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A Common DIO2 Polymorphism And Alzheimer's Disease Dementia in African And European Americans

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Context: A common single nucleotide polymorphism in *DIO2*, Thr92AlaD2, has been associated with a transcriptome typically found in neurodegenerative diseases in postmortem human brain tissue.

Objective: To determine whether Thr92AlaD2 is associated with incident Alzheimer's disease (AD).

Design: Population-based study; human brain tissue microarray.

Setting: Community-based cohorts from Chicago, Illinois and northeastern Illinois, as well as religious clergymen from across the U.S. made up the primary population. A representative sample of the U.S. population was used for secondary analyses.

Participants: 3054 African (AA) and 9304 European Americans (EA).

Main Outcome Measure: Incident AD.

Results: In the primary population, AAs with Thr92AlaD2 had 1.3 times (95% CI, 1.02 to 1.68; P=0.048) higher odds of developing AD. AAs from a second population with Thr92AlaD2 had a trend towards increased odds of dementia (odds ratio (OR) 1.33; 95% CI, 0.99 to 1.78; P=0.06); they also exhibited 1.35 times higher odds of developing cognitive impairment not demented (CIND, 95% CI, 1.09 to 1.67; P=0.006). Meta-analysis showed that AAs with Thr92AlaD2 had 1.3 times increased odds of developing AD/dementia (95% CI, 1.07 to 1.58; P=0.008). In EAs there was no association between Thr92AlaD2 and AD, dementia, or CIND. Microarray of AA brain tissue identified transcriptional patterns linked to AD pathogenesis.

Conclusions: Thr92AlaD2 is associated with molecular markers known to underlie AD pathogenesis in AAs; this translates to an observed phenotype of increased odds of developing AD/dementia in AAs in these populations. Thr92AlaD2 may represent one factor contributing to racial discrepancies in incident AD.

McAninch et al evaluated populations with longitudinal cognitive outcomes and found Thr92AlaD2 to be associated with incident Alzheimer's disease in African Americans but not European Americans.

Introduction

The type II deiodinase (D2) activates thyroxine (T_4) to triiodothyronine (T_3) in peripheral tissues, including the cerebral cortex where it is highly expressed (1). There is a common single nucleotide polymorphism (SNP), rs225014, in *DIO2* with a minor-allele frequency (MAF) of about 40% (2). This SNP results in a single amino acid substitution of threonine (Thr) for alanine (Ala) at position 92 in the D2 protein (Thr92AlaD2) (3); the substitution is distant from D2's catalytic site, within the instability loop (4). Yet the impact of this polymorphism on D2 activity have been not been consistently replicated; some studies suggest that Thr92AlaD2 kinetics are largely intact when transiently expressed in cells (2) and that carriers (i) exhibit normal markers of T_3-responsiveness in brain tissue (5), (ii) have normal thyroid function tests (2,6,7), and (iii) require equivalent replacement doses of levothyroxine in hypothyroidism (8,9), while other studies suggest that Thr92AlaD2 does impair T_4-to-T_3 conversion *in vitro* and *in vivo* (10).

The clinical relevance of Thr92AlaD2 has been controversial as it has been associated with diverse metabolic and cognitive phenotypes (11). These associations have not been consistently replicated and although this could be due to multiple pathway mechanisms or other gene-gene interactions (12), study design heterogeneity and lack of statistical power likely contribute to poor reproducibility and lend these studies prone to false-positives (11). At least some of the molecular consequences of Thr92AlaD2 expression have been described; Thr92AlaD2 accumulates in cells (5,10) and, when stably expressed, instead of remaining in the endoplasmic reticulum escapes to the Golgi apparatus that exhibits a perturbed morphology (5). Human temporal lobe samples from Thr92AlaD2 carriers exhibit transcriptional alterations in processes typically associated with neurodegenerative diseases, such as amyloid-beta (Aβ) peptide processing (5).

