Esaki E, Adachi H, Hirai Y, Yamagishi S, Kakuma T, Enomoto M, Fukami A, Kumagai E, Ohbu K, Obuchi A, Yoshimura A, Nakamura S, Nohara Y, Fujiyama T, Fukumoto Y, Imaizumi T. Serum vaspin levels are positively associated with carotid atherosclerosis in a general population. Atherosclerosis 2013 [in press]

2. 学会発表

H. 知的財産権の出願・登録状況（予定を含む。）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

作成上の留意事項

１．「Ａ．研究目的」について

・厚生労働行政の課題との関連性を含めて記入すること。

２．「Ｂ．研究方法」について

(1) 実施経過が分かるように具体的に記入すること。

(2) 「（倫理面への配慮）」には、研究対象者に対する人権擁護上の配慮、研究方法による研究対象者に対する不利益、危険性の排除や説明と同意（インフォームド・コンセント）に関わる状況、実験動物に対する動物愛護上の配慮など、当該研究を行った際に実施した倫理面への配慮の内容及び方法について、具体的に記入すること。倫理面の問題がないと判断した場合には、その旨を記入するとともに必ず理由を明記すること。

なお、ヒトゲノム・遺伝子解析研究に関する倫理指針（平成１６年文部科学省・厚生労働省・経済産業省告示第１号）、疫学研究に関する倫理指針（平成１９年文部科学省・厚生労働省告示第１号）、遺伝子治療臨床研究に関する指針（平成１６年文部科学省・厚生労働省告示第２号）、臨床研究に関する倫理指針（平成２０年厚生労働省告示第４１５号）、ヒト幹細胞を用いる臨床研究に関する指針（平成１８年厚生労働省告示第４２５号）、厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針（平成１８年６月１日付厚生労働省大臣官房厚生科学課長通知）及び申請者が所属する研究機関で定めた倫理規定等を遵守するとともに、あらかじめ当該研究機関の長等の承認、届出、確認等が必要な研究については、研究開始前に所定の手続を行うこと。

３．「Ｃ．研究結果」について

・当該年度の研究成果が明らかになるように具体的に記入すること。

４．「Ｆ．健康危険情報」について

研究分担者や研究協力者の把握した情報・意見等についても研究代表者がとりまとめて総括研究報告書に記入すること。

５．その他

(1) 日本工業規格Ａ列４番の用紙を用いること。

(2) 文字の大きさは、１０～１２ポイント程度とする。

Ⅱ. 分担研究報告書

平成25年度厚生労働科学研究費補助金（厚生労働科学特別研究事業）
「『認知症予防のための戦略研究』研究実施計画書作成に関する研究」
分担研究報告書

研究分担者　池内　健　　（新潟大学脳研究所　教授）

○研究要旨

認知症の高齢者数は激増しつつあるのに根本的な治療法も予防法もない。しかし多くの研究で、有酸素性運動等の運動の有効性が報告されてきた。そのような背景において、今後全国規模で地域住民を対象に運動習慣をつけることによる認知症予防介入研究が実施される。そこでは、私は被検者の認知機能低下および認知症発症に関する諸因子および被検者の背景情報を得るために採血による生体試料の収集を行う方法等について立案した。本プロジェクトで採取された生体試料は、今後の認知症研究における貴重なバイオリソースになることが期待され、その保管および管理を適切に行う必要性についても考えた。

A. 研究目的

運動習慣作りを介した行動変容によって認知症予防の可能性を探る。そのためにどのような介入、実施体制、成果物作成のための具体的な方法について実行可能で有用性の高い計画を作成する。これに関連して被検者の認知機能低下および認知症発症に関する諸因子および被検者の背景情報を得るために採血による生体試料の収集を行うことに関する具体的な方法を明示する。

B. 研究方法

具体的計画を研究班員による研究と討議の中から生みだす。

（倫理面への配慮）

「世界医師会ヘルシンキ宣言」及び「臨床研究に関する倫理指針」を遵守し、必要に応じて「遺伝子研究に関する倫理指針」を遵守する。本研究で実施された一般的な血液・生化学検査の結果は研究協力者に返却する。ただし*APOE*多型についての遺伝子解析の結果は、*APOE* 多型情報を反映しうる予防法は現時点では存在しないので、返却をしない。

C. 研究結果

以下のように手順を定めた。

採血用資材

被検者毎に１ビジット・１パッケージとした資材を用意する。被検者の参加人数を推定し、必要数を事務局に連絡する。採血用資材には以下のものが含まれている。

a）検査伝票

b) 全血用採血管 EDTA-2K
c) 血漿用採血管 EDTA-2Na
d) 血清用採血管 分離剤入り
e) 血清用採血管（保存用）分離剤入り
f) 血糖用採血管

採血のスケジュール連絡

ご施設までサンプルの回収の手配を業者に依頼するので、担当者は、採血の予定人数と日程、おおよその時間を事務局まで連絡する。

採血の手順

通常の静脈採血は空腹時に行う。被験者様匿名化 ID と伝票（図２）と採血管に記載されている匿名化 ID が一致することを確認する。採血後は数回転倒混和する。採血後のスピッツはパッケージに入れ、冷蔵保存とする。

検体の輸送

ご施設まで業者がサンプルの回収に来る。採血実施者数とパッケージ数を確認の上、業者に引き渡す。

D. 考察

認知症予防についてのわが国で初の大規模研究を行うための実務に関する基本方針のうち採血に関する手順を定めた。今後は採血と収集に関して参加自治体等との強力な連携体制作りが必要になる。

E. 結論

研究班員による討議によって血液採取の方法を立案した。その一方で遺伝子情報の返却に関する配慮も行った。

F. 研究発表

1. 論文発表

2. 学会発表

H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

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SORL1 Is Genetically Associated with Late-Onset Alzheimer's Disease in Japanese, Koreans and Caucasians

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Abstract

To discover susceptibility genes of late-onset Alzheimer's disease (LOAD), we conducted a 3-stage genome-wide association study (GWAS) using three populations: Japanese from the Japanese Genetic Consortium for Alzheimer Disease (JGSCAD), Koreans, and Caucasians from the Alzheimer Disease Genetic Consortium (ADGC). In Stage 1, we evaluated data for 5,877,918 genotyped and imputed SNPs in Japanese cases (n = 1,008) and controls (n = 1,016). Genome-wide significance was observed with 12 SNPs in the *APOE* region. Seven SNPs from other distinct regions with p-values $<2\times10^{-5}$ were genotyped in a second Japanese sample (885 cases, 985 controls), and evidence of association was confirmed for one *SORL1* SNP (rs3781834, $P = 7.33\times10^{-7}$ in the combined sample). Subsequent analysis combining results for several SORL1 SNPs in the Japanese, Korean (339 cases, 1,129 controls) and Caucasians (11,840 AD cases, 10,931 controls) revealed genome wide significance with rs11218343 ($P = 1.77\times10^{-9}$) and rs3781834 ($P = 1.04\times10^{-8}$). SNPs in previously established AD loci in Caucasians showed strong evidence of association in Japanese including rs3851179 near *PICALM* ($P = 1.71\times10^{-5}$) and rs744373 near *BIN1* ($P = 1.39\times10^{-4}$). The associated allele for each of these SNPs was the same as in Caucasians. These data demonstrate for the first time genome-wide significance of LOAD with *SORL1* and confirm the role of other known loci for LOAD in Japanese. Our study highlights the importance of examining associations in multiple ethnic populations.

