齢者としました。対象者募集のため、2つの対象地域における大規模なスクリーニング検査を実施して認知機能に軽・中程度の低下がみられる高齢者を抽出し、500名を対象として約1年間の介入による認知機能向上および認知症発症抑制効果の検証を行っていきます。

介入に用いる認知症予防プログラムに関しては、名古屋市緑区では地域資源を用いた包括的なアプローチを実施し、愛知県大府市では行政事業として実施可能なアプローチの効果を検証するためのプログラムを開発しました。内容としては、運動、学習、コミュニケーションを含む複合プログラム（コミュニティ・プログラム）を開発・実施します。

認知症予防スタッフの募集団体選定と養成プログラム開発

今回の研究事業における地域レベルでの取り組みとしては、認知症予防に対して地域の核となる認知症予防スタッフを養成しました。養成事業により、高齢者機能健診と認知症予防プログラムの実施が可能な人材を育成し、地域貢献に資する資源を発掘するとともに、スタッフ本人の役割を創出して健康増進を図ることを目的としました。これらの事業により、脳とからだの健康チェックをはじめとした健診事業や予防教室など、地域での活動参加が可能となり、地域への普及啓発活動などを通じた認知症予防に関する情報発信、地域での波及効果が期待されます。

認知症予防スタッフを養成するために、まず各地域にある地域資源についてヒアリングを行い、リクルート対象と募集方法を自治体と協議の上で決定しました。その結果、大府市ではNPO法人、名古屋市緑区では認知症サポーターキャラバン、既存のボランティアグループを募集団体としました。募集には認知症予防スタッフに関するリーフレットを作成して実施しました（写真1）。各地域にお住いの概ね40歳以上の方を対象に実施し、100名の募集を

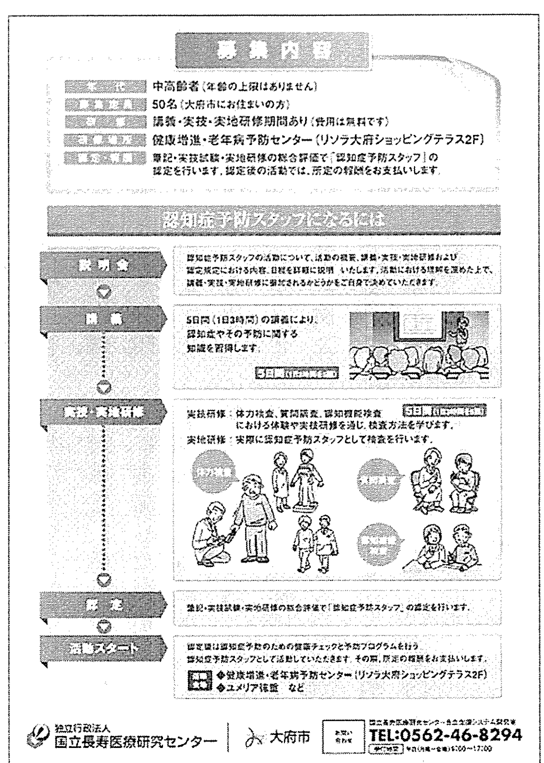

写真1　認知症予防スタッフ募集に使用したリーフレット（大阪市の例）

表1　認知症予防スタッフ養成研修の内容

		内容	回数	時間/日	延時間
講義		認知症やその予防に関する基礎知識および活動に関する知識等を習得（全10章）	5	3	15
実技	教習	体力検査：握力、5 Chair Stand test（5CS）、タンデム歩行 認知機能検査：タッチパネル式デバイスを用いた検査	1	3	3
	ロールプレイ	体力検査：握力、5 Chair Stand test（5CS）、タンデム歩行 質問調査：生活状況などの聞き取り調査 認知機能検査：タッチパネル式デバイスを用いた検査	1	3	3
実地研修	体力検査	握力、5 Chair Stand test（5CS）、Timed Up & Go test（TUG）、開眼片足立ち、タンデム歩行	1	3	3
	質問調査	面接法による生活状況などの聞き取り調査	1	3	3
	認知機能検査	タッチパネルのデバイスを用いた認知機能検査実施	1	3	3
計			10	18	30

表2　養成マニュアルの章立て

章	内容
第1章	認知症について
第2章	認知症予防スタッフの心得
第3章	高齢者機能健診について：運動機能検査
第4章	高齢者機能健診について：認知機能検査
第5章	高齢者機能健診について：質問調査
第6章	認知症予防教室について
第7章	高齢者機能健診と認知症予防教室におけるリスク管理
第8章	居住地域の現状と資源について
第9章	地域におけるサポート・ネットワーク
第10章	認知症予防スタッフの実際の活動

目標としました。

スタッフに対する研修は、本事業に参加を同意した者に対して実施しました。研修日程は講義5日間、実技2日間、実地研修3日間の計10日間で構成され、1日3時間実施しました。講義に関しては、認知症やスタッフとしての心得、地域における資源を考える時間など、講義とワーキンググループ等によって実施しました。また、教室や高齢者機能健診の意義と測定項目に関して学び、実技を行いました。実地研修に関しては、実際に身につけた測定項目を実施する時間としました。実技・実地研修では、高齢者機能健診における検査方法である「体力検査」「質問調査」「認知機能検査」の3分野の習得を主な内容としました（表1、2）。

コミュニティへの波及効果検討

本研究事業では、認知症予防スタッフ養成事業による地域での普及啓発活動、マスメディア（新聞、TVなど）や区民向け講演会、認知症予防教室の実施によるコミュニティへの波及効果を評価することを目的としました。そのために、ベースラインとして、研究事業の介入が始まる前の平成25年6月～8月に、名古屋市緑区に居住する70歳以上の区民のうち、要支援・要介護認定者を除いた24508名（2013年4月1日時点）を対象として郵送による質問紙調査を実施しました。調査内容は、認知症に対する認識、健康状態、地域の実情などとしました。その結果、16276名から回答を得て、回答率は66.4％でありました。今後、波及効果を検証に向けて介入終了時の平成27年6月～8月、同一対象者に対してフォローアップ調査を実施する予定であります。