In the current study, we tested the hypothesis that carriers of the Thr92AlaD2 polymorphism have an increased risk for incident Alzheimer's disease (AD). Although this locus has not been identified in previous genome-wide association studies (GWAS) (13-15), the candidate gene approach could still identify a moderate association that provides novel insight into the multifactorial pathogenesis of AD (16,17). The epidemiology and tissue pathology of AD vary by ethnicity: there may be higher incidence and prevalence of AD in African Americans (AA) compared to European Americans (EA) (18) and AAs are more likely to have mixed tissue pathologies compared with clinically matched EAs (19). Thus well-described populations with AA and EA participants from longitudinal, population-based studies with cognitive outcomes and SNP availability were utilized.

Subjects and Methods

A. Study Populations and Design
Three well-described, longitudinal studies, the Chicago Health and Aging Project (CHAP) (20), the Religious Orders Study (ROS) (21), and the Rush Memory and Aging Project (MAP) (22,23), that used nearly identical methods for clinical diagnosis of AD were utilized for primary investigations.

CHAP has been described previously (20); in brief, residents from a geographically defined, biracial community of AA and EA participants in Chicago from 1993 to 2012 were enrolled. Interviews, including cognitive testing, were performed in approximately three-year cycles over 18 years; genotype data was available for 3656 participants (24).

The ROS began in 1994 and enrolled older Catholic priests, nuns and brothers from groups across the US (21). MAP started in 1997, enrolling older community-based individuals from retirement communities, other housing units, as well as social service agencies and Church

groups in northeastern Illinois (22,23). In ROS and MAP, participants without known dementia were enrolled and underwent annual clinical evaluations; there were available SNP data from 1707 ROS/MAP participants.

For replication of main findings, another large, homogenous cohort with the same outcome measures was not identified; of note, there is a large, biracial cohort but it is a composite of more than 10 smaller, relatively heterogeneous studies (14). Thus, the Health and Retirement Study (HRS) was used as a secondary population. HRS is a longitudinal survey of a representative sample of the US population over age 50 occurring every two years (25). Publically available demographics, cognitive outcomes, and genotyping data for 6995 participants were obtained through the NIH Database of Genotypes and Phenotypes (26).

B. Cognitive Function and AD Diagnosis
A composite cognitive function score for CHAP was generated based on results from four cognitive tests for episodic memory, executive functioning, and the MMSE by averaging the tests together after centering and scaling each to their baseline mean and SD. In a stratified random sample, CHAP participants underwent a uniform clinical evaluation within their homes that included a structured medical history, neurologic examination, and a cognitive assessment consisting of a battery of 19 tests for episodic memory, executive function, general orientation and global cognition (27). A neurologist, who was unaware of previously collected data, reviewed the results and diagnosed mild cognitive impairment (MCI) and AD according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria. These criteria require a history of cognitive decline and evidence of impairment in two or more cognitive domains, one of which must be memory, for a diagnosis of AD.

In ROS/MAP, clinical evaluations were performed during annual home visits; a similar cognitive function test score was created for ROS/MAP with a comprehensive battery of tests for episodic memory, executive functioning, verbal fluency, and visual-spatial tests for cognition. The clinical diagnoses of MCI and AD were determined as in CHAP (28).

In HRS, a neuropsychological test battery was administered and the results reviewed by a geropsychiatrist, neurologist, neuropsychologist, and cognitive neuroscientist, who assigned a preliminary research diagnosis based on composite memory test scores ranging from 0-27; the composite included immediate and delayed recall items, the serial 7s, and backward counting (29). Diagnoses fell within the three categories: normal cognitive function (12-27), cognitive impairment not demented (CIND) (7-11), and dementia (0-6); data on functional impairment was reported by the participant or informant and incorporated into the assessment (29) based on published criteria including the Diagnostic and Statistical Manual of Mental Disorders III-revised and IV (25). Demented HRS participants were not further stratified by etiology (e.g. AD, vascular).