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‡ Membership of The Alzheimer Disease Genetics Consortium is provided in the Acknowledgments.

† Deceased.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive dysfunction and memory loss. Multiple rare mutations in *APP*, *PSEN1*, *PSEN2* and *SORL1* account for most cases of early-onset autosomal dominant AD [1,2]. Risk of late-onset AD (LOAD), the most common type of dementia in the elderly, is associated with complex interactions between genetic and environmental factors. Until recently, *APOE* was the only unequivocally recognized major susceptibility gene for LOAD [1,3]. Several genome-wide association studies (GWAS) each including more than 5,000 Caucasians identified genome-wide significant associations for LOAD with nine other loci including *ABCA7*, *BIN1*, *CD2AP*, *CD33*, *CLU*, *CR1*, *EPHA1*, *MS4A* gene cluster, and *PICALM* [4,5]. To our knowledge, no large GWAS for LOAD has been performed in any Asian population. Because there is a possibility that there exist ethnic-specific LOAD susceptibility variants, we carried out a large-scale GWAS to confirm associations at known loci and identify novel loci for LOAD using a three-stage design including a discovery Japanese cohort and replication cohorts of Japanese, Korean and Caucasian subjects.

Methods

Subjects

Japanese datasets. Clinically defined subjects were recruited by the Japanese Genetic Study Consortium of Alzheimer's Disease (JGSCAD: principal investigator, Y.I.) [6,7]. Probable AD cases were ascertained on the basis of the criteria of the National Institute of Neurological and Communicative Disorders, and Stroke-Alzheimer's Disease and Related Disorders (NINCDS/ADRDA) [8]. The Mini-Mental State Examination [9], Clinical Dementia Rating [10], and/or Function Assessment Staging [11] were primarily used for evaluation of cognitive impairment. Elders living in an unassisted manner in the local community with no signs of dementia were used as controls. DNA was extracted from peripheral blood leukocytes using standard protocols [6]. For the purpose of this study, the Stage 1 genome-wide association study (GWAS) dataset included 2024 subjects (1008 AD cases and 1016

controls) and the Stage 2 dataset included 1870 subjects (885 AD cases and 985 controls).

Korean dataset. A total of 339 subjects with AD were recruited at the Samsung Medical Center in Seoul, Korea. All AD subjects fulfilled NINCDS-ADRDA criteria for probable AD [8]. These subjects underwent a clinical interview and neurological examination that were previously described [12]. The absence of secondary causes of cognitive deficits was assessed by laboratory tests including complete blood count, blood chemistry, vitamin B12/folate, syphilis serology, and thyroid function tests. Conventional brain MRI scans (T1-weighted, T2-weighted, and FLAIR images) confirmed the absence of territorial cerebral infarctions, brain tumors, and other structural lesions. Healthy control subjects (n = 1,129) ages 55 to 85 years were recruited from routine health examination at the same location and showed no evidence of cognitive dysfunction.

Alzheimer Disease Genetics Consortium dataset. Summarized information from tests of genetic association of AD with SNPs located in the candidate gene regions was culled from a recent large GWAS conducted by the Alzheimer Disease Genetics Consortium (ADGC) [5]. Results were computed for SNPs throughout the genome in a sample composed of 11,840 AD cases and 10,931 cognitively normal elders from 15 independent Caucasian data sets. Details of the quality control and statistical analysis procedures and genetic models has been published elsewhere [5].

This study was approved by the Boston University Institutional Review Board, Institutional Review Board of Niigata University, and the Institutional Review Boards of all participating institutions. Written informed consent was obtained from all participants. Next of kin, carer takers or guardians consented on the behalf of participants whose capacity to consent was compromised. All subjects were anonymously genotyped. The basic demographics of the cases and controls before QC in each dataset are presented in Table 1.

Genotyping

GWAS genotyping was performed in the Stage 1 sample using Affymetrix GeneChip 6.0 microarrays containing 909,622 SNPs. Applied Biosystems' (ABI) TaqMan Assays were used to genotype individual SNPs in the Japanese and Korean replication cohorts. *APOE* genotypes in the Japanese and Korean samples were determined by haplotypes derived from rs7412 and rs429358 which were genotyped using TaqMan Assays. Details of *APOE* genotyping in each ADGC dataset were described previously [13].

Quality Control and Population Substructure

In the Stage 1 sample, SNPs with a genotype call rate (GCR) <95%, a minor allele frequency (MAF) <0.05, or significant deviation from the Hardy-Weinberg equilibrium (HWE) in controls ($P<10^{-6}$) were excluded. After excluding 83,673 low quality and 298,304 low frequency SNPs, we removed 196 subjects with a GCR <95% and 41 subjects whose gender as determined by analysis of X-chromosome data using the PLINK program (ver. 1.06) [14] was inconsistent with the reported gender. The same QC procedures were applied to the Japanese and Korean replication datasets. We examined population substructure in the GWAS dataset by analyzing tagging SNPs from the genome-wide panels using the *smartpca* module from EIGENSTRAT software [15] in a manner described previously [5]. Subsequently, we excluded three subjects who were cryptically related to other subjects in the dataset and 49 individuals who were population outliers. The strength of association of the top 10 principal components (PCs) was tested with AD status. The first

Table 1. Sample size and characteristics of the discovery and replication datasets.

Population (Stage)	Total	Alzheimer Disease Cases					Cognitively Normal Controls			
		N	Female (%)	Age at onset (mean ± SD)	Age at exam (mean ± SD)	*APOE* ε2/ε3/ε4 Frequency	N	Female (%)	Age at exam (mean ± SD)	*APOE* ε2/ε3/ε4 Frequency
Japanese Discovery (Stage 1)	2,024	1,008	723 (72%)	73.0 (4.28)	NA	0.02/0.65/0.33	1,016	583 (57%)	77.0 (5.89)	0.04/0.87/0.09
Japanese Replication (Stage 2)	1,870	885	574 (65%)	74.3 (6.98)	NA	0.02/0.69/0.29	985	618 (63%)	73.74 (5.84)	0.05/0.86/0.09
Korean (Stage 3)	1,468	339	245 (72%)	NA	73.67 (9.49)	0.03/0.70/0.27	1,129	550 (49%)	71.04 (4.86)	0.06/0.85/0.09
Caucasian (Stage 3)	22,771	11,840	7168 (61%)	76.37 (5.18)	80.59 (4.92)	0.04/0.61/0.36	10,931	6418 (59%)	76.77 (3.55)	0.08/0.78/0.14
TOTAL	**28,133**	**14,072**					**14,061**			

doi:10.1371/journal.pone.0058618.t001

three PCs were nominally associated with AD status. A total of 574,828 SNPs and 1,735 subjects comprising 891 cases and 844 controls passed the QC and were used for imputation and in further statistical analyses.

Genotype Imputation

Genotypes for all SNPs in Japanese and Caucasians were imputed with the Markov Chain haplotyping (MaCH) software [16] using reference haplotypes in the 1000 Genomes database (version released in February 2012 for Japanese datasets and version released in December 2010 for Caucasian datasets). This procedure also filled in missing data for the genotyped SNPs. Imputation quality was determined as R^2, which estimates the squared correlation between imputed and true genotypes. We applied threshold criteria for quality control assessment of imputed SNPs ($R^2 \geq 0.8$) as recommended for 1000 Genomes imputed data using the IMPUTE2 program [17]. Genotype probabilities for 5,877,918 genotyped and reliably imputed SNPs with a minor allele frequency (MAF) >0.02 were included in the Japanese GWAS.

