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## CHOLESTEROL-RELATED GENETIC RISK SCORES ARE ASSOCIATED WITH HYPOMETABOLISM IN ALZHEIMER'S-AFFECTED BRAIN REGIONS

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## Abstract

We recently implicated a cluster of nine single nucleotide polymorphisms from seven cholesterolrelated genes in the risk of Alzheimer's disease (AD) in a European cohort, and we proposed calculating an aggregate cholesterol-related genetic score (CREGS) to characterize a person's risk. In a separate study, we found that apolipoprotein E (APOE)  $\epsilon$ 4 gene dose, an established AD risk factor, was correlated with fluorodeoxyglucose (FDG) positron emission tomography (PET) measurements of hypometabolism in AD-affected brain regions in a cognitively normal American cohort, and we proposed using PET as a presymptomatic endophenotype to help assess putative modifiers of AD risk. Thus, the objective in the present study is to determine whether CREGS is related to PET measurements of hypometabolism in AD-affected brain regions. DNA and PET data from 141 cognitively normal late middle-aged APOE  $\epsilon$ 4 homozygotes, heterozygotes and noncarriers were analyzed to evaluate the relationship between CREGS and regional PET measurements. Cholesterol-related genetic risk scores were associated with hypometabolism in AD-affected brain regions, even when controlling for the effects of APOE  $\epsilon$ 4 gene dose. The results support the role of cholesterol-related genes in the predisposition to AD, and support the value of neuroimaging in the presymptomatic assessment of putative modifiers of AD risk.

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#### **Keywords**

Alzheimer's Disease; Genetics; Cholesterol; Positron Emission Tomography; Endophenotype

#### INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia in older people. Heritability estimates suggest that genetic variations may account for about 80% of AD risk (Gatz et al., 2006), and researchers continue to search for these genetic risk factors in the hope of contributing to the scientific understanding, early detection and tracking, diagnosis, treatment and prevention of this disorder (Papassotiropoulos et al., 2006). To date, the best established genetic risk factors for AD include relatively rare mutations of the presenilin 1 (PS1), presenilin 2 (PS2), and amyloid precursor protein (APP) genes

(http://www.molgen.ua.ac.be/ADMutations/) and a common variant of the apolipoprotein E (APOE) gene (Corder et al., 1993; Farrer et al., 1997). Whereas the PS1, PS2, and APP mutations account for many cases of AD with dementia onset before age 60 and autosomal dominant inheritance, the APOE  $\varepsilon$ 4 allele increases the susceptibility to (but is not sufficient to cause) many cases of AD with dementia onset after age 60. APOE  $\varepsilon$ 4 gene dose--the number of  $\varepsilon$ 4 alleles in a person's APOE genotype--is associated with a higher risk of late-onset AD and a younger median age at dementia onset (Corder et al., 1993).

While variants of the APOE protein have been shown to differentially influence the accumulation of amyloid in the formation of neuritic plaques (Wisniewski et al., 1994), the phosphorylation of tau in the formation of neurofibrillary tangles (Strittmatter et al., 1994), the vulnerability of neurons to AD pathology (Miyata and Smith, 1996), and other suggested pathogenic processes (Mahley and Huang, 2006), APOE is best known as the major transporter of cholesterol in the blood and central nervous system (Mahley, 1988). The role of APOE in the risk of AD and several other neurobiological, epidemiological, and clinical findings have raised the possibility that dysregulated cholesterol levels may increase the risk of AD, as well as the possibility that statins and other cholesterol-modifying medications may be helpful in the disorder's treatment and prevention (Shobab et al., 2005; Wolozin, 2004; Wolozin et al., 2007).

In a European case-control study, we recently investigated a cluster of cholesterol-related genes in the risk of AD, focusing on cholesterol- and lipid-related single nucleotide polymorphisms [SNPs] implicated in prior single-gene association studies of AD, and used a set-association method to characterize the *combined* contribution of the candidate SNPs (Papassotiropoulos et al., 2005). This compound genetic association study implicated a cluster of nine SNPs from seven genes (including the APOE  $\epsilon$ 4 allele) in the risk of AD, which were used to calculate an aggregate <u>cholesterol-re</u>lated genetic risk <u>s</u>core (CREGS) in each subject. In this sample, CREGS distinguished between 74% of AD cases and controls when the APOE  $\epsilon$ 4 allele was included in the calculation, CREGS distinguished between 66% of cases and controls when the APOE  $\epsilon$ 4 allele was not included in the calculation, and the APOE  $\epsilon$ 4 allele alone distinguished between 66% of cases and controls. Supporting the biological relevance of the proposed score, we also reported a significant positive correlation between CREGS and cerebrospinal fluid levels of 24S-hydoxycholesterol, the major catabolite of cholesterol in the central nervous system, in an independent European sample of non-demented elderly subjects (Papassotiropoulos et al., 2005).