The different diagnostic strategies utilized in CHAP/ROS/MAP and HRS deserve emphasis. Whereas in CHAP/ROS/MAP clinical diagnoses of MCI and AD were determined, in HRS participants with abnormal cognitive function were categorized as either CIND or with nonspecific dementia. CHAP exhibited a higher AD prevalence estimate than HRS due to the higher threshold for dementia diagnosis used in HRS (30). In other words, it is possible that CIND participants in HRS would have been diagnosed with AD in CHAP or ROS/MAP (30). In our analyses we initially assessed for incident dementia in HRS, but then expanded our approach to assess for association at the milder cognitive phenotypes (MCI and CIND) as this was thought to better capture any genotype-phenotype association.

C. Genotyping of DIO2 rs225014

In CHAP, genotyping was performed using the hME Sequenom MassARRAY platform (24). In ROS/MAP, genotyping data was generated on an Affymetrix 6.0 platform (31). Both CHAP and ROS/MAP data were imputed using a multi-ethnic reference population in 1000 Genome Pilot 1 Version 2 reference data in the same quality control pipeline. The overall imputation quality score was more than 0.98 for rs225014. In HRS, SNPs were identified using the Illumina beadchip platform and rs225014 genotype status imputed based on the 1000 Genomes Project (32).

D. Covariates

All of our regression models were adjusted for age (measured in years and centered at 75), sex, and education (measured in number of years of schooling completed centered at 12).

E. Statistical Analysis

Descriptive analysis was performed using means and standard deviations for continuous measures, and percentages for categorical measures. Our initial descriptive analysis was stratified by race for each of the study populations. Descriptive comparisons were made using a two-sample t-test for continuous measures, and chi-squared test statistic for categorical measures. The main objective of this investigation was to examine the association of Thr92AlaD2 genotype status with diagnosis of AD. The HRS classification of dementia was based on the memory test score (0-6), and the cutoff scores provide the prevalence of dementia in HRS rather than time-at-incidence of dementia. The CHAP study also had incident AD diagnosis in three year intervals leading to right censored time of diagnosis. For these reasons, we used a logistic regression model after adjusting for age, sex, education, and self-reported hypertension and diabetes (27).

In order to understand the association of the Thr92AlaD2 polymorphism with AD, we used three different modeling approaches; an additive model to see if the presence of each Ala allele was associated with increased odds of AD, a dominant model was used to compare participants with at least one copy of the Ala allele versus those homozygous for the Thr, and a recessive model to see if those homozygous for the Ala were at an increased odds of AD compared to those with at least one copy of the Thr allele. The reasons for the various approaches were to see if the association of the polymorphism could be understood with greater detail. Following population-specific estimates of association between the Thr92AlaD2 polymorphism and AD/dementia, a meta-analysis of odds ratios was performed using an inverse variance weighted estimation method in a fixed effects model (two AA studies and three EA studies was not enough to fit a random or mixed effects meta-analysis model) using metafor package in R program.

F. Linkage Disequilibrium

Regional linkage disequilibrium (LD) plots were generated using the SNP Annotation and Proxy Search tool (Broad Institute) (33) based on genotype data from the 1000 Genomes Project. Plots were created for rs225014 with data from CEU (Utah residents with Northern and Western European ancestry) and YRI (Yoruba in Ibadan, Nigeria) backgrounds (Figure 1).

G. Microarray analysis

The University of Miami Brain Endowment Bank provided genomic DNA and brain tissue samples from postmortem human donors as in previous studies; genotyping of genomic DNA had been performed according to previously published methods (5). AA brain samples from 11 donors without known thyroid or neurologic disease (four Thr/Thr, four Thr/Ala, and three Ala/Ala) were matched by age (p=0.54), sex (male), and BMI (p=0.82) and chosen for further

studies. Homogenous samples were dissected from Brodmann's Area 38 (temporal cortex) by neuroanatomist. Samples were processed and microarray performed as previous (5). Affymetrix Transcriptome Analysis Console software was used to identify individual genes that demonstrate significant differential expression in the comparison of Ala/Ala versus Thr/Thr samples from AA donors (Appendix figure 1). Differential expression analysis was also performed for data obtained in the previous microarray of structurally identical EA samples; Ala/Ala, n=6, versus Thr/Thr, n=6. Those transcripts exhibiting differential expression (3253 from AA samples and 1676 from EA samples) from were then assessed via Pathway Analysis software (Ingenuity).

