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Risk estimations, risk factors and genetic variants associated with Alzheimer Disease in selected publications from the Framingham Heart Study

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Abstract

The study of Alzheimer Disease (AD) in the Framingham Heart Study (FHS), a multi-generational, community-based population study, began nearly four decades ago. In this overview, we highlight findings from 7 prior publications that examined lifetime risk estimates for AD, environmental risk factors for AD, circulating and imaging markers of aging-related brain injury and explorations on the genetics underlying AD.

Keywords

Cohort Studies; Alzheimer's disease; Risk factors; Cerebrovascular Disorders; Genetic variation

First, we describe estimations of the lifetime risk of AD. These estimates are distinguished from other measures of disease burden and have substantial public health implications. We then describe prospective studies of environmental AD risk factors: one examined the association between plasma levels of Omega-3 fatty-acid and risk of incident AD, the other explored the association of diabetes to this risk in subsamples with specific characteristics. With evidence of inflammation as an underlying mechanism, we also describe findings from a study that compared the effects of serum cytokines and spontaneous production of peripheral blood mononuclear cell cytokines on AD risk. Investigating AD related endophenotypes increases sensitivity in identifying risk factors and can be used to explore pathophysiologic pathways between a risk factor and the disease. We describe here findings of an association between large volume of white matter hyperintensities and a specific pattern of cognitive deficits in non-demented participants. Finally, we summarize our findings from 2 genetic studies: The first used GWA and family-based association methods to explore the genetic basis of cognitive and structural brain traits. The second is a large meta-analysis GWA study of AD, in which novel loci of AD susceptibility were found. Together, these findings demonstratethe FHS multi-directional efforts in investigating dementia and AD.

The Framingham Heart Study began in 1948 as a prospective community-based cohort study to identify risk factors for cardiovascular disease but has since grown into a 3 generational

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study of dementia and Alzheimer's disease and of the preclinical states preceding AD including various types of mild cognitive impairment (MCI) and pre-MCI[1-3]. In 1976 a battery of neuropsychological tests was administered to the Original (Generation 1) cohort and based on these and subsequent follow-up a dementia-free cohort of 3,349 Original cohort participants was established. All Original cohort participants are reassessed biennially at a comprehensive core examination and have been examined 31 times to date[4]. In 1971, offspring of the Original cohort and spouses of these offspring were enrolled into an Offspring (Generation 2) cohort that has been assessed 9 times to date for a core examination[5] and in 1990, a diverse, multiethnic Omni cohort was added[6]. In addition, starting in 1981 the Folstein Mini-Mental State Examination (MMSE) has been administered at the core examinations to allow for ongoing surveillance of cognitive status. Based on a question regarding cognition at the second offspring examination and all available records, a dementia-free cohort for the Offspring was retrospectively established starting in 1979 and numbers 4,460 persons. The Omni cohort was determined to be dementia-free at the time of study entry. A third generation (Gen 3) was enrolled in 2002 (n=4105) and has undergone cognitive screening at their second core examination.

All cohorts are under ongoing surveillance for cognitive decline and dementia. Starting in 1999 Original and Offspring cohort participants have been invited to undergo volumetric brain MRI and were also administered a detailed cognitive battery on the same day; over 2000 persons have undergone 2 rounds of imaging and cognitive testing and a selected subsample (oldest participants, brain donors, persons with suspected cognitive decline) has had multiple rounds of testing. The Gen 3 is also presently undergoing an initial round of cognitive testing and brain MRI. Participants suspected of cognitive impairment are usually evaluated by FHS physicians (neurologists and geriatricians) and undergo neuropsychological testing as required (about every 1-2 years) and then are reviewed at a consensus conference to determine if criteria for dementia are fulfilled. At this evaluation, dates of onset (earliest symptoms) and diagnosis (earliest date when diagnostic criteria are met), dates of transition in disease severity (mild to moderate to severe), dementia diagnosis and subtype are determined. At these reviews data from FHS core and ancillary examinations and health history updates; clinic, home, and nursing home assessments by the neurology and neuropsychology team; family interviews; records from medical contacts and nursing homes; and, when available, detailed brain autopsy findings gathered by the neuropathologist are utilized.

A wealth of lifestyle, vascular, metabolic, circulating biomarker and subclinical disease markers have been collected repeatedly on these participants over the long duration of the study. Multiple concurrent disease events (vascular and pulmonary, cancer, bone and joint disease) are also monitored and most recently, systems biology approaches have been introduced. A wealth of genetic data are also available for these cohorts.