In an American fluorodeoxyglucose (FDG) positron emission tomography (PET) study of cognitively normal, late-middle-aged persons, we demonstrated significant correlations between APOE  $\epsilon$ 4 gene dose (i.e., three levels of genetic risk for AD) and hypometabolism

specifically in the posterior cingulate, precuneus, parietal, temporal, and frontal brain regions previously shown to be preferentially affected in patients with Alzheimer's dementia (Reiman et al., 2005). Based on these findings, we proposed using PET to provide a quantitative presymptomatic endophenotype—a measurable feature that is more closely related to disease-susceptibility than the clinical syndrome itself—to help assess the individual and aggregate effects of putative modifiers of AD risk.

In this study, we used baseline PET data from our cognitively normal American samples to determine the extent to which CREGS is correlated with hypometabolism in AD-affected brain regions, further suggesting the value of brain imaging in the pre-symptomatic assessment of putative modifiers of AD risk. Correlations were performed with and without the contribution of APOE  $\epsilon$ 4 gene dose to the calculation of each person's CREGS.

## METHODS

#### Subjects

Newspaper ads were used to recruit cognitively normal volunteers 47–68 years of age who reported a first-degree family history of probable AD, understood they would not receive any information about their APOE genotype, provided their informed consent, and were studied under guidelines approved by the human subjects committees at Banner Good Samaritan Medical Center and the Mayo Clinic. Venous blood samples were drawn and APOE genotypes characterized with analysis involving restriction-fragment-length polymorphisms (Crook et al., 1994).

Clinical ratings, neuropsychological tests, volumetric magnetic resonance imaging (MRI), and fluorodeoxyglucose PET were performed in APOE  $\epsilon$ 4 homozygotes, heterozygotes, and noncarriers as previously described (Reiman et al., 1996; Reiman et al., 2005). At the time of their initial visit, the subjects had scores of at least 28 on the Folstein Mini-Mental State Examination (MMSE) and less than 10 on the Hamilton Depression Rating Scale (HAM-D), did not satisfy criteria for a current psychiatric disorder using a structured psychiatric interview, did not use centrally acting medications for at least 2 weeks before their PET session, and had a normal neurological exam. At the time of study enrollment, each APOE  $\epsilon$ 4 heterozygote (all with the  $\epsilon$ 3/ $\epsilon$ 4 genotype) and  $\epsilon$ 4 noncarrier was individually matched to an  $\epsilon$ 4 homozygote for his or her gender, age (within 3 years), and educational level (within 2 years).

To correlate CREGS with lower regional CMRgl, CREGS was calculated and PET images analyzed in 141 of the 160 cognitively normal persons with two copies, one copy and no copies of the APOE  $\varepsilon$ 4 allele who had participated in a previous study (Reiman et al., 2005) (DNA from the other 19 subjects in this study was not available for the computation of CREGS). Participants included 29 APOE  $\varepsilon$ 4 homozygotes, 42  $\varepsilon$ 4 heterozygotes (all with the  $\varepsilon$ 3/ $\varepsilon$ 4 genotype) and 70  $\varepsilon$ 4 non-carriers who were individually matched for their gender, age (within 3 years) and educational level (within 2 years).

#### **Brain Imaging**

Volumetric  $T_1$ -weighted MRI and PET were performed as previously described (Reiman et al., 1996; Reiman et al., 2005). PET was performed with the 951/31 ECAT scanner (Siemens, Knoxville, Tenn.), a transmission scan, the intravenous injection of 10 mCi of <sup>18</sup>F-fluorodeoxyglucose a 60-min dynamic sequence of emission scans as the subjects, who had fasted for at least 4 hours, lay quietly with eyes closed in a darkened room. Regional analyses were performed using the PET images (counts relative to the whole brain uptake) acquired during the last 30 min.

