Report of the National Institute on Aging Advisory Panel on Exceptional Longevity

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CONTENTS

EXECUTIVE SUMMARY

The National Institute on Aging (NIA) supports research to identify factors that contribute to long healthy life and protect against age-related diseases and disabilities. As part of its ongoing efforts in this area, NIA convened the Advisory Panel on Exceptional Longevity (APEL) to obtain recommendations on the feasibility of studies to identify genetic factors (and other factors that might interact with genetic factors) that contribute to exceptional longevity (EL). APEL included geneticists, epidemiologists, gerontologists, demographers, and statisticians. The panel explored desirable phenotypes to characterize EL and ways to analyze them; strengths and limitations of different subject groups to study EL; feasibility of full-scale studies; study designs and sample size estimates for studies of EL; and special logistical and ethical issues pertinent to EL studies.

The panel made recommendations on needs for analyses of existing data, for new cell and data collection, and/or for pilot or full-scale studies that are unlikely to be met through the standard NIH process for research project applications, and that could be met more efficiently or effectively by one or more NIA solicitations for proposals. Additionally, it suggested other ways for NIA to advance research on EL. The panel also identified opportunities for NIA to collaborate with ongoing efforts in other countries for collection of cells and data from exceptionally long-lived people. The Report is summarized below. Detailed discussion of the topics the panel addressed and its recommendations can be found in the full Report.

Exceptional longevity phenotypes. Exceptional longevity (EL) can be defined in numerous ways. The definition should take into account secular trends in life expectancy, gender effects, and social, environmental and behavioral factors, because survival to a given age is less exceptional in some subgroups than others. Definitions of EL of interest include survival past a specified extreme age, and disability-free survival past a specified age. The identification of intermediate phenotypes that predict longevity, and which could be used in genetic studies of EL, would be useful. A key question is whether there are homogenous subgroups that comprise the long-lived population. Identification of such subgroups would increase the power of studies to identify contributory genes. Analyses of genetic effects on longevity can be based either on variation of risk of death by a given age, or on survival times characterized as a continuous trait.

Population groups in which to study EL. Among different subject groups (unrelated individuals, relative pairs, and families) for studies to identify genetic effects on EL, each approach has strengths and limitations. All these approaches offer the opportunity to test candidate loci and to identify genomic regions that may harbor previously unknown loci.

Association studies in unrelated individuals have greater power for some analyses, are more feasible for large-scale studies, and for identifying population effect sizes, gene-gene interactions, and gene-environment interactions. However, these approaches are at risk for false-positive results due to population stratification (which can cause differences in frequencies of

noncontributory alleles), and to heterogeneity among contributory alleles, which can lower statistical power. New approaches to identify population stratification and allelic heterogeneity and incorporate them into genetic analyses have been developed to address these problems. Association studies in unrelated individuals also require a large number of markers, which poses statistical issues related to the large number of comparisons required.

In relative pairs and families, linkage approaches have advantages and disadvantages compared to association studies. Advantages include the fact that, in contrast to studies in unrelated individuals, whose power can be affected by the degree of linkage disequilibrium in the population, which is variable and often difficult to determine, loci that are physically close on a chromosome are inherently linked within families. Other advantages include well-developed analytic methods and readily available marker sets. Disadvantages include low power for loci with modest effects, limited mapping resolution, challenges to replication studies posed by population and etiologic heterogeneity, and limited ability to estimate population effect sizes. Even though linkage studies alone are likely to be insufficient to ensure identification of trait loci, they provide a viable and practical approach to narrow the scope of the search to genomic regions that contain loci affecting EL. Many limitations of association studies are alleviated when such studies are directed at specific regions or positional candidate loci identified via linkage.

Relative pairs and extended pedigrees each have advantages and disadvantages with respect to each other. Compared to studies on extended pedigrees, studies on relative pairs have greater ease of recruitment, less susceptibility to birth cohort effects, and high power to detect recessive loci for uncommon traits, but have the disadvantages of usually lacking parental genotype information for studies of traits expressed in old age, and lower mapping resolution and statistical power (in part due to the greater likelihood of genetic etiologic heterogeneity).

Evidence on the feasibility of studies to identify specific contributory factors to EL. Current evidence on the strength and pattern of genetic and other factors' contribution to EL is suggestive but not conclusive regarding the feasibility of such studies. Most studies of heritability of longevity have found it to be between ten and thirty percent. Other studies have found an elevated recurrence risk for EL among first-degree relatives of exceptionally long-lived persons. These data are of limited value for establishing the feasibility of identifying specific genetic factors, because they provide estimates of the aggregate effects of genes rather than the effects of individual genes, and may reflect shared environmental factors as well as genetic factors. However, recent data on the pattern of familial recurrence of excess longevity among near and distant relatives (consistent with effects of one or a few genes) suggest that efforts to map genes related to longevity could succeed, but these data should be interpreted cautiously, particularly since replication studies have not yet been conducted. The detection of effects on longevity of alleles at a specific locus (ApoE) in reasonably sized studies is also reassuring, but it is unknown whether additional genes exist with effects of comparable magnitude in the general population.

Because the characteristics of the genetics of longevity are incompletely known, feasibility estimates and power calculations must be based on estimates of characteristics that seem reasonable. Each of the aforementioned study approaches (unrelated persons, relative pairs, and families) is strong under some plausible conditions and weak under others:

In unrelated persons, case-control comparisons of extremely long-lived with younger persons increase in power with the age of the long-lived group and the frequency of the candidate allele. They require very large sample sizes for genotypes with frequencies less than 15 percent. The approach is very effective for genotypes associated with relative risks of 1.2 or greater, especially if the older sample is over age 90. Studies of survival in age-cohorts of octogenarians require relatively large sample sizes, but are more efficient if the rare genotype is protective. Linkage studies in long-lived relative pairs using markers with a 10 cM mininum density have power to detect genes with relatively modest effects (relative risks of 1.3-1.5) in a sample of between 300 to 500 pairs, provided there is minimal or no genetic heterogeneity. For large-family designs the question of feasibility is too data-dependent to be addressed from theory. Simulated linkage in selected pedigrees is generally needed to estimate power. In general, however, large multi-generation families yield greater power to detect linkage or association than do affected relative pairs.

Each of the above approaches also has a weak point; for studies of unrelated persons, rare alleles drastically reduce power; for studies of relative pairs, locus heterogeneity can be problematic; for large families, scarcity of families and the uncertainty of recruiting key individuals can be prohibitive. It is important to keep options open, rather than specify ideal approaches now, as ongoing active methodologic research is likely to have an impact on ideal methods and designs. Additional research with long-lived individuals and families may also help guide analytic choices by clarifying some of the underlying genetics.

Additional information needed to clarify the feasibility of studies to identify genetic factors and other factors contributing to EL. Such information can be gained from registries, including several European databases that can be searched for EL individuals. Registry data are likely to help in estimating the prevalence of recurrent EL in families, and in exploring the impact of different sampling strategies and phenotype definition on feasibility. Where registry members have been previously surveyed, the role of behavioral and other factors in longevity could also be explored. Twin registers are particularly useful for these purposes. Population registers, including Census, Social Security, and Medicare records, are another source of information about EL, and could also serve as sampling frames to identify individuals who could be surveyed for additional information, though issues regarding permission to use these registers would need to be addressed.

Active efforts to ascertain families that demonstrate recurrent EL are needed. Because such families are likely to be scarce, such efforts should be done in tandem with sample collection and

epidemiological questionnaires. In addition, particularly in regard to possible association studies, the genetic history of the study population needs to be known and taken into account to avoid potential bias. Historic-demographic and historic-epidemiologic information are also needed to interpret EL data appropriately.

More data are also needed on the relationship of survival rates to functional status and environmental factors, to clarify phenotyping questions. For EL phenotypes including functional status, designing studies requires more information on change in functional status with age.

Steps that NIA could take to facilitate EL research (beyond the standard NIH process for research grant submissions). Because the sample size requirements for any genetic EL study are likely to be substantial, multicenter collaborations may be useful. In particular, because the feasibility of large-kindred EL studies depends on the prevalence of such kindreds, and because finding and recruiting these kindreds is time-consuming and tedious, involving a combination of population-based, clinic-based, and self-referred ascertainment, followed by additional data collection, non-standard funding mechanisms may be useful to find such kindreds and determine their prevalence.

If there is good evidence for the familiality of EL, an RFA would likely be necessary. It should be broad to allow investigators to use novel approaches that are particularly appropriate for their study populations. Multicenter collaboration should be encouraged. Standardization of some phenotype definitions and a set of common protocols (in addition to any study-specific ones) might be developed prior to any data collection to assure as high a level of comparability across diverse studies as possible, allowing for potential pooled analyses. Any EL RFA should include a specific budget item to create cell lines. Despite the substantial cost, securing adequate future samples of such rare genetic materials is essential.

