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Cortical binding of Pittsburgh compound B, an endophenotype for genetic studies of Alzheimer's disease

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Abstract

Background—To date, all known Alzheimer's disease genes influence amyloid beta (A β). The development of *in vivo* imaging of A β deposition in the human brain using Pittsburgh compound B (PIB) offers the possibility of using cortical PIB binding as a quantitative endophenotype for genetic studies of late-onset Alzheimer's disease (LOAD).

Methods—Heritability of A β deposition was determined using 82 elderly siblings from 35 families. Correlation with other A β related traits was determined using an unrelated sample of 112 individuals. For both samples, *APOE* ϵ 4 was genotyped and PET imaging was performed using the PIB ligand. Mean cortical binding potential (MCBP) was computed from several regions-of-interest.

Results—MCBP has a high heritability (0.61, $p=0.043$). Furthermore, most of the heritable component (74%) cannot be explained by *APOE* ϵ 4 genotype. Analysis of the unrelated sample reveals that a third of the variance of MCBP cannot be predicted by other biological traits, including CSF A β 42 levels.

Conclusions—These findings demonstrate that MCBP is a genetic trait and that other more easily measured A β related traits such as CSF A β 42 do not fully explain the variance in MCBP. Thus, mean cortical PIB binding is a useful trait for large-scale genetic studies of LOAD.

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Introduction

Studies of genetic factors of complex disorders are often confounded by complex etiologies and idiosyncratic disease presentation. To partially alleviate this problem it can be useful to study endophenotypes (or biomarkers) that may have fewer genes underlying trait variability. A useful disease biomarker for genetic studies should be heritable and possess independent variation from other phenotypes and endophenotypes that are easier to assess.

The most common cause of dementia, late-onset Alzheimer's disease (LOAD), has two characteristic pathological features in addition to neuronal loss: neurofibrillary tangles and senile plaques composed of amyloid beta ($A\beta$) (1). Most dominantly inherited forms of Alzheimer's disease are characterized by mutations leading to elevated levels of the $A\beta$ peptide with 42 amino acids ($A\beta_{42}$) thought to preferentially aggregate into fibrils found in plaques (1). A variety of genetic factors have been reported to influence risk for LOAD, but the only well-replicated finding has been with allelic variants in *apolipoprotein E* (*APOE*). In addition to disease status, various studies have examined other phenotypes related to LOAD, such as age-of-onset (2) and cerebrospinal fluid (CSF) levels of $A\beta$ (3,4). Until recently, plaques and tangles themselves could only be studied through autopsy. However, the Positron Emission Tomography (PET) ligand, Pittsburgh compound B (PIB), allows one to safely visualize and quantify $A\beta$ deposition in a living human being (5,6).

Using the PIB ligand, recent studies have shown that a significant proportion of cognitively normal individuals above 70 years of age exhibit detectable $A\beta$ deposition (7-9). There is also evidence that PIB mean cortical binding potential (MCBP) is associated with decreased CSF levels of $A\beta_{42}$, but not with other CSF peptides (10). Longitudinal studies suggest that positive PIB binding as well as decreased levels of CSF $A\beta_{42}$ in cognitively normal individuals are precursors to clinical LOAD (10-12). Thus, genes that promote changes in these biomarkers (increased MCBP or decreased CSF $A\beta_{42}$) at an earlier age will likely be risk factors for LOAD.

We recruited two samples to determine heritability and test for independent variation for quantified $A\beta$ deposition. Although twin studies are the gold standard for establishing heritability, because of the difficulty of recruiting elderly twins we instead undertook a study of elderly sibships (the "Family Sample"). Heritability in the broad sense is estimated by dividing the measured intraclass correlation coefficient (ICC) of the phenotype by the fraction of shared genes (0.5 for full siblings). Since a recent study (13) demonstrated that number of *APOE* $\epsilon 4$ was significantly associated with MCBP, *APOE* genotypes were also measured in this sample and counted in this fashion.

A second sample of unrelated individuals with MCBP, *APOE* genotypes, clinical diagnosis and CSF $A\beta_{42}$ measurements (the "Unrelated Sample") was used to assess independent variation. Since these other phenotypes are easier and less expensive to assess than MCBP, independent variation would suggest the existence of genetic factors that affect $A\beta$ deposition directly and have secondary effects for other phenotypes and biomarkers, demonstrating the merit of direct analysis of MCBP.

Subjects and Methods

Subjects

All subjects gave written informed consent and all studies were approved by the Human Studies Committee of Washington University School of Medicine.

Family Sample: Elderly sibships were recruited by advertisement from the community without regard to dementia status. One sibling was required to be between the ages of 70 and 75 and all other siblings were within 5 years of age of the index sibling. There were 35 pedigrees and 82 individuals (Table 1).

Unrelated Sample: Subjects are participants in the Memory and Aging Project at the Washington University Alzheimer's Disease Research Center (ADRC) and had participated in studies that include lumbar puncture for CSF, clinical assessment and PET imaging with PIB (Table 1).

Imaging

Details of the PIB PET imaging procedures have been reported previously (9). Briefly, human brain PET imaging was conducted using a Siemens PET scanner (CTI, Knoxville, KY) in a darkened, quiet room. [^{11}C]PIB was administered intravenously simultaneous with initiation of a 60-minute dynamic PET scan in three dimensional mode. A high-resolution, low noise anatomic image data-set for region-of-interest (ROI) determination was created for each participant by aligning and averaging two MPRAGE sequences (9,14). Alignment of PET-MR within a participant was accomplished using an in-house cross-modal registration algorithm (9). A binding potential (BP; (15)) was calculated using the estimated distribution volume (relative to the cerebellum). Mean BP values from the prefrontal cortex, gyrus rectus, lateral temporal, and precuneus ROIs were used to calculate MCBP.