Statistical analysis

Genotyped and imputed SNPs were tested for association with AD in the Stage 1 dataset using a logistic generalized linear model (GLM) controlling for age-at-onset (cases)/age-at-exam (controls), sex and the first three principal components from analysis of of population substructure. Stage 1 analyses were also performed based on a model adjusting for these covariates and the number of *APOE* ε4 alleles. SNPs in the *APOE* region (between map positions 45,000 kb and 45,800 kb on chromosome 19) were also tested for association in ε3/ε3 and ε3/ε4 subgroups. Genotyped SNPs were coded as 0, 1, or 2 according to the number of minor alleles under the additive genetic model. For imputed SNPs, a quantitative estimate between 0 and 2 for the dose of the minor allele were used to incorporate the uncertainty of the imputation estimates. All analyses were performed using PLINK. SNPs attaining a P value below 5×10^{-5} were considered for replication in Stage 2. Initially, only one SNP per region was tested in the replication sample to minimize the penalty for multiple testing. Additional SNPs from regions meeting the signifcance threshold in the replication sample were also evaluated. SNPs with a P value below 1×10^{-5} in the combined Stages 1 and 2 samples and nominally significant in Stage 2 ($P<0.05$) were advanced to Stage 3.

SNP association results obtained from individual datasets were combined by meta-analysis using the inverse variance method implemented in the software package METAL (http://www.sph.umich.edu/csg/abecasis/Metal/index.html) [18]. An additive model was assumed and the association results across datasets were combined by summing the regression coefficients weighted by the inverse variance of the coefficients. The meta-analysis P-value of the association was estimated by the summarized test statistic, after applying a genomic control within each individual study. Effect sizes were weighted by their inverse variance and a combined estimate was calculated by summing the weighted estimates and dividing by the summed weights.

Results

The quantile-quantile plot indicated limited genomic inflation ($\lambda = 1.04$in the Stage 1 GWAS results (Fig. S1). A total of 125 SNPs from seven distinct regions showed evidence of association with $P<10^{-4}$ (Table S1, Fig. S2). In addition to *APOE* SNP rs429358 ($P=2.46\times10^{-49}$, OR [95% CI] =5.5 [4.4–6.9]), 12 other SNPs in the *APOE* region were associated with LOAD at the genome-wide significance level of $P<5.0\times10^{-8}$. The two most significant results in this group of SNPs were rs12610605 (*PVRL2*: $P=1.38\times10^{-13}$, OR [95% CI] =1.8 [1.5–2.0]) and rs62117161 (between *CEACAM16* and *BCL3*: $P=3.46\times10^{-12}$, OR [95% CI] =0.47 [0.38–0.58]). Since imputation in the *APOE* region using the 1000 Genomes reference panel is unreliable [6], we genotyped nine SNPs from this region, spanning multiple linkage disequilibrium (LD) blocks (Fig. S3) and that were nominally significant in the *APOE* ε3/ε3 subgroup, in the Japanese discovery and replication samples using TaqMan assays (Table S2). Genome-wide significant results were obtained for five of these SNPs, but only the association with *PPP1R37* SNP rs 17643262 remained nominally significant after adjustment for the number of *APOE* ε4 alleles ($P=3.96\times10^{-4}$) or in analyses stratified by *APOE* genotype (ε3/ε3: $P=0.01$; ε3/ε4: $P=0.0016$).

SNPs from six other distinct chromosomal regions met Stage 2 follow-up criteria ($P<5\times10^{-5}$) and the top SNP from each region was genotyped in an independent Japanese sample (Table 2). Two SNPs were nominally significant in the replication sample, however the effect direction for *KIAA0494* SNP rs7519866 differed from the discovery sample. Modest evidence for replication was observed only with *SORL1* SNP rs4598682 ($P\leq0.05$). Subsequently, we selected an additional four *SORL1* SNPs (rs3781834, rs2282647, rs17125523, and rs3737529) for testing in the Japanese replication sample that were among the most significant in the basic or extended models in the discovery sample (Table S1) and not in LD with rs4598682 ($r^2<0.2$, Figure S4). Two of these SNPs (rs3781834 and rs17125523) were chosen also because they were genotyped in the discovery sample and thus would minimize the effects of potential imputation artifacts in meta-analysis of the two Japanese samples. Highly significant results were obtained for *SORL1* SNPs rs4598682 ($P=9.51\times10^{-6}$), rs3781834 ($P=7.33\times10^{-7}$), rs17125523 ($P=5.51\times10^{-6}$), and rs3737529 ($P=4.14\times10^{-6}$) after combining results from the discovery and replication samples (Table S3).

These four *SORL1* SNPs showing significant association in the combined samples from Stages 1 and 2 were considered for further replication in Stage 3. We added rs11218343 to this stage of the analysis because it was the most significant *SORL1* SNP in the large Caucasian dataset ($P=1.0\times10^{-7}$), a result which emerged after pooling the Caucasian discovery GWAS sample and unpublished data in the replication sample from our previously published GWAS [5]. These five SNPs were subsequently evaluated in Stage 3 by meta analysis including the Stage 1 and 2 Japanese, Korean and ADGC Caucasian datasets. SNPs rs11218343 ($P=2.20\times10^{-9}$) and rs3781834 ($P=9.90\times10^{-9}$), attained genome-wide significance in the sample of datasets from all stages (Table 3, Fig. 1). There was modest evidence of replication for rs17125523 (meta $P=3.30\times10^{-6}$) and rs 3737529 (meta $P=5.10\times10^{-6}$). Although the allele frequencies for the top SNPs were very different between the Asian (MAF >0.2) and Caucasian (MAF <0.05) samples (Table 3), there was no evidence of heterogeneity in the magnitude of the odds ratios or effect direction among the population groups ($P>0.15$, Fig. 2). There was no apparent association in the comparably smaller Korean dataset; however, the direction of the effect for each SNP was the same as in the Japanese and Caucasian datsets.

Next, we investigated whether robust genetic associations for LOAD reported previously in Caucasians [4,5] generalize to Japanese. After correcting for 15 tests, SNPs rs3851179 located approximately 90 kb upstream from *PICALM* ($P=1.71\times10^{-5}$) and rs744373 located approximately 30 kb upstream from *BIN1* ($P=1.39\times10^{-4}$) were significantly associated with LOAD risk in the Japanese Stage 1 dataset (Table 4). Nominally significant

Table 2. Top-ranked genome-wide association results in the Japanese discovery (Stage 1) sample ($P<2.5\times10^{-5}$) and their replication in Japanese (Stage 2).