H. IRB Approval

The Rush University Medical Center institutional review board (IRB) approved the CHAP and ROS/MAP studies. The University of Michigan IRB approved the HRS study. The University of Miami (UM) Brain Endowment Bank provided genomic DNA and brain tissue samples; their protocols were IRB-approved. HRS data was obtained from dbGaP with Rush IRB approval. All participants provided written informed consent.

Results

In the primary study populations, AAs were younger, had higher body mass index, lower education, were more likely to have an HbA_{1c} level greater than 6.5%, and exhibited less mortality during the period of observation than EAs (Table 1). ROS/MAP participants were older than CHAP participants, were more likely to be female, had higher levels of education, and higher mortality. HRS participants were younger, more likely to be diabetic, and had less mortality than those from the primary study populations (Table 1). There were less AAs with available cognitive and genotype data in HRS (n=733) than in CHAP (n=2321).

Genotyping of the CHAP AA participants revealed the MAF to be 45.3% (Table 1). In the EA participants from CHAP and ROS/MAP, rs225014 MAF was 35.7% and 34.5% respectively. The racial discrepancy in MAF was similar in HRS. When all of the participants from CHAP, ROS/MAP, and HRS were combined and stratified by race, the MAF in AAs was significantly higher than that in EAs (43.9% vs. 36.5%, P<0.001; Appendix Table 1). There was no deviation from Hardy-Weinberg equilibrium in either racial group.

Additive, dominant, and recessive statistical models were used to assess for association between Thr92AlaD2 genotype and AD (Table 2). CHAP AAs with the Thr92AlaD2 polymorphism had 1.31 times higher odds of AD (95% CI 1.02-1.68; P=0.048) than those without the polymorphism (additive model), and via the dominant model had 1.85 times higher odds of AD (95% CI 1.20 to 2.85; P=0.005). In the AAs from HRS with the Thr92AlaD2 polymorphism there was a trend toward increased odds of dementia (additive model odds ratio (OR) 1.33; 95% CI 0.99-1.78; P=0.06; dominant model OR 1.14; 95% CI 0.75 to 1.73; P=0.54). Meta-analysis showed that AAs with the Thr92AlaD2 polymorphism had higher odds of AD/dementia (additive model OR 1.30; 95% CI 1.07-1.58; P=0.008; dominant model OR 1.60; 95% CI 1.15-2.22; P=0.006). In the EA participants, there was no association between rs225014 genotype and AD/dementia in CHAP, ROS/MAP, or HRS.

There were major differences in AD/dementia diagnostic strategies between the primary study populations and HRS (i.e. (i) the clinical diagnosis of AD [CHAP/ROS/MAP] vs. unspecified dementia categorization [HRS] and (ii) the higher threshold for dementia diagnosis in HRS than for AD in CHAP/ROS/MAP) (30), therefore we broadened our scope to assess for association at milder degrees of cognitive impairment (Table 3). In AAs from HRS with Thr92AlaD2, there was 1.35 times increased odds of developing CIND in the additive model

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(95% CI 1.09-1.67; P=0.006); this trend was preserved in the dominant model (OR 1.35; 95% CI 0.99 to 1.84; P=0.06). CHAP AAs with Thr92AlaD2 did not exhibit increased odds of developing MCI. In the EA participants, there was no association between rs225014 genotype and MCI/CIND in any of the populations.

Thr92AlaD2 genotype status was not associated with BMI or diabetes in the primary study populations (Table 4). Serum T_4 levels were only available in some CHAP participants; there was no association between Thr92AlaD2 genotype status and serum T_4 level. There was no association between Thr92AlaD2 genotype status and mortality.

Genetic regional LD plots of SNPs near rs225014 for persons of African (Fig. 1A) versus European (Fig. 1B) descent show significant differences in haploblock inheritance patterns. Whereas the EA population has many SNPs with high LD ($r^2 > 0.6$) with rs225014 within the region, this is contrary to the AA population in which there is only one SNP (rs225015) in high LD, $r^2 = 0.93$, with rs225014. This indicates that the allelic inheritance patterns in the chromosomal region near rs225014 differ greatly among these racial ethnic groups.