研究開発結果・成果

○スクリーニング検査（高齢者機能健診）

「脳とからだの健康チェック2013」と題したスクリーニング検査は、合計5000名の参加を見込んでいたため、効率よく参加者数を確保するためには1日80～100名の参加者を収容できる会場の確保が必要でした。大府市では、国立長寿医療研究センター健康増進・老年病予防センターを会場としました。名古屋市緑区では、行政の協力を得て講堂や

体育室など、十分な広さの会場を確保しました。スクリーニング検査日程は、各会場の規模と確保状況から6～12月の間、合計69日間の日程を設定しました。

スクリーニング検査の周知においては、周知用ポスターおよびパンフレットを作成しました。ポスターは、行政の協力を得て、福祉会館やコミュニティセンター、各医療機関などにて平成25年5月から掲示しました（写真2）。パンフレットについても福祉会館や窓口などでの配布・回覧ができるようになりました。その他にも、公報、公開講座やイベント時のパネル展示にてスクリーニング検査実施について広報活動を行いました。

スクリーニング検査の項目については平成23年度に行った大規模スクリーニング検査の内容に準じて項目の精選を行い、週1回のペースで検討会を開催し、研究グループ内で検討を進めました。

検査実施の成果を対象地域別にみると、大府市（65歳以上）では1995名に案内状を発送し、計8日間健診を実施して、当日参加者は533名でありました（参加率26.4％）。名古屋市緑区（70歳以上）では24271名に案内状を発送し、計61日間健診を実施して、当日参加者は5257名でありました（参加率21.7％）。

○認知症予防スタッフ養成および成果

リクルート対象者は、募集団体に所属し、地域にお住いの概ね40歳以上の方を対象に実施し、100名の募集に対して147名の応募がありました。認知症予防スタッフへの応募者147名に対して、平成25年4月に養成事業に関する説明会を開催し、同意が得られた者126名を対象に5月より認知症予防スタッフ養成事業を実施しました。研修後には筆記試験を実施し、実技・実地研修の評価とあわせた総合的判断から、認知症予防スタッフとしての認定を行いました。認定の結果、地域団体からの募集による新規84名にセンターの健診に手伝った経験のある既存ボランティア14名を含め、98名がスタッフとして認定を受け、高齢者機能健診に従事しました。

本研究事業における研修を実行すると並行し、スタッフ養成のシステム開発を研究開発実施者および協力者で分担して開始しました。これらの開発により、認知症予防スタッフ活動における勤怠管理が可能となりました。具体的には、活動希望日をスタッフ本人が直接パソコンに入力し、スケジュール管理が自分でできるようにしました。また、出勤・退勤時の時刻が自動で記録できるようにしました。さらに、活動配置に関しても自動化をし、本人の能力に合った分野で活動ができるように調整しました。これらの勤怠管理は全て認定証（IDカード）で管理しました。

今後のスタッフ活動として、平成26年度から開始する認知症予防プログラムでの活動に備えるために、教室運営などに関するフォローアップ研修を実施しました。また、今後の地域での活動を支援するために、認定されたスタッフに関しては本人の同意のうえ、ス

写真2　高齢者機能健診「脳とからだの健康チェック」案内に使用したポスター

タッフリストを各自治体に提供しました。自治体の要請によって、認知症関連事業における補助活動などのボランティア活動をスタッフが実施し、地域での活動の場を広げています。

コミュニティへの波及効果

緑区の認知症予防への取り組みに対する評価に関する状況

緑区の認知症予防への取り組みに関する状況を示す代表的な項目として、「緑区は認知症になりにくいまちだと思う」と「緑区は認知症の予防に対する取り組みが充実していると思う」の2項目を取り上げ、その回答状況を図1および図2にまとめました。回答者の49.7％が「緑区は認知症になりにくいまち」、45.4％が「緑区は認知症の予防に対する取り組みが充実している」と思っていることが明らかとなりました。

認知症予防の実践（認知症予防事業への参加、知的活動の実施、身体活動の実施）

認知症予防の実践として、以下の3種類の行動に着目しました。

まず、認知症予防事業への参加に関しては、「「認知症の予防」をテーマとした普及啓発事業に、これまで参加したことがあるか？」を取り上げました。実施に関しては、「頭を使う生活習慣」を知的活動とし、平均的な1週間での「強い身体活動、中等度の身体活動、10分以上の歩行」を身体活動として、実施有無を質問しました。

認知症の予防をテーマとした普及啓発事業に参加経験のある者は、回答者の13.5％でありました（図3）。頭を使う生活習慣に関しては、毎日実践している者が40.5％、週に数回実践している者が24.6％でありました（図4）。また、身体活動に関する3項目について、強い身体活動を行っている者は33.3％、中等度の

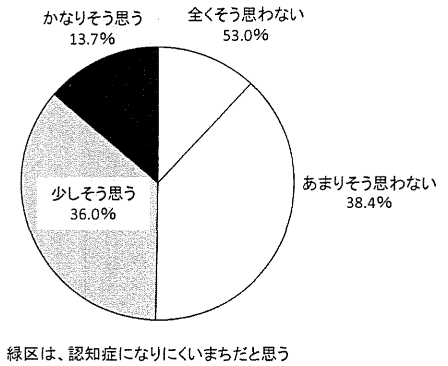

図1　認知症予防への取り組み(1)