SNP	CH:MB	Nearest Gene	MA	MAF	# SNPs	Discovery (Stage 1)		Replication (Stage 2)		Meta-Analysis (Stages 1+2)	
						OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
rs7519866	1:47.0	*KIAA0494*	G	0.37	52	0.71 (0.61–0.83)	9.70×10^{-6}	1.15 (1.01–1.32)	0.04	0.90 (0.57–1.44)	0.67
rs913360	9:111.7	*PALM2*	G	0.28	20	1.56 (1.43–1.70)	1.83×10^{-7}	1.11 (0.96–1.29)	0.16	1.29 (1.15–1.44)	6.6×10^{-6}
rs1273007	10:9.0	*LOC338591*	T	0.27	39	0.68 (0.62–0.74)	3.08×10^{-6}	0.95 (0.81–1.10)	0.47	0.81 (0.73–0.91)	2.2×10^{-4}
rs10898417	11:85.2	*SYTL2*	G	0.15	2	0.59 (0.53–0.66)	1.17×10^{-6}	1.02 (0.85–1.22)	0.83	0.82 (0.71–0.93)	0.003
rs4598682	11:121.1	*SORL1*	G	0.23	11	0.68 (0.57–0.81)	2.25×10^{-5}	0.83 (0.68–1.00)	0.05	0.75 (0.66–0.85)	9.5×10^{-6}
rs11621843	14:92.2	*RIN3*	G	0.26	19	1.47 (1.35–1.60)	5.19×10^{-6}	1.03 (0.88–1.20)	0.72	1.21 (1.08–1.36)	8.1×10^{-4}

CH:MB, chromosome:position (in megabasepairs, build 19); MA, minor allele; MAF, minor allele frequency; # SNPs, the number of SNPs for which $P\leq1\times10^{-4}$ in the discovery (Stage 1) sample; OR, odds ratio; *P* P-value;
Selected SNPs represent the strongest association within each locus.
doi:10.1371/journal.pone.0058618.t002

associations were also observed for SNPs in *CR1*, *CLU*, and *ABCA7*. Of the eight SNPs tested in the small Korean sample, nominally signficant results ($P<0.05$) were obtained for one SNP in *CLU* and *PICALM*, each with the same pattern of association and comparable effect size as in Japanese.

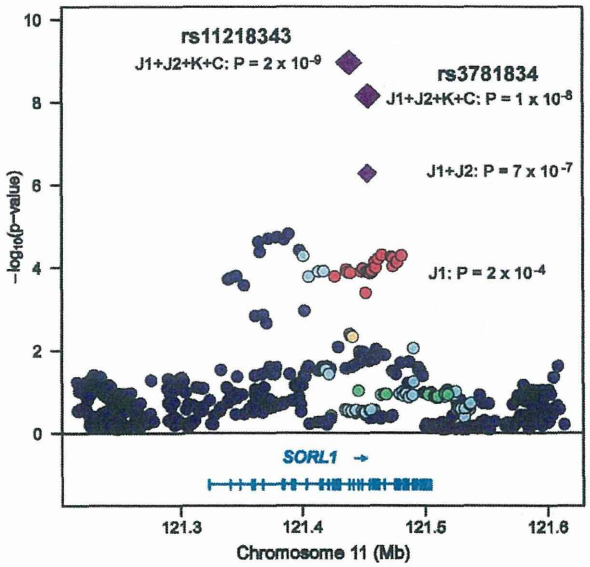

Figure 1. Regional association plot for the *SORL1* region on chromosome 11 in the three-stage design. For each SNP, the chromosomal location is shown on the x-axis and the significance level for association with LOAD is indicated by a $-\log_{10}$P value on the y-axis. P-values are expressed as $-\log_{10}$(P) (y-axis) for every tested SNP ordered by chromosomal location (x-axis). Genomic position was determined using the NCBI database (Build 37.1). Computed estimates of linkage disequilibrium (LD; r^2) between SNPs in this region with the top-ranked SNP (rs3781834) in the Japanese discovery (J1) dataset are shown as red circles for $r^2\geq0.8$, orange circles for $0.5\leq r^2<0.8$, light blue circles for $0.2\leq r^2<0.5$, and dark blue circles for $r^2<0.2$ using hg19/1000 Genomes of Asian populations (ASN; release on November 2010) combined from Han Chinese (CHB) and Japanese (JPT). Meta-analysis *P*-values are shown as purple diamonds for the Japanese datasets (J1+J2) and all datasets (J1+J2+K+C) including Japanese, Korean (K), and Caucasians (C). Two genome-wide significant SNPs in the final stage (rs3781834 and rs11218343) are presented. The gene structure and reading frame are shown below the plot. Exons are denoted with vertical bars. The LD between rs3781834 and rs11218343 is 0.57 in the ASN reference population.
doi:10.1371/journal.pone.0058618.g001

Discussion

Our multi-stage GWAS of LOAD identified for the first-time genome-wide significant association with *SORL1*. Genetic association with *SORL1* was first established in a study focused on genes encoding proteins involved in vacuolar protein sorting [19]. Most, but not all, subsequent studies in Caucasians replicated this finding (summarized in Alzgene database: http://www.alzgene.org/). Confirmatory evidence of association with *SORL1* SNPs has also been reported in comparatively small samples of Chinese and Japanese (reviewed in [20]). These findings are independent of previous candidate gene studies of *SORL1* in Japanese (two subjects in common) and with Caucasians in the Rogaeva et al. study [19] (less than 2% overlap).

The two genome-wide significant *SORL1* SNPs, rs11218343 and rs3781834 are located at chromosome positions 121,435,587 base pairs and 121,445,940 base pairs, respectively, and thus between the two previously reported strongly associated 3-marker haplotypes that extend upstream from rs641120 (121,380,965 base pairs) and downstream from rs1699102 (121,456,962 base pairs) [19]. A recent meta-analysis including more than 30,000 Caucasian and Asian subjects demonstrated that multiple *SORL1* SNPs in distinct regions are associated with AD [20], a finding substantiated in an association study of *SORL1* SNPs with brain MRI traits in LOAD families [21]. Further analysis of our large Caucasian sample suggests that the association peak at rs3781834 is independent of at least one of the two distinct haplotypes previously associated with AD in an independent sample of non-Hispanic Caucasians, Caribbean Hispanics and Israeli-Arabs (Fig. S5) [19], Since all of the SNPs at the association peaks reported in this study and previously are intronic, functional studies are required to determine the identity of pathogenic variants at these locations.

Remarkably, the less frequent alleles at rs11218343 and rs3781834 are protective in both Japanese and Caucasian datasets with very similar odds ratios (range 0.74 to 0.83) despite the fact that these alleles are much rarer in Caucasians (4% and 2%, respectively) than in Japanese (34% and 23%, respectively). The rarity of these SNPs in Caucasians, as well as allelic heterogeneity, may explain why *SORL1* did not previously emerged as a genome-

