Genetic and environmental contributions to amyloid burden in older Australians: a PiB-PET imaging study of twins

Please note: this is the project plan for the larger proposal which we will be submitting to NHMRC in 2012. The DCRC project is a Goldstar-funded pilot study of 10 twin pairs only to demonstrate feasibility and preliminary results.

Aims

β-amyloid (Aβ) plaques are one of the hallmark neuropathologies of Alzheimer's disease (AD). While the presence of these lesions has now been known for over a century, many aspects of amyloid plaques remain poorly understood. Firstly, their aetiology is not fully understood. Converging evidence from autosomal dominant mutations, study of Down's syndrome patients, family studies and the known risk of the *apolipoprotein E ε4* (*APOE ε4*) allele suggests that genetic factors are important (Wisniewski, Wisniewski et al. 1985; Hardy and Selkoe 2002). However, it is not know to what extent this pathology is genetically determined in the sporadic cases of AD, and how much contribution is made by environmental factors. For example, in mouse models, physical and cognitive activity have been shown to significantly reduce amyloid deposition (Mirochnic, Wolf et al. 2009), and in humans there is growing evidence for the importance of vascular risk factors in promoting AD plaques (Carlsson 2010; Dickstein, Walsh et al. 2010). It is possible that there are other small effect genes that interact with environmental factors, or with each other, in determining plaque formation in the human brain. It is therefore important to determine the relative contributions of genetic and environmental factors if we are to seek further risk and protective factors for AD.

Secondly, the relationship between Aβ burden in the brain and cognitive deficits continues to be controversial. In the past, attempts to establish this relationship were confounded by the fact that the amyloid plaque burden could only be determined post-mortem, which was usually remote to the time of the cognitive assessment. Recent developments in neuroimaging now allow amyloid burden to be assessed *in vivo* using positron emission tomography with the Pittsburgh compound B ([^{11}C]PiB-PET), making it possible to examine the relationship between amyloid load and cognitive function in temporal proximity. Potential moderating factors such as genetics or environment need to be considered when examining this relationship however. For example, the brain reserve in an individual could mask the relationship, and the presence of associated pathology, especially cerebrovascular disease, may moderate the relationship.

A powerful strategy to address the above limitations is *the twin design*, which has not been previously exploited in this setting. Twins offer a natural experiment to differentiate the relative contributions of genetic and environmental factors, by virtue of the fact that monozygotic (MZ) twins share 100% of their genes while dizygotic (DZ) twins, like siblings, share only 50% of their genetic inheritance. Moreover, since MZ twins are genetically similar, discordance between such twins are attributable to environmental factors, which is powerful in controlling for confounding factors.

Our group is fortunate to have unique access to a cohort of elderly MZ (identical) and DZ (fraternal) twins who are participating in the longitudinal Older Australian Twins Study (OATS). This cohort has already had extensive medical, neuropsychiatric, and structural brain imaging assessments, with longitudinal data being collected. No *functional* brain imaging has yet been conducted on this group however. They comprise an excellent and

willing sample to examine the genetic and environmental determinants of brain amyloid deposition. The proposed study intends to invite a subset of this cohort (120 people = 30 MZ and 30 DZ twin pairs) to participate in a separate PiB-PET imaging study so that their amyloid burden can be examined and genetic and environmental contributions can be determined. This will be the first PiB-PET study of twins worldwide.

There are two major aims of this project:

1. To determine the relative contribution of genetic and environmental factors to Aβ deposition in the brains of older Australians; and
2. To determine whether discordant cognitive functioning in MZ twins can be explained on the basis of differential Aβ deposition in the brain, and determine the environmental factors that explain these differences.

Background

Neuropathology of Alzheimer's disease

There are three principal neuropathological markers of AD: 1) amyloid plaques; 2) neurofibrillary (tau) tangles; and 3) neurodegeneration, including loss of neurons and synapses, and dendritic arborisation (Braak and Braak 1991; Terry, Masliah et al. 1991). Amyloid plaques are dense extracellular bodies comprised primarily of insoluble $A\beta_{1-42}$ and $A\beta_{1-40}$ (Mathis, Lopresti et al. 2007). There is growing consensus that Aβ-amyloid plays a central role in the pathogenesis of AD (Hardy and Selkoe 2002), and the most important piece of evidence for this "amyloid cascade hypothesis" of AD is the demonstration that several mutations in the Aβ precursor protein (APP) gene on chromosome 21 cause early onset AD. Further support comes from the finding that the most common form of autosomal dominant AD is caused by mutations in the presenilin-1 gene on chromosome 14 which codes for a protein that is strongly implicated to be the "gamma-secretase" enzyme responsible for C-terminal cleavage of Aβ from its precursor, amyloid precursor protein (APP) (Blennow, de Leon et al. 2006).

Previously, post mortem studies were the only way to assess amyloid burden and it has been suggested that deposition of Aβ begins well before clinical symptoms (Price and Morris 1999; Thal, Rub et al. 2002). For example in Down's syndrome, a condition in which AD is very common, amyloid deposition begins over a decade prior to the clinical symptoms of dementia (Wisniewski, Wisniewski et al. 1985). Investigators found little post-mortem evidence of a relationship between β-amyloid plaques and cognitive status, however (Neuropathology Group MRC CFAS 2001). Post-mortem assessment is limited by a number of factors, including: patients who come to pathology have had neuropsychological testing done at varying intervals prior to death; by the time they come to autopsy, their brains have multiple pathologies; and determination of the full burden of pathology is difficult to characterise in the post-mortem brain.