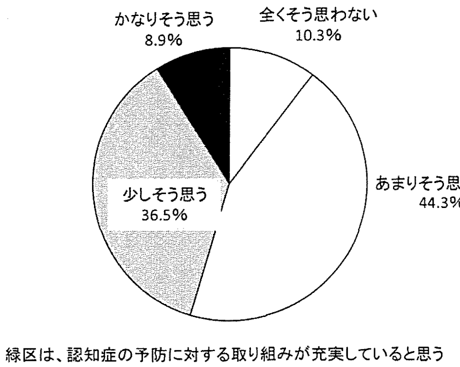

図2　認知症予防への取り組み(2)

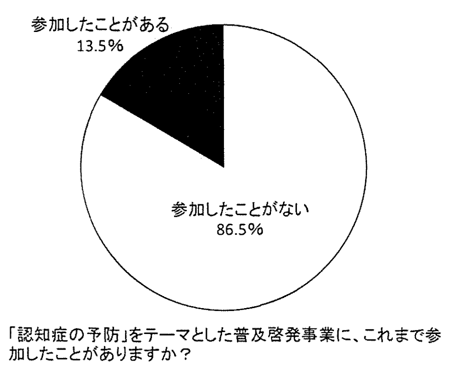

図3　認知症予防事業への参加

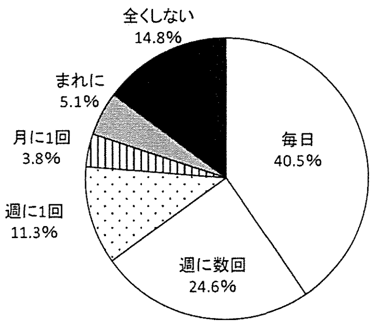

図4　知的活動の実施

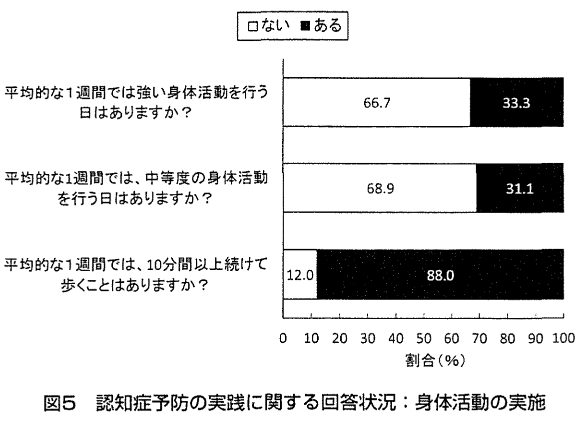

図5　認知症予防の実践に関する回答状況：身体活動の実施

身体活動を行っている者は31・1%、1回10分以上の歩行を行っている者は88・0%でありました（図5）。

※文献

1 Dementia 2010. at http://www.dementia2010.org.)
2 佐々木恵美，朝田隆，茨城県利根町研究の結果から：ADへのコンバージョンを考察する．老年精神医学雑誌2006：17(増刊-II)：55-60
3 Palmer K, Wang HX, Backman L, Winblad B, Fratiglioni L. Differential evolution of cognitive impairment in nondemented older persons: results from the Kungsholmen Project. Am J Psychiatry 2002 : 159 : 436-42
4 Ishikawa T, Ikeda M, Matsumoto N, Shigenobu K, Brayne C, Tanabe H. A longitudinal study regarding conversion from mild memory impairment to dementia in a Japanese community. Int J Geriatr Psychiatry 2006 : 21 : 134-9
5 Forette F, Seux ML, Staessen JA, et al. The prevention of dementia with antihypertensive treatment: new evidence from the Systolic Hypertension in Europe (Syst-Eur) study. Arch Intern Med 2002 : 162 : 2046-52
6 Xiong GL, Benson A, Doraiswamy PM. Statins and cognition: what can we learn from existing randomized trials? CNS Spectr 2005 : 10 : 867-74
7 Thal LJ, Ferris SH, Kirby L, et al. A randomized, double-blind, study of rofecoxib in patients with mild cognitive impairment. Neuropsychopharmacology 2005 : 30 : 1204-15
8 Petersen RC, Thomas RG, Grundman M, et al. Vitamin E and donepezil for the treatment of mild cognitive impairment. N Engl J Med 2005 : 352 : 2379-88
9 Salloway S, Ferris S, Kluger A, et al. Efficacy of donepezil in mild cognitive impairment: a randomized placebo-controlled trial. Neurology 2004 : 63 : 651-7
10 Yoshitake T, Kiyohara Y, Kato I, et al. Incidence and risk factors of vascular dementia and Alzheimer's disease in a defined elderly Japanese population: the Hisayama Study. Neurology 1995 : 45 : 1161-8
11 Scarmeas N, Levy G, Tang MX, Manly J, Stern Y. Influence of leisure activity on the incidence of Alzheimer's disease. Neurology 2001 : 57 : 2236-42
12 Lindsay J, Laurin D, Verreault R, et al. Risk factors for Alzheimer's disease: a prospective analysis from the Canadian Study of Health and Aging. Am J Epidemiol 2002 : 156 : 445-53
13 Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K. Physical activity and risk of cognitive impairment and dementia in elderly persons. Arch Neurol 2001 : 58 : 498-504
14 Verghese J, Lipton RB, Katz MJ, et al. Leisure activities and the risk of dementia in the elderly. N Engl J Med 2003 : 348 : 2508-16
15 Morris MC, Evans DA, Bienias JL, et al. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. JAMA 2002 : 287 : 3230-7
16 Engelhart MJ, Geerlings MI, Ruitenberg A, et al. Dietary intake of antioxidants and risk of Alzheimer disease. Jama 2002 : 287 : 3223-9
17 Barberger-Gateau P, Raffaitin C, Letenneur L, et al. Dietary patterns and risk of dementia: the Three-City cohort study. Neurology 2007 : 69 : 1921-30
18 Wang HX, Karp A, Winblad B, Fratiglioni L. Late-life engagement in social and leisure activities is associated with a decreased risk of dementia : a longitudinal study from the Kungsholmen project. Am J Epidemiol 2002 : 155 : 1081-7
19 Fabrigoule C, Letenneur L, Dartigues JF, Zarrouk M, Commenges D, Barberger-Gateau P. Social and leisure activities and risk of dementia: a prospective longitudinal study. J Am Geriatr Soc 1995 : 43 : 485-90
20 Wilson RS, Mendes De Leon CF, Barnes LL, et al. Participation in cognitively stimulating activities and risk of incident Alzheimer disease. JAMA 2002 : 287 : 742-8
21 Wilson RS, Bennett DA, Bienias JL, et al. Cognitive activity and incident AD in a population-based sample of older persons. Neurology 2002 : 59 : 1910-4
22 Fratiglioni L, Wang HX, Ericsson K, Maytan M, Winblad B. Influence of social network on occurrence of dementia : a community-based longitudinal study. Lancet 2000 : 355 : 1315-9
23 van Uffelen JG, Chinapaw MJ, van Mechelen W, Hopman-Rock M. Walking or vitamin B for cognition in older adults with mild cognitive impairment? A randomised controlled trial. Br J Sports Med 2008 : 42 : 344-51
24 Baker LD, Frank LL, Foster-Schubert K, et al. Effects of aerobic exercise on mild cognitive impairment: a controlled trial. Arch Neurol 2010 : 67 : 71-9
25 Suzuki T, Shimada H, Makizako H, et al. Effects of multicomponent exercise on cognitive function in older adults with amnestic mild cognitive impairment: a randomized controlled trial. BMC Neurol 2012 : 12 : 128
26 Suzuki T, Shimada H, Makizako H, et al. A randomized controlled trial of multicomponent exercise in older adults with mild cognitive impairment. PLoS One 2013 : 8 : e61483
27 Colcombe SJ, Erickson KI, Scalf PE, et al. Aerobic exercise training increases brain volume in aging humans. J Gerontol A Biol Sci Med Sci 2006 : 61 : 1166-70.
28 Erickson KI, Voss MW, Prakash RS, et al. Exercise training increases size of hippocampus and improves memory. Proc Natl Acad Sci U S A 2011 : 108 : 3017-22
29 Gates N, Fiatarone Singh MA, Sachdev PS, Valenzuela M. The effect of exercise training on cognitive function in older adults with mild cognitive impairment: a meta-analysis of randomized controlled trials. Am J Geriatr Psychiatry 2013 : 21 : 1086-97
30 Ball K, Berch DB, Helmers KF, et al. Effects of cognitive training interventions with older adults: a randomized controlled trial. Jama 2002 : 288 : 2271-81