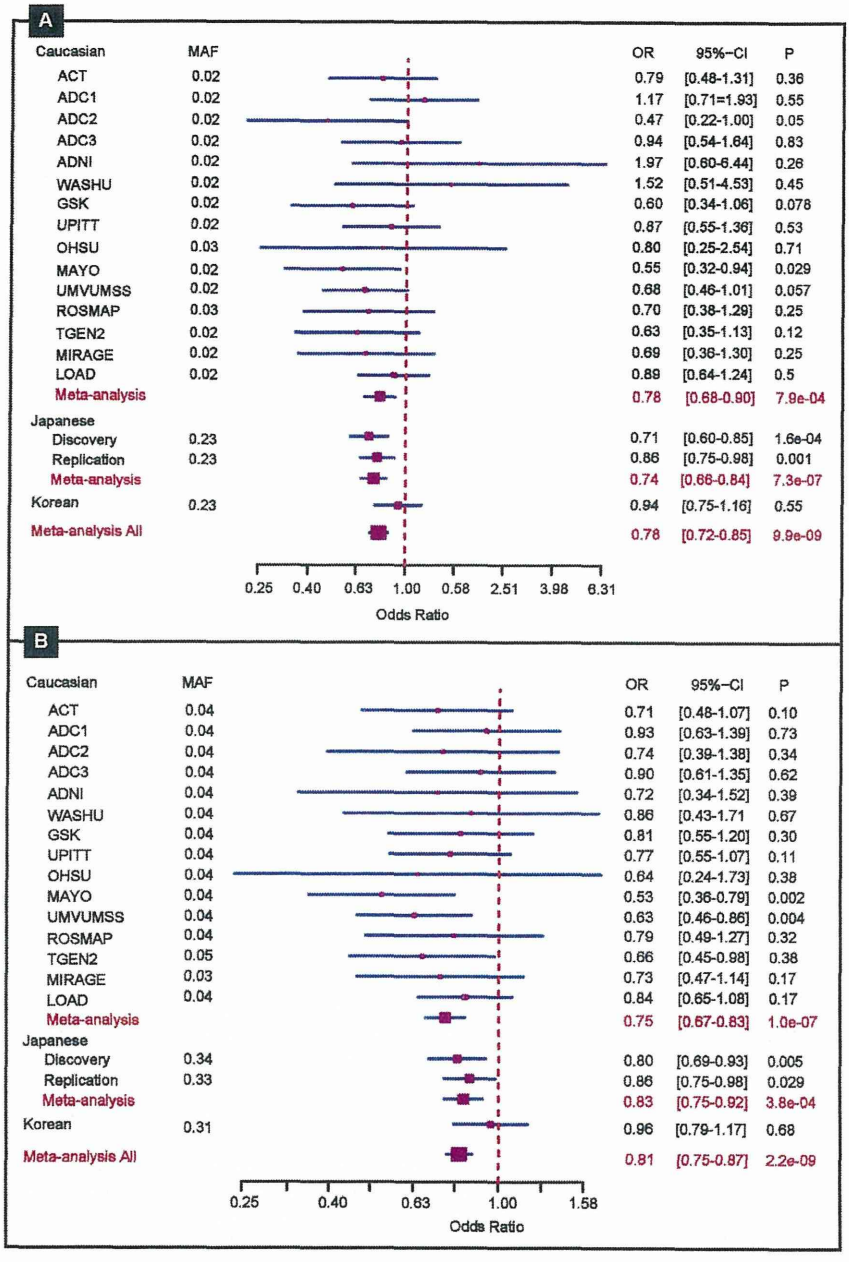

Figure 2. Forest plots of the two most strongly associated SNPs, rs3781834 (A) and rs11218343 (B), in the *SORL1* region showing the strength and pattern of significance in the Japanese discovery and each replication dataset in the model of adjusting for population structure, age, and sex.
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wide significant AD locus in much larger GWAS [4,5]. Given the discovery sample size, effect size (odds ratio [OR] = 0.74) and MAF (0.23) of the top *SORL1* SNP (rs3781834) in the Japanese sample, and a significance level of 2×10^{-5} (i.e., threshold for including a SNP in the Stage 2 replication phase), calculation of power *post hoc* using the PAWE-3D program [22] confirmed that the discovery sample had sufficient power (83.7%). By comparison, the Caucasian sample of 22,771 subjects had only 52.8% power to detect association with this SNP at the observed significance level of 7.9×10^{-4} and OR (0.78) and a much lower MAF (0.02) than in Japanese.

The most significant result in the GWAS in Japanese was obtained for *PALM2* SNP rs913360 ($P=1.8\times10^{-7}$), but this SNP was not significant in the Japanese replication sample ($P=0.16$) and the result for the combined Japanese datasets was less significant than in the discovery sample ($P=6.6\times10^{-6}$). There was no evidence in the large Caucasian dataset supporting association for rs913360 ($P=0.38$) or other *PALM2* SNPs.

Table 3. Meta-analysis of top-ranked association results with *SORL1* in Japanese, Korean, and Caucasian datasets.

SNP	MA	Japanese (Stage 1+2)			Korean (Stage 3)			Caucasian (Stage 3)			Meta-Analysis (Stages 1–3)	
		MAF	OR (95% CI)	P	MAF	OR (95% CI)	P	MAF	OR (95% CI)	P	OR (95% CI)	P
rs4598682	G	0.23	0.75 (0.66–0.85)	9.5×10^{-6}		not available		0.02	1.04 (0.85–1.28)	0.68	0.82 (0.72–0.93)	3.6×10^{-3}
rs11218343	C	0.34	0.83 (0.75–0.92)	3.8×10^{-4}	0.31	0.96 (0.79–1.17)	0.68	0.04	0.75 (0.67–0.83)	1.0×10^{-7}	0.81 (0.75–0.87)	2.2×10^{-9}
rs3781834	G	0.23	0.74 (0.66–0.84)	7.3×10^{-7}	0.23	0.94 (0.75–1.16)	0.55	0.02	0.78 (0.68–0.9)	7.9×10^{-4}	0.78 (0.72–0.85)	9.9×10^{-9}
rs17125523	G	0.25	0.77 (0.68–0.86)	5.5×10^{-6}	0.23	0.96 (0.78–1.19)	0.72	0.02	0.85 (0.74–0.99)	0.034	0.82 (0.76–0.89)	3.3×10^{-6}
rs3737529	T	0.25	0.77 (0.68–0.86)	4.1×10^{-6}	0.26	1.04 (0.84–1.29)	0.70	0.02	0.83 (0.71–0.97)	0.016	0.82 (0.76–0.89)	5.1×10^{-6}

CH:MB, chromosome:position (in megabase pairs, build 19); MA, minor allele; MAF, minor allele frequenc; OR, odds ratio; *P* P-value.
doi:10.1371/journal.pone.0058618.t003

We obtained evidence in Japanese and Korean populations for association of AD with the same SNPs in the *PICALM* and *BIN1* regions that were identified as genome-wide significant in multiple large GWAS in Caucasians [4,5]. There are no previously reported association studies of these loci in Japanese. Several small association studies of *PICALM* in comparatively smaller Chinese samples have yielded conflicting results [23–25]. We also found nominally significant associations in the Japanese sample for previously associated SNPs in *CR1*, *CLU*, and *ABCA7*. Lack of association with *EPHA1, CD2AP, MS4A6A,* and *CD33* may be due to insufficient power, different linkage disequilibrium structure of these regions than in Caucasisans, locus heterogeneity or intragenic heterogeneity.

In addition, our analyses showed numerous highly significant results for imputed SNPs in the *APOE* region (including *CEACAM/BCL3, PVRL2, TOMM40,* and *LOC284352)* even after adjustment for the dose of the ε4 allele. However, recognizing that the reliability of imputation is poor for SNPs in this region [13], we genotyped 10 of the significant SNPs in the Japanese discovery and replication datasets. Only one of these results, a *PPP1R37* SNP, was nominally significant after adjustment for dose of ε4. Association of AD with this SNP, which is located approximately 225 kb from *APOE*, has not been observed previously. *PVRL2* and *APOE* are located in a genomic region sandwiched between two recombination hotspots [26], where strong association signals for LOAD have been reproducibly detected in Caucasians [1,5], but dissipate almost completely for all non-*APOE* loci after conditioning on *APOE*, suggesting that no other loci in this region influence LOAD susceptibility [13]. This conclusion is consistent with the observation of moderate linkage disequilibrium between the SNPs determining *APOE* genotype, rs7412 and rs429358 (Fig. S5), SNPs showing genomewide significant evidence for association with LOAD without adjustment for *APOE* genotype, and our prior LOAD association studies with SNPs in this region among Caucasians [13].

