M.; Allaire, N.; Perrin, S.; Sands, B.; Crowell, T.; Cate, R. L.; McCoy, J. M.; Pepinsky, R. B. LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nat. Neurosci.* **2004**, *7*, 221-228.

[39] Blochl, A.; Blochl, R. A cell-biological model of p75NTR signaling. *J. Neurochem.* **2007**, *102*, 289-305.

[40] von Schack, D.; Casademunt, E.; Schweigreiter, R.; Meyer, M.; Bibel, M.; Dechant, G. Complete ablation of the neurotrophin receptor p75NTR causes defects both in the nervous and the vascular system. *Nat. Neurosci.* **2001**, *4*, 977-978.

[41] Lee, K. F.; Li, E.; Huber, L. J.; Landis, S. C.; Sharpe, A. H.; Chao, M. V.; Jaenisch, R. Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. *Cell* **1992**, *69*, 737-749.

[42] Chapman, B. S. A region of the 75 kDa neurotrophin receptor homologous to the death domains of TNFR-I and Fas. *FEBS Lett.* **1995**, *374*, 216-220.

[43] Baldwin, A. N.; Shooter, E. M. Zone mapping of the binding domain of the rat low affinity nerve growth factor receptor by the introduction of novel N-glycosylation sites. *J. Biol. Chem.* **1995**, *270*, 4594-4602.

[44] Langevin, C.; Jaaro, H.; Bressanelli, S.; Fainzilber, M.; Tuffereau, C. Rabies virus glycoprotein (RVG) is a trimeric ligand for the N-terminal cysteine-rich domain of the mammalian p75 neurotrophin receptor. *J. Biol. Chem.* **2002**, *277*, 37655-37662.

[45] Kanning, K. C.; Hudson, M.; Amieux, P. S.; Wiley, J. C.; Bothwell, M.; Schecterson, L. C. Proteolytic processing of the p75 neurotrophin receptor and two homologs generates C-terminal fragments with signaling capability. *J. Neurosci.* **2003**, *23*, 5425-5436.

[46] Frankowski, H.; Castro-Obregon, S.; del Rio, G.; Rao, R. V.; Bredesen, D. E. PLAIDD, a type II death domain protein that interacts with p75 neurotrophin receptor. *Neuromolecular Med.* **2002**, *1*, 153-170.

[47] Wang, X.; Shao, Z.; Zetoune, F. S.; Zeidler, M. G.; Gowrishankar, K.; Vincenz, C. NRADD, a novel membrane protein with a death domain involved in mediating apoptosis in response to ER stress. *Cell Death Differ.* **2003**, *10*, 580-591.

[48] Murray, E.; Malley, S. E.; Qiao, L. Y.; Hu, V. Y.; Vizzard, M. A. Cyclophosphamide induced cystitis alters neurotrophin and receptor tyrosine kinase expression in pelvic ganglia and bladder. *J. Urol.* **2004**, *172*, 2434-2439.

[49] Davies, A. M.; Lee, K. F.; Jaenisch, R. p75-deficient trigeminal sensory neurons have an altered response to NGF but not to other neurotrophins. *Neuron* **1993**, *11*, 565-574.

[50] Lee, K. F.; Davies, A. M.; Jaenisch, R. p75-deficient embryonic dorsal root sensory and neonatal sympathetic neurons display a decreased sensitivity to NGF. *Development* **1994**, *120*, 1027-1033.

[51] Bentley, C. A.; Lee, K. F. p75 is important for axon growth and schwann cell migration during development. *J. Neurosci.* **2000**, *20*, 7706-7715.

[52] Greferath, U.; Bennie, A.; Kourakis, A.; Bartlett, P. F.; Murphy, M.; Barrett, G. L. Enlarged cholinergic forebrain neurons and improved spatial learning in p75 knockout mice. *Eur. J. Neurosci.* **2000**, *12*, 885-893.

[53] Yeo, T. T.; Chua-Couzens, J.; Butcher, L. L.; Bredesen, D. E.; Cooper, J. D.; Valletta, J. S.; Mobley, W. C.; Longo, F. M. Absence of p75NTR causes increased basal forebrain cholinergic neuron size, choline acetyltransferase activity, and target innervation. *J. Neurosci.* **1997**, *17*, 7594-7605.

[54] Naumann, T.; Casademunt, E.; Hollerbach, E.; Hofmann, J.; Dechant, G.; Frotscher, M.; Barde, Y. A. Complete deletion of the neurotrophin receptor p75NTR leads to long-lasting increases in the number of basal forebrain cholinergic neurons. *J. Neurosci.* **2002**, *22*, 2409-2418.

[55] Bamji, S. X.; Majdan, M.; Pozniak, C. D.; Belliveau, D. J.; Aloyz, R.; Kohn, J.; Causing, C. G.; Miller, F. D. The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. *J. Cell Biol.* **1998**, *140*, 911-923.

[56] Frade, J. M.; Barde, Y. A. Genetic evidence for cell death mediated by nerve growth factor and the neurotrophin receptor p75 in the developing mouse retina and spinal cord. *Development* **1999**, *126*, 683-690.

[57] Ferri, C. C.; Moore, F. A.; Bisby, M. A. Effects of facial nerve injury on mouse motoneurons lacking the p75 low-affinity neurotrophin receptor. *J. Neurobiol.* **1998**, *34*, 1-9.

[58] Troy, C. M.; Friedman, J. E.; Friedman, W. J. Mechanisms of p75-mediated death of hippocampal neurons. Role of caspases. *J. Biol. Chem.* **2002**, *277*, 34295-34302.

[59] Zagrebelsky, M.; Holz, A.; Dechant, G.; Barde, Y. A.; Bonhoeffer, T.; Korte, M. The p75 neurotrophin receptor negatively modulates dendrite complexity and spine density in hippocampal neurons. *J. Neurosci.* **2005**, *25*, 9989-9999.

[60] Rosch, H.; Schweigreiter, R.; Bonhoeffer, T.; Barde, Y. A.; Korte, M. The neurotrophin receptor p75NTR modulates long-term depression and regulates the expression of AMPA receptor subunits in the hippocampus. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 7362-7367.

[61] Woo, N. H.; Teng, H. K.; Siao, C. J.; Chiaruttini, C.; Pang, P. T.; Milner, T. A.; Hempstead, B. L.; Lu, B. Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. *Nat. Neurosci.* **2005**, *8*, 1069-1077.

[62] Sato, T.; Doi, K.; Taniguchi, M.; Yamashita, T.; Kubo, T.; Tohyama, M. Progressive hearing loss in mice carrying a mutation in the p75 gene. *Brain Res.* **2006**, *1091*, 224-234.

[63] Young, K. M.; Merson, T. D.; Sotthibundhu, A.; Coulson, E. J.; Bartlett, P. F. p75 neurotrophin receptor expression defines a population of BDNF-responsive neurogenic precursor cells. *J. Neurosci.* **2007**, *27*, 5146-5155.

[64] Peterson, D. A.; Dickinson-Anson, H. A.; Leppert, J. T.; Lee, K. F.; Gage, F. H. Central neuronal loss and behavioral impairment in mice lacking neurotrophin receptor p75. *J. Comp. Neurol.* **1999**, *404*, 1-20.

[65] Brennan, C.; Rivas-Plata, K.; Landis, S. C. The p75 neurotrophin receptor influences NT-3 responsiveness of sympathetic neurons *in vivo*. *Nat. Neurosci.* **1999**, *2*, 699-705.

[66] Wright, J. W.; Alt, J. A.; Turner, G. D.; Krueger, J. M. Differences in spatial learning comparing transgenic p75 knockout, New Zealand Black, C57BL/6, and Swiss Webster mice. *Behav. Brain Res.* **2004**, *153*, 453-458.

[67] Peterson, D. A.; Leppert, J. T.; Lee, K. F.; Gage, F. H. Basal forebrain neuronal loss in mice lacking neurotrophin receptor p75. *Science* **1997**, *277*, 837-839.

[68] Volosin, M.; Song, W.; Almeida, R. D.; Kaplan, D. R.; Hempstead, B. L.; Friedman, W. J. Interaction of survival and death signaling in basal forebrain neurons: roles of neurotrophins and proneurotrophins. *J. Neurosci.* **2006**, *26*, 7756-7766.

[69] Coulson, E. J.; Reid, K.; Murray, S. S.; Cheema, S. S.; Bartlett, P. F. Role of neurotrophin receptor p75NTR in mediating neuronal cell death following injury. *Clin. Exp. Pharmacol. Physiol.* **2000**, *27*, 537-541.