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A large, cross-sectional observational study of serum BDNF, cognitive function, and mild cognitive impairment in the elderly

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Objective: The clinical relationship between brain-derived neurotrophic factor (BDNF) and cognitive function or mild cognitive impairment (MCI) is not well-understood. The purpose of this study was to identify the relationship between serum BDNF and cognitive function and MCI, and determine whether serum BDNF level might be a useful biomarker for assessing risk for MCI in older people.

Materials and Methods: A total of 4463 individuals aged 65 years or older (mean age 72 years) participating in the study. We measured performance in a battery of neuropsychological and cognitive function tests; serum BDNF concentration.

Results: Eight hundred twenty-seven participants (18.8%) had MCI. After adjustment for sex, age, education level, diabetes, and current smoking, serum BDNF was associated with poorer performance in the story memory, and digit symbol substitution task scores. Serum BDNF was marginally associated with the presence of MCI (odds ratio, 95% confidence interval: 1.41, 1.00–1.99) when BDNF was 1.5 SD lower than the mean value standardized for sex and age, education level, diabetes, and current smoking.

Conclusion: Low serum BDNF was associated with lower cognitive test scores and MCI. Future prospective studies should establish the discriminative value of serum BDNF for the risk of MCI.

Keywords: brain-derived neurotrophic factor, cognition, biomarker, dementia, aged

INTRODUCTION

Mild cognitive impairment (MCI) is a transitional condition between normal cognitive function and a clinical diagnosis of probable Alzheimer's disease (AD). MCI, including amnestic MCI, is a pathologically heterogeneous disorder in which many persons exhibiting mixed pathologies (Schneider et al., 2009). Few studies have investigated biomarkers for MCI. Most work has focused on tau and/or Aβ-42 and their association with neuroimaging results and clinical symptoms in persons at risk for AD. Biomarkers for AD and MCI must be established and validated in larger cohorts, and efforts should be made to investigate markers of other aspects of tau and Aβ pathology, including inflammation and trophic factors (Winblad et al., 2004). Neuronal hypertrophy might constitute an early cellular response to AD pathology or reflect a compensatory mechanism that prevents cognitive impairment despite substantial AD lesions (Riudavets et al., 2007; Iacono et al., 2008, 2009). Neuronal cell growth is modulated by factors such as brain-derived neurotrophic factor (BDNF) (Schindowski et al., 2008). BDNF is highly concentrated in the hippocampus (Phillips et al., 1990), important in synaptic plasticity (Kang and Schuman, 1995; Figurov et al., 1996), and contributes to neurogenesis in the dentate gyrus (Takahashi et al., 1999). BDNF plays a pivotal role in age-related memory impairments and is associated with age-related atrophy of the hippocampus. Previous studies have reported that serum BDNF levels are reduced in AD (Gezen-Ak et al., 2013), MCI (Peng et al., 2005; Yu et al., 2008), major depression disorder, and depressive symptoms (Karege et al., 2002; Shimizu et al., 2003; Cunha et al., 2006; Terracciano et al., 2011). A study of neuronal cell cultures found that amyloid peptide at sub-lethal concentrations interfered with neuronal plasticity mediated by BDNF signaling cascade (Tong et al., 2004; Wang et al., 2006). Neuronally differentiated P19 mouse embryonic carcinoma cells stimulated by BDNF showed a rapid decrease in tau phosphorylation (Elliott et al., 2005). However, clinical studies that report lower serum BDNF levels are difficult to interpret because of limited knowledge of potential confounders and mixed results based on patient's age and sex (Bus et al., 2012). Therefore, there is no normal distribution in serum BDNF level, and this may lead to misinterpretation of BDNF levels in studies that used parametric testing with small sample sizes (Ziegenhorn et al., 2007). To establish a cut-off value for serum BDNF is important for clinical purposes, e.g., for helping to increase diagnostic sensitivity. The purpose of this study was to examine the relationships between serum BDNF level and MCI and evaluate whether serum BDNF level may be useful for assessing MCI risk in older adults using a large sample cohort. We explored the relationship between serum BDNF level and MCI, and various measures of cognitive function in elderly adults.