Brain samples were selected from male, AA donors that were matched by age and BMI and microarray performed as previous (5). Differential expression analysis of the microarray data from AA samples revealed 3253 significantly differentially expressed transcripts in the comparison of Ala/Ala (n=3) versus Thr/Thr (n=4). Then, these 3253 transcripts were run through pathway analysis software that (i) identified the cascade of upstream transcriptional regulators that could explain these observed gene expression changes and (ii) predicted diseases associated with the observed expression patterns. 107 upstream regulators were significant (Appendix Table 2), including APP (amyloid precursor protein, p=0.0007), MAPT (microtubule-associated protein tau, p=0.005), and PSEN1 (presenilin 1, p=0.02), all important in AD pathogenesis. In the predicted disease function of pathway analysis, there was evidence of neurologic disease (Appendix Table 3), specifically AD (p=0.008), neurodegeneration (p=0.009) and tauopathy (p=0.002) (Appendix Table 4).

From the previous EA microarray data (5) a new analysis was performed. 1676 transcripts were significantly differentially expressed in the comparison of Ala/Ala (n=6) versus Thr/Thr samples (n=6). There were 110 significant upstream regulators (Appendix Table 5); this did not identify molecules that were known to be central in the pathogenesis of AD (APP, MAPT, and PSEN1: p=ns). Pathway analysis of differentially expressed genes also didn't reveal any neurodegenerative diseases to be significant (Appendix Table 6).

Discussion

The Thr92AlaD2 polymorphism, more prevalent in AAs than EAs, is associated with the development of AD in AAs in these populations, but not EAs. A major strength of our studies is the utilization of large, well-characterized, populations containing both AA and EA participants in addition to a complimentary molecular analysis of both AA and EA brain samples. Although there were design differences between the population studies that made direct comparison challenging, namely (i) the higher threshold for dementia diagnosis in HRS than for AD in CHAP/ROS/MAP (30), (ii) the nonspecific dementia classification of HRS participants versus the clinical diagnosis of AD in CHAP/ROS/MAP, and (iii) the smaller AA sample size of HRS in comparison to CHAP, we addressed this by assessing not only for incident AD/dementia, but also for incident MCI/CIND. The fact that our findings were consistent among their racial stratifications across these different populations is reassuring; further studies of AA populations, which utilize specific clinical diagnosis of AD, are warranted to replicate our findings. This

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supports the hypothesis that Thr92AlaD2 is a risk factor for neurodegenerative disease in AAs and that Thr92AlaD2 may represent one factor contributing to racial discrepancies in incident AD. This locus may not have been identified in a previous GWAS with AA participants (14), due to its moderate effect size (16,17).

Although the mechanistic explanation for these findings is not implicit, human brain tissue from EA, and now AA, donors with the Thr92AlaD2 risk allele exhibit transcriptional patterns that are associated with neurodegenerative diseases (5). Thr92AlaD2 has been documented *in vitro* to be ectopically located in the Golgi apparatus that exhibits an abnormal morphology with disruption of the expression of many Golgi-related transcripts; the resulting cellular mRNA profile is enriched in transcripts altered in diseases with abnormal Aβ processing (5). Altered Golgi trafficking of APP is implicated in development of AD as Aβ peptide accumulation causes Golgi structural defects that further affect APP trafficking and processing (34). Thus it is conceivable that the cellular and Golgi perturbation associated with Thr92AlaD2 expression promote dysfunction in pathways involved in Aβ peptide processing, contributing to development of AD. It remains possible that there are yet unidentified causal markers that could contribute to the racially-dependent phenotype.

To our knowledge this is the first study to find a Thr92AlaD2-associated phenotype that differs by race, although prior studies of this SNP have been heavily of the Caucasian racial ethnic groups. For instance, in a previous study of non-demented elderly Caucasians, Thr92AlaD2 was not associated with early imaging markers of AD (6).