SorL1, also known as SorLA and LR11, and APP proteins are co-localized in the endosomal and Golgi compartments [27]. SorL1 through its co-dependent interaction with vps26 regulates the intracellular transport and processing of APP, resulting in reduction of amyloid beta (Aß) peptide production [20,27,28]. *SORL1* knock-out mice carrying both pathogenic mutations in the *PSEN1* (exon 9 deletion) and *APP* (Swedish, K595M/N596L)

Table 4. Association of LOAD in Asians with SNPs showing genome-wide significance in Caucasians.

Gene	CH	BP	SNP	MA	Japanese			Korean		
					MAF	P	OR (95% CI)	MAF	P	OR (95% CI)
CR1	1	207,692,049	rs6656401	A	0.04	***9.02E-03***	1.38 (1.08–1.76)	0.04	3.75E-01	1.24 (0.77–1.99)
CR1	1	207,784,968	rs3818361	A	0.39	2.54E-01	0.94 (0.85–1.04)	0.31	4.08E-01	0.92 (0.76–1.12)
BIN1	2	127,894,615	rs744373	G	0.33	***1.39E-04***	1.25 (1.11–1.4)	0.36	8.05E-01	0.98 (0.81–1.18)
CD2AP	6	47,453,378	rs9349407	G	0.14	3.83E-01	0.94 (0.82–1.08)	NT	–	–
EPHA1	7	143,109,139	rs11767557	C	0.17	6.47E-01	1.03 (0.9–1.17)	NT	–	–
CLU	8	27,456,253	rs2279590	T	0.25	***7.01E-03***	0.85 (0.76–0.96)	0.2	9.70E-02	0.82 (0.65–1.04)
CLU	8	27,464,519	rs11136000	T	0.28	***1.09E-02***	0.87 (0.78–0.97)	0.23	***3.61E-02***	0.79 (0.63–0.98)
CLU	8	27,468,862	rs9331888	G	0.41	6.97E-02	1.1 (0.99–1.22)	0.47	1.92E-01	0.89 (0.74–1.06)
MS4A6A	11	59,939,307	rs610932	T	0.3	7.99E-01	0.99 (0.89–1.1)	NT	–	–
MS4A6A	11	59,971,795	rs670139	T	0.4	8.23E-01	0.99 (0.89–1.09)	NT	–	–
MS4A6A	11	60,034,429	rs4938933	C	0.27	3.23E-01	1.06 (0.95–1.18)	NT	–	–
PICALM	11	85,868,640	rs3851179	T	0.39	***1.71E-05***	0.8 (0.73–0.89)	0.34	***1.99E-02***	0.79 (0.66–0.96)
ABCA7	19	1,046,520	rs3764650	G	0.42	***3.66E-02***	1.13 (1.01–1.27)	NT	–	–
EXOC3L2	19	45,708,888	rs597668	C	0.43	***8.23E-03***	0.88 (0.79–0.97)	0.37	7.31E-01	0.97 (0.8–1.17)
CD33	19	51,727,962	rs3865444	A	0.2	4.92E-01	1.04 (0.92–1.18)	NT	–	–

NT not tested; P<0.05 was italized.
doi:10.1371/journal.pone.0058618.t004

exhibited increased production and accumulation of Aß [29]. *SORL1* variants might influence the CSF Aß42 level in AD patients [30]. Recently, Pottier et al. sequenced the exomes of 29 index cases with autosomal dominant early-onset AD who lacked mutations in *APP, PSEN1* and *PSEN2* [2]. Seven of these subjects had private *SORL1* mutations (2 nonsense and 2 missense) that were predicted to have a pathogenic effect. By comparison, the two genome-wide significant SNPs in this study are both intronic. It is expected that future large resequencing studies of *SORL1* will identify the functional variants, thus providing important clues about the mechanisms governing normal and abnormal action of SorL1 on processes leading to LOAD. The emergence of *SORL1* as a genome-wide significant locus for AD confirms existing genetic and functional evidence and elevates the importance of intracellular trafficking involving retromer and the Golgi-to-endosome as a key pathway leading to AD [31,32].

Supporting Information

Figure S1 Quantile-quantile (Q-Q) plot of observed (y-axis) vs. expected (x-axis) *P*-values from tests of association genome-wide (5,877,918 SNPs) adjusted for population structure, age and sex for LOAD in the Japanese discovery sample. Genomic inflation was low ($\lambda = 1.047$).
(TIF)

Figure S2 Manhattan plot of observed $-\log_{10}$ *P*-values for genome-wide SNP association tests for LOAD (y-axis) according to chromosomal location (x-axis) in the Japanese discovery sample adjusted for population structure, age, and sex. All genome-wide significant SNPs (above the horizontal line corresponding to $P = 5 \times 10^{-8}$ on the y-axis) are located in the *APOE* region on chromosome 19.
(TIF)

Figure S3 Linkage disequilibrium (r^2) among SNPs in the *APOE* region genotyped using TaqMan calculated in the Japanese discovery (A) and replication (B) datasets. *APOE* genotype is derived from haplotypes of coding SNPs rs429358 and rs7412.
(TIF)

Figure S4 Linkage disequilibrium (r^2) among SNPs in the *SORL1* region genotyped in the Japanese discovery (A) and replication (B) datasets.
(TIF)

Figure S5 Comparison of *SORL1* association findings in the current study with association signals previously identified by Rogaeva et al. [20]. (**A**) Regional association plot of the *SORL1* region. P-values are expressed as $-\log_{10}$(P) (y-axis) for every tested SNP ordered by chromosomal location (x-axis) and represented as blue rectangles for the Japanese discovery set (J1), light blue diamonds for the ADGC Caucasian set (C), pink circles for meta-analysis of Japanese discovery and Caucasian sets (J1+C), and red circles for meta-analysis of Japanese discovery, Japanese replication (J2), Korean (K), and Caucasian sets (J1+J2+K+C). The numbers below the line showing the orientation of *SORL1* are the designations for associated SNPs in the Rogaeva et al. study: 8 = rs668387, 9 = rs689021, 10 = rs641120, 11 = rs4935775, 19 = rs2070045, 22 = rs1699102, 23 = rs3824968, 24 = rs2282649, and 25 = rs1010159. Recombination hotspots are indicated by the continuous blue line behind the symbols for the SNP P-values. (**B**) Linkage disequilibrium (r^2) of the previously associated SNPs in the *SORL1* region [20] in the HapMap 2 reference Japanese population (JPT). The association signal with rs3781834 (contained in Block 2) appears to be independent of one of the distinct AD-associated haplotypes reported by Rogaeva et al. [20] (including SNPs in Block 1), but not necessarily independent of the other AD-associated haplotype reported by Rogaeva et al which includes rs1699102 in Block 2 and the SNPs in Block 3.
(TIF)

Table S1 Top-ranked GWAS results in the Japanese GWAS dataset ($P<1\times10^{-4}$ and imputation quality ≥0.8) with and withut adjustment for the number of *APOE* ε4 alleles.
(DOCX)