The panel deliberately avoided making firm recommendations on the critical study design questions of ascertainment scheme and choice of EL definition or correlated risk factors. These issues require study- or population-specific optimization and thus may require pilot studies to explore alternative strategies prior to initiation of large-scale genetic studies. Planning grant or pilot project funding would facilitate clarification. Alternatively, providing an extended study design protocol refinement phase in the context of a multicenter EL study could also accomplish this goal.

To advance this field, NIA could also: 1) provide training mechanisms for longevity researchers; 2) provide supplemental sampling funding of existing studies to increase their comparability, and 3) fund testing for various health conditions that could be used to standardize phenotype definitions across studies. NIA could also sponsor a survey of investigators in the field to catalog resources from ongoing studies that might be useful for new EL studies. It might develop a Web site for posting findings (including negative association studies) and protocols for relevant laboratory assays for the study of EL, and could encourage and support replication studies.

Perhaps the single most important prerequisite to planning EL studies is phenotype definition. It would be very useful for NIA to sponsor a consensus development conference precisely on this issue. One strategy would be to identify a basic and common set of EL-related measures that all studies should collect in addition to any specific hypothesis-driven measures. The various definitions of EL would be analyzed in an attempt to determine which are most useful from the standpoint of both biologic meaning and logistics of collection. If several studies are involved, it may be possible to combine information either by pooling the data directly or by using a meta-analytic technique.

Finally, long-term prospective studies may play an important role in defining the genetic factors associated with EL. Longitudinal family designs could provide a wealth of information including surveys of offspring as they age, and information from banked parental DNA. However, these studies might be deferred until there is more experience with the logistics of doing these studies and more information on the specific genetic hypotheses of interest.

Opportunities for international collaboration. Research on EL conducted outside the USA can provide population-based samples that can be identified via extensive and long-standing national registration systems. Many of these populations are ethnically and genetically homogeneous, and many previous studies have achieved high participation rates. NIA could coordinate efforts to define aging and healthy aging across different countries; facilitate replication studies; facilitate validation and standardization of methodologies, and methods to validate ages in different countries; encourage migration studies; catalog resources available in non-USA studies; publish or disseminate a document describing NIA's willingness to collaborate with non-USA funding agencies and scientists; coordinate and facilitate comparisons of genetic and environmental factors' impact on EL across countries, and coordinate and facilitate meta-analyses across countries.

Since families with strong evidence of predisposition to longevity are likely to be rare, it may be important to extend the search for such families as far as possible. Some population databases and studies in Canada, Europe, and Asia could be valuable sources of familial data on longevity. Collaboration between groups with access to or responsibility for management of these resources should be encouraged. Specific countries of interest include ones that maintain national registries allowing ease in identifying aged siblings and linking data on family members (e.g. Scandanavian countries), China (whose large population size has facilitated identification of a large number of centenarians, and countries with founder populations (e.g., Ashkenazi Jews in Israel and Sardinians in Italy) which may have additional advantages under some conditions. Insights into the contribution of genes and environment on EL could ensue from studies on immigrants (e.g., immigrants from southern Italy to various parts of USA).

Ethical issues and logistical pertinent to EL studies. Standard Ethical, Legal, and Social Issues apply to EL. Some are heightened for EL, e.g. the subjects' ability to provide truly

informed consent (since the extreme elderly may suffer from cognitive impairment, sensory loss, or general frailty). EL studies may require informed consent procedures specifically designed for visual/auditory impairments, and/or seek proxy consent for those with significant cognitive impairment. Since a prospective EL study may span several decades, it may require broader permission to future use of biological samples, due to the long time lag between sample collection and data analysis. Many logistical concerns are not unique to EL, such as the need for the dissemination of technologies and free exchange of information among researchers, while protecting individual confidentiality. Cell line establishment may be especially important for rare phenotypes like EL, since they provide indefinite sources of DNA.

INTRODUCTION

The National Institute on Aging (NIA) supports research to identify factors that contribute to long healthy life and protect against age-related diseases and disabilities. As part of its ongoing efforts in this area, representatives of the four NIA extramural programs and the intramural program collaborated to convene the Advisory Panel on Exceptional Longevity (APEL) to obtain recommendations on the feasibility of studies to identify genetic factors (and other factors that might interact with genetic factors) that contribute to exceptional longevity (EL). APEL included geneticists, epidemiologists, gerontologists, demographers, and statisticians.

The Panel explored the following topics:

1. Desirable phenotypes to characterize EL and ways to analyze them;
2. Strengths and limitations of different subject groups to study EL;
3. Feasibility of full-scale studies; suggested study designs and sample size estimates;
4. Needs for preliminary studies;
5. Useful steps that NIA could take to facilitate EL research;
6. Opportunities for international collaboration;
7. Special logistical and ethical issues pertinent to EL studies.

To allow thorough consideration of methodological and practical issues relating to these topics, APEL was divided into three subpanels, one for each of three subject groups for which differing research designs are used in studies on genetic effects: (1) unrelated individuals, (2) relative pairs (e.g., siblings, cousins), and (3) families with high proportions of long-lived members. The subpanels met by conference calls during May through July 2000 and prepared preliminary materials that were presented at the APEL meeting on July 18-19, 2000. Following discussions at this meeting, the APEL report was drafted by the subpanel chairpersons and circulated to the full Panel for review and concurrence and/or suggested changes. Thirty-seven of the 38 APEL members concurred with the report (with their suggested changes included). Their names are listed below. One member, Dr. James Vaupel (Max Planck Institute for Demographic Research, Rostock, Germany) did not concur fully with the report and, in lieu of recommending changes or additions, requested that his name be omitted from the list of authors.

The report is organized as responses to questions posed by NIA to the panel on the seven topics noted above.

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1. Desirable phenotypes to characterize EL and ways to analyze them

What are appropriate strategies for selecting specific phenotypes and analytic strategies (e.g. dichotomous variable, survival analysis, frailty models) for characterizing exceptional longevity (EL)?

1.1. Introduction

Exceptional longevity (EL) can be defined in numerous ways, and the definition selected will strongly influence the approaches used for analysis. It is important to note that in case-control studies, there is only one phenotype to define, but for family studies, there are often differences between the phenotype required for probands and that for additional cases. The strengths and weaknesses of different definitions are highlighted below.

1.2. Phenotypes

1.2.1. Phenotypes based on age alone

The most obvious and simple definition for EL is survival past a specified extreme age. If the age threshold is set high enough (perhaps in the upper 90's or 100), the probability of recurrence in siblings is considerably higher than in underlying populations (Kerber et al. 2001; Perls et al. 1998). The choice of the threshold should consider the availability of a sufficient number of subjects for adequate statistical power, in addition to the recurrence risk in families. An alternative to using a specific age threshold is to treat survival as a quantitative variable, and to study factors associated with the attained ages of a sample of individuals.

The definition of EL should take into account secular trends in life expectancy, gender effects, and social, environmental, and behavioral factors, because survival to a given age is less exceptional in some subgroups than in others. One method for incorporating information on gender and cohort is to evaluate survival past some percentile of the distribution of survival times in an appropriate comparison population. By choosing an appropriate sex- and cohort-specific percentile cutoff, one can produce a definition of EL that controls for some important confounding factors. Information on environmental and behavioral factors can be included in addition to information on sex and cohort. The phenotype of interest would then be the difference between the observed survival and that expected based on sex, birth cohort, and environmental and behavioral factors.

Multigenerational pedigree studies might be particularly susceptible to the confounding effects of secular changes in longevity, whereas this is less of a problem in studies of sibling pairs or

unrelated individuals. In family studies it may be justifiable to use a slightly lower survival cutpoint for relatives than for probands for the benefit of optimizing the sample size. Alternatively, relatives of probands with EL could be followed prospectively to evaluate their survival times.

1.2.2. Phenotypes based on age plus other characteristics

A substantial issue in phenotype determination is whether or not to incorporate health and functional status information at given ages. "Disability-free survival" may be a more useful phenotype than survival *per se*. In general, functional status information should focus on: (1) cognitive abilities, (2) mobility, (3) vision and hearing, and (4) personality factors such as stress response and coping skills. Research is needed to determine the relative independent contributions of these potential phenotypes on EL as well as the additive and interactive impact on genetic contributions.