Understanding the mechanism underlying this association is particularly important in the context of prevention. Mitochondrial dysfunction and Aβ accumulation likely contribute to oxidative stress in AD and cross-sectional data suggest improved cognitive performance among those with higher antioxidant intake, although interventional trials have not consistently demonstrated protection (35). Mitochondrial dysfunction and oxidative stress markers were present in the transcriptomes of human temporal pole samples from Thr92AlaD2 carriers and in the cell model of Thr92AlaD2 expression; some of these alterations in Thr92AlaD2-expressing cells were reversed upon antioxidant treatment (5). Future studies will be needed to determine if antioxidants have a role in prevention of AD amongst AA Thr92AlaD2 carriers.

In previous studies of Brazilian (36), EA (3), Pima Indian (37), Danish (38), and Amish (39) populations the rs225014 MAF was around 40%. In our biracial population, the MAF was higher in AA than EA participants. This will need to be confirmed in other cohorts and further study into any gene-gene interactions or environmental factors that would contribute to the differences in MAF or LD at the rs225014 locus is warranted.

Previous studies have shown an association between Thr92AlaD2 and type 2 diabetes in Brazilians (36), Caucasians (3), and Pima Indians (37) but not in Danish (38) and Amish (39). However, these studies were performed in younger subjects with average ages in the 20's (37)-40's (3,36,38,39). In our biracial, older population there was no association with diabetes or BMI. It is possible that the association was attenuated in these elders due to increased prevalence of type 2 diabetes (4.1% in Americans aged 20-44 versus 25.9% age ≥ 65 years (40)). Longitudinal follow-up of populations in which the association is positive in younger adults would aid in this distinction.

There are several limitations to these studies. That the primary and secondary population studies had different availability of incident outcome data and utilized populations of differing basal characteristics are significant, and thus results need to be interpreted with caution. Further studies with consistent methods performed in large, racially diverse patient populations are

needed to replicate these findings. Also, that the regional LD plots for AA and EA cohorts differed markedly could indicate that the Thr92AlaD2 locus is in LD with a SNP (or SNPs) that may be responsible for the observed association and further studies are needed to define the mechanistic contribution of the two SNPs at a cellular level. Limited numbers of human brain samples were available for transcriptional studies, thus the microarray was not a statistically definitive analysis; future evaluation of more tissue samples by microarray or other technique could be warranted to explore genotype-phenotype associations.

Conclusion

Our results show that in these large, well-characterized, population studies the Thr92AlaD2 polymorphism is associated with development of AD in AAs, but not EAs. This, in addition to concurrent transcriptional evidence, supports the hypothesis that Thr92AlaD2 is a risk factor for neurodegenerative disease. The rs225014 MAF was significantly higher in AA than EA participants and perhaps represents one genetic factor contributing to racial discrepancy in incident AD. It will be important to utilize racially diverse populations in future trials assessing the rs225014 locus as inheritance patterns vary by race, possibly resulting in disease risk stratification as demonstrated in these studies.

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The authors report no conflicts of interest in this work.

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Figure 1. Regional Linkage Disequilibrium Plots at rs225014 by Race. SNPs (diamonds) are indicated by their relative correlation (r^2) with rs225014 (black arrow) plotted against their physical position on chromosome 14. The saturation of the color in the diamond indicates linkage disequilibrium with rs225014 according to scale from $r^2 = 0$ to $r^2 = 1$ based on pairwise r^2 values from the 1000 Genomes Project. The blue line shows the recombination rate across the region. Dashed lines indicate the extent of SNPs within the specified r^2 value of 0.6. Green arrow below shows the location of the known gene in the region (*DIO2*). (A) Plot with SNP data from the YRI (Yoruba in Ibadan, Nigeria) population panel representing African Americans and (B) CEU (Utah residents with Northern and Western European ancestry) population panel representing European Americans. Generated using the SNP Annotation and Proxy Search tool (Broad Institute) (33).

