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Commentary on “A roadmap for the prevention of dementia II. Leon Thal Symposium 2008.” Prevention Trials in Persons At-Risk for Dominantly-Inherited Alzheimer's Disease: Opportunities and Challenges

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Abstract

Autosomal dominant familial Alzheimer's disease (FAD) of young onset due to alterations in the *PSEN1*, *APP*, and *PSEN2* genes is a fully-penetrant and devastating condition. As the subsequent development of AD in persons inheriting such genes is essentially certain, the condition provides a unique opportunity to perform informative studies of interventions with potential for preventing the disease. Though feasible, there are many challenges to such an endeavor including the fact that most persons at-risk for FAD do not desire to know their genetic status. Other challenges include the time course over which a preventative treatment would need to be administered and potential limitations to the degree to which the knowledge gained might be validly generalized to the more common late-onset AD. In this paper we discuss issues of study design including power estimates, protocols in which subjects' genetic status is not revealed to them, and the advantage of one-time interventions such as vaccinations. Though addressed in the context of FAD, many of the issues discussed are relevant to other fully-penetrant autosomal dominant degenerative illnesses such as Huntington's disease. We also discuss important next steps including the performance of pre-clinical studies in model systems appropriate for FAD and the recently funded international Dominantly Inherited Alzheimer Network (DIAN). The goals of the DIAN are to characterize the natural history of FAD and to establish the infrastructure that would be required to perform meaningful studies in this rare, widely dispersed, but informative population.

Introduction

Persons inheriting autosomal dominant mutations causing familial Alzheimer disease (familial AD, or FAD) are essentially certain to develop the illness at a young age that is predictable, at

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least to some extent. Known mutations in the genes encoding for Presenilin-1 (*PSEN1*), Amyloid Precursor Protein (*APP*), and Presenilin-2 (*PSEN2*) result in FAD, an aggressive illness with a more rapidly progressive course than that of late onset Alzheimer's disease (LOAD). FAD accounts for 2% or less of all AD cases. The identification of the genes responsible for this form of the disease has contributed greatly to our understanding of AD in general. Characterization of the normal and abnormal function of the proteins encoded by these genes contributed greatly to the prevailing "amyloid hypothesis" of AD pathogenesis(1). The pathogenic forms of these genes all lead to aberrant metabolism of the amyloid precursor protein, favoring the production of cleavage products more prone to aggregation (particularly the 42-amino acid length beta-amyloid peptide, or A β 42). Many of these mutations have been transfected into mice, producing useful animal models that recapitulate many, though not all, disease characteristics. In addition to leading to breakthroughs in our understanding of the mechanisms of AD pathogenesis, the study of persons with or at-risk for FAD has provided insights into the cognitive(2,3), imaging(4-6), and biochemical changes(7,8) occurring in the earliest stages of the illness. These observations are likely to enhance our ability to diagnose and possibly treat LOAD in its presymptomatic stage. In this paper we discuss the advantages to performing studies for the prevention of AD in the population at-risk for FAD as well as the many challenges to such an endeavor.

With the aging of the baby boomers in the U.S. and with increasing longevity worldwide, the specter of Alzheimer's Disease (AD) as a major public health crisis is looming. Barring major advances in stem cell therapy and neural regeneration, it may be impossible to reverse established disease once it is present. Halting, slowing the progression of, or preventing AD altogether are likely to be more tractable goals. In part due to competing mortality in the aged population, it has been estimated that delaying the onset of AD by 5 years would result in a 50% reduction of AD cases in a generation(9). Therefore, efforts to develop treatments that are effective in preventing AD are of high priority.

It is difficult to definitively establish the efficacy of an intervention in the prevention of AD. Much of the information we have regarding whether or not certain interventions may be helpful in the primary prevention of AD comes from retrospective epidemiological observations(10). Such studies yield important clues as to what types of interventions might be studied further, but they do not offer definitive proof that an intervention causes the observed effect. This level of proof requires the completion of prospective, randomized, double blind, placebo-controlled studies. Such studies of prevention in AD are challenging for several reasons. It is currently difficult to establish *a priori* who in a population will ultimately develop AD and over what time frame. Therefore one must study hundreds or thousands of persons for many years in order to guarantee that a significant number in any treatment arm will develop (or *would* have developed) AD so that meaningful conclusions can be drawn(11,12). For example it has been estimated that it would require between 3,000 and 6,000 total subjects to detect a 30% reduction in the occurrence of AD over 5 years assuming a 5 - 10% cumulative incidence of the disease (13). Such large and long duration studies are prohibitively costly and prone to high drop-out rates. This large scale approach has nonetheless been employed by including the development of dementia or other cognitive variable as outcome measures in controlled studies of interventions thought to have wider health benefits. Examples of this approach include the now-terminated Women's Health Initiative Study that looked at the preventative effects of estrogen and/or a progestational agent(14) and the on-going study of selenium and vitamin E in the prevention of prostate cancer (15).