Intermediate phenotypes are also of interest. Multiple interacting genes and environmental factors are likely to influence extreme longevity. Endophenotypes, risk factors, or other intermediary quantitative traits correlated with extreme longevity are likely to be influenced by only a subset of these genes, and may be more proximal to gene action than a disease or longevity outcome, making it easier to localize genes for them.

Simulation studies suggest that genetic studies of known quantitative intermediaries are statistically more powerful than studies of complex endpoints (Wijsman and Amos, 1997). Additionally, new developments in multivariate genetic methodology have made possible simultaneous analyses of quantitative intermediaries and dichotomous outcomes (Williams et al., 1999), which permit a formal test of whether genes localized through intermediaries influence the complex trait of interest. If pleiotropic genetic effects simultaneously influence the measures that define EL then we would expect a genetic correlation between these phenotypes, and analysis of correlated phenotypes also increases the power to detect linkage (Williams et al., 1999).

For this approach to be feasibly applied to genetic studies of extreme longevity, quantitative intermediaries correlated with extreme longevity need to be identified. These indicators should be shown to predict longevity, rather than being simply a consequence of aging. Also, they must be demonstrated to be heritable. It may be of interest to identify genetic factors underlying function at older ages as a way of identifying genes that affect EL. However, although physical and cognitive functioning predict survival well on a group level, it is less clear that they predict EL on an individual level. Moreover, it may be important to reduce etiologic heterogeneity of EL by classifying the phenotype into more homogeneous sub-groups. If suitable classification variables and values are not known then multivariate approaches, such as cluster or factor analysis, may be used to develop suitable phenotype definitions.

1.2.3. Other phenotype considerations

Some longevous individuals may show robust mental and physical function while others may be extremely old and fragile. Therefore, a key question is whether there are homogenous subgroups that comprise the long-lived population. Phenotypically, these subgroups may be defined by different patterns and trajectories of aging. Identification and characterization of these typologies would require multidimensional assessments that cut across functional and health domains. Genotypically, these subgroups may vary at important loci and refined typologies would help to reduce the background genetic variation and increase power to detect QTL.

Several papers describe the application of multivariate models for QTL detection based on data from genetically informative samples (Boomsma, 1996, Martin et al., 1997, Boomsma and Dolan, 1998). Pleiotropic genetic effects that influence several functional domains would imply genetic sources of covariation between the phenotypes measuring longevity. Individual genotypic values at the QTL can be estimated by analyzing the covariance structure between these quantitative traits. As demonstrated in simulation analyses, this method increases the power to detect linkage in sibling analyses.

1.3. Analytic strategies

1.3.1. General considerations

The data pertaining to genetic factors in longevity provide a number of analytic challenges. Methods will need to be able to handle considerable missing data: DNA samples (and thus marker genotypes) are likely to be available primarily for living individuals, for whom longevity cannot yet be determined, while precise estimates of longevity will be available primarily for deceased, unsampled individuals. Longitudinal studies to determine survival times of sampled individuals may require many years of follow-up, and this could have considerable logistical and ethical/legal implications (see section 7). Fortunately, observed marker genotypes on living individuals, coupled with information on their relationships and estimates of population-specific allele frequencies, provide considerable information with which to estimate marker genotypes on deceased individuals.

Most of the genetic analysis methods that currently exist or are under development can accommodate missing data to some extent. Most methods that incorporate specification of a trait model can handle censored survival data, including traditional lod score, variance component, and Bayesian Markov chain Monte Carlo methods. However, some methods may require extensions in order to make efficient use of the available data. This may be particularly true for censored survival data and analysis methods based on marker allele scoring methods (e.g., sib-pair methods). It is also likely to be true for all methods employing multivariate phenotypes, e.g., longevity plus other, correlated, inherited, covariates.

Analytic methods that incorporate multi-locus models are likely to be more effective for analysis of longevity than are simpler methods. Both variance component methods and Monte Carlo Markov chain methods are providing capabilities for such analysis, including incorporation of covariate data. Both approaches are based on continuous trait data, and can include censored age data. In addition, approaches that may be infeasible today may become feasible soon because of improvements in computer technology and/or computational algorithms.

1.3.2. Dichotomous age cut-points and survival/ hazard functions

The genetic effects on longevity may be viewed either in terms of variation in the relative risk of death, or in terms of variation in rates of aging. For analysis of variation in the relative risk of death, proportional hazards models with "frailty" terms are useful (Vaupel 1988), with the frailty estimates themselves as the phenotype of interest. Although most of the applications of genetic frailty models have either been in assessing heritability for longevity or associations with candidate genes (Yashin et al., 1999a, 1999b; Siegmund et al., 1999), these methods are now beginning to incorporate linkage information as well (Li, 1999).

Analyses of variation in rates of aging would treat survival times as a continuous trait. This eliminates the problem of setting an upper threshold for calling an individual "long-lived." However, it may still be useful to set a lower age threshold below which individuals are considered censored, because variation in age at death below that threshold is unlikely to be due to variation in rates of aging. Intermediate phenotypes that predict the risk of eventually being long-lived might be usefully measured in young and middle-aged adults.

2. Strengths and limitations of different subject groups to study EL

What are the advantages and disadvantages of different subject groups (unrelated individuals, relative pairs, or families) for studies with the following goals?

(a) Find genomic regions linked to EL

(b) Find specific loci/candidate genes associated with EL

(c) Test previously identified candidate regions for linkage to EL

(d) Test previously identified candidate genes for association with EL

(e) Characterize interactions of specific "risk" factors for EL (GxG, GxE, ExE, etc.)

(f) Other analyses to determine specific genetic effects

For the above goals, are there differences in advantages and disadvantages of the subject groups for studies to determine the presence of a relationship vs. studies to estimate the magnitude of the relationship? What, if any, are effective design strategies to alleviate or correct for the disadvantages?

2.1. Introduction

The study of complex traits can benefit from using a wide variety of approaches, especially early on when little is known about trait etiology. As with other complex traits, it appears likely that any individual genes affecting life span will have small population effects; that is, they may have a small effect in a large number of people or they may have large effect in a small number of people. Because longevity is an outcome downstream of every biological process affecting an individual's life, it seems particularly likely that many loci could contribute to EL. However, even though the population effects of any particular locus may be small, elucidation of *any* relevant factors can provide insight into the underlying mechanisms and can improve the power to detect the remaining loci.

There are two basic approaches that are commonly used to identify loci influencing complex traits: linkage and association studies. In the case of the former, sets of genetically-related subjects are required, while family data is not required for the latter. Linkage studies can be carried out using, as a minimum, sib pairs, to nuclear families, sets of extended relative pairs (e.g. cousin pairs, avuncular pairs), up to full extended pedigrees. In this report, we focus on the sampling units of unrelated individuals, sibships and extended pedigrees, along with the strengths and limitations of each kind of sample for gene discovery. Some of the essential features of this comparison are summarized in Table 1 at the end of this section, assuming particular analytic approaches; the full discussion follows (see also Lander & Schork, 1994 or Schork & Chakravarti, 1996). Both approaches – linkage and association – offer the opportunity

to test candidate loci (chosen for their presumed involvement in a relevant pathway) and to identify genomic regions that may harbor previously unknown loci.

An issue of general importance for the study of any complex trait is the confounding effect of heterogeneity. There are two types of importance to this discussion: etiologic or genetic heterogeneity is where different sets of factors or genes can produce the identical phenotype, and population heterogeneity where populations differ in the distributions and frequencies of the relevant etiologic factors. The effect of the former is particularly detrimental for linkage studies when unrecognized – the power to detect trait loci can be often dramatically reduced. The effect of population heterogeneity is comparably detrimental for association studies; however, the effect of unrecognized stratification is rather an increased rate of false positive findings. The overall effect of heterogeneity is to decrease power to detect true signals. Any steps to minimize the potential effects of heterogeneity are crucial. Simple approaches such as focusing initial efforts on more homogeneous populations (e.g. Amish, Ashkenazi Jews, Finns) to reduce the potential of genetic heterogeneity can be adopted. Although results from such studies may not be broadly generalizable, loci detected in these special populations may also affect EL in other groups and, even if they do not, they may provide structural or functional clues to longevity in other populations. Additionally, attempts to refine the phenotype by taking into account other features of the aging process – such as health status, disability, or declining general health – to devise more homogeneous phenotype definitions also can be useful.