An automated algorithm (SPM, Wellcome Department of Cognitive Neurology, London, U.K.) was used to linearly and non-linearly deform each person's PET image into the coordinates of a standard brain atlas, normalize for absolute measurements using proportionate scaling, and generate statistical parametric maps of the correlations between CREGS and lower regional CMRgl with and without including the contribution of APOE  $\varepsilon$ 4 gene dose in the calculation of CREGS (P<0.005, uncorrected for multiple comparisons). This statistical map was superimposed onto a map of CMRgl reductions in previously studied patients with Alzheimer's dementia (Alexander et al., 2002) and a spatially standardized, volume-rendered MRI. Significance levels were then adjusted for the number of resolution elements in the AD-affected posterior cingulate, precuneus, parietotemporal, and frontal brain regions postulated to be preferentially affected in cognitively normal persons at genetic risk for AD using the smallvolume correction procedure in SPM. In subsequent sub-group analyses, statistical parametric maps were then generated and superimposed onto the map of AD-related CMRgl reductions to determine whether observed correlations between CREGS and lower CMRgl in AD-related regions were present in the aggregate APOE ɛ4 carrier subgroup (with and without the contribution of APOE ɛ4 gene dose in the CREGS score) and in the homozygote, heterozygote and non-carrier subgroups.

#### CREGS

Calculation of each person's CREGS was based on a cluster of polymorphisms associated with AD in an exclusive European sample as previously described (Papassotiropoulos et al., 2005). The SUMSTAT program (http://www.genemapping.cn/sumstat.html), which was developed for statistical analysis with the set-association method (Hoh et al., 2001), was used for the definition of the polymorphic cluster. The set-association method uses relevant sources of genetic information, such as allelic association and Hardy-Weinberg disequilibrium (HWD). Information is combined over multiple markers and genes in the genome, quality control is improved by trimming SNPs with high HWD values, and permutation testing limits the overall false-positive rate (Hoh et al., 2001). For each candidate marker, two chi-square statistics were computed: one for the allelic association with disease status and one for the HWD in cases. The products of these statistics for each marker were then ranked from largest to smallest. Progressively larger sums  $(S_i)$  are then calculated over the *j* largest chi-square statistics. For example,  $S_1$  is the largest chi-square statistic of association.  $S_2$  is the sum of the largest and second largest. S3 is the sum of the largest, second largest and third largest, etc. The empirical significance level  $(P_i)$  for each  $S_i$  is evaluated by permutation methods carried-out under the null hypothesis of no genetic association with AD. The smallest of the empirical significance levels (i.e. P<sub>imin</sub>) identifies the best and most parsimonious model predicting disease status. Importantly, the set-association method has been shown to be of superior power compared with conventional locus-by-locus analyses and to successfully capture statistical interactions between genes (Hoh et al., 2001; Kim et al., 2003; Maitland-van der Zee et al., 2005; Ott and Hoh, 2003; Papassotiropoulos et al., 2005).

After detection of the AD-associated SNPs, calculation of the CREGS was based upon the setassociation statistic for each significant SNP. Thus, CREGS mirrors the number of individual risk alleles weighted by the contribution of each risk allele to the overall cluster. Hence, CREGS would be 0 in persons not carrying any risk allele; CREGS would be equal to the corresponding set-association statistic for the particular SNP (e.g., 1.8 for *APOE4*, 0.3 for *OLR1*) in persons carrying only one risk allele; and CREGS would be computed by adding the set-association statistics of the corresponding SNPs in person with two or more risk alleles. Depending on whether the *APOE* genotype was included to or excluded from CREGS, the maximal possible CREGS value is 2.5 (*APOE4* not included), 4.3 (*APOE4* included using the dominant model, which accounts for the presence or absence of any £4 alleles in the person's *APOE* genotype) and 6.1 (*APOE4* included using the additive model, which accounts for the number of £4 in

the person's *APOE* genotype [i.e., his or her *APOE* gene dose]). An adjustment for a possible protective role of the *APOE2* allele was not performed because *APOE2* failed to be part of the gene cluster exclusively of *APOE4* in the European population.

SNPs in the APOE, SOAT1, APOE 5'-untranslated region, OLR1, CYP46A1, LPL, LIPA and APOA4 genes, which were implicated and used to calculate CREGS in our previous study, were used to calculate CREGS in the current study. APOE genotyping was performed as previously described. SNPs rs1044925 (SOAT1), rs405509 (APOE 5'-untranslated region), rs1050286 (OLR1), rs754203 (CYP46A1), rs268 (LPL), rs13500 (LIPA), rs328 (LPL) and rs5104 (APOA4) were analyzed by pyrosequencing<sup>TM</sup>. The following forward, reverse and sequencing primers were used for these SNPs: 5'-biotin-CAT TTC CTT GAA ACC AAT CAC A-3', 5'-TGA CTT CTC TTG AAT TAT TTT GCA-3', 5'-TGC AGA CTA GTG AGT CTG TA-3' for rs1044925; 5'-AGG TGG GGC ATA GAG GTC TT-3', 5'-biotin-ATT CAT CCA GGC ACA GGA AA-3', 5'-ACA CCT CGC CCA GT-3' for rs405509; 5'-biotin-TGC TAT AAT TCC GAA ACA TAC AAG A-3', 5'-TCC ATC TGT TTC TAT TCA GCG A-3', 5'-ATT TGC ATA CCT GGC T-3' for rs1050286; 5'-AAC GAG TTT CCC GTC CCA GA-3', 5'-biotin-TCG GCA AGT GAG CAA CAG G-3', 5'-CTG GGG CCC AGG AGC-3' for rs754203; 5'-biotin-TGC TTG AGT TGT AGA AAG AAC CG-3', 5'-GAG AAC GAG TCT TCA GGT ACA TTT T-3', 5'-TCT TTT GGC TCT GAC TTT-3' for rs268; 5'-ATA CAC CTG CTT CAC GTC CC-3', 5'-biotin-CGC CCT GCC TAT ATT AAC CA-3', 5'-CAT TTC CTT ACA ATA CTG TC-3' for rs13500; 5'-biotin-ATT TGC AGA AAG GAA AGG CA-3', 5'-GGA TGC CCA GTC AGC TTT AG-3', 5'-GAA TGC TCA CCA GCC-3' for rs328; 5'-CAA CCT GCG AGA GCT TCA G-3', 5'-biotin-ACT CTC TCC ATG CGC TGT G-3', 5'-CGC ACC CAG GTC A-3' for rs5104.

Each person's CREGS was calculated as the risk-weighted summation of his or her implicated SNPs, with or without the contribution of APOE ɛ4 gene dose, as follows:

- APOE4 dose effect  $(1.78 \times 0, 1, \text{ or } 2)$
- + SOAT1 effect (rs1044925 C allele) +  $(1.51 \times 0 \text{ or } 1)$
- + APOE promoter effect (rs405509 A allele) +  $(0.30 \times 0 \text{ or } 1)$
- + OLR1 effect (rs1050286 T allele) +  $(0.28 \times 0 \text{ or } 1)$
- + CYP46 effect (rs754203 TT genotype) +  $(0.19 \times 0 \text{ or } 1)$
- + LPL effect (rs268 TT genotype) +  $(0.07 \times 0 \text{ or } 1)$
- + LIPA effect (rs13500 T allele) +  $(0.07 \times 0 \text{ or } 1)$
- + LPL effect (rs328 GG genotype) +  $(0.06 \times 0 \text{ or } 1)$
- + APOA4 effect (rs5104 AA genotype) +  $(0.05 \times 0 \text{ or } 1)$

## RESULTS

#### Correlations between CREGS and Hypometabolism in AD-Affected Brain Regions

To test the hypothesis that higher CREGS is associated with lower measurements of the cerebral metabolic rate for glucose (CMRgl) in AD-affected brain regions, we analyzed data from 141 cognitively normal subjects whose PET images and DNA remained available from a recent study. The subjects had APOE  $\epsilon$ 4-inclusive mean  $\pm$  SD CREGS of 2.77  $\pm$  1.62 and APOE  $\epsilon$ 4-exclusive CREGS of 1.51  $\pm$  0.78. As expected, the distribution of CREGS-related SNPS in our American sample (which was primarily Caucasian) was not significantly different from that in the previously studied European sample. The baseline demographic features, clinical ratings, neuropsychological test scores, and APOE  $\epsilon$ 4-inclusive and APOE  $\epsilon$ 4-

As predicted, CREGS was significantly correlated with lower regional-to-whole brain PET measurements of CMRgl in the posterior cingulate, precuneus, parietotemporal, and frontal regions previously implicated in patients with Alzheimer's dementia (Figure 1a; Table 2a). Supporting the relevance of CREGS to the risk of AD, the genetic risk scores continued to be significantly correlated with lower regional-to-whole brain CMRgl in the precuneus, parietotemporal, and frontal regions when the contribution of APOE £4 gene dose was excluded (Figure 1b, Table 2b). Indeed, CREGS was not significantly associated with lower CMRgl outside the vicinity of the AD-affected brain regions and was not significantly associated with higher CMRgl anywhere in the brain, whether or not the contribution of APOE £4 gene dose was excluded.

In the subsequent sub-group analyses, CREGS was significantly correlated with lower regional-to-whole brain CMRgl in AD-affected precuneus and parietotemporal regions in the aggregate APOE  $\varepsilon$ 4 carrier group (even when the contribution of APOE  $\varepsilon$ 4 gene dose was excluded); CREGS was significantly correlated with lower regional-to-whole brain CMRgl in AD-affected precuneus and parietotemporal regions in the  $\varepsilon$ 4 homozygotes, with lower regional-to-whole brain CMRgl in AD-affected posterior cingulate, precuneus, parietotemporal, and frontal regions in the  $\varepsilon$ 4 heterozygotes, and with lower regional-to-whole brain CMRgl in AD-affected parietal and frontal regions in the  $\varepsilon$ 4 non-carriers (p < 0.005, uncorrected for multiple comparisons); and CREGS was not significantly associated with increased CMRgl in AD-affected regions in any of these sub-groups. (Brain maps corresponding to these subsequent sub-group comparisons are available upon request.) As noted in Table 1, the APOE  $\varepsilon$ 4 homozygotes, heterozygotes, and non-carriers did not differ significantly in their APOE  $\varepsilon$ 4-exclusive CREGS (ANOVA, p=0.86), suggesting that the significant correlations between APOE  $\varepsilon$ 4-exclusive CREGS and regional hypometabolism were not indirectly affected by the subjects' APOE genotype.

## DISCUSSION

This study examined inverse correlations between CREGS, a putative measure of AD risk, and PET measurements of regional CMRgl in cognitively normal late-middle-aged persons. CREGS was significantly correlated with lower CMRgl in brain regions previously found to be preferentially affected by AD. Supporting the biological relevance of these findings to the risk of AD, these inverse correlations were confined to the vicinity of AD-affected regions, were present whether or not the contribution of APOE ɛ4 gene dose was included in the calculation of CREGS, and were observed in separate analyses of the aggregate APOE ɛ4 carrier, homozygote, heterozygote and non-carrier groups despite the smaller sizes of these genetic sub-groups. Together, our findings provide further support for the role of brain imaging in the presymptomatic assessment of putative AD risk modifiers, provide further support for the role of a cluster of cholesterol-related genes in the risk of AD, and may support the promising role of cholesterol-lowering treatments in the primary prevention of AD.

In our prior study (Papassotiropoulos et al., 2005), we used a set-association algorithm (Hoh et al., 2001) to examine the *combined* contribution of different candidate SNPs to the risk of AD, an approach that may be more suitable to the genetic study of common multigenic disorders than the traditional analysis of individual SNPs. Capitalizing on the evidence that an impairment in cholesterol metabolism may contribute to the risk of AD, we implicated a cluster of 9 cholesterol related genes in the risk of AD, proposed the calculation of an aggregate genetic

risk score, and supported the biological relevance of this aggregate score by showing its relationship to cerebrospinal fluid levels of the major metabolite of cholesterol in the brain. The brain imaging findings from our current study supports the computation of aggregate genetic scores for the risk of AD.

Whereas we previously proposed the calculation of CREGS from the study of European subjects, we now support the relevance of this genetic risk score in an independent sample of American subjects. Our findings underscore the importance and validity of CREGS in particular and aggregate genetic risk scores in general. Indeed, it may be possible to extend the calculation of aggregate genetic risk scores to include these and other yet unrecognized genes which have been estimated to account for about 80% of AD risk, and to extend this approach to the calculation of aggregate genetic risk scores for other common multigenic disorders.