[70] Curtis, R.; Adryan, K. M.; Stark, J. L.; Park, J. S.; Compton, D. L.; Weskamp, G.; Huber, L. J.; Chao, M. V.; Jaenisch, R.; Lee, K. F.; Lindsay, R.M.; Distefano, P.S. Differential role of the low affinity neurotrophin receptor (p75) in retrograde axonal transport of the neurotrophins. *Neuron* **1995**, *14*, 1201-1211.

[71] Yamashita, T.; Tucker, K. L.; Barde, Y. A. Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. *Neuron* **1999**, *24*, 585-593.

[72] Bergmann, I.; Priestley, J. V.; McMahon, S. B.; Brocker, E. B.; Toyka, K. V.; Koltzenburg, M. Analysis of cutaneous sensory neurons in transgenic mice lacking the low affinity neurotrophin receptor p75. *Eur. J. Neurosci.* **1997**, *9*, 18-28.

[73] Scott, A. L.; Borisoff, J. F.; Ramer, M. S. Deafferentation and neurotrophin-mediated intraspinal sprouting: a central role for the p75 neurotrophin receptor. *Eur. J. Neurosci.* **2005**, *21*, 81-92.

[74] Jansen, P.; Giehl, K.; Nyengaard, J. R.; Teng, K.; Lioubinski, O.; Sjoegaard, S. S.; Breiderhoff, T.; Gotthardt, M.; Lin, F.; Eilers, A.; Petersen, C. M.; Lewin, G. R.; Hempstead, B. L.; Willnow, T. E.; Nykjaer, A. Roles for the pro-neurotrophin receptor sortilin in neuronal development, aging and brain injury. *Nat. Neurosci.* **2007**, *10*, 1449-1457.

[75] Bartus, R. T.; Dean, R. L., 3rd; Beer, B.; Lippa, A. S. The cholinergic hypothesis of geriatric memory dysfunction. *Science* **1982**, *217*, 408-414.

[76] Coyle, J. T.; Price, D. L.; DeLong, M. R. Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science* **1983**, *219*, 1184-1190.

[77] Perry, E.; Walker, M.; Grace, J.; Perry, R. Acetylcholine in mind: a neurotransmitter correlate of consciousness? *Trends Neurosci.* **1999**, *22*, 273-280.

[78] Van der Zee, C. E.; Ross, G. M.; Riopelle, R. J.; Hagg, T. Survival of cholinergic forebrain neurons in developing p75NGFR-deficient mice. *Science* **1996**, *274*, 1729-1732.

[79] Selkoe, D. J. Alzheimer's disease is a synaptic failure. *Science* **2002**, 298, 789-791.

[80] Yuan, J.; Yankner, B. A. Apoptosis in the nervous system. *Nature* **2000**, *407*, 802-809.

[81] Caughey, B.; Lansbury, P. T. Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. *Annu. Rev. Neurosci.* **2003**, *26*, 267-298.

[82] Fahnestock, M.; Michalski, B.; Xu, B.; Coughlin, M. D. The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease. *Mol. Cell Neurosci.* **2001**, *18*, 210-220.

[83] Peng, S.; Wuu, J.; Mufson, E. J.; Fahnestock, M. Increased proNGF levels in subjects with mild cognitive impairment and mild Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **2004**, *63*, 641-649.

[84] Counts, S. E.; Mufson, E. J. The role of nerve growth factor receptors in cholinergic basal forebrain degeneration in prodromal Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **2005**, *64*, 263-272.

[85] Pedraza, C. E.; Podlesniy, P.; Vidal, N.; Arevalo, J. C.; Lee, R.; Hempstead, B.; Ferrer, I.; Iglesias, M.; Espinet, C. Pro-NGF isolated from the human brain affected by Alzheimer's disease induces neuronal apoptosis mediated by p75NTR. *Am. J. Pathol.* **2005**, *166*, 533-543.

[86] Cuello, A. C.; Bruno, M. A. The failure in NGF maturation and its increased degradation as the probable cause for the vulnerability of cholinergic neurons in Alzheimer's disease. *Neurochem. Res.* **2007**, *32*, 1041-1045.

[87] Kuner, P.; Schubenel, R.; Hertel, C. Beta-amyloid binds to p57NTR and activates NFkappaB in human neuroblastoma cells. *J. Neurosci. Res.* **1998**, *54*, 798-804.

[88] Sotthibundhu, A.; Sykes, A. M.; Fox, B.; Underwood, C. K.; Thangnipon, W.; Coulson, E. J. Beta-amyloid(1-42) induces neuronal death through the p75 neurotrophin receptor. *J. Neurosci.* **2008**, *28*, 3941-3946.

[89] Yaar, M.; Zhai, S.; Fine, R. E.; Eisenhauer, P. B.; Arble, B. L.; Stewart, K. B.; Gilchrest, B. A. Amyloid beta binds trimers as well as monomers of the 75-kDa neurotrophin receptor and activates receptor signaling. *J. Biol. Chem.* **2002**, *277*, 7720-7725.

[90] Hashimoto, Y.; Kaneko, Y.; Tsukamoto, E.; Frankowski, H.; Kouyama, K.; Kita, Y.; Niikura, T.; Aiso, S.; Bredesen, D. E.; Matsuoka, M.; Nishimoto, I. Molecular characterization of neurohybrid cell death induced by Alzheimer's amyloid-beta peptides *via* p75NTR/PLAIDD. *J. Neurochem.* **2004**, *90*, 549-558.

[91] Costantini, C.; Della-Bianca, V.; Formaggio, E.; Chiamulera, C.; Montresor, A.; Rossi, F. The expression of p75 neurotrophin receptor protects against the neurotoxicity of soluble oligomers of beta-amyloid. *Exp. Cell Res.* **2005**, *311*, 126-134.

[92] Tsukamoto, E.; Hashimoto, Y.; Kanekura, K.; Niikura, T.; Aiso, S.; Nishimoto, I. Characterization of the toxic mechanism triggered by Alzheimer's amyloid-beta peptides *via* p75 neurotrophin receptor in neuronal hybrid cells. *J. Neurosci. Res.* **2003**, *73*, 627-636.

[93] Zhang, Y.; Hong, Y.; Bounhar, Y.; Blacker, M.; Roucou, X.; Tounekti, O.; Vereker, E.; Bowers, W. J.; Federoff, H. J.; Goodyer, C. G.; LeBlanc, A. p75 neurotrophin receptor protects primary cultures of human neurons against extracellular amyloid beta peptide cytotoxicity. *J. Neurosci.* **2003**, *23*, 7385-7394.

[94] Susen, K.; Blochl, A. Low concentrations of aggregated beta-amyloid induce neurite formation *via* the neurotrophin receptor p75. *J. Mol. Med.* **2005**, *83*, 720-735.

[95] Perini, G.; Della-Bianca, V.; Politi, V.; Della Valle, G.; Dal-Pra, I.; Rossi, F.; Armato, U. Role of p75 neurotrophin receptor in the neurotoxicity by beta-amyloid peptides and synergistic effect of inflammatory cytokines. *J. Exp. Med.* **2002**, *195*, 907-918.

[96] Binder, D. K. The role of BDNF in epilepsy and other diseases of the mature nervous system. *Adv. Exp. Med. Biol.* **2004**, *548*, 34-56.

[97] Kunugi, H.; Hashimoto, R.; Yoshida, M.; Tatsumi, M.; Kamijima, K. A missense polymorphism (S205L) of the low-affinity neurotrophin receptor p75NTR gene is associated with depressive disorder and attempted suicide. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **2004**, *129*, 44-46.

[98] McGregor, S.; Strauss, J.; Bulgin, N.; De Luca, V.; George, C. J.; Kovacs, M.; Kennedy, J. L. p75(NTR) gene and suicide attempts in young adults with a history of childhood-onset mood disorder. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **2007**, *144*, 696-700.

[99] Marenco, S.; Weinberger, D. R. The neurodevelopmental hypothesis of schizophrenia: following a trail of evidence from cradle to grave. *Dev. Psychopathol.* **2000**, *12*, 501-527.

[100] Weinberger, D. R. From neuropathology to neurodevelopment. *Lancet* **1995**, *346*, 552-557.

