

Exploring the genetics of longevity in the Old Order Amish

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Abstract

Lifespan is a complex phenotype determined by the interaction of genetic and environmental factors. This makes the identification of variants in genes that influence longevity challenging. We believe that the Old Order Amish (OOA) of Lancaster, Pennsylvania is an excellent population for studying the genetics of longevity. They are a closed population derived from a limited number of founders. They have large families and maintain extensive genealogic records dating to the 1700 s. They eschew modern technology; their lifestyle is little changed over the last 250 years. Homogeneity of environment and lifestyle factors across time and across the OOA population minimizes the influence that environmental factors have in determining the differences in lifespan between individuals. We hypothesize that this reduction in environmental variability will make it easier to identify the genetic factors that influence lifespan. In this article, we describe our strategy for identifying variants in genes that influence longevity in the Amish and present the results of our studies to date.

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1. Introduction

Although a number of studies have identified genetic, biological and environmental factors that contribute to diseases that decrease lifespan, we know much less about characteristics and genes that contribute to longevity. Family studies suggest that a long lifespan is heritable. The longest documented lifespan, 122 years, was achieved by Jeanne Calment, who died in 1997. Madame Calment’s brother survived to 97 years of age. Twenty-four percent of her immediate ancestors survived to age 80 or older compared to 2% in a control population (Robine and Allard, 1998). Perls et al., 1998 found that siblings of centenarians are four times more likely to live into their early 90 s. These examples and studies of twins reared apart (Herskind et al., 1996; Ljungquist et al., 1998) suggest that extreme longevity is determined, at least in part, by genetic factors.

Human lifespan may be determined by a “biological clock” and a variety of chronic external stressors. Some people will suffer early mortality due to an acute extrinsic stress, such as an accident. This might be viewed as a “wrong place, wrong time” death in which genetics plays little or no role. Other people will succumb to diseases whose lethality is determined by the interaction of environmental factors and disease susceptibility genes. Thus one mechanism by which putative longevity genes may work is by protecting the individual from common fatal diseases, such as atherosclerosis, cancer or infection. Alternatively, gene variants that increase lifespan might slow the aging process (the so-called biological clock) by mechanisms that delay cellular senescence, limit oxidative damage to cellular proteins and DNA, or extend the limit of cellular replications (Bodnar et al., 1998; Hayflick, 1965).

Unlike diseases and traits that are caused by a pathogenic mutation in a single gene, longevity is probably polygenic; variants in multiple genes carried by the same individual are probably required to express the trait. Each gene variant

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probably has only a small effect on lifespan, and there may be many combinations of genetic variants that increase the likelihood of longevity. The distribution of particular sets of genetic variants may vary from one population to the next and even within families of a given population. Additionally, there likely is great inter-individual and inter-population variability in the environmental stressors required for these gene variants to express themselves phenotypically. These complexities make the search for longevity genes quite challenging.

Any search for variants in genes that influence longevity must include a study design that reduces complexity and potential biases. Potential strategies include focusing on special populations with less genetic diversity and identifying and studying longevity-associated phenotypes whose genetic underpinnings may be less complex, but which may still contribute genetic variation in lifespan.

2. General methods for studying genetics of complex traits

Most approaches to discovering a gene variant that influences a trait involve the identification of a sequence variant in the genome, assaying (genotyping) the variant in DNA samples from a large number of individuals, followed by statistical analysis to determine if the variant occurs more frequently in a group of individuals possessing the trait (cases) than in a comparison group of individuals lacking the trait (controls). This approach is referred to as an association analysis. Alternatively, one might ask if family members carrying the trait are more likely to have inherited the same genotype from a common ancestor than would be expected by chance. This approach is referred to as a linkage analysis. Although conceptually straightforward, these approaches are difficult in practice given that the human genome consists of three billion base pairs and there exist approximately 3–6 million common sequence variants in the human genome.

3. The Old Order Amish—an excellent population for genetic studies

The Old Order Amish (OOA) are an anabaptist sect that originated in 1639 in Berne, Switzerland. An estimated 250 Amish families emigrated from Switzerland to Lancaster, Pennsylvania in the 1700 s. Marriage into the OOA is exceedingly rare. Amish families are large (mean sibship size approximately seven children). The Amish agrarian lifestyle has changed little over the past 250 years. The OOA have no electricity in their homes, do not drive cars, and perform largely manual labor. The relative constancy in lifestyle and environmental exposures both between individuals and over the years should greatly enhance our ability to dissect genetic underpinnings of complex traits.