2.2. Unrelated Individuals and Association Studies

Some of the more pronounced **advantages** of using association analysis of unrelated individuals to dissect the genetic basis of EL include:

- ***Greater power in certain analysis settings.*** Association studies have been shown to have greater power for gene discovery than traditional linkage approaches when true functional variants can be measured or when the polymorphism is in linkage disequilibrium with the functional variant (Risch and Merikangas 1996). In addition, although it is possible to conduct association analyses with related individuals (such as in Transmission-Disequilibrium Testing (TDT) scenarios) it has been shown that greater power for association analyses can be obtained in many situations using samples of unrelated individuals (e.g., as in case-control samples) (Schaid and Rowland 1998; Rich and Teng 1998; Teng and Risch 1999).
- ***Flexibility of Design.*** The use of unrelated individuals can facilitate the implementation and conduct of a wide variety of very sophisticated study designs for studies of aging and longevity. For example, one could more easily conduct prospective cohort, case-control, and cross-sectional studies using unrelated individuals than with related individuals because it is easier to easier large numbers of unrelated individuals, than related individuals, and thus to increase statistical power.

- ***Estimation of Population Effect Sizes and Assessment of Epistasis and Gene x Environment Interaction.*** The use of unrelated individuals in traditional genetic-epidemiologic designs allows one to efficiently estimate the size of the effect (e.g., attributable risk) of a particular genetic variant on a longevity phenotype in the population at large. Traditional epidemiologic designs can be used for assessing epistasis and the relations between genetic and environmental influences on EL once relevant measured genotypes are available (Ottman 1990; Ottman 1996).

There are many **disadvantages** of the use of association analyses involving unrelated individuals for the genetic studies of exceptionally long-lived individuals, including:

- ***Overt and Cryptic Stratification.*** Stratification refers to admixture of populations with an inherent allele frequency difference. Stratification, when unrecognized, can increase false-positive rates in genetic case-control studies (Ewens and Spielman 1995; Lander and Schork 1994; Spielman et al. 1993) and cause heterogeneity and reduced effect sizes in other designs using unrelated individuals. Fortunately, methods exist to not only identify stratification, but also accommodate it in relevant genetic association analyses (Bacanu et al. 2000; Devlin and Roeder 1999; Devlin and Roeder 1999; Pritchard and Rosenberg 1999; Pritchard et al. 2000; Schork et al. 2000).
- ***Allelic Heterogeneity***. Since association studies investigate the relationship between particular allelic variant (or haplotypes) and a disease or outcome, if there are many polymorphisms within a genomic region, then one might need unrealistically large sample sizes to detect the relatively weak associations any one of the polymorphisms might have with the disease. Haplotype analysis can overcome this problem to a great degree, but not without multiple comparisons and sparse-data analysis problems arising (Schork et al. 2000). Moreover, the trait may be associated with more than one of the polymorphisms, depending upon the evolutionary history of the genomic region. Cladistic analysis holds promise as powerful method to identify relevant markers and haplotypes for association analysis (Templeton et al. 2000).
- ***The Need for a Dense Map of Markers.*** Since linkage disequilibrium is not likely to extend to distances beyond 250-1000 kilobases (Clark et al. 1998; Collins et al. 1999; Jorde 1995; Jorde et al. 1993; Kruglyak 1999), association analyses of unrelated individuals – which would require linkage disequilibrium among adjacent locus alleles for mapping purposes – require a much denser map of DNA markers than those necessary for the initial stages of linkage analysis based genetic studies, potentially involving hundreds of thousands of markers to cover the human genome.
- ***False positives and interpretation of genome association scans.*** Screening such a large number of markers poses serious multiple comparisons problems and questions as to how to assess the significance of the results. The problem is less serious for assessments of candidate genes in limited regions. Nonetheless, novel statistical approaches will have to be developed to aid in the interpretation of such studies.

- ***Unrelated subjects provide NO information for linkage or segregation.*** Without information on relatives, it is impossible to investigate Mendelian transmission phenomena, such as the sharing of alleles identical-by-descent, that form the basis of traditional linkage analyses. Linkage analyses are known to be more powerful in certain situations (e.g., great allelic heterogeneity, or disease genes with very strong effects on the phenotype) than association-based methods.

With the exception of an inability to conduct traditional linkage analysis and the requirement for a dense map of markers, the remaining disadvantages of using unrelated individuals described above can be overcome (Schork et al. 2000).

2.3. Linkage studies – general considerations

Whether using relative pairs or extended pedigrees, there are some advantages and disadvantages to using a linkage approach as compared with an association study. The advantages are as follows.

- ***Linkage is a universal phenomenon.*** Loci that are physically close on a chromosome are linked in all humans and all populations. Association studies rely on having measured the functional variant or on linkage disequilibrium between a measured marker and the functional variant. Linkage disequilibrium is a population-specific phenomenon and may or may not exist, or be sufficiently strong, in the region of interest.
- ***Analysis methods available.*** Linkage information can be extracted from a sample of almost any set of related individuals. Epistasis and gene x environment interaction can be modeled, however the power to detect these effects when indexing the trait genotype by a linkage relationship is lower than if an associated or causal variant can be identified.
- ***Readily available marker sets.*** Over the last decade, informative polymorphic marker sets have been developed that are reliable and suitable for genome-wide scans by linkage. Approximately 400 markers provide adequate coverage of the genome.

There are also certain limitations.

- ***Insufficient power for loci with modest effect.*** Linkage studies may not be sufficiently powerful to detect loci with modest effects on longevity, even in very large samples. There appears to be a lower limit of resolution for linkage studies, although there is likely to be sufficient power in certain data sets to detect loci whose marginal effects are appreciable and of biomedical importance in understanding EL.
- ***Limited mapping resolution.*** Linkage studies are likely to identify a genomic region that may comprise several million bases and contain many positional candidate genes to evaluate. Unless a kindred is very large and spans multiple generations, or multiple kindreds are available that share a predisposing locus, linkage studies alone are generally

insufficient to identify the precise chromosomal locations where predisposing genetic variants reside. While dense multipoint linkage maps assessed in such regions can help narrow the likely location of a trait locus, subsequent steps toward gene identification usually involve linkage disequilibrium mapping and association studies for fine localization and testing of specific variants. Thus, association studies inevitably come into the picture.

- ***Replication of linkage signals.*** Interpreting the results of a genome scan continues to pose challenges. One must find an appropriate balance between type I and type II statistical errors to assure adequate power to find true signals, while limiting the number of false positives (Rao, 1998). Replication, the traditional way to sort out the true positives from false positives, can be problematic because of population and genetic heterogeneity often present for complex traits. Moreover, it may be difficult to replicate a linkage because a different hypothesis is being tested in a replication experiment – one is looking for a *particular* gene, rather than *any* gene as in the original experiment (Suarez et al. 1994).
- ***Compromised ability to estimate population effect sizes.*** Population-based assessment of the magnitude of genetic influences on EL, e.g. attributable proportion and allele frequency, depends upon accurate characterization of the ascertainment process. In many cases, there are both ambiguities in specifying the actual manner in which the family material is ascertained and in applying suitable ascertainment corrections that preserve sufficient information to estimate the parameters of interest.

Even though linkage studies alone are likely to be insufficient to ensure identification of trait loci, they provide a viable and practical approach to narrow the scope of the search to those genomic regions that likely harbor loci with appreciable effect on EL. Many of the limitations of association studies are alleviated when such studies are directed at specific regions or positional candidate loci identified via linkage.

2.4. Relative pairs

Specific considerations regarding the use of relative pairs as a sampling unit include the following advantages:

- ***Relative ease of ascertainment and recruitment.*** It is far easier to recruit sib pairs through a longevous proband than to locate and recruit multiple, more distant relatives to construct pedigrees.
- ***Existing registers can aid in recruitment.*** There are several extant twin registries with DZ (i.e., full sib) twin pairs that could be utilized to identify "affected" sib pairs.
- ***High power to detect recessive loci for uncommon traits.*** Power calculations demonstrate that very few affected sib pairs are needed to detect recessive loci, even

with low penetrance and low attributable risk, provided the trait is not very common (i.e., population prevalence < 1-5%).

Limitations include:

- ***Lack of parental genotypes.*** There is a drop in information using allele-sharing methods for linkage analysis if identity-by-descent cannot be determined when parental genotypes are missing or unavailable. However, linkage analyses based rather on identity-by-state methods maintain decent power for gene discovery under a wide variety of models with feasible sample sizes (see Power calculations by Li, Question 3, section 3). Also, the loss of power is minimized when using highly polymorphic markers.
- ***Localization of putative loci limited.*** The interval of the region likely to contain a trait locus estimated from a sample of sib pairs is considerably wider than that obtained from analysis of extended pedigrees, often spanning tens of centimorgans.