MATERIALS AND METHODS

STUDY POPULATION

Our study assessed 5104 individuals who were enrolled in the Obu study of health promotion for the elderly (OSHPE). Each individual was recruited from Obu, Japan, which is a residential suburb of Nagoya. To be included in this study, each participant was 65 years or older at the time of examination (2011 or 2012), resided in Obu city, and had not participated in another study. We excluded participants who had missing BDNF data and characteristics, diagnosed neurological disorders included stroke, Parkinson's disease, AD, and depression, certified long-term care insurance, or functional decline of activities of daily living (ADL). **Figure 1** shows the flow of participants (**Figure 1**). Six hundred forty-one of the 5104 participants were excluded and 4463 older adults (range 65–97 years) were included in this study. The data of 4463 individuals were used to analyze in the present study. Informed consent was obtained from all participants prior to their inclusion in the study, and the Ethics Committee of the National Center for Geriatrics and Gerontology approved the study protocol.

BDNF MEASUREMENT

Whole blood samples were collected from each patient by venipuncture. To obtain serum, whole blood samples were allowed to coagulate at room temperature (RT) for 30 min and then centrifuged at RT for 15 min at $1000 \times g$. The collected serum was stored in polypropylene tubes at −80°C until assayed. BDNF concentrations were quantitatively determined by enzyme-linked immunosorbent assay (ELISA) using the DuoSet ELISA Development Kit from R&D Systems (Minneapolis, MN, USA). Assays were performed using a specific human BDNF antibody (Minneapolis, MN, USA); no significant cross reactivity or interference was observed in this assay. Serum samples were diluted 1:50. Sample BDNF concentrations were then determined by non-linear regression from the standard curves. Measurements were performed in duplicate and averaged to give a value in picogram per milliliter, which was then expressed in nanogram per milliliter after correcting for sample dilution. "Low" and "High" concentration quality control pools were prepared by adding 10 or 100 ng to 5 ml portions of human serum (Innovative Research, Novi, MI, USA), giving nominal concentrations of 2 and 20 ng/ml, respectively. The assays were performed by one laboratory (SRL Inc., Tokyo, Japan). The repeatability of the BDNF ELISA, as measured by intra-assay precision was 3.8%, and the reproducibility, as measured by inter-assay precision, was 7.6%.

MCI CRITERIA AND COGNITIVE FUNCTION TESTS

We defined MCI based on previous studies (Hanninen et al., 2002; Jungwirth et al., 2005; Yaffe et al., 2011), using the following criteria: (1) subjective memory complaints; (2) objective cognitive impairment [indicated by an age-adjusted score at least 1.5 SD below the reference threshold of any of the tests, all of which are commonly used for detailed neuropsychological assessments] but no general cognitive impairment; (3) no evidence of functional dependency (no need for supervision or external help in performing daily activities); and (4) exclusion from the clinical criteria for dementia. Screening for MCI included a standardized personal interview for collecting sociodemographic and

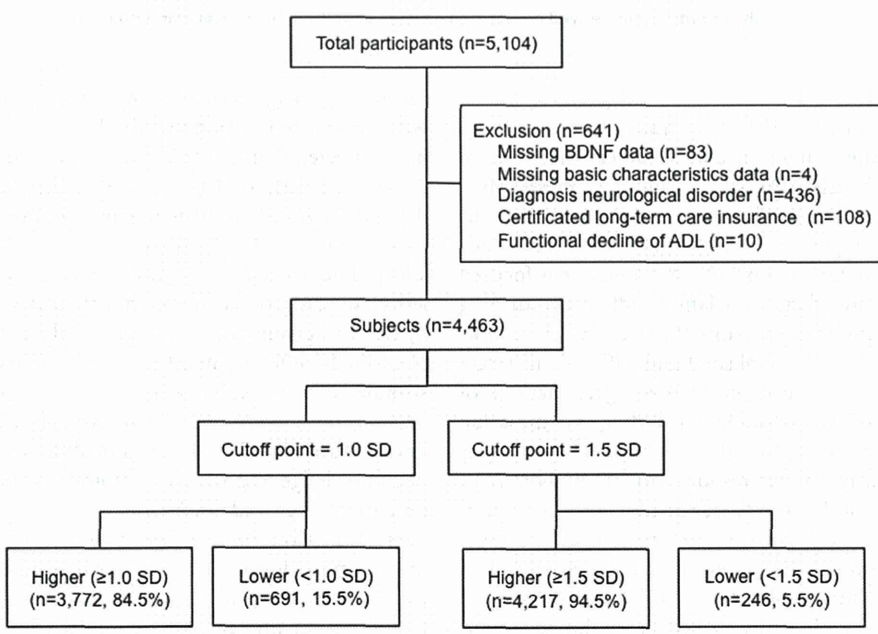

FIGURE 1 | Flow of participants. A total of 4463 participants >65 years of age were included in the study. BDNF levels were measured and the participants were divided into those with higher or lower BDNF levels below 1.0 and 1.5 standard deviations from the mean age- and sex-adjusted BDNF value.