Life-Time Risk of AD and stroke

With the growing number of people living to older ages, the number of affected people and the costs associated with Alzheimer's disease (AD) are projected to rise around the world. Lifetime risk (LTR) of a particular disorder is an aggregate estimate of actual risk during the remaining lifespan that is applicable to people of a particular age and sex. Estimates of LTR of AD and all-cause dementia from the FHS Original cohort were described in 1997 [7]. In 2006, this observation was extended utilizing longer follow-up and additional cases that had accrued in the interim[8]. The latter findings were reported together with estimates of LTR of stroke and enabled for the first time the comparison of this measure between these two related diseases within the same cohort. In this paper we studied 2794 participants who reached 65 years of age free of dementia and were followed for up to 29 years (42,233

person years). Sex-specific 10-, 20-, and 30-year risks and the LTR of developing dementia and AD were estimated at ages 65, 75, and 85 years. We estimated that approximately 1 in 5 women and 1 in 10 men who reached 65 years of age would develop AD during their remaining lifetime, and more than 1 in 5 women and 1 in 6 men would develop dementia. The LTR of AD was less than or equal to the LTR of stroke at all ages, except for in women aged >85 years for whom LTR of AD was significantly higher than that of stroke. The combined LTR of developing either dementia or stroke was approximately 30% in men, and 40% in women, which is above the LTR for developing symptomatic coronary artery disease. The implications of these findings are further discussed in a subsequent paper by Seshadri et al. [9].

Although LTR estimates were widely used in other fields such as oncology, the FHS was the first to provide cohort-based estimates of LTR of AD and dementia. In one study, the Baltimore Longitudinal Study [10], LTR of AD was reported; however their sample was small with only 27 incident cases that were later reported as possibly misclassified [11]. Estimation of the cumulative risk of dementia and AD is different from the LTR estimates in that it does not account for the competing risk of death. While cumulative risk estimates treat subjects who die during the follow-up period as censored (and thus inflate the estimates), LTR estimates recognize that the risk of the disease after death is zero and thus is more appropriate when assessing individual risk, for example in estimating the value of a risk predictor using Bayesian principles. However, cumulative incidences remain appropriate in evaluating whether a given variable is associated with the risk of disease, that is in determining biological associations. LTR should also be distinguished from other descriptive statistical measures such as incidence, prevalence, relative risk or cause-specific mortality. Estimation of LTR allows a person who is free of a disease to be aware of the probability of acquiring it at some time during his remaining expected lifespan. Similarly, this measure is essential for public health planners for estimating disease burden in a population while considering average expected life span, and it may serve as a valuable tool for public education because it is easy to interpret and can be put in perspective in comparison to LTR estimates for developing other diseases. The need for such an epidemiological measure in AD was recognized by Seshadri et al. after the apolipoprotein E (APOE) ε4 allele was discovered as a major AD risk factor. A Bayesian analysis was then used to determine the risk of developing AD, with and without an APOE ε4 allele, for unaffected 65-year-olds [12]. Recently, Genin et al. used a bootstrap method for the calculation of AD LTR and the evaluation of the impact of APOE ε4 allele on this risk using lifetime cumulative incidences based on the Rochester data [13].

Metabolism and inflammation

To date, no effective treatment is available for prevention or cure of dementia. In order to reduce the burden of Alzheimer's disease (AD) and dementia, it is crucial to understand the mechanisms underlying the disease and to identify potentially modifiable risk factors. Accumulating evidence suggests that multiple vascular and environmental risk factors are associated with late-life cognitive impairment, however consistent findings in epidemiological studies for the association with dementia exist only for age, family history and ApoE4 genotype, and all these are not modifiable. This inconsistency may be attributed to differences in population sample, definitions of variables and study design. In exploring risk factors associated with dementia it is notably important to use a prospective design with long follow-up periods, since this disease has a long sub-clinical phase. In FHS, we had the opportunity to explore various potential risk factors for AD and dementia in a large population-based cohort with long and intensive follow-ups.