With recent developments in neuroimaging, we are now able to examine amyloid plaques *in vivo* using positron emission tomography with the Pittsburgh compound B ([^{11}C]PiB-PET) during the preclinical stages of AD when only mild cognitive impairment (MCI) or no cognitive impairment is observable. Initial findings from CI Rowe's group support some of the general conclusions of post-mortem studies including: a) extensive uptake of PiB in neocortical areas known to accumulate Aβ in participants with AD; b) extensive uptake in asymptomatic elderly participants with documented recent decline in cognitive test scores but whose performance still fell within normal limits for age; c) variable uptake in 22% of normal elderly participants of mean age 74 years, consistent with the expected prevalence

of AD in this group 10 years hence; d) extensive PiB binding in 56% of participants with MCI consistent with the expected future prevalence of AD in this condition (Neuropathology Group MRC CFAS 2001; Pike, Savage et al. 2007; Rowe, Ng et al. 2007; Villemagne, Fodero-Tavoletti et al. 2008).

Heritability of Amyloid

There is converging evidence for the heritability of amyloid pathology. A neuropathological study found concordance between MZ twins in amyloid plaques, in spite of differences in clinical presentation (Brickell, Leverenz et al. 2007). In a separate study, plasma $A\beta_{1-42}$ was found to be highly heritable, even after adjusting for *APOE-ε4* status (Ertekin-Taner, Graff-Radford et al. 2001). Further evidence for a genetic component in amyloid deposition comes from individuals with increased genetic susceptibility to AD. Carriers of the *APOE-ε4* allele had higher PiB binding than non-carriers (Rowe, Ng et al. 2007; Villemagne, Fodero-Tavoletti et al. 2008; Drzezga, Grimmer et al. 2009; Reiman, Chen et al. 2009), and those with familial autosomal AD mutations (presenilin 1 and APP) had higher striatal PiB binding than non-carriers (Villemagne, Ataka et al. 2009).

Hinrichs and colleagues examined amyloid deposition in non-twin sibling pairs, and concluded that there is a genetic component to amyloid binding which 'may be a useful phenotype for large-scale genetic studies to identify risk factors for late onset Alzheimer's disease' (p. 583, Hinrichs, Mintun et al. 2010). The authors acknowledged that whilst twin studies were the gold standard for establishing heritability, they used siblings and unrelated pairs due to the difficulty in recruiting elderly twins. We will improve on this study by investigating MZ and DZ twin pairs from an already established cohort of elderly twins to identify more accurately genetic contributions to amyloid deposition.

If amyloid burden is indeed heritable, and amyloid accumulation is a key marker of the pathogenesis of AD, then brain amyloid load would serve as an excellent endophenotype for genetic studies of neurocognitive disorders with Alzheimer type pathology, especially genome-wide association studies in which a highly heritable phenotype is extremely valuable. Our study will help establish in vivo amyloid burden measure as an endophenotype of AD.

Environmental factors contributing to amyloid deposition

A number of environmental determinants of amyloid deposition have been identified. Our focus is on two important factors; vascular risk factors and complex mental activity.

In animal models, a relationship has been established between Aβ levels and the vascular risk factors such as hypertension (Gentile, Poulet et al. 2009) and cholesterol clearance (Deane, Wu et al. 2004). Vascular risk factors were found to be associated with plasma Aβ levels in cognitively normal individuals (Lopez, Kuller et al. 2008) and first-degree relatives of patients with AD (Abdullah, Luis et al. 2009). The authors of the latter study concluded that '(the) increased risk of AD associated with family history may be mediated in part through the enrichment of individuals with vascular risk factors' (p. 99, Abdullah, Luis et al. 2009). We will investigate this further by examining the relative influence of genetics and vascular risk factors on amyloid deposition.

The effect of an enriched environment on amyloid deposition in mice has been contentious, with some investigators finding reduced amyloid deposition (Lazarov, Robinson et al. 2005) and subsequent protection against cognitive impairment (Cracchiolo, Mori et al. 2007); but

some finding *increased* amyloid deposition as a result of environmental enrichment (Jankowsky, Xu et al. 2003). Different transgenic mouse models have been used across the various studies, highlighting the influence of genetics and the need to control for this when examining this relationship in humans, as we propose to do by examining MZ twins.