Another method used during the drug development process that may be adapted to prevention protocols is the use of small short-duration studies in which alternative outcome measures such as changes in cognition, biochemical markers, or imaging variables are the primary outcome

measures. Until such measures have been validated and accepted as surrogate disease markers by the U.S. Food and Drug Administration, however, alternative methods will be required.

In recent years, there have been significant advances in our understanding of clinical, genetic, imaging, and biochemical markers that predict who is more likely to develop clinical AD in the near future. Such indices may be used to identify a sub-group, or “enriched” population at increased risk for the development of AD that might be entered into prevention trials(16). The “oldest old,” persons with amnesic mild cognitive impairment (MCI), persons with medial temporal lobe atrophy on magnetic resonance imaging (MRI), people carrying the $\epsilon 4$ allele of the Apolipoprotein E gene, or persons with other “risk factors” have been proposed as populations at increased risk for developing AD that might be identified and enrolled in prevention trials. Though these strategies might reduce the size of the population and the duration required for prevention studies, these measures are still imperfect predictors of the subsequent development of AD. Inclusion of persons who are not destined to develop AD in prevention trials increases the number of people required and the duration over which they need to be followed. This adds to the costs of such studies and requires unnecessary exposure of persons who will not develop the disease to potential risks of the intervention. Therefore, alternative mechanisms by which potential preventative therapies can be more efficiently tested should be considered.

Prevention Trials in Persons Inheriting FAD Mutations: the Opportunity

Persons inheriting pathogenic *PSEN1*, *PSEN2*, and *APP* mutations are essentially certain to develop the disease. The age of onset within families with *PSEN1* mutations may be quite consistent(17,18). Some variability in the age of onset does occur, particularly with regard to *PSEN2* mutations(19), and cases of incomplete or delayed penetrance have been reported (20). Such occurrences, however, are thought to be rare overall and FAD is generally considered to be a fully-penetrant condition. Persons inheriting FAD mutations, in whom the presence of such a mutation can be determined at any point in their lifespan, therefore represent a population that provides a unique opportunity to test interventions for the prevention of AD in an informative and relatively efficient manner.

The number of persons at-risk for FAD that would need to be enrolled in a prevention study and the time period over which they would need to be treated would depend on the specific goals of the study. Assuming that the outcome of interest was prevention of the development of diagnosable dementia (a binary outcome), enrollment is restricted to those known to be carrying pathogenic FAD mutations (see below), and all subjects could be followed to the point when they would have developed dementia, one would require 96 subjects per treatment arm to have 80% power to detect a 30% reduction in the incidence of AD with a 95 percent confidence interval. Though this is far fewer subjects than the 3000 to 6000 persons required in studies to prevent late-onset AD, it is still a large number considering the paucity of persons at-risk for FAD who are aware of their (positive) gene status. Furthermore, the time period over which they would have to be studied would be influenced by their age and variability in the age of symptom onset and the numbers above assume that all mutation carriers would have developed the disease over the time period of the study had they not received the intervention. Therefore, the number of appropriate subjects that are available and willing to participate and the time period over which they would need to be studied in such a primary prevention study present further challenges. Alternative study designs are discussed below.

Prevention Trials in Persons Inheriting FAD Mutations: the Challenges

Testing interventions for the prevention of AD in presymptomatic persons inheriting FAD mutations initially sounds like an obvious and attainable goal from both scientific and ethical standpoints. Persons from FAD families participating in our observational studies repeatedly

inquire about the availability of treatment studies. Persons affected by FAD, however, typically cannot take part in treatment trials for LOAD because of age cut-offs or enrollment criteria specifically excluding them. Preventative trials for presymptomatic persons with FAD mutations present a number of obstacles, both scientific and logistical, regarding how they would be performed. These stem mainly from two main issues: 1) the appropriate design of a preventative study in which an agent may have to be administered over the course of years, decades, or even a lifetime to be effective and 2) the majority of persons who know they are at-risk for a determinant FAD mutation they can be tested for do not desire such testing.