Table 1. Characteristics of Primary and Secondary Cohorts According to Race

		Primary			Secondary	
		CHAP		ROS/MAP	HRS	
		African Americans (n=2321)	European Americans (n=1335)	European Americans (n=1707)	African Americans(n=733)	European Americans (n=6262)
Age at evaluation (years)		77.8 ± 6.1	81.2 ± 7.0	86.3 ± 6.8	75.5±7.2	76.5±7.2
Female sex—no. (%)		1450 (62.6)	822 (61.6)	1181 (69.2)	475 (64.8)	3622 (57.8)
Body-mass index (kg/m²)†		29.6 ± 6.2	27.4 ± 5.2	27.0 ± 5.2	28.6 ± 6.4	27.5 ± 5.6
Highest education—yr		12.0 ± 3.2	14.4 ± 3.2	16.4 ± 3.6	11.6 ± (3.5)	12.9 ± 2.9
Serum total T4 (µg/dL) ‡		7.5 ± 1.6	7.4 ± 1.7	--	--	--
Diabetes status—no. (%)§		772 (21.1)	216 (16.2)	338 (20.2)	284 (38.7)	1707 (27.3)
HbA$_{1C}$ ≥ 6.5%—no. (%)		457 (28.8)	111 (11.4)	142 (16.3)	--	--
Mortality—no. (%)		725 (31.2)	496 (37.2)	1050 (61.5)	121 (16.6)	889 (14.2)
Genotype at rs225014—no. (%)	Thr/Thr	714 (30.8)	556 (41.7)	726 (42.5)	265 (36.1)	2471 (39.5)
	Ala/Thr	1110 (47.8)	604 (45.2)	783 (45.9)	356 (48.6)	2922 (46.7)
	Ala/Ala	497 (21.4)	175 (13.1)	198 (11.6)	112 (15.3)	869 (13.9)
Minor allele frequency (%)		45.3	35.7	34.5	41.6	37.3

Plus-minus values are means ± SD. Mortality was assessed from the Social Security Master Death File and confirmed by the National Death Index through 12/31/2012. CHAP: Chicago Healthy Aging Project; ROS/MAP: Religious Orders Study/Memory and Aging Project; HRS: Health and Retirement Study; Ala: alanine; Thr: threonine; HbA$_{1C}$: glycosylated hemoglobin. †The body-mass index is the weight in kilograms divided by the square of the height in meters. ‡The serum total T4 level is micrograms per deciliter. §Diabetes status was determined by self-report and/or use of a diabetes medication.

Table 2. The Thr92AlaD2 Polymorphism and Odds Ratios for Incident Alzheimer's Disease or Dementia Relative to No Cognitive Impairment.

	Primary		Secondary	Meta-analysis
	CHAP (AD diagnosis)	ROS/MAP (AD diagnosis)	HRS (dementia diagnosis)	(AD/dementia)
African Americans				
Additive Model	1.31 (1.02–1.68)	--	1.33 (0.99–1.78)	1.30 (1.07–1.58)
Dominant Model	1.85 (1.20–2.85)	--	1.14 (0.75–1.73)	1.60 (1.15–2.22)
Recessive Model	1.12 (0.73–1.71)	--	2.15 (1.26–3.70)	1.33 (0.95–1.87)
European Americans				
Additive Model	0.84 (0.59–1.18)	0.98 (0.83–1.16)	0.95 (0.83–1.09)	0.94 (0.84–1.05)
Dominant Model	0.89 (0.56–1.40)	0.99 (0.79–1.24)	0.95 (0.78–1.15)	0.94 (0.81–1.09)
Recessive Model	0.59 (0.26–1.31)	0.95 (0.67–1.35)	0.92 (0.70–1.20)	0.93 (0.42–1.44)

Numbers presented are odds ratios and their 95% confidence intervals. All odds ratios are for the comparison of participants carrying the minor allele at the rs225014 locus versus those of genotype Thr/Thr (referent), where the additive statistical model denotes the comparison of (Ala/Thr +2[Ala/Ala]) versus Thr/Thr, the dominant statistical model denotes the comparison of (Ala/Thr +Ala/Ala) vs. Thr/Thr and the recessive model denotes the comparison of Ala/Ala vs. (Ala/Thr+Thr/Thr). Ala: alanine; Thr: threonine. CHAP: Chicago Healthy Aging Project; ROS/MAP: Religious Orders Study/Memory and Aging Project; HRS: Health and Retirement Study.

Table 3. The Thr92AlaD2 Polymorphism and Odds Ratios for Incident Mild Cognitive Impairment or Cognitive Impairment-Not Demented, Relative to No Cognitive Impairment.

	Primary		Secondary	Meta-analysis
	CHAP (MCI diagnosis)	ROS/MAP (MCI diagnosis)	HRS (CIND diagnosis)	(MCI/CIND)
African Americans				
Additive Model	1.02 (0.86–1.21)	--	1.35 (1.09–1.67)	1.27 (1.07–1.52)
Dominant Model	1.03 (0.79–1.34)	--	1.35 (0.99–1.84)	1.28 (0.99–1.64)
Recessive Model	1.04 (0.77–1.39)	--	1.74 (1.17–2.58)	1.23 (0.97–1.56)
European Americans				
Additive Model	0.88 (0.67–1.12)	0.99 (0.83–1.20)	0.99 (0.91–1.08)	0.98 (0.91–0.95)
Dominant Model	0.88 (0.62–1.24)	0.90 (0.71–1.15)	0.98 (0.87–1.10)	0.95 (0.86–1.06)
Recessive Model	0.75 (0.44–1.28)	1.19 (0.82–1.75)	1.00 (0.85–1.18)	1.00 (0.86–1.15)

Numbers presented are odds ratios and their 95% confidence intervals. All odds ratios are for the comparison of participants carrying the minor allele at the rs225014 locus versus those of genotype Thr/Thr (referent), where the additive statistical model denotes the comparison of (Ala/Thr +2[Ala/Ala]) versus Thr/Thr, the dominant statistical model denotes the comparison of (Ala/Thr +Ala/Ala) vs. Thr/Thr and the recessive model denotes the comparison of Ala/Ala vs. (Ala/Thr+Thr/Thr). Ala: alanine; Thr: threonine. CHAP: Chicago Healthy Aging Project; ROS/MAP: Religious Orders Study/Memory and Aging Project; HRS: Health and Retirement Study.

Table 4. Participant Characteristics and the Thr92AlaD2 Polymorphism by Race.

	African Americans CHAP		European Americans CHAP		European Americans ROS/MAP	
	Ala/Ala +Ala/Thr	Thr/Thr	Ala/Ala +Ala/Thr	Thr/Thr	Ala/Ala +Ala/Thr	Thr/Thr
	(n=1607)	(n=714)	(n=779)	(n=556)	(n=981)	(n=726)
Serum total T4 (μg/dL)‡	7.56 ± 1.68	7.48 ± 1.54	7.37 ± 1.59	7.46 ± 1.72	--	--
Mortality—no. (%)	483 (30.1)	242 (33.9)	288 (37.0)	208 (37.4)	458 (63.1)	592 (60.4)
Body-mass index (kg/m^2)†	29.6 ± 6.1	29.4 ± 6.3	27.3 ± 5.2	27.6 ± 5.1	27.0 ± 5.2	27.0 ± 5.2
Diabetes status—no. (%)§	536 (33.3)	236 (33.1)	125 (16.1)	91 (16.4)	180 (18.7)	158 (22.2)
HbA$_{1C}$ ≥ 6.5%—no. (%)	321 (28.8)	136 (28.5)	59 (10.4)	59 (10.4)	86 (17.3)	56 (14.9)

Plus-minus values are means ± SD. Ala: alanine, Thr: threonine, and HbA$_{1C}$: glycosylated hemoglobin. ‡The serum total T4 level is micrograms per deciliter. †The body-mass index is the weight in kilograms divided by the square of the height in meters. §Diabetes status was determined by self-report and/or use of a diabetes medication.

Figure 1