Neurocognitive performance and amyloid deposition in vivo

Generally the evidence for a relationship between PiB uptake and cognition has been equivocal (Rabinovici and Jagust 2009). Some studies found an inverse relationship between PiB uptake and episodic memory in normal controls (Pike, Savage et al. 2007) and MCI (Pike, Savage et al. 2007; Forsberg, Engler et al. 2008), and in individuals with cognitive decline (Villemagne, Pike et al. 2008; Storandt, Mintun et al. 2009). Further, several studies reported that individuals who showed cognitive decline over time (Villemagne, Pike et al. 2008), or those who converted from normal to AD (Morris, Roe et al. 2009) or from MCI to AD (Forsberg, Engler et al. 2008; Villemagne, Fodero-Tavoletti et al. 2008; Okello, Koivunen et al. 2009; Wolk, Price et al. 2009), were more likely to have a "PiB positive" scan at baseline. However contrary to this, several studies found no relationship in cognitively unimpaired individuals (Mintun, Larossa et al. 2006; Rowe, Ng et al. 2007; Aizenstein, Nebes et al. 2008) or across diagnostic groups (Jack, Lowe et al. 2009; Jagust, Landau et al. 2009)

In patients with AD, this relationship has been even less certain. Whilst one study found a significant correlation between PiB uptake and the general cognition measure 'CDR sum of boxes' (Grimmer, Henriksen et al. 2009), others found only a tenuous or no relationship between PiB uptake and cognition in AD (Engler, Forsberg et al. 2006; Edison, Archer et al. 2007; Pike, Savage et al. 2007; Rowe, Ng et al. 2007). To explain this lack of a relationship between PiB uptake and cognition in AD, or even in at-risk controls and MCI, it was speculated that either amyloid accumulation plateaus prior to the emergence of clinical symptoms of AD (discussed in Jack, Lowe et al. 2009); progresses very slowly (Rabinovici and Jagust 2009); or the relationship between Aβ and memory reaches a plateau and other pathologies (e.g. ischaemic neuronal damage) take over to cause ongoing cognitive decline (Pike, Savage et al. 2007). Others have posited that factors such as cognitive reserve (Roe, Mintun et al. 2008), hippocampal atrophy (Mormino, Kluth et al. 2009) or age (Grimmer, Henriksen et al. 2009) may be mediating the relationship. AD patients with high education had higher levels of PiB uptake than patients with low education at the same stage of the disease (Kemppainen, Aalto et al. 2008), supporting the notion that greater cognitive reserve could be compensating for, or masking to some degree, amyloid deposition, raising the neuropathological threshold at which clinical signs are observed. Therefore in any study examining the relationship between neuropathology and neurocognitive performance, moderating factors such as genetic variants, disease stage (pre-MCI, MCI, AD), cognitive reserve, and other cerebral pathology need to be considered. The best way to tease apart such genetic and environmental factors is within a twin study, as proposed here.

Twin studies in Alzheimer's research

Twin studies can be used to determine the heritability of a trait by comparison of MZ and DZ twins (de Geus, Goldberg et al. 2008). Using the twin study design, investigators have established a genetic contribution to volumes of various brain structures (Peper, Brouwer et al. 2007); brain activation (Koten, Wood et al. 2009); and the relationship between cognition and neuropathology such as white matter hyperintensities (Carmelli, Reed et al. 2002).

Twin studies estimate that the concordance of AD in MZ twins is 59% (Gatz, Fratiglioni et al. 2005), however a number of studies have looked at monozygotic twins who are discordant for dementia. Differences between twin pairs such as hippocampal atrophy (Jarvenpaa, Laakso et al. 2004) and complexity of work (Andel, Crowe et al. 2005) were found, indicating that environmental factors could be contributing to dementia onset. Non-demented co-twins were more likely to suffer from non-pathological cognitive deficits than a control (Gatz, Fiske et al. 2005). Age of onset of AD may also have a genetic component as it was found to be more similar in MZ than DZ twins (Gatz, Fiske et al. 2005; Gatz, Reynolds et al. 2006).

Premorbid brain reserve, associated pathology, education, and medical diseases all confound the interpretation of the relationship between amyloid deposition and cognitive decline. Since MZ twins are genetically identical and in most cases share the same environment in the developing phase of their lives, twin studies offer a unique level of control for confounding factors.

Pittsburgh Compound B

Pittsburgh Compound B, or [^{11}C]PiB, (*N*-Methyl-[^{11}C])2-(4=-methylamino-phenyl)-6-hydroxy-benzothiazole ([^{11}C]6-OH-BTA-1), is a carbon-11-labeled derivative of the thioflavin-T amyloid dye. It binds with high affinity and high specificity to neuritic Aβ plaques (Rowe, Ng et al. 2007). PiB binds to fibrillar Aβ, and much less to soluble Aβ, however these represent less than 1% of the total brain Aβ (Kuo, Emmerling et al. 1996; McLean, Cherny et al. 1999; Villemagne, Fodero-Tavoletti et al. 2008). Correlations between *in vivo* PiB measurements and post-mortem Aβ pathology have been reported (Bacskai, Frosch et al. 2007; Ikonomovic, Klunk et al. 2008). An inverse correlation has been found between PiB binding and CSF $A\beta_{1-42}$ in MCI (Forsberg, Engler et al. 2008), and across diagnostic groups (Jagust, Landau et al. 2009).

Using a PiB SUVR (standardised uptake value ratio) threshold of 1.60 (Ng, Villemagne et al. 2007), sensitivity, specificity, and accuracy for diagnosing AD have all been reported as over 0.90 (Villemagne, Fodero-Tavoletti et al. 2008). PiB imaging may therefore contribute to earlier and more accurate diagnosis of AD, better differential diagnosis, identification of appropriate individuals for anti-amyloid therapy, and tracking of such therapy (Villemagne, Fodero-Tavoletti et al. 2008). Understanding the relationship between PiB uptake and cognition is crucial to its usefulness in these roles.