Design of a Trial to Prevent FAD

A major challenge to the successful performance of prevention studies in persons at-risk for FAD is the question of how long a preventative treatment would have to be administered in order to be effective. Interventions that act directly on the cascade of events causing neurodegeneration would be preferable as they hold the most promise in halting the progression of disease and might be effective when treatment is initiated later in adult life when some degree of pathology is already established. Current examples are active and passive immunization approaches and gamma- and beta-secretase inhibitors. However, in persons who are constitutively over-producing A β , one would assume that such interventions would have to be continued to provide ongoing protection from disease throughout what would hopefully be a long and productive life. Compliance and the unknown risks of chronic exposure then become potential issues. Interventions such as vaccines in which the treatment can be given once or at least intermittently to provide long-term protection are therefore appealing(21).

A more tractable experimental goal than preventing the insidious development of dementia from the presymptomatic state might be to perform smaller short-term studies using changes in cognition, biochemical markers, or imaging variables as the primary outcome measures. Imaging (22) and biochemical(8) indicators of disease status that might be appropriate for study in this population have been identified. We(8) and others(7) have demonstrated that levels of tau and phosphorylated tau in the cerebrospinal fluid (CSF) are elevated decades prior to the onset of dementia in FAD. Such indices therefore might be of utility as alternative outcome measures throughout the course of FAD. The ability to detect an effect of a drug on such surrogate measures would also be maximized by studying the population in whom this would be expected to be changing most rapidly. The longitudinal behavior of most potential FAD biomarkers, however, is currently largely unknown.

Some data are available regarding decline in cognitive function and changes in brain volume in FAD(22). From our own unpublished data in which Mexican persons at-risk for *PSEN1* mutations underwent neuropsychological testing, 11 of 72 such subjects underwent more than one annual assessments, were not demented when first evaluated, and were of age within 15 years of the typical age of dementia diagnosis in their family. Using performance on delayed recall of a 10-word list, a mean decline of only 0.15 words per year was observed. Based on this data and assuming a two-arm (1:1 ratio of drug:placebo), 3-year treatment study with annual cognitive assessments, a sample size of 3,800 such subjects per arm would be necessary to provide 80% power to detect a 30% reduction in rate of worsening on this test(23). Our cognitive data are clearly not ideal in that 6 of the 11 subjects were not tested across the age during which maximal cognitive decline would be expected to occur and thus some subjects did not decline and some even improved on this measure. The data therefore underestimate the amount of decline in performance on this test that would be seen in subjects appropriately enrolled in a prevention study according to their age. This emphasizes the importance of taking into account subjects' ages in relation to the typical age of dementia onset in their family, variability in the age of onset and other inter- and intra-family differences as well as the

characteristics of the chosen outcome variable when designing a prevention study in this population.

Alternatively, based on an annual decline in hippocampal volume on MRI of 3.23% (standard deviation = 1.6%) that was observed by Ridha et al (22) in 9 FAD mutation carriers near the time of conversion to MCI and dementia, 97 such subjects per treatment arm would provide 80% statistical power to detect a 20% reduction in rate of hippocampal atrophy. If the effect size were as large as 30%, only 43 subjects per treatment arm would be required. Though the ability of an intervention to induce a favorable change in such biomarkers might provide “proof-of-concept” that the intervention in question should be developed further(24), such studies inevitably leave open the question as to whether or not it actually prevents AD.

Another critical issue is the generalizability of both efficacy and safety data from FAD to LOAD. There are certainly differences in the pathogenesis of these forms of the illness and it is possible that a drug (e.g. a gamma-secretase inhibitor) that might be effective in FAD in which overproduction of A β 42 appears to be etiological may not be effective in LOAD in which the causative chain of events is less clear. Conversely, it cannot be assumed that drugs effective in preventing late-onset AD would necessarily be helpful in treating or preventing FAD. In-vitro studies and studies in transgenic mice suggest that at least some mutant forms of Presenilin-1 are resistant to the effects of gamma-secretase inhibitors(25). Lack of efficacy of such a drug in a clinical study of FAD therefore might not necessarily predict lack of efficacy in LOAD. Preclinical invitro work exploring the effects of experimental agents on cell lines derived from persons with FAD might therefore serve an important function in screening compounds and understanding their pharmacology prior to their administration in humans (see below).