2.5. Extended pedigrees

- ***Good power for gene discovery and localization.*** Large multiplex kindreds generally give high power to identify linked loci for complex traits (Wijsman and Amos 1997), especially as compared with relative pairs or other simpler family designs for the same number of subjects. However, for particular genetic models, certain pedigree relationships are more informative than others, so that if the mode of inheritance was known with confidence, this could be used to further refine the study design.
- ***Heterogeneity within extended pedigrees is minimized.*** It is unlikely (although not impossible) that there will be genetic heterogeneity within a particular kindred, thus, power to detect the operating loci will be optimized. However, quite large pedigrees or a few pedigrees with a similar etiologic models may be required to ensure adequate power for gene discovery.
- ***Good power to detect associations.*** What is sometimes less appreciated is that there can also be increased power to model association to candidate genes in extended pedigrees over that obtained from the same number of unrelated individuals, if one properly models the degree of dependence between family members. In some sense, this is counterintuitive, since it is often argued that the use of non-independent related family members decreases the amount of "independent" information that can be brought to bear to assess association compared to that from the simpler "unrelated individuals" case. However, simulation studies (Province et al., 2001) have demonstrated an increase in power whenever modeling the association effects in families decreases the overall error variance (compared to the unrelated individuals case) which thus boosts the relative power to detect any effect.

The limitations are as follows:

- ***Low power to detect recessive traits.*** Large kindreds are rarely useful in situations where recessive alleles play a major role in phenotypic variability. In principle, this can be addressed in highly inbred populations, but in practical terms it is probably far more reasonable to examine what evidence might exist for the influence of recessive alleles on EL, and consider alternative designs (e.g., sib pairs) if such effects seems to be important.
- ***Ascertainment of "loaded" pedigrees may be counterproductive.*** A potential problem is that "more" is not always "better" in selecting multiplex families for genetic studies. For example, simulation studies have shown that there can actually be *less* power to detect linkage using affected quartets (four affecteds) than by using the same number of affected sib pairs (two affecteds), because quartets are more likely to arise from homozygous by homozygous matings which are uninformative for linkage (Holmans, 1998). The basic genetic paradigm depends upon there being a certain strong degree of within-pedigree variability in expression of the phenotype and lack of variability in phenotypic expression can be a hindrance to gene finding. Pedigrees that show a strong degree of variability in survival may actually be preferable.

2.6. How special aspects of EL affect preference for study design

- ***Practical Sampling.*** Exceptionally long-lived individuals are not likely to have many living relatives and especially not likely to have living parents. Children of longevous probands will not be old enough to demonstrate their longevity phenotype. Therefore, designs that require extensive DNA and phenotype evaluations of relatives of long-lived probands (such as for extended pedigrees) are impractical for cross-sectional studies. However, prospective longitudinal studies of the children of long-lived individuals who are members of longevous families could provide rich opportunities for identifying both strictly genetic effects (major, oligogenic, or epistatic), and gene-environment interactions, especially if additional data on health status or changes in functioning can be obtained.
- ***Phase Information and Haplotype Analysis*.** Haplotype analysis is desirable for gene localization. Haplotypes are readily determined in extended pedigrees or any sample for which parental genotypes are available. This will generally not be the case for EL probands. Determination of haplotypes can also be aided by the availability of additional siblings that are informative for phase – the larger the sibships, the better. In unrelated individuals, one must resort to complicated DNA sequencing protocols or construct haplotype-based tests using estimated population haplotype frequencies (Schork et al. 2000).

- ***Cohort Effects.*** Cohort effects are minimized using twin and sibling pairs, since longevity is defined on a relatively narrow birth cohort.
- ***Prospects for Association Studies in Relative Pairs.*** Relative pair data is not readily adapted for tests of association because such studies require a "control" of some sort, either constructed or actual. In the transmission disequilibrium test, considering the parental alleles *not* transmitted to affected offspring forms a matched control. This requires either the parental genotypes or, with some loss of power, discordant siblings. In either case, samples from these subjects generally will not be available as they will be deceased. Prospective, longitudinal designs could overcome such difficulties. Additionally, it should be noted that if the endpoint is altered from EL *per se* to alternate predictor phenotypes that can be assessed in living subjects, then such association studies can be considered.

Table 1. Advantages and Disadvantages of DNA Marker-Based Statistical Gene Mapping Paradigms

Issue	Parametric Pedigree *Linkage* Analysis	Non-Parametric Sibpair *Linkage* Analysis	*Association* Analysis of Unrelated Individuals
Sampling Units (e.g.)	1-5 Large pedigrees	200+ sibpair units	100-200 cases/controls
Sampling Ease	Difficult	Moderately difficult	Simple
Marker Density	Sparse (5-10 cM)	Sparse (5-10 cM)	Dense (1-50 kb)
Marker Type	Microsatellites	Microsatellites	SNPs
Available Maps	Genome-wide	Genome-wide	Candidate genes/regions
Mapping Resolution	1-2 cM for large pedigrees	5-25 cM (200 sibpairs)	1-150 kb
Mapping Power	Excellent (Monogenic only)	Poor generally	Excellent (high resolution)
Genetic Heterogeneity	Not problematic	Problematic	Problematic
Allelic Heterogeneity	Not problematic	Not problematic	Problematic
Overt Stratification	(Not applicable)	(Not applicable)	Problematic
Crytpic Stratification	Not problematic	Moderately problematic	Problematic
Bilineality	Problematic	Moderately problematic	Not problematic
Replication Ease(given true locus)	Problematic	Problematic	Not problematic
Population Generalization	Problematic	Moderately problematic	Not problematic
Parameter Assumptions	Many	Few	Few
Epistasis Modeling	Computationally intense	Problematic	Not problematic
Covariates	Computationally intense	Computationally intense	Not problematic
Haplotype Analysis	Not problematic	Moderately problematic	Problematic
Multiple Phenotype Analysis	Computationally intense	Computationally intense	Not problematic
Computational Burden	Heavy	Slight	Slight

3. Feasibility of full-scale studies and suggested study designs and sample size estimates

Is current information on the strength and pattern of genetic and other factors' contributions to EL (e.g., heritability, sibling relative risk, familial aggregation), sufficient to indicate that studies with a reasonable chance of identifying or determining effects of specific genetic factors contributing to it (e.g., goals a-f from question 1) are feasible, or are not feasible for the subpanel's subject group?

If data indicate that such studies are feasible, what designs and analytic approaches are especially desirable, and what are the best estimates of sample sizes needed?

3.1. Introduction (approaches to judging feasibility of studies)

Numerous studies of the heritability of longevity have been conducted during the last century, most of them yielding heritability estimates in the range of ten to thirty percent (Beeton and Pearson 1899, Bocquet-Appel and Jakobi 1990, Phillipe 1978, Kerber, et al. 2001). These studies, while establishing a general pattern of recurrence in the phenotype of longevity, are only of limited value for establishing the feasibility of efforts to discover longevity-associated genetic variability. This is because heritability studies provide estimates of the aggregate effects of genes rather than estimates of the effects of individual genes. In addition, the results of these studies are widely variable because of changes in the impact of environmental factors on longevity in different environments (across time and space), along with the great heterogeneity of longevity phenotypes and differences in study design and methodology.

In general, the same criticism applies to the limited but growing number of studies on familial recurrence risks for longevity (Perls, et al. 1998, Kerber, et al. 2001). However, the extension of familial recurrence risk estimates to more distant relatives allows some additional information about mode of inheritance and the potential to reduce confounding caused by shared environment (Risch 1990a, Kerber, et al. 2001).

Biodemographic and/or epidemiologic studies of the familial aggregation of longevity, however, can only provide indirect information about the feasibility of linkage or association studies of a complex trait. Conclusive information about feasibility will ultimately be provided only by studies that attempt to characterize variable longevity in relation to observed genetic variation. For example, the detection of ApoE effects in reasonably sized studies is reassuring, because it suggests that associations with some genes are large enough to be detected. However it is unknown whether there are additional genes that exert effects of comparable magnitude on complex phenotypes like longevity in the general population.

3.2. Evidence relating to feasibility of studies

3.2.1. Heritability results

A Scandinavian study of monozygotic and dizygotic twins calculated the heritability of life expectancy to be only 20-30% (Ljungquist, et al. 1998). However, the oldest subjects in the twin study were in their mid to late 80s and the majority lived to average life expectancy. Other studies of twins, siblings, and parent-offspring pairs have produced heritability estimates ranging from zero (Phillipe 1978) to 0.33 (McGue, et al. 1993). Analyses of larger kindreds have been carried out by Bocquet-Appel and Jakobi (1990) and Kerber, et al. (2001). The heritability estimates resulting from these studies are generally consistent with those confined to first-degree relatives.