[101] Pilowsky, L. S.; Kerwin, R. W.; Murray, R. M. Schizophrenia: a neurodevelopmental perspective. *Neuropsychopharmacology* **1993**, *9*, 83-91.

[102] Seeman, P. Dopamine receptors and the dopamine hypothesis of schizophrenia. *Synapse* **1987**, *1*, 133-152.

[103] Laruelle, M.; Abi-Dargham, A.; van Dyck, C. H.; Gil, R.; D'Souza, C. D.; Erdos, J.; McCance, E.; Rosenblatt, W.; Fingado, C.; Zoghbi, S. S.; Baldwin, R. M.; Seibyl, J. P.; Krystal, J. H.; Charney, D. S.; Innis, R. B. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 9235-9240.

[104] Braff, D.; Stone, C.; Callaway, E.; Geyer, M.; Glick, I.; Bali, L. Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology* **1978**, *15*, 339-343.

[105] Braff, D. L.; Grillon, C.; Geyer, M. A. Gating and habituation of the startle reflex in schizophrenic patients. *Arch. Gen. Psychiatry.* **1992**, *49*, 206-215.

[106] Parwani, A.; Duncan, E. J.; Bartlett, E.; Madonick, S. H.; Efferen, T. R.; Rajan, R.; Sanfilipo, M.; Chappell, P. B.; Chakravorty, S.; Gonzenbach, S.; Ko, G. N.; Rotrosen, J. P. Impaired prepulse inhibition of acoustic startle in schizophrenia. *Biol. Psychiatry* **2000**, *47*, 662-669.

[107] Kunugi, H.; Tanaka, M.; Hori, H.; Hashimoto, R.; Saitoh, O.; Hironaka, N. Prepulse inhibition of acoustic startle in Japanese patients with chronic schizophrenia. *Neurosci. Res.* **2007**, *59*, 23-28.

[108] Davies, A. M. Nerve growth factor synthesis and nerve growth factor receptor expression in neural development. *Int. Rev. Cytol.* **1991**, *128*, 109-138.

[109] Rajakumar, N.; Leung, L. S.; Ma, J.; Rajakumar, B.; Rushlow, W. Altered neurotrophin receptor function in the developing prefrontal cortex leads to adult-onset dopaminergic hyperresponsivity and impaired prepulse inhibition of acoustic startle. *Biol. Psychiatry* **2004**, *55*, 797-803.

[110] McGrath, J. J.; Feron, F. P.; Burne, T. H.; Mackay-Sim, A.; Eyles, D. W. Vitamin D3-implications for brain development. *J. Steroid Biochem. Mol. Biol.* **2004**, *89-90*, 557-560.

[111] Gao, X.; Daugherty, R. L.; Tourtellotte, W. G. Regulation of low affinity neurotrophin receptor (p75(NTR)) by early growth response (Egr) transcriptional regulators. *Mol. Cell Neurosci.* **2007**, *36*, 501-14.

[112] Yamada, K.; Gerber, D. J.; Iwayama, Y.; Ohnishi, T.; Ohba, H.; Toyota, T.; Aruga, J.; Minabe, Y.; Tonegawa, S.; Yoshikawa, T. Genetic analysis of the calcineurin pathway identifies members of the EGR gene family, specifically EGR3, as potential susceptibility candidates in schizophrenia. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 2815-2820.

[113] Geuze, E.; Vermetten, E.; Bremner, J. D. MR-based *in vivo* hippocampal volumetrics: 2. Findings in neuropsychiatric disorders. *Mol. Psychiatry* **2005**, *10*, 160-184.

[114] Honea, R.; Crow, T. J.; Passingham, D.; Mackay, C. E. Regional deficits in brain volume in schizophrenia: a meta-analysis of voxel-based morphometry studies. *Am. J. Psychiatry* **2005**, *162*, 2233-2245.

[115] Steen, R. G.; Mull, C.; McClure, R.; Hamer, R. M.; Lieberman, J. A. Brain volume in first-episode schizophrenia: systematic review and meta-analysis of magnetic resonance imaging studies. *Br. J. Psychiatry* **2006**, *188*, 510-518.

[116] Campbell, S.; Macqueen, G. The role of the hippocampus in the pathophysiology of major depression. *J. Psychiatry Neurosci.* **2004**, *29*, 417-426.

[117] Czeh, B.; Lucassen, P. J. What causes the hippocampal volume decrease in depression? Are neurogenesis, glial changes and apoptosis implicated? *Eur. Arch. Psychiatry Clin. Neurosci.* **2007**, *257*, 250-260.

[118] Hof, P. R.; Haroutunian, V.; Friedrich, V. L., Jr.; Byne, W.; Buitron, C.; Perl, D. P.; Davis, K. L. Loss and altered spatial distribution of oligodendrocytes in the superior frontal gyrus in schizophrenia. *Biol. Psychiatry* **2003**, *53*, 1075-1085.

[119] Uranova, N. A.; Vostrikov, V. M.; Orlovskaya, D. D.; Rachmanova, V. I. Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: a study from the Stanley Neuropathology Consortium. *Schizophr. Res.* **2004**, *67*, 269-275.

[120] Longo, F. M.; Vu, T. K.; Mobley, W. C. The *in vitro* biological effect of nerve growth factor is inhibited by synthetic peptides. *Cell Regul.* **1990**, *1*, 189-195.

[121] Longo, F. M.; Manthorpe, M.; Xie, Y. M.; Varon, S. Synthetic NGF peptide derivatives prevent neuronal death *via* a p75 receptor-dependent mechanism. *J. Neurosci. Res.* **1997**, *48*, 1-17.

[122] Turner, B. J.; Murray, S. S.; Piccenna, L. G.; Lopes, E. C.; Kilpatrick, T. J.; Cheema, S. S. Effect of p75 neurotrophin receptor antagonist on disease progression in transgenic amyotrophic lateral sclerosis mice. *J. Neurosci. Res.* **2004**, *78*, 193-199.

[123] Massa, S. M.; Xie, Y.; Yang, T.; Harrington, A. W.; Kim, M. L.; Yoon, S. O.; Kraemer, R.; Moore, L. A.; Hempstead, B. L.; Longo, F. M. Small, non-peptide p75NTR ligands induce survival signaling and inhibit proNGF-induced death. *J. Neurosci.* **2006**, *26*, 5288-5300.

[124] Pehar, M.; Cassina, P.; Vargas, M. R.; Xie, Y.; Beckman, J. S.; Massa, S. M.; Longo, F. M.; Barbeito, L. Modulation of p75-dependent motor neuron death by a small non-peptidyl mimetic of the neurotrophin loop 1 domain. *Eur. J. Neurosci.* **2006**, *24*, 1575-1580.

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Impaired Secretion of Brain-Derived Neurotrophic Factor and Neuropsychiatric Diseases

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Abstract: Recent studies have elucidated mechanisms of brain-derived neurotrophic factor (BDNF) secretion, and impaired secretion of BDNF may be involved in the pathogenesis of several neuropsychiatric diseases. The huntingtin gene, for example, has been shown to regulate vesicular transport of BDNF, which may play a role in the neurodegeneration present in Huntington's disease. In animal studies, mice lacking calcium-dependent activator protein for secretion 2 (CADPS2), which is involved in the activity-dependent release of BDNF, showed several phenotypes including autistic behavior. A single nucleotide polymorphism that results in an amino-acid change (Val66Met) in the BDNF gene has been shown to cause a decline in the function of BDNF vesicular sorting and has been reported to be associated with behavioral and intermediate phenotypes (e.g., episodic memory) in humans. In this review, we introduce recent progress in the molecular mechanisms of BDNF secretion and discuss its possible role in the pathophysiology and treatment of neuropsychiatric diseases.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, has been implicated in a broad range of processes that are important for neuronal survival and synaptic plasticity in the central nervous system (CNS) [1-3]. Early in the 1950s, nerve growth factor (NGF) was discovered by Levi-Montalcini and Hamburger and Cohen [4,5] as a soluble factor that induced fiber outgrowth of chicken sympathetic neurons. Subsequently, Barde *et al.* [6] isolated BDNF, which was later found to be homologous to NGF [7], from pig brain as a neuronal survival factor. These discoveries motivated homology-based searches for additional family members of which there are currently a total of four in mammals – NGF, BDNF, neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). Additional members are conserved in fish – neurotrophin-6 (NT-6) and neurotrophin-7 (NT-7) [8,9]. All neurotrophins are secreted from neuronal (partially glial) cells and bind to their receptors in an autocrine/paracrine manner. In the last two decades, a bulk of studies have suggested that neurotrophins, especially BDNF, are involved in the pathophysiology of neuropsychiatric diseases through their role in the regulation of synaptic efficacy (synaptic plasticity) and synaptogenesis in the CNS. In this review, we focus on recent findings of secretion mechanisms of BDNF and their relationship with neuropsychiatric diseases.