CI Rowe's group has conducted one of the largest studies of PiB in older adults, however interpretation can be confounded by a number of factors, including associated pathology, differing genetic susceptibility to AD, and brain reserve. None of his work to date has looked at twins which would enable examination of heritability versus environmental factors.

The benefits of twin research to the study of AD are clear however to the best of our knowledge, a study of amyloid burden using PiB-PET in elderly MZ and DZ twins would be the first of its kind. We have received approval from the OATS chief investigator (AI Sachdev) and the Australian Twins Registry (ATR) to approach OATS participants with the prospect of recruiting them into this proposed new PiB-PET study.

The hypotheses to be tested are:

1. That amyloid burden in older individuals is highly heritable. The amyloid burden also relates to cognitive deficits of the Alzheimer-type (i.e. with prominent episodic memory disturbance), making it an endophenotype of AD.
2. That a number of risk factors including cognitive reserve; concomitant neuropathology (e.g. cerebrovascular pathology present on MRI); and cardiovascular health will have an influence on the level of Aβ in the brain.
3. The concordance and discordance of cognitive function in MZ twins is explained by the amyloid burden in their brains.

Research plan

Subject recruitment

Twin pairs will be recruited from the Older Australian Twins Study (OATS; Sachdev, Lammel et al. 2009) and invited to undertake a PiB-PET scan as a new and separate component of their current research participation. At the time of writing this proposal, the OATS cohort consists of 553 individuals (MZ 134 pairs, DZ 105 pairs, undetermined zygosity 2 pairs; singletons: MZ 20, DZ 27; Sibs 24), with a target of 150 pairs each of MZ and DZ twins. OATS recruitment and two year follow-up are still in progress. We aim to recruit 30 pairs of MZ twins and 30 pairs of DZ twins into the proposed PiB-PET study. As PiB-PET imaging is only available in Victoria, we will initially concentrate on recruiting MZ twins (47 twin pairs in Vic) and DZ twins (35 twin pairs in Vic) from there, and expand to NSW if required (transporting participants to Melbourne). The initial inclusion/exclusion criteria for the proposed PiB-PET study are as follows:

Inclusion criteria: aged 65 years or older, ability to consent, having a consenting MZ or DZ co-twin, having completed some education in English, and being at least of low average estimated premorbid IQ.

Exclusion criteria: inadequate English to complete a neuropsychological assessment; history of epilepsy, significant head injury (loss of consciousness > 1hr), other neurological disorder or a systemic disease with impact on cognitive functioning; current diagnosis of an acute psychotic disorder or major depression; participants in whom magnetic resonance imaging (MRI) is contraindicated including, but not limited to, those with a pacemaker, presence of metallic fragments near the eyes or spinal cord, or cochlear implant (dental fillings do not present a risk for MRI); incapable of giving informed consent due to severe cognitive impairment or any other reason.

In line with Australian Twin Registry (ATR) policy, a letter describing the additional PET measurement and the purpose of the study will be sent via the ATR to the current participants to invite them to participate. Twins will then respond to the ATR to indicate their interest in the additional component, and formal contact will then be made by the PiB-PET research team.

Neuropsychological assessment

Extensive data on neurocognitive performance have already been collected on elderly twins by OATS. This neuropsychological assessment includes general measures of cognitive/mental ability (e.g. the Mini Mental State Exam) and performance on the following cognitive domains: verbal and non-verbal memory, frontal-executive function, attention/working memory/processing speed, language, and visuo-spatial ability. A measure of general cognitive ability can also be obtained using the first principal component of all

neuropsychological test scores. Our group published details of this neuropsychological test battery (Sachdev, Lammel et al. 2009).

Cognitive Reserve
Years of education and the Lifetime Experiences Questionnaire (LEQ, an instrument for estimating brain reserve that has been shown to be reliable and valid) (Valenzuela and Sachdev 2007) will be used as measures of cognitive reserve.

Physical Health Measures
Cardiovascular health will be assessed with current blood pressure and history of hypertension (hypertension defined by >140/90 mmHg) (World Health Organisation 2006); current blood sugar levels and history of diabetes (diabetes defined by fasting plasma glucose concentration ≥7.0 mmol l^{-1}) (World Health Organisation 2006); current lipid status and history of hypercholesterolemia (defined by overall serum cholesterol of >200 mg/dL or 5.2 mmol/l) (NCEP Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults 2002; Heart and Stroke Foundation of Canada 2003); history of heart disease (myocardial infarction, atrial fibrillation, ECG abnormality); history of smoking; body mass index (BMI) [All data are routinely collected as part of the OATS assessment].

MRI protocol
All participants will require a 3D-MRI scan within 6 months prior to the PET scan, as the PiB-PET imaging data will be co-registered to each individual's T1 MRI scan. Whilst some twins will have had an MRI at their follow-up OATS assessment, this is estimated at only 50% of participants.