3.2.2. Recurrence risk estimates

Because heritability studies rely on correlation coefficients, they are most useful in situations in which the phenomenon of interest is broadly expressed throughout a population in variable degrees. EL, depending on how it is defined, might be confined to less than 1% of a population. Recurrence risk estimates provide a more sensitive means of assessment of the possibility that rare phenomena recur in families more often than would be expected by chance, and offer opportunities for making preliminary estimates of power of linkage studies using affected relative pairs as well as mode of inheritance (Risch 1990a,b,c).

Perls, et al (1998) compared the longevity of siblings of 102 centenarians and siblings of a control group (n=77) who were from a similar birth cohort born in 1896 but who died 27 years earlier at the age of 73 (3). The siblings of the centenarians were about 4 times as likely to survive to age 91 as the siblings of controls. The relative risk for survival to older age continued to rise beyond age 91 (RR=10 at age 95 and RR=15 at age 100), though these larger differences were not statistically significant because of small numbers of siblings at these extreme ages. Kerber, et al. (2001) examined patterns of recurrence risk among first- through fifth-degree relatives drawn from the Utah Population Database. They reported smaller relative risks among first-degree relative pairs than did Perls, et al. (RR = 2.3 at the 97^{th} percentile of excess longevity, approximately 97 years for women and 95 years for men). However, the recurrence risks among more distant relatives remained significantly greater than 1.0 for all classes of relatives out to second cousins, in a manner generally consistent with single-gene effects.

The persistence of familial recurrence of excess longevity among distant relatives suggests perhaps more strongly than the other evidence cited here that efforts to map genes related to longevity could succeed. It is less likely that second cousins (as compared with siblings) share either childhood or adult environments that would influence longevity in important ways, and particularly unlikely that the effects of such sharing would decay at a rate consistent with that of single-gene effects. However, there are at least two reasons for caution in interpreting these

results: 1) they have not yet been replicated in any other study; and 2) a model that posits an infinite number of loci with small multiplicative effects on risk (i.e., a classic polygenetic model), effectively a nightmare for gene discovery, can also be fit to the Utah data, though it fits less well than the single-gene model.

3.3. Types of studies that are feasible with regard to phenotype (age alone vs. age plus other characteristics, dichotomous vs. survival/hazard function characterization of phenotype) and subject group (unrelated, relative pairs, families). (Sample size calculations included where possible)

The feasibility of the various study designs under consideration depends heavily on the actual characteristics of the genetics of longevity. Because these characteristics are incompletely known, we must rely for our estimates of feasibility on assumed characteristics that seem to be

and particularly weak under others. Moreover, hybrid designs (e.g., "sib-pair" designs that incorporate data on other relatives, family-based association studies) may also be attractive.

Power calculations are problematic when they are undertaken in the context of so much uncertainty regarding both the phenotype and the underlying genetics. We will focus this discussion on power to detect linkage or association of EL, defined as either a continuous or discrete trait, to variation either at a chromosomal location or within a defined gene. It should be noted that associating variability in a particular gene with a characteristic is generally more valuable than linking the characteristic to variability in a chromosomal region. Thus, given nominally equivalent power, the information obtained in an association study may be more valuable than that from a linkage study.

Finally, note that the sample size calculations for both unrelated individuals and relative pairs employ a significance threshold of 0.05. This is not ordinarily considered sufficiently stringent for studies of large numbers of loci, because the large number of comparisons undertaken in such a study increases the probability that one or more tests will produce a "significant" result by chance. Estimates of required sample sizes given below should be considered minimal.

3.3.1. Unrelated Individuals

Case-Control studies compare gene frequencies at the oldest ages (often centenarians or nonagenarians) with the frequencies at a younger age (under age 50). The sample size calculation is based on a two-sided t-test comparing the proportions with the risky genotype at younger and older ages. We use a 5% level of significance and a power of 80%. The calculations assume that the relative risk is constant with age. The proportions surviving were calculated using the life tables for U.S. males born in 1900, 1910, and 1920 for ages 100, 90, and 80 (Wilmoth, 2000).

Table 2 shows the required sample sizes at three exact ages: 80, 90, and 100. The first row provides estimates based on a relative risk of death and gene frequencies similar to those found in Europe for the Apolipoprotein-E ε4 allele. In that case, only 83 centenarians (individuals at age 100) and a similar number of younger individuals are required to test for a significant effect. Over sampling the younger cohort by a factor of 2 reduces the required number of centenarians by about 23% and increases the total sample size by about 15%.

Table 2. Sample Size Requirements at Three Exact Ages

Relative Risk	Frequency of Risky Genotype at Younger Ages	Sample Size Required at Exact Age:		
		80	90	100
1.25	25%	1,544	295	83
1.10	25%	3,132	636	197
1.10	10%	23,207	4,071	909

Cohort survival. We assume the population is divided into two genotypes with relative sizes of $S_1(20)$ and $S_2(20)$ at age 20. At later ages, the number still alive for genotype i is determined by the age-specific mortality rates, $\mu_i(x)$. Given a relative risk of death, r_2, we can use an iterative process to calculate the age-specific mortality rate for the low mortality group at age x, $\mu_1(x)$, which matches the U.S. life table for males born in 1910, $\mu(x)$, using the equation:

$$\mu_1(x) = \frac{\mu(x)}{S_1(x) + r_2 S_2(x)} \quad .$$

Then we calculate the proportion of each genotype that would survive from age 85 to 90. The required sample size is determined using a test for a significant difference between the proportions surviving using a power of 80% and a 5% significance level. With a relative risk of 1.25 and a gene frequency of 25% at the youngest ages requires a total sample size (including both genotypes) of 2,315. The sample size increases to 11,260 for a relative risk of 1.10. Part of the problem here is that the risky genotype becomes more rare at the oldest ages and the sample is not equally balanced between the two genotypes. This works to our advantage if the more rare genotype is protective. With a relative risk of 0.75 and an initial gene frequency of 25%, we only require a sample of 1,040. If we followed an equal number of individuals with the two genotypes the total sample size for a relative risk of 1.25 drops to 1,222, about half as large as before.

These calculations are based on the assumption that the relative risk is the same at all ages. This may not be true in general because of variations in frailty (unobserved heterogeneity). If the relative risk moves towards 1.0 at the oldest ages, the differences in mortality will be smaller at ages 85-90. In addition, we would need a larger relative risk at the youngest ages to achieve the same average relative risk over the age interval 50-100. This would lead to a greater reduction in

the proportion of the population that has the more rare, riskier genotype at age 85. These two factors combine to increase the required sample size, probably by a factor or two or three.

These sample sizes can be compared with the samples used to test the relationship between APOE genotype and mortality in three studies. The largest was the study by Vogt et al. (1997) based on the Study of Osteoporotic Fractures Study in the U.S. They followed 1751 women aged 65 and over for about 6.4 years. The differences in mortality by APOE genotypes were not statistically significant. However, the difference was significant among those over age 75. Skoog et al (1998) followed a sample of 412 85-year-olds in Sweden for three years. They found no difference in mortality by APOE genotype. Tilvis et al. (1998) followed 550 individuals aged 75-85 for five years in Finland. They did find a statistically significant effect of APOE genotype on survival.

In conclusion, the case-control comparison of gene frequencies at younger and older ages requires very large sample sizes for genotypes with frequencies less than about 15%. Even for common genotypes (25%) with relatively high relative risks of death (1.25), the sample sizes using 80-year olds are relatively large. However, this approach is very effective for genotypes associated with relatively large relative risks (1.2 or greater), especially if the older sample is over age 90. This holds true for genotypes that are more rare than APOE ε3/4.

The cohort survival method for a five-year follow-up period requires relatively large sample sizes for follow-up studies of octogenarians. However, this approach is more efficient if the rare genotype is protective. These estimated sample sizes are sensitive to the relative risk, the assumed initial gene frequency, and the assumption that the relative risk is constant with age.

3.3.2. Relative Pairs

Assuming that a genetic map with a minimum density of 10 cM will be used, the maximum distance between a linked marker and an EL gene is 5 cM. These calculations utilize identity-by-state probabilities because of the lack of parental genotypes for markers with different levels of polymorphic information content (PIC). We consider a PIC level of 0.86 – most contemporary microsatellite linkage markers have PIC values in the range of 0.7-0.9. Multipoint linkage analysis can also be employed to increase the informativeness of the marker data. Given the above assumptions, it would be possible to detect a locus with a variety of different effects with reasonable sample sizes, particularly major loci with large effect. As shown in Table 3 below, to detect genes with relatively modest effect, (e.g., $1.3 \leq \lambda_s \leq 1.5$), perhaps 300-500 exceptionally long-lived sib-pairs would suffice provided there is minimal or no genetic heterogeneity.