Using OOA genealogies (Agarwala et al., 1998, 1999) we have identified a large number of Amish who lived past the

age of 90 years, all of whom can be connected into a single pedigree.

4. Evidence for a limited number of founders

Genealogical records indicate that the Lancaster County OOA have a relatively small number of founders. The Lancaster County Amish address book lists over 5000 households with only 42 different surnames. Eight surnames account for over 80% of households. Pedigree studies in over 2500 individuals show that fewer than 100 founders account for over 95% of the current OOA gene pool. To further examine the founder structure of the OOA, we examined genotypes from nine-microsatellite short tandem repeat (STR) markers on the Y-chromosome in 739 men (Pollin et al., 2003). In 23 male lineages, we identified 22 distinct founder haplotypes. In all but one instance, each haplotype corresponded to a separate surname and lineage. Seven haplotypes accounted for 83% of the men in the cohort. Preliminary results of mitochondrial DNA in the OOA similarly support a small number of female founders. These results confirm the small number of founders and the accuracy of the genealogy, and they support our belief that the OOA are a young, closed relatively genetically homogeneous population.

5. Characteristics and heritability of lifespan in the Amish

We studied the heritability of lifespan in 1655 OOA who survived to age 30, had a known date of death, and were born between 1749 and 1890. Heritability, h^2 , was defined as the proportion of the total trait variance, σ_T^2 , attributable to the additive effects of genes, σ_G^2 , i.e., “narrow sense” heritability $h^2 = \sigma_G^2 / \sigma_T^2$. Our estimate of the heritability of lifespan was 0.25 ± 0.05 ($h^2 \pm$ S.E.), indicating that the additive effects of genes accounted for 25% of the total variation in age at death (Mitchell et al., 2001). Heritability was similar for men and women. There was little evidence for a parent of origin effect. The heritability estimate measures the familial influence on variation in lifespan, which may differ somewhat from the familial influence on exceptional longevity. Of note, there was no evidence for a secular increase in age of death in the OOA (Fig. 1). The mean age of death of an 1890 cohort who lived to the age of 30 years or older was 70.2 years, which was not significantly different from that of earlier cohorts going back as far as 1745. Additionally, there was no difference between the age-specific mortality rates of men and women (Fig. 2).

6. Strategy for identifying longevity genes in the Amish

We are currently applying two approaches to the identification of longevity genes in the Amish. First, we

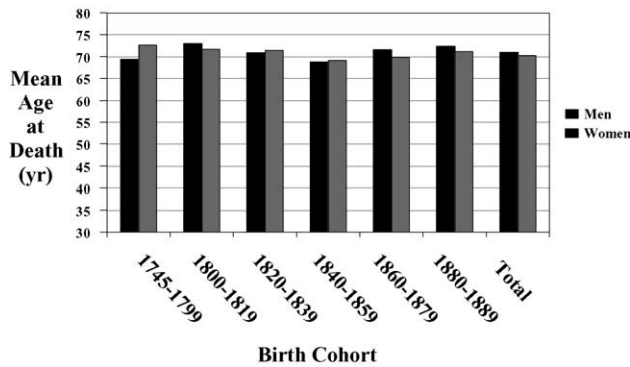


Fig. 1. Mean age of death of birth cohorts of adults who lived to the age of 30 years or older. Remarkably, the mean age at death has not changed over the last 250 years in the old order Amish. Adapted from Mitchell et al., 2001.

are recruiting long-lived (older than 90 years) Amish probands. These individuals, estimated to be at least 30 in number, can be included in a single pedigree. This multiplex longevity pedigree will be used in genome-wide linkage and association analyses to identify chromosomal regions harboring longevity-related genes.

Second, in order to identify candidate intermediate phenotypes associated with longevity for which genetic influences can then be elucidated, we are also recruiting the offspring of the long-lived probands and the offsprings' spouses. The rationale for this approach is that the genomes of many of the offspring of long-lived probands, ages ~60–80 years, may be enriched in longevity genes possibly resulting in favorable (longevity-associated) phenotypes. By contrast, the offsprings' spouses, who have been exposed to similar lifestyle factors, whose genomes have not been enriched with longevity genes, will not express longevity-associated phenotypes. Thus, phenotypes that are found to differ significantly between offspring and offsprings' spouses will be designated as candidate longevity associated intermediate traits. These traits will be used in both genome-wide linkage analysis and candidate gene analysis to identify gene variants that influence those traits, and thus longevity.

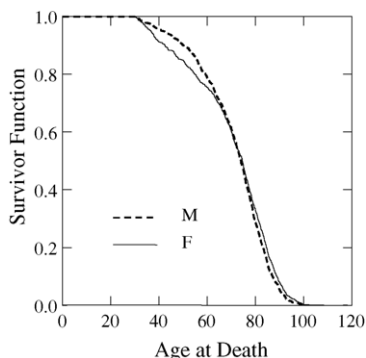


Fig. 2. Survival curves in OOA men and women. Unlike the general US population, where adult women survive approximately six to eight years longer than do men, in the Amish, there is no difference in survival between males (M) and females (F). Adapted from Mitchell et al., 2001.

Our phenotypic assessment includes detailed evaluations of a number of age-related traits, primarily focusing on cardiovascular, endocrinologic and frailty measures. Phenotypes include questionnaires to measure physical activity, depression, cognition, and dietary intake. Blood tests assess insulin and glucose metabolism, thyroid function, plasma lipid and subfraction concentrations, renal function, and inflammatory markers. We also measure blood pressure, pulmonary function, body composition and body fat distribution.

Since the leading cause of death in developed nations is cardiovascular disease, to achieve longevity, one must be protected from atherosclerotic vascular disease. We are examining measures of subclinical atherosclerosis including carotid intima media thickness (IMT) by B-mode ultrasound, and coronary calcification by electron beam CT (EBCT) scanning. These non-invasive measures are known to be potent predictors of the risk for cardiovascular events (Hoff et al., 2001; Meaume et al., 2001; O'Leary et al., 1999; Raggi et al., 2000). A widened pulse pressure reflects structural changes in the central arterial walls resulting in arterial stiffness (decreased arterial compliance). We are also measuring pulse wave velocity (PWV), a measure of vascular stiffness, which reflects the speed of the pulsatile component of arterial flow and increases with age. Increased PWV is associated with cardiovascular risk independent of blood pressure in individuals over the age of 70 years. Our hypothesis is that offspring of long-lived probands will have less coronary artery calcification, lower carotid IMT and slower pulse wave velocity than their spouses.

7. Phenotypic data from the Amish: preliminary analysis

To date we have recruited 19 probands, age 90 years and older, all of whom can be connected into a single pedigree. In addition, we have recruited 76 offspring (mean age 63.3 years) and 55 spouses (mean age 63.5 years). The number of subjects recruited to date is small, precluding any definitive interpretations of our data. Initial analysis of this small sample has revealed no significant differences between offspring and spouse with respect to BMI, waist circumference, glucose, or hemoglobin A1c levels. However, offspring appeared to have lower systolic and diastolic blood pressure than spouses, suggesting that blood pressure might be a relevant longevity-associated intermediate trait. Differences in blood pressure between offspring and spouses are of interest given our recently published genome-wide scan of blood pressure in 694 subjects from the Amish Family Diabetes Study, in which we identified a locus on chromosome 2q31–q34 that influences systemic blood pressure (LOD = 3.36; $p = 0.00004$) (Hsueh et al., 2000). This is an example of a quantitative trait locus (QTL) that could be related to longevity. Positional cloning of the putative blood pressure (and possibly longevity) gene is

currently underway. We hope that the identification of this gene will provide insights into blood pressure regulation and possibly the mechanism by which variation in this gene might increase longevity. These insights may lead to interventional therapies to prolong meaningful human life.

8. Summary and conclusions

The identification of variants in genes that are associated with longevity is complicated by the interplay of genes and environment and by the small “signal” that any one gene is likely to have on longevity. In genetically heterogeneous populations the small signal of any particular longevity gene will be difficult to identify. We believe that the task of locating variants in genes that influence longevity will be simplified by studying the Old Order Amish, a genetically and environmentally homogenous population.

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