Further, pathology will be measured on MRI scans including lacunes on T1 images (as per Chen, Wen et al. 2009), and white matter hyperintensities (WMHs) on T2 FLAIR images (as per Wen and Sachdev 2004), for entry into analyses as a possible modifying factor.

PET image acquisition & analysis
Participant preparation consists of intravenous cannulation. The [^{11}C]PiB scan requires an i.v. bolus administration over 30 sec of 10 mCi of high-specific activity [^{11}C]PiB. GMP standard precursor chemical for this compound is purchased from ABX Advanced Chemicals, Germany, and labelled with carbon-11 by the radiochemists of the Austin Health Centre for PET. Carbon-11 is produced in the cyclotron of the Austin Hospital.

The Austin Health Centre for PET has over 10 years experience in the labelling of compounds for PET studies in humans. Labelling is done to Good Laboratory Practice (GLP) with validation of sterility, lack of pyrogenicity and purity prior to human administration. [^{11}C]PiB is produced at high specific activity so that only a tracer dose, containing less than 5micrograms of PiB, is administered. CI Rowe and his group have already established a significant track record in PiB imaging in elderly individuals (e.g. Pike, Savage et al. 2007; Rowe, Ng et al. 2007; Villemagne, Fodero-Tavoletti et al. 2008; Villemagne, Pike et al. 2008; Villemagne, Ataka et al. 2009) and have the skills and experience necessary to complete data collection for this project.

The participant is positioned in the PET scanner and a 20 minute scan is acquired starting 40 minutes post injection of the PiB. Binding will be quantified by the standard uptake value ratio (SUVR, see below for details). Informed consent will be obtained prior to commencement of screening visit procedures. We will only accept clearly valid and

informed consent from patients. The exclusion of patients with severe AD suggests that patient consent will be valid in most cases screened. If it is considered by a Chief or Associate Investigator that consent is not valid, the subject will not be enrolled.

MRI images will be used for defining regions of interest (ROIs) and co-registration of the PET data. Activity in ROIs will be corrected for body weight and dose injected to produce Standard Uptake Value (SUV) data. SUV is defined as the decay-corrected brain radioactivity concentration of PiB and this quantitative measure will be used for subsequent analyses. The Standard Uptake Value Ratio (SUVR) will be calculated and used for analysis using the formula: SUVR = $SUV_{ROI}/SUV_{CEREBELLUM}$. ROIs will be examined in the following cortical areas: frontal (consisting of dorsolateral prefrontal, ventrolateral prefrontal and orbitofrontal regions), superior parietal, lateral temporal, lateral occipital, plus anterior and posterior cingulate, consistent with previous research on PiB-PET. The cerebellar ROI is defined in the cerebellar cortex. Activity in ROIs is normalised against cerebellar activity as it is relatively devoid of senile plaques and does not appear to uptake PiB (Tolboom, Yaqub et al. 2009). Regional amyloid burden is defined as the mean SUVR for each ROI. Total amyloid burden will be defined as the mean SUVR across all ROIs.

Analytic strategy

Heritability estimates will be explored with Mx (Neale 2003) which utilises quantitative genetic modelling of twin data (Neale and Cardon 2003). Three sources of variation in a structural equation model can be identified: additive genetic (A), common environment (C), and unique environment (E). Different combinations of these components can then be hypothesised to account for the pattern of variation in twin data. Speculating that for heritability of amyloid deposition, genetic variance is 0.6 (moderate heritability), and that common and unique environment are both 0.2, 30MZ and 30DZ twin pairs would provide 80% power for detection of the additive genetic variance at $\alpha=0.05$ (Visscher 2004; Visscher, Gordon et al. 2008).

We will then investigate the relationship between total amyloid burden and potentially modifying factors using hierarchical multiple regression analyses. Our sample size of 60 pairs of twins satisfies power analyses to detect a moderate effect of an individual predictor (amyloid burden), with six control variables (cognitive reserve, lacunes, WMHs, cerebral atrophy, BP, and cholesterol; $\alpha=0.05$; $f^2=0.15$; power=80%). We will then examine the relationship between cognition and amyloid burden using hierarchical multiple regression analyses. Our sample size of 60 pairs of twins also satisfies power analyses to detect a moderate effect of an individual predictor (cognition), with six control variables (amyloid burden, cognitive reserve, cerebral atrophy, WMHs, BP and cholesterol; $\alpha=0.05$; $f^2=0.15$; power=80%) (Cohen 1988; Cohen, Cohen et al. 2003).

OATS has a longitudinal design and twins are followed-up for repeat medical, neuropsychological and health assessments. As such the opportunity exists to follow-up on the progressive cognitive status of the PiB-PET study participants, which would allow for an assessment of the prognostic value of PiB-PET in the progression of cognitive decline.

Participant safety

Participant confidentiality will be maintained together with the principles of Good Clinical Research Practice and the Declaration of Helsinki. Immediately following the PiB-PET scan, participants will be questioned for adverse events and will be instructed to contact the investigators if unexpected illness develops in the month following the PiB scan.