Table 3. Number of Sibling Pairs Required to Detect Linkage between a Given Genetic Marker Locus and an EL Trait Locus

θ	λs								
	1.1	1.2	1.3	1.4	1.5	2.0	2.5	3.0	3.5
0.01	2327	689	358	232	170	74	50	40	35
0.05	3273	971	504	328	240	105	72	58	50

θ represents a recombination fraction, or, the probability that an odd number of crossover events will take place between two loci

λs represents the estimate of recurrence risk for siblings

Using dense marker maps such that the maximum expected distance from any EL is minimized also can provide considerable advantage (contrast required sample sizes for 1 cM vs. 5 cM distances). Beyond detection, it may not be possible to achieve a tight localization of any loci identified by linkage. The use of relatively homogenous populations can favorably impact the ability to localize putative genes. The analysis above relies on a dichotomization of EL, which may result in a loss of power under some circumstances. Gu and Rao (1997), however, describe an approach to relative-pair linkage for quantitative traits based on a simple categorization of the data with power similar to that described above.

3.3.3. Families

For the large-family design the question of feasibility is too data-dependent to be addressed from theory. Simulated linkage in selected pedigrees is generally required in order to estimate power to detect linkage. In general, however, large multigenerational families yield greater power to detect linkage or association of a trait on a person-for-person basis than do affected relative pair studies (Wijsman and Amos 1997). This is equally true for quantitative and discrete phenotypes. For example, to have 80% power to detect a QTL accounting for 20% of the residual trait variance at a LOD score of 3 would require 8065 individuals in randomly ascertained nuclear families with two sibs, 2616 subjects in nuclear families with four sibs, or 959 individuals in 3 to 4 generation pedigrees with an average of 48 people each (Blangero et al., 2001). Ascertaining nuclear families on the basis of a sibling who is in the top 10% of the trait distribution will reduce the sample size required by a factor of 3-4, but the number of individuals required will still be more than twice the number required in *unselected* large families.

3.4. Summary

Each of the contemplated designs has a weak point: for studies of unrelated individuals, rare alleles will drastically reduce power; for studies of relative pairs, locus heterogeneity can be problematic; for large families, the scarcity of the families and the uncertainty of recruiting key

individuals (particularly if they are very old) can be prohibitive. Importantly, we have little information on any of these factors.

When subjects are rare, the case can be made for collaborative or multi-center studies. Although there are at least 30,000 centenarians in the United States, recruitment is still difficult, particularly for sibships and other relative pairs likely to be used for linkage studies. Another very rare group are super-centenarians, those individuals age 110 and older. These individuals, one per 100,000 in the population, represent even more demographic selection than centenarians and may be useful in determining alleles that have a particularly strong association with survival. Collaboration among multiple sites, adhering to standardized protocols and procedures, is a means to generate the larger sample sizes necessary to detect genes of modest effect.

It is important to keep options open both in terms of analysis approaches and study designs that result in data to be used in such analyses, rather than to specify ideal approaches now. Currently there is considerable active research on both analytic methods and on study design issues, both of which are likely to have an impact on ideal analysis methods and designs. Additional research with long-lived individuals and families may also help guide analytic choices by clarifying some of the underlying genetics.

4. Needs for preliminary studies

For studies that could be useful, but for which available information is insufficient to determine whether or not such studies are feasible in the subpanel's subject group, what additional information is needed to clarify feasibility?

Although genetic studies of longevity have been carried out by some groups, there is limited experience with identification and recruitment of probands who have survived to EL, and moreover, their family members. Identification of potential probands has usually relied on some type of registry. For this reason, studies conducted in European populations may have an advantage where a number of databases exist that can be searched for EL individuals or families. The use of such data is also likely to be helpful in estimating the prevalence of recurrent EL in families. Although such registries tend to have limited information on extended family members, they can be used as a sampling frame for EL studies.

Twin registers are a rich potential resource for EL studies, and are available in Scandinavia, Italy, and Australia, among other countries. Whether these registers provide large enough sample sizes for analysis would depend in part on how the phenotype is defined. In fact, such registries are quite useful in exploring various sampling strategies and the impact of phenotype definition on feasibility. In addition, when the twins themselves have been previously surveyed, the role of behavioral factors (diets, exercise, smoking) could be examined. The United States does not sponsor a national twin register. Some data on U.S. twins are available from registries in Minnesota, of twin white male WWII veterans born in 1917-1927, and Vietnam War records on twins. However, currently, these cohorts are too young to be considered for EL studies.

Population registers provide another potential source of information about EL individuals. Permission to utilize these registers for EL sampling would have to be established, especially for registry relatives, but such national registers already are in use for other research purposes in Europe. In the United States, elderly individuals potentially could be identified through Social Security and Medicare records. However, confidentiality restrictions may effectively preclude such use. In certain states, such as Massachusetts, annual censuses conducted by the electoral offices of each town can provide publicly available data including age and address of citizens. Because of the high mortality rate of centenarians, the specificity of the census list is about 50%, however, the sensitivity approaches 100%. An alternative strategy to identify elderly (male) sibling pairs in the US is to begin with the 1920 Census data from which information can be obtained on individuals who were children at the time of the Census. This information could then be used to match these individuals to current Social Security and Medicare records. Through this match, surviving individuals could be identified who could then be surveyed for additional information, including information on surviving relatives. This approach is presently under investigation. (Note, this approach is limited to males because matching female records from the

1920 census to current data sources is problematic because of changes in last names for married women.)

Active efforts to ascertain families that demonstrate recurrent EL are needed. Because such families (and in particular their EL members) are likely to be scarce, such an effort should probably proceed in tandem with sample collection and epidemiological questionnaires. Segregation analysis, either as a first step or jointly with linkage analysis, should be performed.

It may be important to design studies in such a manner that ascertainment corrections are possible. In general, analytic methods for extended pedigrees are likely to involve a trait model at some level, therefore requiring some amount of model fitting, either before or during linkage analysis. For extreme phenotypes, the correction is most readily achieved by ascertaining through a single proband. Pedigrees can then be extended through either fixed-structure or sequential sampling designs, using clearly defined criteria for additional sampling based on phenotypes of individuals rather than sets of individuals. An ascertainment correction that is based on the original probands' phenotypes can then be devised. This suggests that the case-control design could complement the extended pedigree design in providing a sample of individuals for use in estimating ascertainment probabilities. This approach could be particularly useful for multivariate phenotypes.

The reliability of association studies using genetic markers depends upon the genetic homogeneity of the study population. The underlying assumption is that all individuals in the population are fundamentally related to each other, with the degree of relationship undiluted by mutation rates or recombination between the marker and the functional variant. Therefore the genetic history of the population must be known and taken into account to avoid potential bias. Moreover, because of the nature of EL, historic-demographic and historic-epidemiological information is also required.

More data are also needed on rates of survival by functional status and environmental factors to clarify phenotyping questions. If the EL phenotype of most relevance concerns functional status, then designing genetic studies requires more information on the change in functional status with age. Knowledge of these data is also crucial in order to correctly interpret replication studies.

5. Useful steps that NIA could take to facilitate EL research

What, if any, are needs for analyses of existing data, for new cell and data collection, and/or for pilot or full-scale studies, that are unlikely to be met through the standard NIH process for research project applications, and that could be met more efficiently or effectively by one or more NIA solicitations for proposals? In addition to possible solicitations, what else could NIA do to advance research in this field?

5.1. Needs unlikely to be met through standard NIH grant application process, that could be met via NIA solicitation(s)

The sample size requirements for any genetic EL study are likely to be substantial. The recruitment challenge is compounded by 1) the relative scarcity of EL individuals, 2) the lack of central registries for US populations, and 3) the need to recruit family members. One strategy for assembling large datasets for rare outcomes is multicenter collaborations, which also assures common protocols and standardized procedures. A model successfully used at other Institutes (e.g. NHLBI Family Heart Study) is to solicit proposals and choose 3-5 clinical centers, a data coordinating center, and any specialized laboratories or reading centers, as necessary.

The feasibility of large-kindred EL studies depends upon the prevalence of such kindreds. In many populations, finding and recruiting kindred will be a tedious and time-consuming process involving some combination of population-based, clinic-based, or self-referred ascertainment of individuals who have exceeded some aging threshold, followed by interviews and vital records searches to establish family histories. Many such probands will not have family histories of EL. Non-standard funding mechanisms may prove helpful in this case. Alternatively, a multicenter study might begin with an initial population sampling phase to identify EL kindreds via mailed questionnaires, followed by a clinic phase in which selected kindreds are extensively examined (e.g. NHLBI Family Heart Study). However, a caveat to this two-phased approach is that critical family members may dies before the clinical phase begins. Thus, if a promising family is identified, it may be appropriate to obtain blood samples and other relevant clinical data immediately.