One example is a study in which the relationship between levels of Plasma Phosphatidylcholine (PC) Docosahexaenoic Acid (DHA), an Omega-3 fatty-acid and risk of dementia and AD was assessed[14]. In this study, plasma samples were obtained from 899 dementia-free participants of the Original cohort (median age was 79), and plasma PC DHA were measured. Then, these participants were followed-up for the development of AD or dementia over a mean duration of 9.1 years. In addition, dietary data on DHA and fish intake was available in a subsample. In this study, subjects with baseline plasma PC DHA levels in the upper quartile experienced a significant lower risk of dementia (Relative Risk =0.53; 95% confidence interval, 0.29-0.97) compared to participants with levels in the lower 3 quartiles, independent of age, sex, ApoE4 genotype, plasma homocysteine concentration, and education level. In addition, a correlation between plasma PC DHA content and fish intake was found (both with p value<0.001), however fish intake accounted for less than half of the variability in DHA levels. This study was the first to use a prospective design in assessing the predictive value of plasma PC DHA content in the occurrence of dementia and AD, and it set the background for subsequent research, including clinical trials and gene expression studies. Recently, further exploration of these findings in the Offspring cohort revealed lower brain volumes and a “vascular” pattern of cognitive impairment among dementia free persons with lower red blood cell DHA levels[15].

Diabetes is another example of a modifiable risk factor that was explored within the Original cohort of the FHS. Although diabetes is known to be a risk factor for cognitive impairment and dementia as reported in numerous population-based studies, it remains controversial in relation to AD; for instance it has been suggested that the association observed is with the vascular disease that co-exists in persons with clinical AD[16]. The purpose of the study by Akomolafe et al. [17] was not just to explore whereas such an association exists, but also to define a sub-population in which this relationship might be more apparent. Thus, 2210 dementia-free participants (mean age 70 years) were followed for a mean duration of 12.7 years. The Relative Risk (RR) of AD comparing diabetic patients to non-diabetics was not statistically significant (1.15; 95% Confidence interval, 0.65-2.05). However, among participants with neither the ApoE4 allele nor elevated plasma homocysteine levels, the RR (95% Confidence interval) was 2.98 (1.06-8.39). Moreover, the effect was strongest among younger individuals (<75 years) (RR=4.77; 95% confidence interval, 1.28-17.72). This study suggested that diabetes should be considered as a risk factor for AD, and emphasized the need to further explore the predictive value of diabetes and other risk factors in specific subgroups such as in those with an initial low risk for developing AD.

Though the pathological mechanisms that relate diabetes, DHA levels and other risk factors to the development of dementia are not clear, strong evidence supports the role of inflammation as a common link. Diabetes is associated with low-grade inflammation [18] and Omega-3 fatty acids have a variety of anti-inflammatory effects [19], and they influence the expression of many genes which are involved in inflammation [20]. Yet, clinical observations on the potential role of inflammation in AD have yielded inconsistent results. In a study by Tan et al., serum cytokines and spontaneous production of peripheral blood mononuclear cell (PBMC) cytokines were related to the risk of incident AD 7±3 years later, in 691 dementia-free participants of the Original FHS cohort [21]. Participants in the second tertile of IL-1 production levels were at increased risk of developing incident AD compared to those in the lowest tertile (Hazard Ratio=2.84; 95% Confidence Interval, 1.09-7.43) and individuals within the highest tertile of production showed a trend toward an increased risk but with borderline significance (Hazard Ratio=2.61; 95% Confidence Interval, 0.96-7.07). High production of TNF-α was associated with future AD (Hazard Ratio=2.59; 95% Confidence Interval, 1.09-6.12 comparing highest to lower two tertiles). In contrast, levels of circulating inflammatory cytokines (CRP, IL-6) were not associated with the risk of AD. This study demonstrates the possible importance of spontaneous production of cytokines by

PBMC in reflecting intracerebral inflammation as opposed to serum cytokine levels which reflect systemic inflammation, but due to the blood brain barrier may not adequately reflect intracerebral inflammation. PBMCs cross the blood brain barrier and are detected as brain macrophages that impact neuronal and glial function.

Structural evidence of brain aging in a community-based cohort

Abnormalities of the white matter of the brain are identified as areas of signal hyperintensity on magnetic resonance imaging (MRI). White Matter Hyperintensities (WMH) occur not only among stroke and AD patients but also are remarkably common in healthy individuals beginning in middle age [22]. We tested the association between WMH and cognitive performance in stroke- and dementia-free participants from the Framingham Heart Study's relatively young, non-demented Offspring cohort [23]. Participants were categorized as having large WMH for their age group, adjusting for head size, as follows: we created, within each 5 year age group. cutoffs at 1 SD above the mean ratio of WMH to head size. A participant was categorized as having large WMH if their ratio of WMH to head size was greater than their age-specific cutoff. Our findings suggested that individuals with a ‘large volume’ of WMH performed significantly worse on measures of visual memory and organization ($p=0.04$), attention and executive function ($p=0.01$) and new learning (immediate recall score; $p=0.04$) compared to others. This pattern of cognitive deficits is suggestive of subcortical frontal system involvement, and may indicate a higher risk of developing AD or vascular dementia in the future. Indeed, in a recent follow-up study within the Offspring cohort, WMH as well as brain infarcts were shown to be important predictors of amnestic mild cognitive impairment and dementia [24] .

The causes of WMH remain unclear. Previous studies among the Offspring cohort have demonstrated an association of various vascular risk factors [25] and subclinical disease markers such as carotid artery intimal-medial thickness [26] with WMH volumes, implying an important vascular component. However, the relationship between WMH and cognition in the current study was independent of concurrent levels of vascular risk factors. There are several possible explanations for this observation: the brain changes of higher WMH and lower cognition may both be due to non-vascular processes related to normal or pathological aging or WMH may be a marker of a longer duration of exposure to cerebrovascular risk factors. The fact that WMH were associated with poorer cognitive performance even among relatively young, non-demented individuals is important. It emphasizes that pathological and imaging changes precede clinical dementia by at least a decade during which prevention strategies may be beneficial.

Genetics of AD and related phenotypes

The heritability of AD is high [27]. For many years, the only gene definitively associated with late-onset AD was apolipoprotein E (*APOE*) region, but this gene could not fully explain the genetic variation of the disease. Other candidate gene studies suggested that changes in expression or function in the neuronal sortilin-related receptor gene (SORL1) are causally linked to the pathogenesis and risk of AD[28]. In order to understand better the genetic basis of AD, it is important to investigate the effect of genes on graded susceptibility to developing the disease, using subclinical endophenotypes that may be manifest years before clinical and pathological criteria for the disease are met. A study among 705 stroke and dementia-free Framingham participants used both linear models adjusting for family relationships and family based association tests to explore the genetic basis of 6 cognitive traits and 9 brain MRI traits [29]. Several phenotype-SNP associations within biologically interesting genes were found. The strongest phenotype-SNP association in linear models analyses was between a SNP on the retinal cadherin gene *CDH4* and TCBV (rs1970546; p =

3.7 × 10-8) and in family based association test, between a SNP on the *SORL1* gene (rs1131497; p = 3.2 × 10-6) and performance in a test of abstract reasoning. This early genome wide association (GWA)study showed that genes previously associated with clinical AD might also be associated with cognitive and brain MRI subclinical endophenotypes. A subsequent study by Seshadri et al. [30] was among the early, large, genome wide association studies in AD, which combined data from several population-based cohort studies and thus had the statistical power to detect genes with small effects. Three stages of GWA analyses were performed: Stage 1 was a meta-analysis combining new GWA data from white participants in the large, population-based Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium samples. In stages 2 and 3, significant results from previous stages were pooled with additional data from two recently published datasets [31, 32] and at the end of this process, 4 SNPs with p-value $<1.7 \text{ X } 10^{-8}$ were identified. Two of them were novel loci on chromosomes 2 and 19 (*BIN1* and *EXOC3L2*), and the others had been reported earlier that year (*CLU* and *PICALM*)[31, 32]. All of the 4 loci identified were successfully replicated in an independent Catalan sample as part of this study. BIN1 is the second major gene underlying late-onset AD (after APOE) in terms of the consistency of association, strength of association, effect size and population attributable risk fraction after adjusting for APOE genotype.[33] This gene has been associated with risk of AD not only in Caucasians but also in Caribbean Hispanics and within large late-onset AD families.[34, 35] Interestingly, BIN1 is also a tumor suppressor and scaffolding protein and reduced expression has been associated with metastatic cancer and cardiomyopathies which is an intriguing fact since an inverse association has been described between the risks of cancer and AD.[36-38] The active gene at the second locus is likely MARK4 but because of the close proximity of this locus to the APOE locus, the independent significance of this locus remains uncertain.[39]

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