The use of [^{11}C]PiB is considered to be generally safe and effective as approved by the University of Pittsburgh's Radioactive Drug Research Committee in accordance with US Food and Drug Administration regulations (21 CFR 361.1) and also by the Human Research Committees of University of Uppsala and the Imperial College of London (Hammersmith). Preclinical toxicity studies of [^{11}C]PiB have demonstrated no toxic effects. These include acute pharmacology and toxicity (effects on heart rate and blood pressure at doses a 100 times higher than those used in the PET study, as well as histological evaluation at 2 days and 2 weeks), genotoxicity (in bacteria and mammalian cells with maximal concentrations up to 50,000 times the estimated average tissue concentration in the PET study); and plasma protein binding and biodistribution studies on rats and monkeys. No pharmacological effects have been reported in humans and PiB therefore meets criteria for early phase studies on humans of the US Radioactive Drug Research Committee.

No adverse reactions to [^{11}C]PiB have been observed in over 400 human participants studied at Austin Health. Approximately 2000 participants worldwide have had [^{11}C]PiB with no reports of adverse reactions.

Timeframe

Recruitment and training of staff is expected to take 3 months. Since our invitees have already been assessed by OATS, we could begin to identify potential participants immediately after the recruitment and training phase. Enrolment into the proposed study must be mediated by the ATR (the ATR has already approved our proposal to re-contact the OATS cohort) and we expect that this will proceed quickly, taking approximately 2 months. PiB-PET scans can begin as soon as formal consent has been received. We expect that we could scan 30 pairs of twins per year, taking a total of 2 years. Data analyses and write up will then take 7 months.

Participant Monitoring Plan

Participants will be questioned for adverse events and responses recorded at the completion of the scans. They will be instructed to contact the investigators if unexpected illness develops in the month following the PiB scan. All adverse events will be recorded in the Clinical Record Forms (CRF's) and in the participant's clinical file. Serious Adverse Events (SAEs) will be reported to the Secretary and the Chairman of the Austin Health Human Research Ethics Committee as soon as practicable and to the Therapeutic Goods Administration (TGA), within the specified reporting time lines.

References

Abdullah, L., C. Luis, et al. (2009). "High serum Abeta and vascular risk factors in first-degree relatives of Alzheimer's disease patients." Molecular Medicine **15**(3-4): 95-100.

Aizenstein, H. J., R. D. Nebes, et al. (2008). "Frequent amyloid deposition without significant cognitive impairment among the elderly." Archives of Neurology **65**(11): 1509-1517.

Andel, R., M. Crowe, et al. (2005). "Complexity of work and risk of Alzheimer's disease: a population-based study of Swedish twins." Journals of Gerontology Series B-Psychological Sciences & Social Sciences **60**(5): P251-258.

Bacskai, B. J., M. P. Frosch, et al. (2007). "Molecular imaging with Pittsburgh Compound B confirmed at autopsy: a case report." Archives of Neurology **64**(3): 431-434.

Blennow, K., M. J. de Leon, et al. (2006). "Alzheimer's disease." Lancet **368**(9533): 387-403.

Braak, H. and E. Braak (1991). "Neuropathological stageing of Alzheimer-related changes." Acta Neuropathologica **82**(4): 239-259.

Brickell, K. L., J. B. Leverenz, et al. (2007). "Clinicopathological concordance and discordance in three monozygotic twin pairs with familial Alzheimer's disease." Journal of Neurology, Neurosurgery & Psychiatry **78**(10): 1050-1055.

Carlsson, C. M. (2010). "Type 2 diabetes mellitus, dyslipidemia, and Alzheimer's disease." Journal of Alzheimer's Disease **20**(3): 711-722.

Carmelli, D., T. Reed, et al. (2002). "A bivariate genetic analysis of cerebral white matter hyperintensities and cognitive performance in elderly male twins." Neurobiology of Aging **23**(3): 413-420.

Chen, X., W. Wen, et al. (2009). "Prevalence, incidence, and risk factors of lacunar infarcts in a community sample." Neurology **73**(4): 266-272.

Cohen, J. (1988). Statistical power analysis for the behavioral sciences New York, LEA.

Cohen, J., P. Cohen, et al. (2003). Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences. Mahwah, NJ, Lawrence Earlbaum Associates.

Cracchiolo, J. R., T. Mori, et al. (2007). "Enhanced cognitive activity--over and above social or physical activity--is required to protect Alzheimer's mice against cognitive impairment, reduce Abeta deposition, and increase synaptic immunoreactivity." Neurobiology of Learning & Memory **88**(3): 277-294.

de Geus, E., T. Goldberg, et al. (2008). "Imaging the genetics of brain structure and function." Biological Psychology **79**(1): 1-8.

Deane, R., Z. Wu, et al. (2004). "RAGE (yin) versus LRP (yang) balance regulates alzheimer amyloid beta-peptide clearance through transport across the blood-brain barrier." Stroke **35**(11 Suppl 1): 2628-2631.

Dickstein, D. L., J. Walsh, et al. (2010). "Role of vascular risk factors and vascular dysfunction in Alzheimer's disease." Mount Sinai Journal of Medicine **77**(1): 82-102.

Drzezga, A., T. Grimmer, et al. (2009). "Effect of APOE genotype on amyloid plaque load and gray matter volume in Alzheimer disease." Neurology **72**(17): 1487-1494.