Assuming good evidence for the familiality of EL, an RFA would likely be necessary. It should not be too specific so that investigators can be creative and suggest novel approaches that are particularly appropriate for their study populations. But even if a set of thematically related proposals are funded, the RFA might require an initial joint protocol design phase for all awardees, where standardization of phenotype definition and a set of common protocols (in addition to any study-specific ones) might be developed prior to any data collection. This would assure a minimal level of comparability across the diverse studies, so that pooled analyses could take place. However, one danger here is that some awardees may be too committed to their

original designs or definitions and might not be able to make the necessary compromises for common protocols. Care would have to be taken to assure compliance with common goals, via a central data coordinating center, and an Observational Study Monitoring Board (OSMB). A good model for this approach is the NIA's Frailty and Injuries Cooperative Studies (FICSIT).

Any EL RFA should include a specific budget item to create cell lines. Despite the substantial cost, securing adequate future samples of such rare genetic materials is essential.

We have deliberately avoided making firm recommendations on the critical study design questions of ascertainment scheme and choice of EL definition or correlated risk factors. These issues require study- or population-specific optimization and thus may require pilot studies to explore alternative strategies prior to initiation of large-scale genetic studies. Planning grant or pilot project funding would facilitate clarification. Alternatively, providing an extended study design protocol refinement phase in the context of a multicenter EL study could also accomplish this goal (e.g. the NHLBI Family Heart Study had a one year design phase prior to any new data collection).

5.2. Other Ways NIA can facilitate and advance research on EL

To advance this field, NIA could also: 1) provide training mechanisms for longevity researchers (e.g. short courses, training grants); 2) provide supplemental sampling funding of existing studies to increase their comparability (e.g. adding a sample of the institutionalized to studies that had excluded them) and 3) fund testing for various health conditions that could be used to standardize phenotype definitions across studies (e.g. cognitive testing of subjects for whom genetic material is available).

The NIA could also sponsor a survey of investigators in the field to catalog existing resources from ongoing studies (e.g., large cohort studies, longitudinal studies, family studies, or clinical trials) that might be useful for new studies of EL, including (1) sufficiency of the informed consent, (2) availability of genetic materials (DNA, cells, sera), (3) age distribution of enrollees, and (4) clinical measures. It might develop a WEB site for posting findings (including negative association studies) and posting protocols for relevant laboratory assays for the study of EL. It could encourage and support replication studies, by sponsoring consensus conferences to discuss results and select candidates for replication, or provide funds to encourage sharing of genetic materials.

Perhaps the single most important prerequisite to planning EL studies is phenotype definition. It would be very useful for NIA to sponsor a consensus development conference precisely on this issue. One strategy would be to identify a basic and common set of EL related measures that all studies should collect in addition to any specific hypothesis-driven measures (e.g., markers of immunologic functioning). The various definitions of EL would be analyzed in an attempt to

determine which are most useful from the standpoint of both biologic meaning and logistics of collection. If several studies are involved, it may be possible to combine information either by pooling the data directly or by using a meta-analytic technique.

Finally, long-term prospective studies may ultimately play an important role in defining the genetic factors associated with EL. Longitudinal family designs, such as following the offspring of affected sib pairs, could provide a wealth of information including surveys of the offspring as they age, banking DNA on the long-lived parent(s) of the offspring for association studies, and increased information content for genetic studies by virtue of the ascertainment through affected sib pairs. However, because of the long-term commitment over the period of decades that would likely be required, these studies might be deferred until there is considerably more experience with the logistics of doing these studies and more information on the specific genetic hypotheses of interest. If such studies were undertaken, it would be important to design them so that some basic questions could be answered right away, like good prevalence estimates of various definitions of EL (which will almost surely itself change over time), even if the ultimate goals of finding the EL genes would not be achievable until the final stages.

6. Opportunities for international collaboration

What, if any, are useful opportunities for NIA to collaborate with ongoing efforts in other countries for collection of cells and data from exceptionally long-lived people?

6.1. Introduction

Research on EL conducted in other countries than the USA can provide population based samples that can be identified via extensive and long-standing national registration systems. Many of these populations are ethnically (and therefore genetically) homogeneous, and many previous studies have achieved high participation rates. Another advantage of conducting family studies in other countries is that people tend to be less mobile, and family members (who span several generations) live geographically close to one another.

6.2. NIA role (initiatives, use of NIA Webpage, publications, etc.)

- Coordinate efforts to define aging and healthy aging across different countries
- Facilitate replication studies
- Facilitate validation and standardization of methodologies, and methods to validate ages and birth years in different countries
- Encourage migration studies
- Catalog resources available in non-USA studies
- Publish or disseminate a document describing the NIA's willingness to collaborate with non-USA funding agencies and scientists
- Coordinate and facilitate genetic and cross-cultural environmental factor comparison impact on EL across countries
- Coordinate and facilitate meta-analysis studies of existing data across countries

6.3. Specific countries/populations of interest (including relevant information/data appropriate to EL research)

While the USA offers a large population for the selection of families with EL, there are unique populations across the world that also offer outstanding opportunities. In particular, searching for genes for extreme longevity in founder populations may have additional advantages under some conditions when families are studied. For example, the Ashkenazi Jewish population in Israel,

and the Sardinian populations are examples where such efforts have been conducted. Due to its sheer size, a large number of centenarians have been identified in China. In countries that maintain national registries, it is easy to identify aged siblings and to link data on family members. Scandinavia is ideal for identifying old siblings because the countries have a long tradition of maintaining population-based registries (i.e. censuses, birth registries, death registries, twin registries), and data linkage between these registries is routinely conducted.

Insights into the contribution of genes and environment on EL could ensue from studies on people who emigrated from their land into a new country at the beginning of the century, e.g. a number of people migrated from southern Italy, especially from Calabria and Sicily, to various parts of USA.

Because families with strong evidence of predisposition to longevity are likely to be rare, it may be particularly important to extend the search for such families as far as possible. Some population databases and studies that exist in Canada, Europe, and Asia could be valuable sources of familial data on longevity (see citations in Hadley et al. 2000). Collaboration between groups with access to or responsibility for management of these resources should be encouraged.

7. Special logistical and ethical issues pertinent to EL studies

What are the implications of ethical and logistical considerations regarding genetic studies on exceptionally long-lived persons for the organization of cell- and data-collection projects?

7.1. Ethical, legal, and social issues (ELSI) and their implications for EL Studies

Standard ELSI apply to EL, including: informed consent process, privacy and confidentiality, use of genetic material, rights and obligations in reporting results to subjects and their physicians, and risks of research participation (e.g. stigmatization; loss of health care/employment, etc.). For good discussions of these issues with extensive lists of relevant publications, see the web sites for various institutes and genetics societies (NHGRI, 2000; OHRP, 2000; ASHG, 2000; IGES, 2000; FASEB Genetics Societies, 2000). ELSI standards are evolving and becoming more stringent, particularly for genetic studies. Some ELSI are heightened for EL, e.g. the subjects' ability to provide truly informed consent (since the extreme elderly may suffer from cognitive impairment, sensory loss, or general frailty). EL studies may require informed consent procedures specifically designed for visual/auditory impairments, and/or seek proxy consent for those with significant cognitive impairment. Since a prospective EL study may span several decades, it may require broader permission to future use of biological samples, due to the long time lag between sample collection and data analysis. Standards for the future use of biological samples have changed radically over the years, from "blanket consent" to more stringent, explicit specifications. Since so little is known about the genetics of EL, having a too restrictive informed consent on the use of DNA could especially hamper studies.

7.2. Logistical issues and their implications for EL Studies

Many logistical concerns are not unique to EL, such as the need for the dissemination of technologies (e.g., DNA chips, high-throughput genotyping, etc.), and the need for free exchange of information among researchers, while protecting individual confidentiality. Cell line establishment may be especially important for rare phenotypes like EL, since they provide indefinite sources of DNA. Although initial sample needs may be small, fine mapping and SNP typing can quickly explode, especially as the number of subphenotypes increases. To allow for future studies, cell lines should be established because the EL population is unlikely to be available for later re-sampling. A laboratory with a long history of successful transformation and storage of cell lines as well as dependable isolation, storage, and distribution of DNA is preferable. Viral contamination and inadequate growth of lines prior to freezing are just two of the reasons rare and valuable samples should be handled by the most experienced laboratories. Although cell lines can be expensive (approximately $400 per sample), the cost is well justified.

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