Table S2 Association of individually genotyped SNPs in the *APOE* region in models with and without adjustment for the number of *APOE* ε4 alleles.
(DOCX)

Table S3 Association results for *SORL1* SNPs genotyped in the Japanese replication sample.
(DOCX)

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References

1. Ertekin-Taner N (2010) Genetics of Alzheimer disease in the pre- and post-GWAS era. Alzheimer's Res Ther 2: 3.
2. Pottier C, Hannequin D, Coutant S, Rovelet-Lecrux A, Wallon D, et al. (2012) High frequency of potentially pathogenic SORL1 mutations in autosomal dominant early-onset Alzheimer disease. Mol Psychiatry 17: 875–879.
3. Farrer LA, Cupples LA, Haines JL, Hyman BT, Kukull WA, et al. (1997) Effects of age, gender and ethnicity on the association of apolipoprotein E genotype and Alzheimer disease. JAMA 278: 1349–1356.
4. Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, et al. (2012) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 43: 429–435.
5. Naj AC, Jun G, Beecham GW, Wang L-S, Vardarajan BN, et al. (2011) Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet 43: 436–441.
6. Kuwano R, Kuwano R, Miyashita A, Arai H, Asada T, et al. (2006) Dynamin-binding protein gene on chromosome 10q is associated with late-onset Alzheimer's disease. Hum Mol Genet 15: 2170–2182.
7. Miyashita A, Arai H, Asada T, Imagawa M, Matsubara E, et al. (2007) Genetic association of CTNNA3 with late-onset Alzheimer's disease in females. Hum Mol Genet 16: 2854–2869.
8. McKhann G, Drachman D, Folstein M, Katzman R, Price D, et al. (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34: 939–944.
9. Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12: 189–198.
10. Morris JC (1993) The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology 43: 2412–2414.
11. Reisberg B (1988) Functional assessment staging (FAST). Psychopharmacol Bull 24: 653–659.
12. Seo SW, Im K, Lee JM, Kim YH, Kim ST, et al. (2007) Cortical thickness in single- versus multiple-domain amnestic mild cognitive impairment. Neuroimage 36: 289–297.
13. Jun G, Vardarajan BN, Buros J, Yu C-E, Hawk MV, et al. (2012) A comprehensive search for Alzheimer disease susceptibility loci in the APOE region. Arch Neurol 69: 1270–1279.
14. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559–575.
15. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, et al. (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 38: 904–909.
16. Li M, Boehnke M, Abecasis GR (2006) Efficient study designs for test of genetic association using sibship data and unrelated cases and controls. Am J Hum Genet 78: 778–792.
17. Donnelly P, Marchini J (2009) A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genetics 5: e100529.
18. Willer CJ, Li Y, Abecasis GR (2010) METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics 26: 2190–2191.
19. Rogaeva E, Meng Y, Lee JH, Gu Y-J, Zou F, et al. (2007) The sortilin-related receptor SORL1 is functionally and genetically associated with Alzheimer's disease. Nat Genet 39: 168–177.

20. Reitz C, Rogaeva E, Lee JH, Tokuhiro S, Bettens K, et al. (2011) Association of SORL1 gene variants with Alzheimer's disease: A meta-analysis. Arch Neurol 68: 99–106.
21. Cuenco KT, Lunetta K, Baldwin CT, McKee AC, Guo J, et al. (2008) Distinct variants in SORL1 are associated with cerebrovascular and neurodegenerative changes related to Alzheimer disease. Arch Neurol 65: 1640–1648.
22. Gordon D, Haynes C, Blumenfeld J, Finch SJ (2005) PAWE-3D: visualizing power for association with error in case-control genetic studies of complex traits. Bioinformatics 21: 3935–3937.
23. Li HL, Shi SS, Guo QH, Ni W, Dong Y, et al. (2011) PICALM and CR1 variants are not associated with sporadic Alzheimer's disease in Chinese patients. J Alzheimers Dis 25: 111–117.
24. Yu JT, Song JH, Ma T, Zhang W, Yu NN, et al. (2011) Genetic association of PICALM polymorphisms with Alzheimer's disease in Han Chinese. J Neurol Sci 300: 78–80.
25. Chen LH, Kao PY, Fan YH, Ho DT, Chan CS, et al. (2012) Polymorphisms of CR1, CLU and PICALM confer susceptibility of Alzheimer's disease in a southern Chinese population. Neurobiol Aging 33: 210.e1–7.
26. Takei N, Miyashita A, Tsukie T, Arai H, Asada T, et al. (2009) Genetic association study on in and around the APOE in late-onset Alzheimer disease in Japanese. Genomics 93: 441–448.
27. Andersen OM, Reiche J, Schmidt V, Gotthardt M, Spoelgen R, et al. (2005) Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. Proc Natl Acad Sci USA 102: 13461–13466.
28. Fjorback AW, Seaman M, Gustafsen C, Mehmedbasic A, Gokool S, et al. (2012) Retromer binds the FANSHY sorting motif in SorLA to regulate amyloid precursor protein sorting and processing. J Neurosci 32: 1467–1480.
29. Dodson SE, Andersen OM, Karmali V, Fritz JJ, Cheng D, et al. (2008) Loss of LR11/SORLA enhances early pathology in a mouse model of amyloidosis: evidence for a proximal role in Alzheimer's disease. J Neurosci 28: 12877–12886.
30. Kölsch H, Jessen F, Wiltfang J, Lewczuk P, Dichgans M, et al. (2008) Influence of SORL1 gene variants: association with CSF amyloid-beta products in probable Alzheimer's disease. Neurosci Lett 440: 68–71.
31. Vardarajan BN, Breusegem SY, Harbour ME, Rivka Inzelberg, Friedland R, et al. (2012) Identification of Alzheimer disease associated variants in genes that regulate retromer function. Neurobiol Aging 33: 2231.e15–2231.e30.
32. Choy RW-Y, Cheng Z, Schekman R (2012) Amyloid precursor protein (APP) traffics from the cell surface via endosomes for amyloid β (Aβ) production in the trans-Golgi network. Proc Natl Acad Sci USA 109: 2077–2082.

平成25年度厚生労働科学研究費補助金（厚生労働科学特別研究事業）
「『認知症予防のための戦略研究』研究実施計画書作成に関する研究」
分担研究報告書

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○研究要旨

高齢化社会の進行とともに、認知症の高齢者数も激増しつつある。ところが根本的な治療法は現在のところない。一方で認知症の発症予防に関しても確実に有効とされるものはない。しかし多くの研究で、有酸素性運動等の運動の有効性が報告されてきた。そこで多数の地域住民において運動習慣をつけることによる認知症予防介入研究が今後実施される。そこでは、どのような介入方法を考え、実施体制を作り、成果物作成に関するプランニングを行うべきかについて討議を重ね、計画を作成した。

A. 研究目的

運動習慣作りを介した行動変容によって認知症予防の可能性を探る。そのためにどのような介入、実施体制、成果物作成のための具体的な方法について実行可能で有用性の高い計画を作成する。

B. 研究方法

戦略研究に関する指導的立場の委員によって定められた研究方針に則って作業を進める。本研究の目標を達成するためにはいかなる介入方法で、どのような実施体制構築し、さらに優れた成果物を生み出すための具体的計画を研究班員による研究と討議の中から生みだす。本研究班では、この介入研究を機能させるための組織として部会と委員会を置いて、これらの機能を個々に定めてゆき、併せて横の連携を考える中から具体案を策定する。