As we previously proposed, PET could provide a quantitative presymptomatic endophenotype to help evaluate the individual and aggregate effects of putative genetic and non-genetic modifiers of AD risk (Reiman et al., 2005). As a complement to observational studies of older Alzheimer's dementia cases and controls, our proposed endophenotype could provide prospective evaluation of putative risk modifiers, help address the potentially confounding effects of differential survival related to the putative risk modifier (e.g., the differential effects of CREGS on mortality due to cardiovascular and cerebrovascular causes), provide information about the individual or aggregate effects of the putative risk factor (e.g., CREGS with or without the contribution of APOE E4 gene dose, as shown here, or in conjunction with other putative risk modifiers, such as serum cholesterol levels or statin treatment), and could permit the accurate measurement and real-time evaluation of a putative risk factor (e.g., mid-life serum cholesterol levels, which may be more strongly associated with AD risk and neuropathology than late-life levels) (Kivipelto and Solomon, 2006) years before the onset of symptoms. As a complement to prospective cohort studies, the endophenotype could provide information about the putative risk modifier without having to study many more healthy subjects or wait many years to determine whether or when they show clinical decline.

This study adds to the evidence that impairments in cholesterol metabolism may increase the AD pathology and risk, and it supports that possibility that cholesterol-lowering therapies may help in the treatment and prevention of this disorder (Puglielli et al., 2003; Shobab et al., 2005; Wolozin, 2004). Experimental studies suggest that cholesterol may promote the accumulation of the amyloid- $\beta$  peptide (A $\beta$ ) and amyloid plaques, a cardinal feature of AD (Refolo et al., 2000; Sparks et al., 1994), and that cholesterol-lowering therapies may reduce the accumulation of A $\beta$  and amyloid plaques (Fassbender et al., 2001; Howland et al., 1998; Hutter-Paier et al., 2004; Refolo et al., 2001; Sparks, 1996). Several longitudinal studies (Kalmijn et al., 2000; Kivipelto et al., 2002; Notkola et al., 1998; Whitmer et al., 2005), but not all (Tan et al., 2003), and one neuropathological study (Pappolla et al., 2003) have reported an association between higher mid-life serum cholesterol levels and a higher risk of subsequent AD. However, epidemiological studies investigating the relationship between late-life serum cholesterol levels and subsequent AD risk have generated conflicting findings (Kivipelto and Solomon, 2006; Mielke et al., 2005), suggesting that our proposed PET endophenotype might have particular value in the assessment of putative mid-life risk factors for AD, reducing the possibility of potentially confounding effects of older age or pre-clinical AD on the putative risk factor and reducing the time and number of subjects needed in a longitudinal study. Similarly, one longitudinal and several cross-sectional studies (Jick et al., 2000; Wolozin et al., 2000) have reported an association between the use of cholesterol-lowering hydroxymetholglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) and a lower risk of AD. However, other observational studies have failed to find an association on the incidence of dementia and have raised the possibility of potentially confounding effect of AD on statin treatment (Rockwood, 2006; Zandi et al., 2005), suggesting that our proposed PET

endophenotype might be able to address this confound by permitting the assessment of putative AD protective factors many years before the possible onset of AD. A small clinical trial has suggested that the statin atorvastatin may slow clinical decline in patients with AD and multicenter clinical trials of this treatment are now in progress (Sparks et al., 2005). Findings from a recent prospective study using a large population database to examine the association between different statins and the risk of dementia suggested that, in particular, simvastatin may be associated with a reduced rate of incident dementia (Wolozin et al., 2007). To date, randomized clinical trials of non-demented elderly persons have failed to detect an association between statin use and a lower risk of cognitive decline or dementia, but these studies were not primarily intended to investigate the effects of treatment on AD risk (Shepherd et al., 2002). For example, subject selection criteria, clinical ratings, and neuropsychological assessments were not optimally suited for an AD prevention study, the number of subjects and study duration appear to be associated with limited statistical power, and the benefits of statins in late-middle-aged persons have not been studied.

Just as we have proposed the use of brain imaging as a presymptomatic endophenotype to assess the effects of putative AD risk modifiers, we have proposed the development of brain imaging measurements as presymptomatic surrogate therapeutic endpoints to help in the costeffective evaluation of putative primary prevention therapies (Reiman et al., 2001). Indeed, we have suggested how PET could be used in cognitively normal APOE ɛ4 carriers to evaluate the effectiveness of putative primary prevention therapies to slow down progressive regional CMRgl declines without having to study thousands of healthy late-middle-aged persons or wait many years to determine whether or when persons in the clinical trial develop symptoms. Since an even modestly effective therapy would have a major impact on the incidence of AD, since cholesterol-lowering treatments have other benefits on the heart and brain, and since these treatments tend to be well tolerated and commonly used, we suggest the value of using PET to evaluate the efficacy of cholesterol-reducing treatments in the primary prevention of AD.

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# Figure 1a. Correlations between CREGS, Including the Contribution of APOE ɛ4 Gene Dose, and Lower CMRgl in Alzheimer's-Affected Brain Regions

Negative correlations between CREGS, a) including and b) excluding the contribution APOE  $\epsilon$ 4 gene dose, and lower CMRgl (shown in blue, p<0.005, uncorrected for multiple comparisons) in cognitively normal, late middle-aged persons are projected onto the lateral and medial surfaces of the left and right cerebral hemispheres and shown in relationship to brain regions preferentially affected in an earlier PET study of patients with Alzheimer's dementia (Alexander et al., 2002) (shown in purple). a) Significant correlations between APOE  $\epsilon$ 4-inclusive CREGS and lower CMRgl were observed bilaterally in the posterior cingulate (PC), precuneus (PCu), and parietotemporal (PT) cortex bilaterally and in the left frontal (F) cortex. b) Significant correlations between APOE  $\epsilon$ 4-exclusive CREGS and lower CMRgl were observed bilaterally and in the left frontal (F) cortex. b) Significant correlations between APOE  $\epsilon$ 4-exclusive CREGS and lower CMRgl were observed bilaterally and in the left frontal (F) cortex.



## Figure 1b. Correlations between CREGS, Excluding the Contribution of APOE ɛ4 Gene Dose, and Lower CMRgl in Alzheimer's-Affected Brain Regions

Negative correlations between CREGS, a) including and b) excluding the contribution APOE  $\epsilon$ 4 gene dose, and lower CMRgl (shown in blue, p<0.005, uncorrected for multiple comparisons) in cognitively normal, late middle-aged persons are projected onto the lateral and medial surfaces of the left and right cerebral hemispheres and shown in relationship to brain regions preferentially affected in an earlier PET study of patients with Alzheimer's dementia (Alexander et al., 2002) (shown in purple). a) Significant correlations between APOE  $\epsilon$ 4-inclusive CREGS and lower CMRgl were observed bilaterally in the posterior cingulate (PC), precuneus (PCu), and parietotemporal (PT) cortex bilaterally and in the left frontal (F) cortex. b) Significant correlations between APOE  $\epsilon$ 4-exclusive CREGS and lower CMRgl were observed bilaterally and in the left frontal (F) cortex. b) Significant correlations between APOE  $\epsilon$ 4-exclusive CREGS and lower CMRgl were observed bilaterally and in the left frontal (F) cortex.

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	ε4 NON- CARRIERS N = 70 (mean ± SD)	ε4 HETEROZYGOTES N = 42 (mean ± SD)	ε4 HOMOZYGOTES N= 29 (mean ± SD)	ANOVA p- value
Age (years)	$56.9 \pm 4.6$	55.8 ± 3.9	55.6 ± 5.1	0.29
Gender (F / M)	42/28	28/14	20/9	0.63
Years of Education	$15.9 \pm 1.5$	$15.4 \pm 1.6$	$15.9 \pm 1.3$	0.14
MMSE	$29.7 \pm 0.6$	$29.9 \pm 0.4$	$29.8 \pm 0.6$	0.40
HAM-D	$2.25 \pm 2.7$	$1.7 \pm 1.8$	$3.5 \pm 3.8$	0.03
AVLT	-			
Total Learning	$47.4 \pm 8.0$	$48.6 \pm 9.5$	$50.1 \pm 9.8$	0.37
Short Term Memory	$9.4 \pm 2.6$	$10.4 \pm 2.4$	$10.2 \pm 3.4$	0.18
Long Term Memory	$8.7 \pm 3.0$	$10.0 \pm 2.7$	9.8 ± 3.3	0.08
Complex Figure Test	-			
Сору	$35.0 \pm 1.5$	$33.8 \pm 2.9$	$34.3 \pm 1.9$	0.02
Recall	$18.5 \pm 6.1$	$18.8 \pm 6.0$	$17.9 \pm 6.6$	0.83
Boston Naming Test	57.1 ± 3.3	$56.7 \pm 3.0$	57.3 ± 2.5	0.64
WAIS-R	-			
Information	$12.1 \pm 2.2$	$12.3 \pm 2.1$	$11.2 \pm 2.0$	0.43
Digit Span	$11.3 \pm 2.2$	$11.4 \pm 2.8$	$11.2 \pm 2.8$	0.94
Block Design	$12.1 \pm 2.7$	$12.2 \pm 2.7$	$12.3 \pm 2.5$	0.92
Arithmetic	$12.1 \pm 2.3$	$12.5 \pm 2.2$	$11.5 \pm 2.8$	0.25
Similarities	$12.6 \pm 2.2$	$12.6 \pm 2.0$	$12.1 \pm 1.7$	0.50
COWAT	$44.0 \pm 11.0$	$42.8 \pm 11.4$	47.3 ± 9.1	0.79
WMS-R Orientation	$13.8 \pm 0.4$	$13.9 \pm 0.4$	$13.8 \pm 0.4$	0.51
CREGS, including APOE e4 gene dose	$1.5\pm0.8$	$3.3\pm0.8$	$5.1\pm0.8$	< 0.001
CREGS, excluding APOE e4 gene dose	$1.5 \pm 0.8$	1.6 ± 0.8	$1.5 \pm 0.8$	0.86

TABLE 1

Abbreviations: MMSE, Mini-Mental State Exam; HAM-D, Hamilton Depression Rating Scale; AVLT, Auditory Verbal learning Test; WAIS-R, Wechsler Adult Intelligence Scale-Revised; COWAT, Controlled Oral Word Association Test; WMS-R, Wechsler Memory Scale-Revised; CREGS, aggregate cholesterol-related genetic score.

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TABLE 2

TABLE 2a Location and Magnitude of Most Significant Correlations between Cholesterol Related Genetic Risk Scores, Including APOE & Gene Dose Effects, and Lower Regional-to-Whole P-Value<sup>+</sup> **Correlation Coefficient** Atlas Coordinates $^{\dot{\tau}}$ Brain CMRgl<sup>\*</sup> Brain Region

Brain Region		Atlas Coordinates $^{\dagger}$		Correlation Coefficient	P-Value <sup>+</sup>
	X	Y Millimeters	Z		
Precuneus	-2	-76	39	-0.29	$2.8  imes 10^{-4}$
Left Parietotemporal	-67	-32	16	-0.24	$1.8  imes 10^{-3}$
a	-57	-49	30	-0.23	$3.4  imes 10^{-3}$
	-51	-62	43	-0.25	$1.7  imes 10^{-3}$
Right Parietal	63	-22	23	-0.28	$3.1 imes 10^{-4}$
3	61	-39	41	-0.26	$1.0  imes 10^{-3}$
	57	-56	45	-0.25	$1.7  imes 10^{-3}$
Left Frontal	-48	10	36	-0.22	$4.3 imes10^{-3}$

The data were extracted from voxels associated with the most significant correlations in each of the brain regions previously found to be associated with abnormally low CMRg in patients with Alzheimer's dementia (Alexander et al., 2002). + The reported significance levels are uncorrected for multiple comparisons; each of these correlations remained significant (p<0.05) after correcting for multiple comparisons in the relevant AD-related search regions.

tThe coordinates were obtained from Talairach and Tournoux (1998). X is the distance to the right (+) or left (-) of the midline, Y is the distance anterior (+) or posterior (-) to the anterior commissure, and Z is the distance superior (+) or inferior (-) to a horizontal plane through the anterior and posterior commissures. + The reported significance levels are uncorrected for multiple comparisons; correlations in the precuneus and left frontal regions remained significant (*p*<0.05) after correcting for multiple comparisons in the relevant AD-related search regions. fThe coordinates were obtained from Talairach and Tournoux (1998). X is the distance to the right (+) or left (-) of the midline, Y is the distance anterior (+) or posterior (-) to the anterior commissure, and Z is the distance superior (+) or inferior (-) to a horizontal plane through the anterior and posterior commissures.