Genetic Testing for FAD Mutations in Presymptomatic Individuals

It has been shown that the majority of persons at-risk for fully-penetrant autosomal neurodegenerative diseases of onset in adulthood for which there are (currently) no or only minimally effective treatments opt not to undergo genetic testing. In Huntington's disease, an autosomal dominant neurodegenerative disease for which specific genetic testing has been available since 1993, only a minority of at-risk persons decide to undergo testing in most series (26). In a study in France published in 2002(27), 57% of 712 persons who initially *requested genetic counseling* went through with the test. In a clinical series of 251 persons at-risk for FAD or frontotemporal lobar degeneration due to mutations in the *MAPT* gene, only 8.4% requested such testing(28). This number corresponds to our experience in which only 3 (7.9%) of 38 non-demented persons at-risk for FAD have asked for and undergone genetic counseling and testing. Such attitudes are perhaps understandable, considering the lack of a substantively effective treatment for these disorders and the psychological impact that might accompany positive test results. When such testing is undertaken it is typically due to the desire to make reproductive and career decisions and other personal or financial plans. An individual's decision to whether or not to undergo such testing ideally would represent the outcome of an informed analysis of the costs and benefits of such testing.

How does one therefore perform a blinded, randomized, placebo-controlled prevention trial in a population that typically does not desire to know whether or not they are actually appropriate to participate? We envision three possible solutions to this problem: 1) open enrollment to all at-risk subjects and confine the primary outcome analyses to FAD mutation carriers, 2) perform quasi-randomization such that only FAD mutation carriers receive active drug or, 3) only enroll subjects who desire to undergo revealing testing. Each of these approaches has both advantages and disadvantages, the importance of each of which varies with the degree of potential efficacy and toxicity of a given compound. These are discussed below.

1) Enroll all at-risk subjects—Approaching all persons known to be at-risk for FAD for enrollment in a preventative trial has the advantage of avoiding the issue of whether or not subjects have made the decision as to undergo revealing testing. Efficacy analyses in such a trial would be limited to comparisons between the treatment and placebo groups among the mutation carriers. It has been our experience that many persons from families with FAD, even those who have apparently “escaped” the disease by virtue of aging beyond the typical age of disease onset without symptoms, are eager to participate in research if there is hope that the research will benefit others in their family. This altruism might enable study investigators to enroll adequate numbers of mutation-carrying subjects in addition to the non-mutation carrying volunteers.

A potential issue with this design is whether or not it is ethical for researchers to expose persons who are not going to develop FAD to the potential risks of an experimental intervention. Involvement of non-mutation carrying persons in such a study might be considered akin to the exposure of volunteers to experimental drugs in Phase I studies. Unlike typical Phase I studies, however, the exposures in a preventative study would be of much greater duration, increasing the possibility of adverse outcomes. Nonetheless, such exposure would yield additional information on long-term tolerability, information that is critical for a putative preventative treatment. Furthermore, non-carriers of FAD mutations are still prone to develop LOAD and therefore might still benefit from a potentially prophylactic intervention. The known or suspected toxicity of an agent would have to be considered carefully by investigators and all persons enrolling in a study with such a design. If attempted, such studies might best be reserved for interventions with toxicity profiles that are known or expected to be relatively benign.

2) Quasi-randomize so that only mutation carriers receive active drug—A study design that would avoid both the need for revealing genetic testing of prospective subjects and exposure of non-mutation carriers to potential toxicity is to “quasi-randomize” subjects depending on their mutation status. Non-revealing genetic testing would be performed and only mutation carriers would be assigned to active drug while all non-mutation carriers would receive placebo. In order to have a control arm, at least some FAD mutation carriers would have to be assigned to placebo. This could be carried out such that the likelihood of mutation carriers receiving drug is maximized while sufficient statistical power is maintained (e.g. a 3:2 ratio of active drug to placebo).

A potential drawback to this method would be the potential for unblinding due to physiological effects of the drug. That is, if a subject were to have an adverse effect in such a study, they could surmise (possibly incorrectly!) that they were receiving active drug and therefore must be an FAD mutation carrier. This might well be information that they had already made the active decision to not know.

3) Restrict enrollment to subjects who know they are FAD mutation carriers—Ideally, all persons who make the decision whether or not to participate in a placebo-controlled trial of a potential therapeutic agent do so in as fully an informed capacity as is possible. This includes, of course, knowing whether or not you are actually destined to develop the illness for which the putative intervention is intended to prevent (of course, persons who have not inherited FAD mutations nonetheless remain “at-risk” for LOAD but we will forego this discussion). Unfortunately, as we are *heretofore* lacking preventative treatments demonstrated to be effective and the majority of at-risk persons do not therefore desire such testing, the ability to enroll substantial numbers of FAD mutation carriers is markedly decreased.

Persons at-risk for FAD undoubtedly have great hope that effective preventative treatments will be developed and will have a low threshold for participating in what is perceived as a promising study. If such a study were to only enroll persons whose positive genetic status was

known to them, we anticipate the possibility that potential subjects would decide to undergo testing with the knowledge that if they are carriers they would be eligible to participate. That is, there could be an element of unintentional *implicit coercion* in which the existence of a study of a *potential* therapy might bias a person at-risk into being tested. At best, the decision as to whether or not to undergo testing is complicated by the existence of the clinical trial. At worst, in the case when an agent whose efficacy is unknown and has significant toxicity (whether known or unknown), the person deciding to undergo testing may not be making the right decision. Even for investigators experienced with AD, FAD, clinical research methodology, and the drug in question, it would be difficult to counsel potential subjects appropriately and objectively. A further concern is the possibility that an at-risk subject, interested in participating in a promising study, undergoes (positive) testing, only to be assigned to placebo. One method that is commonly used to insure that all subjects have some exposure to the experimental treatment is a cross-over design. Unfortunately, this is impractical in the context of a prevention study. The use of historical controls is another option that might be employed though differences between the trial and control populations and the ways they are assessed can plague the interpretation of such studies.

Current Efforts in FAD

To our knowledge, there is only one prevention study currently being performed in FAD(29). Six presymptomatic persons known (by themselves and investigators) to carry *PSEN1* mutations are being treated in an open-label fashion with HMG-CoA reductase inhibitors (either atorvastatin and simvastatin) and are being followed prospectively. In addition to cognitive outcome measures, CSF indices (A β 42, tau, p-tau₁₈₁, sAPP α , and sAPP β) are being obtained. Though of small scale, this trial represents an important first step towards larger efforts to explore the effects of interventions in FAD.

The National Institute of Aging recently funded an international network of sites to collectively study persons with and at-risk for FAD (the *Dominantly-Inherited Alzheimer Network*, or “DIAN”). The aims of this project, which is funded for 6 years, are to:

1. Establish an international, multicenter registry of individuals (mutation carriers and noncarriers; presymptomatic and symptomatic) who are biological adult children of a parent with a known causative mutation for AD in the *APP*, *PSEN1*, or *PSEN2* genes in which the individuals are evaluated in a uniform manner at entry and longitudinally thereafter with standardized instruments.
2. In presymptomatic individuals, compare mutation carriers and non-carriers to determine the order in which changes in clinical, cognitive, neuroimaging, and biomarker indicators of AD occur prior to the occurrence of dementia.
3. In symptomatic individuals, compare the clinical and neuropathological phenotypes of autosomal dominant AD to those of late-onset “sporadic” AD.
4. Maintain an integrated database incorporating all information obtained from individuals in the registry to permit analyses within, between, and among the various data domains and also to disseminate the data to qualified investigators in a user-friendly manner.
5. Provide genetic counseling to all DIAN participants and, for those who after counseling wish to learn their mutation carrier status, provide genetic testing by Clinical Laboratory Improvement Amendments-approved laboratories.

Data obtained through the DIAN will enhance our understanding of the natural history of FAD and will provide invaluable information to be used in the design and statistical powering of prevention and treatment studies in this population. Additionally, white blood cells will be

forwarded to the National Cell Repository for Alzheimer's Disease (NCRAD) to establish immortalized lymphoblastoid cell lines that may be used in a variety of investigations, including those to characterize the pharmacodynamic properties of putative anti-AD agents the infrastructure critical for the recruitment and retention of subjects necessary for the successful performance of clinical trials in this rare, widely dispersed, and informative population.

Conclusion

FAD simultaneously represents an aggressive disease variant and a scientific and therapeutic opportunity. Already responsible for much medical knowledge pertaining to AD, the study of FAD may also provide strategic and critical steps towards developing new therapies for this global healthcare crisis. Such studies, however, are not without ethical and methodological concerns and must be approached cautiously. If they are to occur, studies of investigational therapies in FAD will require close collaboration between academic, industrial, and regulatory bodies.

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