I. BIOLOGICAL FUNCTIONS OF BDNF

i. Survival and Synaptic Plasticity

Neurotrophins exert their biological effects through the binding of secreted homodimeric neurotrophins to two types of transmembrane receptor proteins: the tyrosine kinase Trk (tropomyosin-related kinase) receptors and the low affinity common neurotrophin receptor (p75NTR). Neurotrophins are expressed in a precursor form (pro-neurotrophins) and are proteolytically processed to a mature form. Mature neurotrophins preferentially bind to their specific Trk receptor: NGF to TrkA, BDNF and NT-4/5 to TrkB and NT-3 to TrkC. Pro-neurotrophins, however, bind to p75NTR with higher affinity than mature neurotrophins, although they have a lower affinity for Trk receptors [10]. Binding of neurotrophins to Trk receptors immediately generates receptor dimerization and autophosphorylation of tyrosine residues in the intracellular kinase domain. Trk receptor phosphorylation activates intracellular signaling regulated by mitogen-activated protein kinase (MAPK), phosphoinositide-3 (PI3)-kinase/Akt and phospholipase C-γ (PLC-γ) pathways as well as several small G proteins, including Ras, Rap-1, and the CDC-42-Rac-Rho family [11-13]. These intracellular signaling cascades modulate expression of genes and are responsible for most of the long-term effects of neurotrophins related to neuronal growth, survival, and differentiation [14]. On the other hand, binding of pro-neurotrophins to p75NTR leads to antagonistic effects to Trk receptor signaling. Several of these p75NTR-dependent signaling are pro-apoptotic and can be suppressed by Trk receptor-initiated signaling [15,16]. The first evidence of a significant relationship between neurotrophins and synaptic plasticity was obtained by Lohof *et al.* [17]; exogenous BDNF and NT-3 increased synaptic efficacy at the *Xenopus* neuromuscular junction. Subsequently, these neurotrophins were shown to facilitate glutamatergic synaptic transmission, even in the hippocampus of the mammalian CNS [18-20]. There is now substantial evidence implicating BDNF in activity-dependent long-term synaptic plasticity [21,22]. The neurotrophin-binding Trk receptor activates many kinds of signaling pathways that promote neuronal survival and synaptic efficiency, although it is still unclear how the complex signaling pathways are

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systematically integrated to generate many biological functions.

ii. Activity-Dependent Expression of Neurotrophins

Among neurotrophins, BDNF shows the most ubiquitous expression in the developing and adult mammalian brain. BDNF expression levels are increased dramatically during the first few weeks of postnatal development. Expression of neurotrophins in neurons is linked to neuronal activity. BDNF and NGF mRNA levels are rapidly increased by seizure activity in the hippocampus and the cerebral cortex [23-25]. In contrast, blockade of visual input causes rapid down-regulation of BDNF mRNA in the rat visual cortex of dark-reared animals [26]. A similar phenomenon has been found in cultured neurons. The introduction of glutamate with high concentration potassium-induced depolarization increase levels of BDNF and NGF mRNA, while blockade of neuronal activity with γ-aminobutyric acid (GABA) decreases such levels [27,28].

iii. Processes of BDNF Secretion

BDNF is synthesized as a 32 kDa precursor protein (proBDNF) and proteolytically cleaved to generate the mature BDNF (13 kDa). The synthesis of the pro-BDNF occurs at the rough endoplasmic reticulum (ER). Following this, pro-BDNF is transported to the Golgi apparatus and concentrated in membrane stacks of the *trans*-Golgi network (TGN). Finally, BDNF-containing vesicles bud off the TGN to eventually transport to the releasing sites. Recent studies clarified some of the details of BDNF vesicular sorting. Specifically, the pro-region of BDNF has been implicated as a regulator of BDNF sorting to secretory vesicles [29]. Moreover, fusing the pro-region of BDNF to NT-4, which is rarely sorted into secretory vesicles, allowed NT-4 to sort more efficiently into specific vesicles [30]. These data support the importance of the BDNF pro-region as a potential target to help guide secretory granules. Furthermore, binding of BDNF to the lipid-raft-associated sorting receptor carboxypeptidase E (CPE) in the TGN is also important for sorting into secretory vesicles of the regulated pathway [31]. Sortilin, a trans-membrane protein, has also been implicated in the sorting of BDNF to secretory glanules. Sortilin is expressed in secretory granules and interacts specifically with the pro-region of BDNF. Interestingly, the truncated form of sortilin results in missorting of BDNF to the secretory vesicles [32].

It is still controversial as to where and how pro-neurotrophins are processed into mature neurotrophins in the CNS. Originally, it had been thought that pro-neurotrophins are prototypically cleaved by furin and pro-protein convertases (PCs) in the TGN or in secretory granules before secretion [33]. However, recent studies have indicated that a considerable amount of BDNF is secreted in the pro-form from neurons. Released pro-BDNF is subsequently processed to mature BDNF extracellularly by proteases such as plasmin or matrix metalloproteinases [34,35]. More recently, however, it was shown that pro-BDNF is rapidly converted into mature BDNF intracellularly and almost all BDNF was secreted as the mature form from hippocampal neurons [36].

iv. Constitutive and Regulated Secretion

Secretion of neurotrophins is classified into "constitutive" and "regulated" pathways, depending on whether the secretion occurs spontaneously or in response to neuronal activity, respectively. In hippocampal neurons, BDNF appears to be sorted primarily into the regulated pathway [37-39]. In the regulated pathway, BDNF-containing vesicles are transported into either presynaptic axon terminals or postsynaptic dendrites along microtubules for activity-dependent secretion [40-43]. Recently, Lessmann and colleagues conducted an elegant study that provided the long-awaited understanding of BDNF secretion. The activity-dependent postsynaptic secretion of neurotrophins critically depends on Ca^{2+} influx *via* ionotropic glutamate receptors or voltage-gated Ca^{2+} channels, Ca^{2+} release from internal stores, activation of Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), and intact protein kinase A (PKA) signaling. Trk signaling and activation of Na^{+} channels, on the other hand, are not required for BDNF secretion [44-46]. Furthermore, recent reports suggest that the Golgi apparatus exists in dendrites as well as the cell soma, and have gone so far as to identify a local BDNF secretory pathway in neuronal dendrites [47,48]. Future works may reveal more details concerning the secretory systems of neurotrophins at the subcellular level and that may be more complex and dynamic than we can presently imagine.

II. IMPAIRED SECRETION OF BDNF AND NEUROPSYCHIATRIC DISEASES

i. Huntington's Disease

Huntington's disease (HD) is a fatal, dominantly inherited, neurodegenerative disease that usually presents during midlife. It is characterized by relatively selective degeneration of striatal neurons which lead to psychiatric, cognitive and motor dysfunction. Polyglutamine expansion (polyQ) in the protein huntingtin (htt) is thought to be the principal mechanism for the neuronal toxicity in HD. Recently, evidence has indicated the possible link between HD and BDNF. Wild type htt plays a role as a transcription factor and facilitates expression of BDNF [49]. Furthermore, htt has been implicated in BDNF-containing vesicle transport. Mutant (PolyQ)-htt perturbs post-Golgi trafficking of BDNF in the regulated secretory pathway, though it does not influence the constitutive pathway [50,51]. Conversely, the exogenously transfected BDNF gene generated increased BDNF levels and TrkB signaling in the striatum, which resulted in improved symptoms in HD model mice [52]. These findings suggest that mutation of htt reduces levels of BDNF in the striatum by inhibiting gene expression and perturbing anterograde transport of BDNF-containing vesicles from cortex to striatum. Therefore, the development of therapies focused on the reduction of BDNF release should be important for future studies.

ii. Rett Syndrome

Rett syndrome (RTT) is an X-linked disorder characterized by arrested neurological development and subsequent cognitive decline. Methylation of DNA in vertebrates occurs preferentially on cytosine residues of dinucleotides in which the cytosine is followed by a guanine residue (CpGs). Meth-

ylated CpGs bind a variety of proteins. One of these proteins, methyl-CpG binding protein 2 (MeCP2), has been implicated in the long-term silencing of gene expression. Inactivating mutations in MeCP2 is caused in the majority of cases with Rett syndrome. Chen *et al.* showed that MeCP2 selectively binds to the BDNF promoter III and represses expression of BDNF [53]. Membrane depolarization triggers the calcium-dependent phosphorylation and release of MeCP2 from the BDNF promoter III, thereby facilitating transcription [54]. A conditional BDNF transgene increased BDNF expression in the MeCP2 mutant brain, which resulted in rescue of locomotor defects, recovery of electrophysiological deficits, and extension of lifespan in MeCP2 mutant animals [55]. Although MeCP2 null mice exhibited a slightly decreased content of BDNF in some brain areas, mutant neurons demonstrated equivalent secretion levels of BDNF compared to wild-type in response to high-frequency electrical stimulation [56]. Furthermore, BDNF expression in MeCP2 null neurons was significantly improved by chronic ampakine treatment, which was administered to facilitate AMPA receptor activation [57]. These results suggest that the expression of BDNF is still plastic in the MeCP2 null condition and manipulating the BDNF level or the BDNF signaling pathways may provide therapeutic opportunities for RTT patients.

iii. Autism

Autism is a severe neurodevelopmental disorder with a childhood onset, characterized by profound disturbances in socialization, language skills, communicative, and behavioral functions. BDNF is expressed abnormally in individuals with autism and, as a result, may be involved in the pathogenesis of autism [58,59]. Elevated levels of BDNF and NT4/5 measured by archived neonatal blood samples of autistic patients were reported [60]. Elevation of BDNF was also reported in a study of 18 Japanese children with autism compared with controls [61]. These findings suggest that excess BDNF during childhood may be involved in the neurobiological abnormalities observed in autism. The specific molecular mechanisms involving BDNF and autism remain unknown, though one report suggests that genetic changes in autistic individuals may account for altered neurotrophin levels [62]. Ca^{2+}-dependent activator protein for secretion 2 (CAPS2/CADPS2) is a secretory granule-associated protein that is abundant at the parallel fiber terminals of granule cells in the mouse cerebellum and is involved in the release of BDNF and NT-3. The human CAPS2/CADPS2 gene is located on chromosome 7q31.32 within a critical autism susceptibility locus 1 (AUTS1). CAPS2 knock-out mice demonstrate autistic-like behavioral phenotypes and deficient release of BDNF and NT-3. Moreover, phosphorylation of Trk receptors is decreased in the cerebellum, which may play a role in the pronounced impairment of cerebellar development and function, including neuronal survival, differentiation and migration of postmitotic granule cells, that these mice exhibit [63]. Although there have been few reports suggesting the relation between autism and BDNF secretion, further investigation may result in novel insights.

iv. Epilepsy

Epilepsy is a neurological disorder characterized by recurrent and unpredictable seizures. Various studies have shown that BDNF increases neuronal excitability and is up-regulated in areas implicated in epileptogenesis. Seizure activity increases expression of BDNF mRNA and protein, and recent studies have shown that interfering with BDNF signal transduction inhibits the development of the epileptic state *in vivo* [64]. Half of all drug-resistant individuals experience seizure control with dietary manipulation, such as isocaloric substitution of carbohydrates with fats and protein referred to as the 'ketogenic diet'. Daley *et al.* reported that an inhibitor of glycolysis is shown to have antiepileptic effects in the rat kindling model, which may be related to NADH-dependent regulation of BDNF expression [65]. This result may explain how the 'ketogenic diet' treatment works. Although it is unclear whether the up-regulation of BDNF is the cause or the consequence of epilepsy, the reduction of BDNF expression or BDNF signaling can be a useful tool for the treatment of epilepsy.

v. Psychiatric Disorders

Mood and anxiety disorders are the most common psychiatric diseases. BDNF has been implicated in these disorders, because decreased levels of BDNF in the hippocampus are correlated with stress-induced depressive behaviors [66]. Other studies also showed decreased plasma levels of BDNF in patients with major depression [67]. Many classes of antidepressants, including selective serotonin reuptake inhibitors, significantly increase BDNF mRNA expression in the hippocampus and prefrontal cortex [68,69]. The time course of such increase is consistent with the slow onset of therapeutic effects of antidepressants. More recently, striking evidence for the involvement of TrkB-dependent neurogenesis in the antidepressant effect has been reported. Mice lacking TrkB in the hippocampal neuron progenitor cells had impaired neurogenesis and proliferation induced by antidepressant treatment. These mice also demonstrated increased anxiety-like behavior and decreased sensitivity to antidepressants [70,71]. Taken together, BDNF may play a key role in the brains of recovering patients during antidepressant treatment [72,73].

Many reports have isolated the possible association between BDNF levels and schizophrenia in several brain regions [74]. However, results from these studies are contradictory in that some demonstrate decreased BDNF levels in the postmortem brain or serum, while others report that the BDNF level in patients was not significantly different from that in normal controls [75]. Moreover, samples used in each experiment differ in age, species (rodents, primates, human) and regions (i.e., hippocampus, frontal cortex, CSF and blood) [76]. Although there have been many studies examining the possible role of BDNF in schizophrenia, integrated knowledge concerning this has not been produced. Despite this, the neurobiological vulnerability paradigm remains an attractive concept, supporting that increased susceptibility may be a consequence of reduced expression of BDNF (neurotrophins) at a certain point of life [77].

In the region encoding BDNF's pro-region, a SNP was identified at amino acid 66 (Val66Met). Egan and colleagues reported that the met allele was associated with decreased episodic memory and abnormal hippocampal activation as assayed with fMRI in human subjects [29]. Furthermore, neurons transfected with met-BDNF-GFP showed lower

depolarization-induced secretion, while constitutive secretion was unchanged. Met-BDNF-GFP failed to localize to secretory granules or synapses [29]. Following this, a number of association studies of this polymorphism with psychiatric disorders have been done. Unexpectedly, the Met66 allele, which reduces BDNF release, has been suggested to be protective against developing bipolar disorder [78], although this association was not confirmed by large-scale studies [79,80]. The Met66 allele has also been implicated in other disorders like anorexia [81]. Future research is required to assess how the Val66Met is associated with particular psychiatric disorders.

CONCLUSIONS

The biological mechanisms of neurotrophins are critically important for neuronal functions that affect brain functions and behavior. Growing evidence has implicated BDNF in the pathophysiology of many neuropsychiatric diseases. Genetic variations leading to deranged expression or secretion due to altered transcription, vesicular sorting, vesicular trafficking and secretion of BDNF seem to play an important role in several neuropsychiatric diseases and related behavioral phenotypes. In order to develop treatment strategies for these diseases through targeting neurotrophins and their receptors, however, clarification of more detailed mechanisms is needed. Studies that reveal not only an increase/decrease in expression of neurotrophins, but also accurate spatiotemporal secretion profiles of neurotrophins are necessary.

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REFERENCES

[1] Lu B. Acute and long-term synaptic modulation by neurotrophins. Prog Brain Res 2004; 146: 137-50.

[2] Poo MM. Neurotrophins as synaptic modulators. Nat Rev Neurosci 2001; 2(1): 24-32.

[3] Barde YA. Trophic factors and neuronal survival. Neuron 1989; 2(6): 1525-34.

[4] Levi-Montalcini R, Hamburger V. A diffusible agent of mouse sarcoma, producing hyperplasia of sympathetic-ganglia and hyperneurotization of viscera in the chick embryo. J Exp Zool 1953; 123: 233-87.

[5] Cohen S, Levi-Montalcini R. A nerve growth-stimulating factor isolated from snake venom. Proc Natl Acad Sci USA 1956; 42: 571.

[6] Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. EMBO J 1982; 1: 549-53.

[7] Leibrock J, Lottspeich F, Hohn A, *et al.* Molecular cloning and expression of brain-derived neurotrophic factor. Nature 1989; 341: 149-52.

[8] Gotz R, Koster R, Winkler C, *et al.* Neurotrophin-6 is a new member of the nerve growth factor family. Nature 1994; 372: 266-69.

[9] Lai KO, Fu WY, Ip FCF, Ip NY. Cloning and expression of a novel neurotrophin, NT-7, from carp. Mol Cell Neurosci 1998; 11: 64-76.

[10] Lee R, Kermani P, Teng KK, Hempstead BL. Regulation of cell survival by secreted proneurotrophins. Science 2001; 294: 1945-48.