（倫理面への配慮）

「世界医師会ヘルシンキ宣言」及び「臨床研究に関する倫理指針」を遵守し、必要に応じて「遺伝子研究に関する倫理指針」を遵守する。

C. 研究結果

予め定めた以下の7重要項目について班員間で検討した。具体的にはⅠ目標登録症例数の設定、Ⅱ学術サイトの役割・責務、Ⅲ運動介入の実際、Ⅳ座学の実行：認知症予防の学習、Ⅴ戦略研究中央事務局機能、Ⅵ評価項目と臨床判定、Ⅶ生化学的データである。これらの成果については、現在要約して研究指針を示すプロトコールとして完成させつつある。また個々の実務や規約については、マニュアルとして個々にまとめている。加えて学術サイトとして、参加候補となる鳥取県下の自治体の担当者との相談を行った。

D. 考察

認知症予防についてのわが国で初の大規模研究を行うための実務に関する基本方針を定めた。今後はまず参加自治体等との強力な連携体制作りが必要になる。

E. 結論

運動習慣をつけることで認知症発生を防御するための介入方法、実施体制を計画するとともに、世界レベルの成果物を出すための方略を研究班員による討議によって立案した。その一方で参加の可能性がある自治体との実務的な相談も行った。

F. 研究発表

1. 論文発表

2. 学会発表

H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

平成25年度厚生労働科学研究（厚生労働科学特別研究事業）
「認知症予防のための戦略研究」研究実施計画書作成に関する研究
分担研究報告

地域高齢住民における認知症の有病率調査：久山町研究

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○研究要旨　本調査では，2012年に福岡県久山町の65歳以上の高齢住民を対象として行われた認知症の有病率調査の未受診者に対する追加調査を行った．対象者は529名で，そのうち383名（受診率74.4%）が調査を受診した．調査では2段階方式の調査法を用いた．追加調査における認知症の粗有病率は17.1%（88名）であり，MCIの粗有病率は3.1%（18名）だった．追加調査者における介護保険認定率は13.7%（71名）で，介護認定を受けた認知症者における認知症高齢者の日常生活自立度Ⅰ度以上の頻度は92.2%(47名)だった．追加調査者は初調査者と比べて有意に高齢で，認知症の粗有病率および介護保険認定率も有意に高く（すべてP<0.01），重症例ほど調査を受けないことが明らかとなった．

前年度の調査成績と合わせた最終的な受診率は93.5%だった．全認知症の粗有病率は17.9%であり，MCI10.0%を含めると27.9%の高齢者が何らかの認知機能障害を有していた．認知症の病型別粗有病率をみるとアルツハイマー病は12.3%，血管性認知症は3.0%，その他の認知症は2.6%だった．本調査における介護保険の認定率は12.8%(243名)であり，介護認定を受けた認知症者の90.6%(163名)が認知症高齢者の日常生活自立度Ⅰ度以上と診断されていた．

A. 研究目的

わが国は超高齢社会を迎え，それに伴い認知症高齢者が急増しており，認知症対策が大きな医療・社会問題となっている．福岡県久山町では精度の高い認知症の疫学調査が継続中である．我々は厚生労働科学研究「都市部における認知症有病率と認知症の生活機能障害への対応」(研究代表者：朝田隆)の分担研究において，2012年5月末から12月末までに実施した認知症有病率調査(受診率74.4%)を報告した．その結果，認知症の粗有病率は16.6%と高かったが，受診者と未受診者の違いを比べたところ，女性および施設入所または病院入院中の頻度に違いはなかったが，未受診者の方が有意に高齢で（受診者75.7歳，未受診者77.7歳，$p<0.01$），介護保険の認定率も有意に高く(受診者11.3%，未受診者17.9%，$p<0.01$)，認知症の実態を過小評価している可能性が高かった．そこで，2013年に追加調査として行った認知症の有病率について検討した．

B. 研究方法

本研究の対象者は，2012 年 4 月 1 日時点で福岡県久山町に在住する 65 歳以上の全住民 2,083 名のうち，調査を開始した同年 5 月から同年 12 月までに実施した初調査を未受診であった 529 名とした．調査では 2 段階方式の調査法を用い，第 1 段階のスクリーニング調査では，原則的に医師が各対象者を直接面接し，Mini-Mental State Examination，改訂版長谷川式簡易知能評価スケール，改訂版 Wechsler memory scale logical memory A，および Clinical Dementia Rating を用いて認知機能低下者を抽出した．認知機能低下が疑われる者に対して 2 次調査を行い，家族あるいは主治医からの病歴聴取，神経・理学的所見，The Psychogeriatric Assessment Scales，および The Geriatric Depression Scale -Short Form -Japanese より，臨床的な認知症や軽度認知障害(MCI)の有無，重症度，および認知症の病型を判定した．認知症，アルツハイマー病（AD），血管性認知症（VaD）の診断には，それぞれ DSM-ⅢR，NINCDS-ADRDA，NINDS-AIREN の基準を用いた．

（倫理面の配慮）

本研究は，「疫学研究に関する倫理指針」に基づき研究計画書を作成し，九州大学医学部倫理委員会の承認を得て行われた．本研究は，すべての対象者または代諾者からインフォームドコンセントを取得したうえで実施した．研究者は，対象者の個人情報の漏洩を防ぐうえで細心の注意を払い，その管理に責任を負っている．

C. 研究結果

2013 年度の追加調査には 383 名（男性：143 名，女性：240 名）が受診した．追加調査の受診率は，調査期間中の転出・死亡者 14 名を除くと 74.4%だった．追加調査の受診者のうち，認知症を有する高齢者は 88 名，MCI を有する高齢者は 18 名だった（全認知症の粗有病率 17.1%，MCI の粗有病率 3.1%）．認知症を病型別にみると，AD は 51 名，VaD は 5 名，その他の認知症は 32 名であり，その粗有病率はそれぞれ 9.9%，1.0%，6.2%だった．追加調査者における介護保険認定率は 13.7%（71 名）であり，介護認定を受けた認知症者の 92.2%(47 名)が認知症高齢者の日常生活自立度Ⅰ度以上と診断されていた．

2012 年度の調査成績と合わせると，調査期間中に死亡・転出した 47 名を除いた 2,036 名のうち最終的に 1,904 名（受診率 93.5%）が有病率調査を受診した．全認知症の粗有病率は 17.9%であり，MCI を含めると 27.9%の高齢者に何らかの認知機能障害が認められた．認知症を病型別にみると，AD，VaD，およびその他の認知症の粗有病率はそれぞれ 12.3%，3.0%，2.6%だった．また，有病率調査受診者における介護保険の認定率は 12.8%（243 名）であり，介護保険認定者の 74.1%(180 名)に認知症を認めた．介護認定を受けた認知症者における認知症高齢者の日常生活自立度Ⅰ度以上の頻度は 90.6%(163 名)だった．

D. 考察

今回の久山町における断面調査の成績では，認知症の有病率は 17.9%と極めて高く，最近の高齢者では，5.6 人に 1 人が認知症を有すると推定される．この成績を全国に当てはめると，現在では認知症高齢者は 550 万人に達する可能性がある．久山町で 1985 年，1992 年，