Edison, P., H. A. Archer, et al. (2007). "Amyloid, hypometabolism, and cognition in Alzheimer disease: an [11C]PIB and [18F]FDG PET study." Neurology **68**(7): 501-508.

Engler, H., A. Forsberg, et al. (2006). "Two-year follow-up of amyloid deposition in patients with Alzheimer's disease." Brain **129**(Pt 11): 2856-2866.

Ertekin-Taner, N., N. Graff-Radford, et al. (2001). "Heritability of plasma amyloid beta in typical late-onset Alzheimer's disease pedigrees." Genetic Epidemiology **21**(1): 19-30.

Forsberg, A., H. Engler, et al. (2008). "PET imaging of amyloid deposition in patients with mild cognitive impairment." Neurobiology of Aging **29**(10): 1456-1465.

Gatz, M., A. Fiske, et al. (2005). "Performance on neurocognitive tests by co-twins to dementia cases compared to normal control twins." Journal of Geriatric Psychiatry & Neurology **18**(4): 202-207.

Gatz, M., L. Fratiglioni, et al. (2005). "Complete ascertainment of dementia in the Swedish Twin Registry: the HARMONY study." Neurobiology of Aging **26**(4): 439-447.

Gatz, M., C. A. Reynolds, et al. (2006). "Role of genes and environments for explaining Alzheimer disease." Archives of General Psychiatry **63**(2): 168-174.

Gentile, M. T., R. Poulet, et al. (2009). "Beta-amyloid deposition in brain is enhanced in mouse models of arterial hypertension." Neurobiology of Aging **30**(2): 222-228.

Grimmer, T., G. Henriksen, et al. (2009). "Clinical severity of Alzheimer's disease is associated with PIB uptake in PET." Neurobiology of Aging **30**(12): 1902-1909.

Hardy, J. and D. J. Selkoe (2002). "The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics." Science **297**(5580): 353-356.

Heart and Stroke Foundation of Canada (2003). The Growing Burden of Heart Disease and Stroke in Canada.

Hinrichs, A. L., M. A. Mintun, et al. (2010). "Cortical binding of pittsburgh compound B, an endophenotype for genetic studies of Alzheimer's disease." Biological Psychiatry **67**(6): 581-583.

Ikonomovic, M. D., W. E. Klunk, et al. (2008). "Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease." Brain **131**(Pt 6): 1630-1645.

Jack, C. R., Jr., V. J. Lowe, et al. (2009). "Serial PIB and MRI in normal, mild cognitive impairment and Alzheimer's disease: implications for sequence of pathological events in Alzheimer's disease." Brain **132**(Pt 5): 1355-1365.

Jagust, W. J., S. M. Landau, et al. (2009). "Relationships between biomarkers in aging and dementia." Neurology **73**(15): 1193-1199.

Jankowsky, J. L. P., G. P. Xu, et al. (2003). "Environmental Enrichment Exacerbates Amyloid Plaque Formation in a Transgenic Mouse Model of Alzheimer Disease." Journal of Neuropathology & Experimental Neurology **62**(12): 1220-1227.

Jarvenpaa, T., M. P. Laakso, et al. (2004). "Hippocampal MRI volumetry in cognitively discordant monozygotic twin pairs." Journal of Neurology, Neurosurgery & Psychiatry **75**(1): 116-120.

Kemppainen, N. M., S. Aalto, et al. (2008). "Cognitive reserve hypothesis: Pittsburgh Compound B and fluorodeoxyglucose positron emission tomography in relation to education in mild Alzheimer's disease." Annals of Neurology **63**(1): 112-118.

Koten, J. W., Jr., G. Wood, et al. (2009). "Genetic contribution to variation in cognitive function: an FMRI study in twins." Science **323**(5922): 1737-1740.

Kuo, Y. M., M. R. Emmerling, et al. (1996). "Water-soluble Abeta (N-40, N-42) oligomers in normal and Alzheimer disease brains." Journal of Biological Chemistry **271**(8): 4077-4081.

Lazarov, O., J. Robinson, et al. (2005). "Environmental Enrichment Reduces A[beta] Levels and Amyloid Deposition in Transgenic Mice." Cell **120**(5): 701-713.

Lopez, O. L., L. H. Kuller, et al. (2008). "Plasma amyloid levels and the risk of AD in normal subjects in the Cardiovascular Health Study." Neurology **70**(19): 1664-1671.

Mathis, C. A., B. J. Lopresti, et al. (2007). "Impact of amyloid imaging on drug development in Alzheimer's disease." Nuclear Medicine & Biology **34**(7): 809-822.

McLean, C. A., R. A. Cherny, et al. (1999). "Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease." Annals of Neurology **46**(6): 860-866.

Mintun, M. A., G. N. Larossa, et al. (2006). "[11C]PIB in a nondemented population: potential antecedent marker of Alzheimer disease." Neurology **67**(3): 446-452.

Mirochnic, S., S. Wolf, et al. (2009). "Age effects on the regulation of adult hippocampal neurogenesis by physical activity and environmental enrichment in the APP23 mouse model of Alzheimer disease." Hippocampus **19**(10): 1008-1018.