Dementia Assessment

Family Sample: There was no formal assessment for dementia. Gross cognitive status was assessed using the Short Blessed Test (SBT; (16,17)) (Table 1).

Unrelated Sample: All subjects were unrelated community-dwelling volunteers enrolled in longitudinal studies of healthy aging and dementia through the ADRC. Cognitive status was assessed using the Clinical Dementia Rating (CDR) (18). CSF collection and biomarker measurement have been described previously (8,10).

Genotyping

APOE genotyping was performed using a Taqman assay (from Applied Biosystems).

Analyses

Family Sample: Because MCBP is highly non-normal (Shapiro-Wilk p -value $< 10^{-4}$), we used the software SAGE (19) which implements a robust estimate of ICC (20) (specifically, the k -score Winer reliability where k is the number of individuals in a pedigree). We computed a parametric and an empirical p -value through permutation testing with and without controlling for significant covariates.

Unrelated Sample: To examine variability in MCBP, we used generalized additive models. Spline models were constructed and degrees of freedom were determined by generalized cross validation. Potential predictors of MCBP were age, gender, CDR, *APOE* genotype, and CSF levels of A β 42, tau, and phospho-tau₁₈₁ (p-tau181). Multivariate models were built manually using forward selection. In particular, linear models were first tested and spline models were used only when linear models were significant and spline models improved the fit significantly.

Results

Family Sample: The heritability estimate was 0.61 with significant p -values for both the parametric ($p=0.043$) and empirical ($p=0.009$) tests. We also computed the variance of MCBP

explained by number of *APOE* $\epsilon 4$ alleles, age, gender, and the SBT. The results show highly significant association for *APOE* and significant association for age and SBT (Table 2). Controlling for just *APOE* and then controlling for all significant factors yields heritability estimates of 0.45 and 0.52, respectively, with non-significant parametric p-values ($p=0.14$ and $p=0.096$) and significant empirical p-values ($p=0.034$ and $p=0.027$). Calculating the ratio of the smallest and largest heritability estimates (0.45 and 0.61, respectively), we see that while *APOE* genotype and other covariates are important factors, 74% of the heritable component MCBP remains unexplained.

Unrelated Sample: We first performed regression for each variable separately with MCBP (Table 2). As previously reported (8,13), MCBP is positively correlated with CDR and number of *APOE* $\epsilon 4$ alleles and negatively correlated with CSF A β 42. In this larger sample, we also see a positive correlation with tau and p-tau181 (Table 2).

The best multivariate model contains CSF A β 42, CSF tau, and CDR; no other predictors are significant once these are included. Including these three factors explained 65.5% of the variance of MCBP leaving approximately one third of the variance unexplained.

Discussion

The sibship analysis indicates that A β deposition as measured by MCBP is a highly heritable trait. We observe significance for both the nominal p-value (0.043) and the empirical p-value ($p=0.009$). We anticipate that the empirical p-value is more accurate because of the relatively small sample size; we conservatively report the nominal p-value. After controlling for covariates, the trait remains heritable as measured by the empirical p-value ($p=0.034$) and 74% of the heritable component of the variance remains unexplained.

Comparing regression analyses in the family sample with the sample of unrelated individuals show a much less important role for number of *APOE* $\epsilon 4$ alleles and a much more important role for age. This may be due to the lower mean age of the unrelated sample (65.5y vs 71.7y), given that the impact of *APOE* $\epsilon 4$ genotype on AD risk extends up to the mid-seventies. Further, since the family sample has a much narrower age range than the unrelated sample, age has reduced variability and consequently lower predictive power.

In the unrelated sample, we see that a third of the variance of MCBP cannot be predicted by other phenotypes and endophenotypes already being measured in our studies. However, since we lack CSF data on the family sample, we cannot determine how much of the independent variance is heritable. The unexplained variance from the regression model of MCBP could arise from factors not measured or measurement error. Despite this limitation, these results demonstrate that MCBP is a genetic trait with significant unique variability and may be a useful phenotype for large-scale genetic studies to identify risk factors for LOAD.

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Table 1

Sample characteristics

	Family Sample	Unrelated sample
	82	112
Number of pedigrees	35	-
Mean pedigree size (SD) [min-max]	2.3 (0.68) [2 - 4]	-
Male (%) / Female (%)	33 (40%) / 49 (60%)	31 (28%) / 81 (72%)
Mean Age (yrs) (SD) [min - max]	71.7 (3.4) [64 - 80]	65.5 (11.8) [45 - 88]
Mean SBT (SD) [min - max]	2.6 (3.0) [0 - 12]	
CDR: 0 (%) / 0.5 (%) / 1 (%) / 2 (%)	-	96 (86%) / 12 (11%) / 3 (3%) / 1 (1%)
Mean MCBP (SD) [min-max]	0.13 (0.23) [-0.06 – 0.93]	0.16 (0.28) [-0.16 – 1.24]

Table 2

Proportion of variance of MCBP predicted by other factors

	Family Sample	Unrelated Sample
Gender	0.01	0.0004
Age	0.07 *	0.21 †
# of APOE ε4 alleles	0.30 †	0.07 *
Short Blessed Test Score	0.06 *	
CDR		0.23 †
CSF Aβ42		0.52 †
CSF tau		0.31 †
CSF p-tau		0.25 †

* p-value between 0.01 and 0.05;

† p-value < 0.0001