[11] Mazzoni IE, Said FA, Aloyz R, Miller FD, Kaplan D. Ras regulates sympathetic neuron survival by suppressing the p53-mediated cell death pathway. J Neurosci 1999; 19: 9716-27.

[12] Grewal SS, York RD, Stork PJ. Extracellular-signal-regulated kinase signalling in neurons. Curr Opin Neurobiol 1999; 9: 544-53.

[13] Vaillant AR, Mazzoni I, Tudan C, Boudreau M, Kaplan DR, Miller FD. Depolarization and neurotrophins converge on the phosphatidylinositol 3-kinase-Akt pathway to synergistically regulate neuronal survival. J Cell Biol 1999; 146: 955-66.

[14] Blum R, Konnerth A. Neurotrophin-mediated rapid signaling in the central nervous system: mechanisms and functions. Physiology 2005; 20: 70-8.

[15] Majdan M, Miller, FD. Neuronal life and death decisions functional antagonism between the Trk and p75 neurotrophin receptors. Int J Dev Neurosci 1999; 17: 153-61.

[16] Yoon SO, Casaccia-Bonnefil P, Carter B, Chao MV. Competitive signaling between TrkA and p75 nerve growth factor receptors determines cell survival. J Neurosci 1998; 18: 3273-81.

[17] Lohof AM, Ip NY, Poo MM. Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. Nature 1993; 363: 350-53.

[18] Lessmann V, Gottmann K, Heumann R. BDNF and NT-4/5 enhance glutamatergic synaptic transmission in cultured hippocampal neurones. NeuroReport 1994; 6: 21-25.

[19] Kang H, Schuman EM. A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. Science 1996; 273: 1402-06.

[20] Levine ES, Dreyfus CF, Black IB, Plummer MR. Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons *via* postsynaptic tyrosine kinase receptors. Proc Natl Acad Sci USA 1995; 92: 8074-77.

[21] Korte M, Carroll P, Wol E, Brem G, Thoenen H, Bonhoeffer T. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc Natl Acad Sci USA 1995; 92: 8856-60.

[22] Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER. Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron 1996; 16: 1137-45.

[23] Gall C M, Isackson PJ. Limbic seizures increase neuronal production of messenger RNA for nerve growth factor. Science 1989; 245: 758-61.

[24] Zafra F, Hengerer B, Leibrock J, Thoenen H, Lindholm D. Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. EMBO J 1990; 9: 3545-50.

[25] Ernfors P, Bengzon J, Kokaia Z, Persson H, Lindvall O. Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis. Neuron 1991; 7: 165-76.

[26] Castraen E, Zafra F, Thoenen H, Lindholm D. Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. Proc Natl Acad Sci USA 1992; 89: 9444-48.

[27] Lindholm D, Castren E, Berzaghi M, Blochl A, Thoenen H. Activity-dependent and hormonal regulation of neurotrophin mRNA levels in the brain - implications for neuronal plasticity. J Neurobiol 1994; 25: 1362-72.

[28] Berninger B, Marty S, Zafra F, Berzaghi MP, Thoenen H. GABAergic stimulation switches from enhancing to repressing BDNF expression in rat hippocampal neurons during maturation *in vitro*. Development 1995; 121: 2327-35.

[29] Egan MF, Kojima M, Callicott JH, *et al.* The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 2003; 112(2): 257-69.

[30] Brigadski T, Hartmann M, Lessmann V. Differential vesicular targeting and time course of synaptic secretion of the mammalian neurotrophins. J Neurosci 2005; 17; 25(33): 7601-14.

[31] Lou H, Kim SK, Zaitsev E, Snell CR, Lu B, Loh YP. Sorting and activity-dependent secretion of BDNF require interaction of a specific motif with the sorting receptor carboxypeptidase E. Neuron 2005; 45(2): 245-55.

[32] Chen ZY, Ieraci A, Teng H, *et al.* Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. J Neurosci 2005; 25(26): 6156-66.

[33] Seidah NG, Benjannet S, Pareek S, *et al.* Cellular processing of the nerve growth factor precursor bythe mammalian pro-protein convertases. Biochem J 1996; 314: 951-60.

[34] Pang PT, Teng HK, Zaitsev E, *et al.* Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science 2004; 306(5695): 487-91.

[35] Lu B, Pang PT, Woo NH. The yin and yang of neurotrophin action. Nat Rev Neurosci 2005; 6(8): 603-14.

[36] Matsumoto T, Rauskolb S, Polack M, *et al.* Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. Nat Neurosci 2008; 11(2): 131-3.

[37] Farhadi HF, Mowla SJ, Petrecca K, Morris SJ, Seidah NG, Murphy, RA. Neurotrophin-3 sorts to the constitutive secretory pathway of hippocampal neurons and is diverted to the regulated secretory pathway by coexpression with brain-derived neurotrophic factor. J Neurosci 2000; 20: 4059-68.

[38] Goodman LJ, Valverde J, Lim F, *et al.* Regulated release and polarized localization of brain-derived neurotrophic factor in hippocampal neurons. Mol Cell Neurosci 1996; 7: 223-28.

[39] Mowla SJ, Farhadi HF, Pareek S, *et al.* Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. J Biol Chem 2001; 276: 12660-66.

[40] Kojima M., Takei N, Numakawa T, *et al.* Biological characterization and optical imaging of brain-derived neurotrophic factor-green fluorescent protein suggest an activitydependent local release of brain-derived neurotrophic factor in neurites of cultured hippocampal neurons. J Neurosci Res 2001; 64: 1-10.

[41] Kohara K, Kitamura A, Morishima M, Tsumoto T. Activity-dependent transfer of brain-derived neurotrophic factor to postsynaptic neurons. Science 2001; 291(5512): 2419-23.

[42] Gartner A, Staiger V. Neurotrophin secretion from hippocampal neurons evoked by long-term-potentiation-inducing electrical stimulation patterns. Proc Natl Acad Sci USA 2002; 99: 6386-91.

[43] Adachi N, Kohara K, Tsumoto T. Difference in trafficking of brain-derived neurotrophic factor between axons and dendrites of cortical neurons, revealed by live-cell imaging. BMC Neurosci 2005; 6(1): 42.

[44] Hartmann M, Heumann R, Lessmann V. Synaptic secretion of BDNF after high-frequency stimulation of glutamatergic synapses. EMBO J 2001; 20(21): 5887-97.

[45] Kolarow R, Brigadski T, Lessmann V. Postsynaptic secretion of BDNF and NT-3 from hippocampal neurons depends on calcium calmodulin kinase II signaling and proceeds *via* delayed fusion pore opening. J Neurosci 2007; 27(39): 10350-64.

[46] Lessmann V, Gottmann K, Malcangio M. Neurotrophin secretion: current facts and future prospects. Prog Neurobiol 2003; 69(5): 341-74.

[47] Horton AC, Ehlers MD. Dual modes of endoplasmic reticulum-to-Golgi transport in dendrites revealed by live-cell imaging. J Neurosci 2003; 23(15): 6188-99.

[48] Horton AC, Rácz B, Monson EE, Lin AL, Weinberg RJ, Ehlers MD. Polarized secretory trafficking directs cargo for asymmetric dendrite growth and morphogenesis. Neuron 2005; 48(5): 757-71.

[49] Zuccato C, Cattaneo E. Role of brain-derived neurotrophic factor in Huntington's disease. Prog Neurobiol 2007; 81(5-6): 294-330.

[50] Gauthier LR, Charrin BC, Borrell-Pagès M, *et al.* Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell 2004; 118(1): 127-38.

[51] del Toro D, Canals JM, Ginés S, Kojima M, Egea G, Alberch J. Mutant huntingtin impairs the post-Golgi trafficking of brain-derived neurotrophic factor but not its Val66Met polymorphism. J Neurosci 2006; 26(49): 12748-57.

[52] Gharami K, Xie Y, An JJ, Tonegawa S, Xu B. Brain-derived neurotrophic factor over-expression in the forebrain ameliorates Huntington's disease phenotypes in mice. J Neurochem 2008; 105(2): 369-79.

[53] Chen WG, Chang Q, Lin Y, *et al.* Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science 2003; 302(5646): 885-9.

[54] Martinowich K, Hattori D, Wu H, *et al.* DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science 2003; 302(5646): 890-3.

[55] Chang Q, Khare G, Dani V, Nelson S, Jaenisch R. The disease progression of Mecp2 mutant mice is affected by the level of BDNF expression. Neuron 2006; 49(3): 341-8.

[56] Wang H, Chan SA, Ogier M, *et al.* Dysregulation of brain-derived neurotrophic factor expression and neurosecretory function in Mecp2 null mice. J Neurosci 2006; 26(42): 10911-5.

[57] Ogier M, Wang H, Hong E, Wang Q, Greenberg ME, Katz DM. Brain-derived neurotrophic factor expression and respiratory function improve after ampakine treatment in a mouse model of Rett syndrome. J Neurosci 2007; 27(40): 10912-7.

[58] Tsai SJ. Is autism caused by early hyperactivity of brain-derived neurotrophic factor? Med Hypotheses 2005; 65(1): 79-82.

[59] Nelson PG, Kuddo T, Song EY, *et al.* Selected neurotrophins, neuropeptides, and cytokines: developmental trajectory and concentrations in neonatal blood of children with autism or Down syndrome. Int J Dev Neurosci 2006; (1): 73-80.

[60] Nelson KB, Grether JK, Croen LA, *et al.* Neuropeptides and neurotrophins in neonatal blood of children with autism or mental retardation. Ann Neurol 2001; 49: 597-606.

[61] Miyazaki K, Narita N, Sakuta R, *et al.* Serum neurotrophin concentrations in autism and mental retardation: a pilot study. Brain Dev 2004; 26: 292-95.

[62] Sadakata T, Kakegawa W, Mizoguchi A, *et al.* Impaired cerebellar development and function in mice lacking CAPS2, a protein involved in neurotrophin release. J Neurosci 2007; 27(10): 2472-82.

[63] Sadakata T, Mizoguchi A, Sato Y, *et al.* The secretory granule-associated protein CAPS2 regulates neurotrophin release and cell survival. J Neurosci 2004; 24(1): 43-52.

[64] Binder DK, Croll SD, Gall CM, Scharfman HE. BDNF and epilepsy: too much of a good thing? Trends Neurosci 2001; (1): 47-53.

[65] Daley TJ, Pfender RM, Morrison JF, *et al.* 2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP-dependent metabolic regulation of chromatin structure. Nat Neurosci 2006; (11): 1382-7.

[66] Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry 2006; 59: 1116-27.

[67] Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res 2002; 109: 143-48.

[68] Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 1995; 15: 7539-47.

[69] Russo-Neustadt AA, Beard RC, Huang YM, Cotman CW. Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus. Neuroscience 2000; 101: 305-12.

[70] Li Y, Luikart BW, Birnbaum S, *et al.* TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. Neuron 2008; 59(3): 399-412.

[71] Bergami M, Rimondini R, Santi S, Blum R, Götz M, Canossa M. Deletion of TrkB in adult progenitors alters newborn neuron integration into hippocampal circuits and increases anxiety-like behavior. Proc Natl Acad Sci USA 2008; 105(40): 15570-5

[72] Martinowich K, Manji H, Lu B. New insights into BDNF function in depression and anxiety. Nat Neurosci 2007; (9):1089-93.

[73] Angelucci F, Brenè S, Mathé AA. BDNF in schizophrenia, depression and corresponding animal models. Mol Psychiatry 2005; (4): 345-52.

[74] William RP, Cynthia SW, Mayada A, Joel EK. Postmortem investigations of the pathophysiology of schizophrenia: the role of susceptibility genes. J Psychiatry Neurosci 2004; 29: 287-93.

[75] Ikeda Y, Yahata N, Ito I, *et al.* Low serum levels of brain-derived neurotrophic factor and epidermal growth factor in patients with chronic schizophrenia. Schizophr Res 2008; 101(1-3): 58-66.

[76] Buckley PF, Mahadik S, Pillai A, Terry A Jr. Neurotrophins and schizophrenia. Schizophr Res 2007; 94(1-3): 1-11.

[77] Castrén E. Neurotrophic effects of antidepressant drugs. Curr Opin Pharmacol 2004; (1): 58-64.

[78] Ribasés M, Gratacós M, Armengol L, *et al.* Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. Mol Psychiatry 2003; (8): 745-51.

[79] Kunugi H, Iijima Y, Tatsumi M, *et al.* No association between the Val66Met polymorphism of the brain-derived neurotrophic factor gene and bipolar disorder in a Japanese population: a multicenter study. Biol Psychiatry 2004; 56: 376-8.

[80] Green EK, Raybould R, Macgregor S, *et al.* Genetic variation of brain-derived neurotrophic factor (BDNF) in bipolar disorder: case-control study of over 3000 individuals from the UK. Br J Psychiatry 2006; 188: 21-5.

[81] Groves JO. Is it time to reassess the BDNF hypothesis of depression? Mol Psychiatry 2007; (12): 1079-88.

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BIOLOGICAL PSYCHIATRY - ORIGINAL ARTICLE

Failure to confirm an association between *Epsin 4* and schizophrenia in a Japanese population

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Abstract Previous studies suggested that genetic variations in the 5′ region of *Epsin 4*, a gene encoding enthoprotin on chromosome 5q33, are associated with schizophrenia. However, conflicting results have also been reported. We examined the possible association in a Japanese sample of 354 patients and 365 controls. Seventeen polymorphisms of *Epsin 4* [3 microsatellites and 14 single nucleotide polymorphisms (SNPs)] were selected. A microsatellite marker (D5S1403) demonstrated a significant difference in the allele frequency between patients and controls (uncorrected $P = 0.04$). However, there was no significant difference in the genotype or allele frequency between the two groups for the other microsatellites or SNPs. Haplotype-based analysis provided no evidence for an association. The positive result at D5S1403 no longer reached statistical significance when multiple testing was taken into consideration. Our results suggest that the examined region of *Epsin 4* does not have a major influence on susceptibility to schizophrenia in Japanese.

Keywords Association study · *Epsin 4* · Enthoprotin · Schizophrenia · Polymorphism

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Introduction

Schizophrenia is a debilitating psychiatric disorder that affects approximately 1% of the world's population (Fenton et al. 2003; Hyman 2000). Patients may suffer from delusions, hallucinations, disorganized speech and behavior, as well as impairment in short-term verbal and nonverbal memory. The complete etiology of the disease remains unknown, though twin and adoption studies have demonstrated that schizophrenia is highly heritable (estimated heritability of >80%) (Cardno et al. 1999). While the contributing genes and pathophysiological mechanisms remain elusive, identifying the susceptibility genes is essential in discovering the true pathogenesis of schizophrenia. The mode of schizophrenia transmission is complex and is thought to be polygenic (Owen et al. 2004). Thus far, linkage and association studies have been successful approaches in searching for complex disease genes, discovering such candidate genes as neuregulin 1, dysbindin, G72, and D-amino acid oxidase (DAAO). Through such analysis, several chromosomal regions have been identified and investigated as potential sources for schizophrenia susceptibility genes (Chumakov et al. 2002; Stefansson et al. 2002; Straub et al. 2002a). As a result, the long arm of chromosome 5q has been identified as a putative chromosomal region of interest and subsequently investigated for susceptibility loci (Lewis et al. 2003; Straub et al. 2002b).

An association study of English, Irish, Welsh, and Scottish populations found *Epsin 4* on chromosome 5q33 as a strong candidate for schizophrenia susceptibility (Pimm et al. 2005). Four associated polymorphisms were discovered at the 5′ end of *Epsin 4*, a gene encoding the clathrin-associated protein enthoprotin. These included two microsatellite markers, D5S1403 and AAAT11, and

two single-nucleotide-polymorphism (SNP) markers, rs10046055 and rs254664. Enthoprotin plays a critical role in the formation, transport and stability of clathrin-coated vesicles (CCVs) and is therefore thought to regulate the transport and storage of neurotransmitters in the brain (McPherson and Ritter 2005; Wasiak et al. 2002). Neuronal CCVs are critical in the insertion and recycling of neurotransmitter receptors at the postsynaptic membrane and have been implicated as a regulatory mechanism for synaptic plasticity (Blanpied et al. 2002; Wang and Linden 2000). Specifically, CCVs facilitate AMPA receptor trafficking on the postsynaptic membrane, therefore affecting overall glutamatergic neurotransmission (Malinow and Malenka 2002; Man et al. 2002). Dysfunction of enthoprotin could, therefore, stimulate disturbances in glutamatergic brain signaling as well as synaptic plasticity, both postulated to be integral components of schizophrenia pathophysiology (Christison et al. 1989; Carter et al. 2006). Moreover, it has been demonstrated that enthoprotin interacts with the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex found in hippocampal pyramidal cells (Antonin et al. 2000; Chidambaram et al. 2004). This suggests a possible role for enthoprotin in calcium-mediated vesicle fusion of excitatory neurotransmitters released from the hippocampus. If the SNARE complex does not function properly in this way, abnormal neural connectivity may result, which is another characteristic of schizophrenia (Honer et al. 2002).

Thus far, two replication studies have been conducted in the Han Chinese population (Liou et al. 2006; Tang et al. 2006). Both studies found no association between the four markers previously reported (D5S1403, AAAT11, rs10046055, or rs254664) and schizophrenia. Tang et al. (2006), however, did detect haplotypes near the 5′ end of *Epsin 4* (252/T consisted of AAAT11 and rs10046055, global $P = 0.0021$; T/T of rs1145603 and rs254664, $P = 0.0033$) showing an association with schizophrenia in Han Chinese family trios. Liou et al. (2006) analyzed nine SNPs on *Epsin 4* in a case–control design and found a significant difference in the allele frequency of a SNP on the 5′ upstream region of *Epsin 4* (rs1186922); however, this difference was not significant after multiple testing. These conflicting results prompted us to examine *Epsin 4* for an association with schizophrenia in a Japanese sample.

Materials and methods

Subjects

Subjects were 354 patients with schizophrenia [212 males, mean age of 44.0 years (SD 13.7)] and 365 healthy controls [113 males, mean age of 39.7 years (SD 14.1)]. All subjects were biologically unrelated Japanese and recruited from the same geographical area (Western part of Tokyo Metropolitan). Consensus diagnosis by at least two psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria (American Psychiatric Association 1994) on the basis of unstructured interviews and information from medical records. The controls were healthy volunteers recruited from hospital staffs and their associates. Control individuals were interviewed and those with a current or past history of psychiatric treatment or regular use of psychotropic agents were not enrolled in the study. Participants were excluded from both the patient and control groups if they had prior medical histories of central nervous system disease or severe head injury, or if they met criteria for alcohol/drug dependence or mental retardation. The study protocol was approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. After description of the study, written informed consent was obtained from every subject.

Genotyping

Venous blood was drawn from the subjects and Genomic DNA was extracted from peripheral leukocytes using the Wizard Genomic DNA Purification System kit (Promega, Madison, WI, USA) based on the solution-based method according to the manufacturer's instructions. Fourteen SNPs (rs1186922, rs10046055, rs1894962, rs6556290, rs7735412, rs1145585, rs1186930, rs1145603, rs1186934, rs11744778, rs1145602, rs1186998, rs17055032, rs254664) and three microsatellites (D5S1400, D5S1403, AAAT11) were genotyped. Five SNPs (rs1186922, rs10046055, rs1186930, rs1145603, rs254664) were selected based on previous studies conducted by Liou et al. (2006), Tang et al. (2006), and Pimm et al. (2005). The three microsatellites demonstrated to be significantly associated with schizophrenia by Pimm et al. (2005) and Tang et al. (2006) were selected in order to replicate their positive findings. The other nine SNPs (rs1894962, rs6556290, rs7735412, rs1145585, rs1186934, rs11744778, rs1145602, rs1186998, rs17055032) were selected from the international HapMap project (http://hapmap.org/index.html.en) using Paul de Bakker's Tagger algorithm in the Haploview V 3.32 program. The 17 studied polymorphisms cover approximately 193 kb of *Epsin 4* from the 5′ upstream region to intron 1 (Fig. 1).

The SNPs were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay. TaqMan probes of the assay on demand (rs1186922, rs10046055, rs6556290, rs7735412, rs1145585, rs1186930, rs1145603, rs1186934, rs11744778, rs1145602, rs1186998, rs17055032, rs254664)

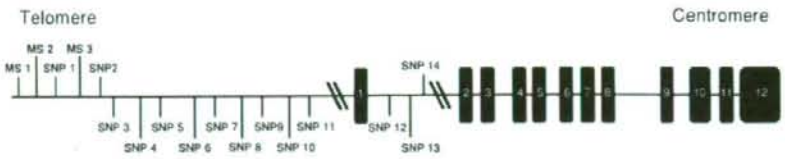

Fig. 1 The genetic structure of *Epsin 4* and location of studied markers. Upper six markers were studied in the previous reports. Lower 11 markers were additionally selected for Tag SNPs in this area

and assay by design (rs1894962) with Universal PCR Master Mix were obtained from Applied Biosystems (Foster City, CA, USA). Thermal cycling conditions for polymerase chain reaction (PCR) were one cycle at 95°C for 10 min followed by 50 cycles of 92°C for 15 s and 60°C for 1 min. After amplification, the allelic specific fluorescence was measured on ABI PRISM 7900 Sequence Detector Systems (Applied Biosystems, Foster City, CA, USA). Genotype data were read blind to the case–control status. Ambiguous genotype data were not included in the analysis.

Primer sequences for PCR amplification of the microsatellite markers D5S1400 and D5S1403 were obtained from the uniSTS database in NCBI (http://www.ncbi.nlm.nih.gov/), while the primer sequence of microsatellite marker AAAT11 was obtained from Pimm et al. (2005). PCR amplification of microsatellite markers was performed using primers fluorescently labeled with Beckman dyes. PCR products were subject to electrophoresis on Beckman CEQ 8000 (Beckman Coulter, Fullerton, CA, USA). As the PCR fragments of microsatellites were not the same length as those in the original work, we adjusted the sizes of our PCR fragments to Pimm et al. (2005).

Statistical analysis

Deviations of genotype distributions from Hardy–Weinberg equilibrium were assessed with the χ^2 test for goodness of fit. Genotype and allele distributions of each SNP were compared between patients and controls by using the χ^2 test for independence. These tests were performed with the SPSS software ver. 11 (SPSS Japan, Tokyo, Japan). The allelic association of microsatellite markers with schizophrenia was examined by use of the CLUMP program (Sham and Curtis 1995), which assesses the significance of departure between the observed and expected values in a $2 \times N$ contingency table using a Monte Carlo approach. The standardized measure of linkage disequilibrium (LD), (D') and r^2, were estimated using the online software SHEsis (http://202.120.7.14/analysis/myAnalysis.php). Haplotype-based association analyses were examined with the COCAPHASE software ver. 2.4 (http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/) (Dudbridge et al. 2000). The expectation–maximization (EM) and "droprare" options were used. Haplotypes with frequencies less than 3% were considered to be rare.

Results

All investigated SNPs and microsatellite markers for case and control groups resulted in distributions that did not significantly deviate from Hardy–Weinberg Equilibrium. LD estimates of pairwise markers, expressed in D' and r^2, are presented in Table 1. Pairs in LD are represented as gray-shaded values, with estimates of $D' > 0.8$ and $r^2 > 0.8$. Pairwise LD analysis and haplotype block determination demonstrated that there were two blocks across the studied genomic region (Fig. 2). Measurement of pairwise LD showed that the middle seven SNPs (rs1145585, rs1186930, rs1145603, rs1186934, rs11744778, rs1145602, and rs1186998) were in strong LD with each other and were located in the sample block (haplotype block 2). The remaining block, haplotype block 1, consisted of three SNPs (rs1186922, rs10046055, and rs1894962) in the 5′ upstream region. In the haplotype-based analysis, the haplotypes in the two blocks were analyzed separately.

In the single-marker analysis, the microsatellite 2 marker (D5S1403) demonstrated a significant difference in allele frequency between case and control groups (Table 2: Allele 203, $\chi^2 = 4.26$, $df = 1$, $P = 0.04$ from the "T3" analysis of CLUMP). The remaining microsatellite markers and SNPs did not show any significant allelic association with schizophrenia (Table 2, all $P > 0.05$). The obtained evidence for an association at microsatellite 2 with schizophrenia was weak and non-significant when multiple testing was taken into consideration.

Results of the haplotype-based analysis are also presented in Table 2. None of the haplotypes showed a statistically significant difference between case and control groups. Even when haplotypes were examined according to the groupings established by Pimm et al. (2005), no evidence for an association was obtained. Tang et al. (2006) reported a highly significant transmission of haplotypes consisting of AAAT11 and rs10046055 ($P = 0.0048$) in addition to rs254664 and rs1145603 ($P = 0.0047$) to affected offspring in Han Chinese trios. Our results, again, failed to provide evidence to support this finding.