lifestyle data, medical history, and functional status (ADL) data, along with cognitive function testing using the mini-mental state examination (MMSE) (Folstein et al., 1975) and the National Center For Geriatrics And Gerontology-Functional Assessment Tool (NCGG-FAT) (Makizako et al., 2012). Individuals who scored ≤23 points on the MMSE were considered to have general cognitive impairment (Anthony et al., 1982). The NCGG-FAT consists of multidimensional cognitive tasks used to assess word-list memory (delayed recall), story memory (delayed recognition), attention and executive function (tablet version of the Trail Making Test – Part A and B), processing speed (tablet version of the symbol digit substitution test), and visuospatial skill (figure selection). The participants were given 20–30 min to complete the battery of tests and their associated tasks. High test–retest reliability and moderate-to-high validity were previously confirmed in community-dwelling older adults for all components of the NCGG-FAT (Makizako et al., 2012). All tests used in this study had previously established standardized thresholds for the definition of cognitive impairment in the corresponding domain (score <1.5 SD below the age-specific mean) for a population-based OSHPE cohort of healthy older adults.

POTENTIAL CORRELATES

Based on the review articles by Bus et al. (2011, 2012), Ziegenhorn et al. (2007), Knaepen et al. (2010), and Plassman (2010), we selected three demographic variables, one physiological variable, two health status indicators, and three behavioral variables as possible confounding factors of the association between BDNF and cognitive decline (Ziegenhorn et al., 2007; Knaepen et al., 2010; Bus et al., 2011, 2012). The three demographic variables – sex, age, and educational level – were selected as possible confounding factors in determining the association of serum BDNF and MCI. Walking speed – the physiological variable – was measured on a flat and straight surface at a comfortable walking speed. Two markers were used to indicate the start and end of a 2.4-m walkway, with a 2-m section to traverse before passing the start marker so that participants were walking at a comfortable pace by the time they reached the timed path. Participants were asked to continue walking for an additional 2 m past the end of the path to ensure a consistent walking pace while on the timed path. Histories of heart disease and diabetes were obtained as health status indicators. Behavioral factors, including current smoking, regular exercise, and frequency of going outdoors, were identified during the interview. Participants were asked whether they currently smoked or exercised regularly: responses were either "yes" or "no." Participants were asked how often they traveled to places outside their town during a week.

STATISTICAL ANALYSIS

Student's t-test was used to compare BDNF concentrations between men and women. Differences in serum BDNF concentrations were analyzed among four age-groups (65–69, 70–74, 75–79, 80–84, and ≥85 years) by one-way analysis of variance (ANOVA) in both sexes. A linear regression was used to analyze the relationships between BDNF concentration and age and education in both sexes. Participants were divided into two groups according to 1.0 or 1.5 SD from age- and sex-specific mean values among the four age-groups (**Figure 1**). Independent sample t-tests or Chi-square tests were used to compare the potential correlates and cognitive performance between: (a) participants who had BDNF levels below 1.0 SD and above 1.0 SD; and (b) participants who had BDNF levels below 1.5 SD and above 1.5 SD. Linear regression analyses (forced-entry) were used to reveal the relationships between BDNF concentration and cognitive performance. Multivariate logistic regression analyses, forced-entry, were used to determine adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs), and to assess independent associations between the serum BDNF levels and MCI. The covariates of sex, age, and educational level, and significant variables in univariate analyses were added to the regression models to evaluate independent associations between BDNF and cognitive performances or MCI. Logistic regression models determined the crude OR and the adjusted OR of BDNF for 1.0 and 1.5 SD. Sensitivity, specificity, and positive and negative likelihood ratios of the BDNF values with MCI were calculated. We excluded the participants who scored ≤23 points on the MMSE and did not complain of memory loss. We used the data of MCI ($n = 827$) and cognitive healthy ($n = 2533$) elderly adults in the logistic regression analyses. All statistical comparisons were made at the 0.05 level of significance, and all data management and statistical computations were performed using the IBM SPSS Statistics 20.0 software package (SPSS Inc., Chicago, IL, USA).

RESULTS

The mean BDNF concentrations were statistically significantly different in men (20.8 ± 5.6 ng/ml) and women (21.2 ± 5.2 ng/ml; $t = 2.162$, $df = 4394$, $P = 0.031$). BDNF concentrations declined with increasing age in both sexes ($F = 24.822$, $df = 3$, $P < 0.001$) (**Table 1; Figure 2**). Linear regression found that serum BDNF was

Table 1 | Serum BDNF levels among the four age-groups.

	Men		Women	
	BDNF values 1.0 SD lower than the mean	BDNF values 1.5 SD lower than the mean	BDNF values 1.0 SD lower than the mean	BDNF values 1.5 SD lower than the mean
65–69 years	16.08	13.34	16.75	14.15
70–74 years	15.20	12.52	15.85	13.16
75–79 years	14.82	11.84	15.12	12.57
80 years and over	13.30	10.27	15.05	12.63

associated with age in men ($\beta = -0.123$, $t = -5.750$, $P < 0.001$) and women ($\beta = -0.154$, $t = -7.475$, $P < 0.001$). Education level was associated with serum BDNF in women ($\beta = 0.045$, $t = 2.149$, $P = 0.032$), but not in men ($\beta = 0.012$, $t = 0.564$, $P = 0.573$).

The comparison between participants who had BDNF levels below 1.0 SD and above 1.0 SD, revealed that the participants below 1.0 SD had a higher prevalence of diabetes, a lower proportion of smokers, higher scores of story memory, and a symbol digit substitution task, compared with participants who had BDNF levels above 1.0 SD. The results were similar for the comparison between the participants who had BDNF levels below 1.5 SD and above 1.5 SD. A comparison of MCI prevalence found no significant difference between the participants who had serum BDNF below and above 1.0 SD. In contrast, when serum BDNF was dichotomized according to 1.5 SD below the mean, a significant difference was found in MCI (**Table 2**). The mean BDNF concentrations did not show significant differences between MCI participants (20.9 ± 5.3 ng/ml) and non-MCI participants (21.2 ± 5.4 ng/ml; $t = 1.362$, $df = 3358$, $P = 0.173$).

Table 3 shows the association between serum BDNF and performance on various cognitive function tests using multiple linear regression, adjusted for sex, age, education level, diabetes, and current smoking status. Serum BDNF levels were associated with a decline in story memory ($\beta = 0.027$, $t = 1.958$, $P < 0.05$) and digit symbol substitution test scores ($\beta = 0.027$, $t = 2.172$, $P < 0.05$). There was no significance between BDNF and MMSE for word-list memory, the tablet version of the Trail Making Test – Part A and B, or figure selection.

In all, 827 participants (18.8%) had MCI. A total of 691 participants (15.5%) had BDNF levels below 1.0 SD from the mean, and 246 participants (5.5%) had levels below 1.5 SD from the mean. **Table 4** shows the association between serum BDNF levels and the diagnosis of MCI using multiple logistic regression, adjusted for sex, age, education level, diabetes, and current smoking status. The crude logistic model showed significant relationships between MCI and BDNF: 1.5 SD (OR, 1.40; 95% CI, 1.00–1.96),

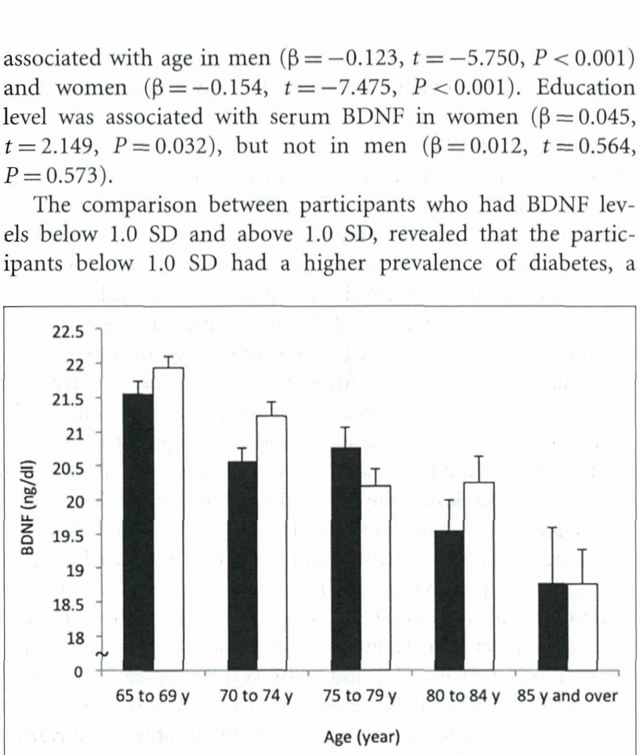

FIGURE 2 | Sex and age differences in serum BDNF concentration. Mean and standard error of serum BDNF levels are shown for each 5-year increment in age. Serum BDNF decreased with aging in men (black bars) and women (white bars; $P < 0.001$) and women showed higher BDNF levels than men ($P = 0.031$).

Table 2 | Comparisons between BDNF levels of 1.0 and 1.5 SD from the mean.

	BDNF levels of 1.0 SD from the mean			BDNF levels of 1.5 SD from the mean		
	Participants above 1.0 SD	**Participants below 1.0 SD**	***P***	**Participants above 1.0 SD**	**Participants below 1.0 SD**	***P***
Sex, women, *n*, %	1919, 50.9	372, 53.8	0.152	2175, 51.6	116, 47.2	0.177
Age, years	71.9 ± 5.4	72.1 ± 5.5	0.395	71.9 ± 5.5	71.8 ± 5.2	0.744
Education level, years, 10[a]	11.4 ± 2.5	11.3 ± 2.5	0.294	11.4 ± 2.5	11.2 ± 2.5	0.237
Walking speed, m/s, 6[a]	1.3 ± 0.2	1.3 ± 0.2	0.236	1.3 ± 0.2	1.3 ± 0.2	0.722
Heart disease, yes, *n*, 2[a]	585, 15.5	124, 17.9	0.109	671, 15.9	38, 15.4	0.844
Diabetes, yes, *n*	474, 12.6	111, 16.1	0.012	538, 12.8	47, 19.1	0.004
Current smoking, yes, *n*, %, 1[a]	392, 10.4	51, 7.4	0.015	430, 10.2	13, 5.3	0.012
Habitual exercise, yes, *n*, 5[a]	2816, 74.8	519, 75.1	0.844	3152, 74.8	183, 74.4	0.876
Going outdoors, times/week, 1[a]	5.9 ± 1.6	5.8 ± 1.7	0.125	5.9 ± 1.7	5.8 ± 1.7	0.841
MMSE score, 6[a]	26.3 ± 2.7	26.2 ± 2.8	0.64	26.3 ± 2.7	26.0 ± 2.8	0.09
Word-list memory score, 19[a]	3.8 ± 2.0	3.8 ± 2.0	0.872	3.8 ± 2.0	3.7 ± 2.0	0.466
Story memory score, 26[a]	6.8 ± 1.9	6.6 ± 1.9	0.029	6.7 ± 1.9	6.4 ± 1.9	0.011
Trail making test – part A, s, 11[a]	21.2 ± 6.9	21.5 ± 7.3	0.261	21.2 ± 7.0	22.0 ± 7.1	0.083
Trail making test – part B, s, 15[a]	43.1 ± 17.9	44.1 ± 18.4	0.173	43.2 ± 17.9	45.3 ± 18.7	0.068
Symbol digit substitution task, 14[a]	38.4 ± 8.4	37.5 ± 8.5	0.013	38.3 ± 8.4	37.2 ± 8.4	0.049
Visuospatial skill score, 85[a]	5.2 ± 1.5	5.2 ± 1.5	0.928	5.2 ± 1.5	5.2 ± 1.4	0.798
Mild cognitive impairment, yes, *n*, %, 73[a]	689, 24.2	138, 24.6	0.244	774, 24.3	53, 31.0	0.047

[a]*Number of missing data.*

Table 3 | Multiple linier regression analyses with serum BDNF, potential confounders, and cognitive tests.

Independent variable	Dependent variables													
	MMSE		Word-list memory		Story memory		Trail making test – part A		Trail making test – part B		Symbol digit substitution task		Visuospatial skill	
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
BDNF, ng/ml	0.011	0.442	0.017	0.229	0.027	0.050	−0.008	0.584	−0.022	0.091	0.027	0.030	−0.011	0.472
Sex, men = 1, women = 2	0.156	<0.001	0.160	<0.001	0.107	<0.001	−0.032	0.027	−0.027	0.050	−0.028	0.030	−0.074	<0.001
Age, years	−0.208	<0.001	−0.316	<0.001	−0.322	<0.001	0.354	<0.001	0.400	<0.001	−0.473	<0.001	−0.167	<0.001
Education, years	0.219	<0.001	0.176	<0.001	0.242	<0.001	−0.167	<0.001	−0.235	<0.001	0.230	<0.001	0.192	<0.001
Diabetes, no = 1, yes = 2	0.009	0.512	−0.016	0.253	−0.015	0.254	0.023	0.091	0.006	0.616	−0.036	0.003	−0.015	0.306
Current smoking, no = 1, yes = 2	−0.042	0.004	−0.009	0.522	0.004	0.781	0.031	0.029	0.057	<0.001	−0.059	<0.001	0.012	0.421

Table 4 | Relationships between MCI and BDNF or selected correlates.

	Crude OR		Adjusted OR in BDNF 1.0 SD		Adjusted OR in BDNF 1.5 SD	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
BDNF 1.0 SD, below/above	1.14 (0.92–1.40)	0.244	1.14 (0.92–1.42)	0.236		
BDNF 1.5 SD, below/above	1.40 (1.00–1.96)	0.048			1.41 (1.00–1.98)	0.050
Sex, women/men	1.00 (0.86–1.17)	0.971	0.85 (0.71–1.00)	0.051	0.85 (0.72–1.01)	0.063
Age, years	1.02 (1.01–1.04)	0.003	1.00 (0.99–1.02)	0.977	1.00 (0.99–1.02)	0.942
Education, years	0.82 (0.79–0.85)	<0.001	0.82 (0.79–0.85)	<0.001	0.82 (0.79–0.85)	<0.001
Diabetes, yes/no	1.11 (0.88–1.39)	0.377	1.04 (0.82–1.31)	0.752	1.03 (0.82–1.31)	0.778
Current smoking, yes/no	1.09 (0.84–1.43)	0.517	1.19 (0.90–1.59)	0.23	1.20 (0.90–1.60)	0.208

age (OR, 1.02; 95% CI, 1.01–1.04), and education (OR, 0.82; 95% CI, 0.79–0.85). The adjusted logistic model for BDNF 1.0 SD showed no significant relationship between serum BDNF and MCI. In contrast, when serum BDNF was dichotomized according to 1.5 SD below the mean, a significant association with MCI was found (OR, 1.41; 95% CI, 1.00–1.98). Education was also associated with MCI (OR, 0.82; 95% CI, 0.79–0.85). Sensitivity and specificity of the BDNF values for 1.5 SD were 6.4% (95% CI: 4.8–8.3%) and 95.3% (95% CI: 94.5–96.1%), respectively. Positive and negative likelihood ratios of the BDNF values of 1.5 SD were 1.38 (95% CI: 1.00–1.88) and 0.98 (0.96–1.00), respectively.

DISCUSSION

In our cross-sectional observational study of 4463 community-living older adults, serum BDNF was associated with a decline in story memory and digit symbol substitution test scores, even when adjusted for sex, age, education, diabetes, and current smoking. Moreover, serum BDNF levels of 1.5 SD lower than the age- and sex-adjusted means were associated with a significant risk of MCI. These results suggest that serum BDNF may be a useful biomarker of cognitive function and MCI status in the elderly.

In demographic variables, serum BDNF was higher in women than men. Similar results were found by Trajkovska et al. (2007) using both serum and whole blood BDNF, whereas they were in contrast to other studies using only serum BDNF (Lang et al., 2004; Ziegenhorn et al., 2007). Another study found a significant interaction of age and menopausal state with BDNF in women, with age-related increases serum BDNF premenopause and age-related decreases postmenopause (Bus et al., 2011). Estrogen levels are significantly associated with BDNF levels (Scharfman and MacLusky, 2006), so the postmenopausal drop in estrogen could result in decreased serum BDNF. Therefore, the differences in serum BDNF levels in men and women might be related to sex hormone differences. However, it is difficult to draw conclusions with cross-sectional approaches, and longitudinal studies are needed.

Among lifestyle measures, diabetes and current smoking showed significant differences between the participants who had high and low serum BDNF levels. Low levels of BDNF accompanied impaired glucose metabolism. Krabbe et al. reported