Mormino, E. C., J. T. Kluth, et al. (2009). "Episodic memory loss is related to hippocampal-mediated beta-amyloid deposition in elderly subjects." Brain **132**(Pt 5): 1310-1323.

Morris, J. C., C. M. Roe, et al. (2009). "Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease." Archives of Neurology **66**(12): 1469-1475.

NCEP Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults (2002). "Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation,

and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report." Circulation **106**(25): 3143-3421.

Neale, M. C. (2003). Mx: statistical modeling. Richmond, VA, Department of Psychiatry, Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University.

Neale, M. C. and L. R. Cardon (2003). Methodology for genetic studies of twins and families. Dordrecht, Germany, Kluver Academic Publisher.

Neuropathology Group MRC CFAS (2001). "Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales." Lancet **357**(9251): 169-175.

Ng, S., V. L. Villemagne, et al. (2007). "Visual assessment versus quantitative assessment of 11C-PIB PET and 18F-FDG PET for detection of Alzheimer's disease." Journal of Nuclear Medicine **48**(4): 547-552.

Okello, A., J. Koivunen, et al. (2009). "Conversion of amyloid positive and negative MCI to AD over 3 years: an 11C-PIB PET study." Neurology **73**(10): 754-760.

Peper, J. S., R. M. Brouwer, et al. (2007). "Genetic influences on human brain structure: a review of brain imaging studies in twins." Human Brain Mapping **28**(6): 464-473.

Pike, K. E., G. Savage, et al. (2007). "Beta-amyloid imaging and memory in non-demented individuals: evidence for preclinical Alzheimer's disease." Brain **130**(Pt 11): 2837-2844.

Price, J. L. and J. C. Morris (1999). "Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease." Annals of Neurology **45**(3): 358-368.

Rabinovici, G. D. and W. J. Jagust (2009). "Amyloid imaging in aging and dementia: testing the amyloid hypothesis in vivo." Behavioural Neurology **21**(1): 117-128.

Reiman, E. M., K. Chen, et al. (2009). "Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease." Proceedings of the National Academy of Sciences of the United States of America **106**(16): 6820-6825.

Roe, C. M., M. A. Mintun, et al. (2008). "Alzheimer disease and cognitive reserve: variation of education effect with carbon 11-labeled Pittsburgh Compound B uptake." Archives of Neurology **65**(11): 1467-1471.

Rowe, C. C., S. Ng, et al. (2007). "Imaging beta-amyloid burden in aging and dementia." Neurology **68**(20): 1718-1725.

Sachdev, P. S., A. Lammel, et al. (2009). "A comprehensive neuropsychiatric study of elderly twins: the Older Australian Twins Study." Twin Research & Human Genetics **12**(6): 573-582.

Storandt, M., M. A. Mintun, et al. (2009). "Cognitive decline and brain volume loss as signatures of cerebral amyloid-beta peptide deposition identified with Pittsburgh compound B: cognitive decline associated with Abeta deposition." Archives of Neurology **66**(12): 1476-1481.

Terry, R. D., E. Masliah, et al. (1991). "Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment." Annals of Neurology **30**(4): 572-580.

Thal, D. R., U. Rub, et al. (2002). "Phases of A beta-deposition in the human brain and its relevance for the development of AD." Neurology **58**(12): 1791-1800.

Tolboom, N., M. Yaqub, et al. (2009). "Test-retest variability of quantitative [11C]PIB studies in Alzheimer's disease." European Journal of Nuclear Medicine & Molecular Imaging **36**(10): 1629-1638.

Valenzuela, M. J. and P. Sachdev (2007). "Assessement of complex mental activity across the lifespan: Development of the literature of Lifetime Experience questionnaire (LEQ)." Psychological Medicine **37**(7): 1015-1025.

Villemagne, V. L., S. Ataka, et al. (2009). "High striatal amyloid beta-peptide deposition across different autosomal Alzheimer disease mutation types." Archives of Neurology **66**(12): 1537-1544.

Villemagne, V. L., M. T. Fodero-Tavoletti, et al. (2008). "The ART of loss: Abeta imaging in the evaluation of Alzheimer's disease and other dementias." Molecular Neurobiology **38**(1): 1-15.

Villemagne, V. L., K. E. Pike, et al. (2008). "Abeta deposits in older non-demented individuals with cognitive decline are indicative of preclinical Alzheimer's disease." Neuropsychologia **46**(6): 1688-1697.

Visscher, P. (2004). "Power of the classical twin design revisited." Twin Research & Human Genetics **7**: 505-512.

Visscher, P., S. Gordon, et al. (2008). "Power of the classical twin design revisited: II Detection of Common Environmental Variance." Twin Research and Human Genetics **11**: 48-54.

Wen, W. and P. S. Sachdev (2004). "Extent and distribution of white matter hyperintensities in stroke patients: the Sydney Stroke Study." Stroke **35**(12): 2813-2819.

Wisniewski, K. E., H. M. Wisniewski, et al. (1985). "Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome." Annals of Neurology **17**(3): 278-282.

Wolk, D. A., J. C. Price, et al. (2009). "Amyloid imaging in mild cognitive impairment subtypes." Annals of Neurology **65**(5): 557-568.

World Health Organisation (2006). Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation.