Increasing Genetic Contribution to Exceptional Longevity with Increasing Age

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Presented at the Living to 100 Symposium
Orlando, Fla.
January 5-7, 2011

Revised January 15, 2012 following corrected results and publication of Sebastiani P., et al., Genetic Signatures of Exceptional Longevity in Humans, PLoS ONE, 2012, http://dx.plos.org/10.1371/journal.pone.0029848.

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Introduction

Based primarily upon calculations from twin studies, the heritability of "longevity" has been noted to be around 20 percent (Herskind et al. 1996; Hjelmborg et al. 2006b; McGue et al. 1993). Unfortunately, many papers that have cited these results have interpreted them to reflect the heritability of extreme old age or even life span, which is incorrect. The oldest subjects in these studies were in their mid- to late-80s, and thus the results say little about the relative importance of genes and environment or behaviors in the ability to live to much more exceptional ages such as 100 (centenarians), 105 (semi-supercentenarians) or even 110 years and older (supercentenarians). Given the log scale difference in the odds of survival between these oldest groups, it would seem particularly unlikely that the relative contributions of genes and environment would be the same across this age spectrum. Furthermore, given the tremendous difference in the propensity of women versus men to achieve the oldest ages and the gender-based differences in the expression of age-associated diseases and disability, it is also very unlikely that heritability estimates for exceptional longevity are similar between women and men.

The increasing body of demographic, genetic and medical data being generated from studies of centenarians suggests an increasingly greater genetic contribution to the ability to survive to ages beyond 100. A possible increasing level of homogeneity in functional history and medical histories amongst centenarians beyond the age of 105 years may lead to increased power to reveal genetic associations with the phenotype of exceptional longevity and subphenotypes such as the delay or escape of specific age-related diseases and syndromes such as dementia.

1. A Strong Familial Component to Exceptional Longevity

(a) Siblings. Siblings of centenarians have an increased risk of exceptional longevity relative to the average survival of their birth cohort. In analyzing data from the first 100 enrolled centenarians of the New England Centenarian Study (NECS), we compared the ages of their 456 siblings to the siblings of people from the same birth cohort (around the year 1900), but who died at the age of the cohort's average life expectancy (73 years) (Perls et al. 1998). The survival rates of the two sibling groups were the same at younger ages of death, but the relative risk of survival became progressively greater for the siblings of centenarians after age 70 years. For the ages 90 to 94 years, the relative risk was 3.9 for female siblings and 5.1 for male siblings. The relative risks continued to climb beyond these ages, but because of the small sample size at ages beyond 95, significance could not be accurately assessed. Similar findings were made with survival data from the Okinawan Centenarian Study (Willcox et al. 2006)**.** More recently, the Leiden Longevity Study found that nonagenarians with nonagenarian siblings have a 40 percent reduced mortality rate compared to sporadic nonagenarians (Westendorp RG et al. 2009).

With the continued NECS enrollment effort, the analysis of sibling survival was performed again, but with 444 families containing 2,092 siblings of centenarians. After accounting for race and education, from age 20 until age 100 years, the siblings of centenarians generally maintained half the mortality risk of their birth cohort (Perls et al. 2002b), a finding that was replicated in the Okinawan study (Willcox et al. 2006). The year-to-year survival advantage translated into very high relative survival probabilities of living to age 100. The net

survival advantage of siblings of centenarians was 16 years greater than the general population from the same birth cohort (Perls et al. 2002b). The male siblings of centenarians had an 18 times greater risk of surviving to age 100 and the female siblings had an 8 times greater risk, compared to the general population born in 1900. These findings also suggest that the genetic component of exceptional longevity plays a greater determinist role in males than in females.

Truly exceptional sibships, in terms of clustering for exceptional longevity, have also been described in the literature. One family in a case series had a sibship consisting of 13 siblings born over a 25-year period, beginning in 1894, and nine of the 13 survived beyond the age of 99 years. The probability of a family like this occurring solely by chance is less than one family per all the families that exist in the world today. Thus, the siblings must have had survival-related factors in common that would confer such a tremendous survival advantage (Perls et al. 2000).

Of course, family members can and often do have more in common than just genetic factors; they can also have behavioral-environmental factors in common (e.g., type and quantity of diet, tobacco or alcohol use, risk taking, years of education, socioeconomic status, access to health care, exercise habits, secondhand smoke, war and violence, toxins, diet, stressors, accident risk, etc.) and thus a familial risk ("familiality") for longevity, rather than a solely genetic or heritable risk, is a more accurate description of a family-related predisposition to longevity. Therefore, studying families that cluster for longevity should facilitate the study of both genetic and environmental factors (and the interactions of those factors) that they and their members have and don't have in common.

(b) Offspring. Another line of evidence supporting at least the familiality of exceptional longevity is the study of the children of centenarians. Terry and colleagues have noted that relative to members of their birth cohort who did not have parents achieving exceptional longevity, the children of centenarians start healthier and stay healthier (Adams et al. 2008; Terry DF 2004). These offspring have a lower prevalence of cardiovascular disease and cardiovascular risk factors (Terry 2002; Terry DF 2007; Terry et al. 2004a; Terry et al. 2004b). Following ~500 centenarian offspring and a similarly aged referent cohort over a four-year period of time, NECS researchers noted a 78 percent lower risk for myocardial infarction, 83 percent lower risk of stroke, and 86 percent lower risk of developing diabetes mellitus (Adams et al. 2008). Centenarian offspring were 81 percent less likely to die than the referent cohort during the follow-up period. Only 1.1 percent of centenarian offspring died, compared with 5.2 percent of the referent cohort. In another study, the NECS showed that the offspring scored unusually low, compared to established population norms, in the personality trait of neuroticism, and high in extroversion, traits that are conducive, respectively, to managing stress well and establishing social connections (Givens et al. 2009).

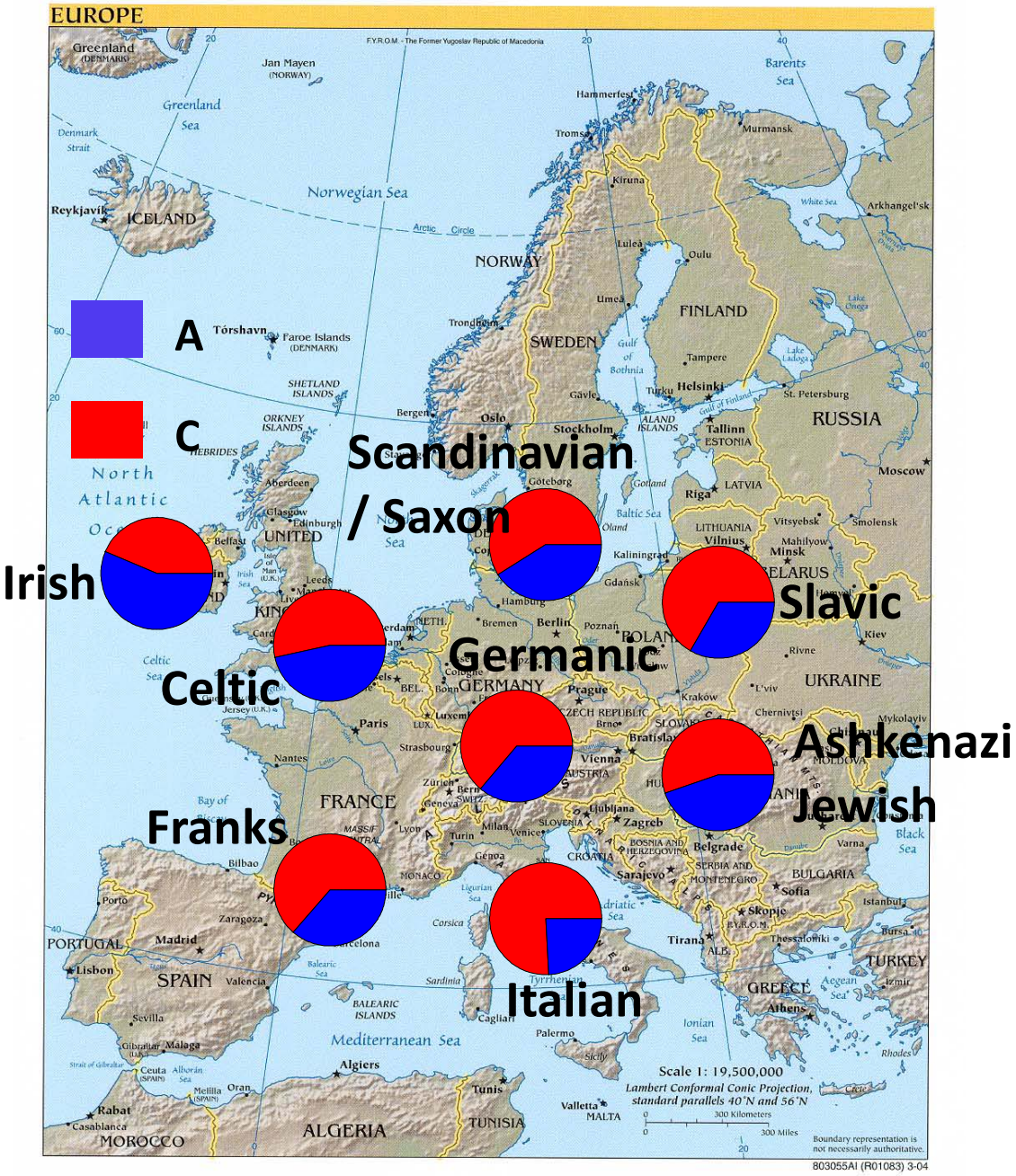
2. Genetics of Exceptional Longevity

(a) A Complex Trait. Exceptional longevity can result from numerous patterns of disability and disease ranging from complete independence and being disease-free to the opposite extreme (Christensen et al. 2008; Hubert et al. 2002; Nusselder and Peeters 2006; Terry et al. 2008; Terry et al. 2005), and as a complex phenotype, it is also likely a complex genetic trait as well, with many gene-gene and gene-environment interactions as determinants (Finch and Tanzi 1997; Perls 2001; Perls, Kunkel and Puca 2002a; Salvioli et al. 2006; Sorkin et al. 2005).

(b) Association Study Findings. The phenotypic and genetic complexity of exceptional longevity may be why relatively few statistically significant genetic associations have emerged from hypothesis-driven genetic association studies and data-driven genome-wide association studies of centenarians. Notable published findings include genetic variants related to lipoprotein metabolism (Barzilai et al. 2003), FOXO proteins (Flachsbart et al. 2009; Willcox et al. 2008) and insulin/IGF-1 signaling (Vijg and Campisi 2008), but these still explain a small proportion of the aging phenotype (Christensen, Johnson and Vaupel 2006), suggesting that the majority of genetic modifiers of exceptional longevity have yet to be discovered (Hjelmborg et al. 2006; Perls et al. 2000; Perls et al. 2002c).

When significant associations do emerge, the additional concern arises as to the reproducibility of findings in other populations. A negative finding for an association in another population should not, however, be proof that a significant association does not exist for the population in which the discovery was made. A good example is the population-based differences observed for the frequencies of the common variations of the Apolipoprotein E gene (APOE). Figure 1 depicts results from the NECS (conducted by Nadia Solovieff for her Ph.D. thesis at Boston University School of Public Health), showing the relative frequencies of two variants, A and C, of a single nucleotide polymorphism (SNP) associated with APOE for subjects of different ethnicities. For example, in the Irish, the A variant predominates, but in the Italians, the C variant is by far the more common.

Figure 1



The relative frequencies of two variants, A and C, of a single nucleotide polymorphism (SNP) associated with APOE for subjects of different ethnicities. For example, in the Irish, the A variant predominates, but in the Italians, the C variant is by far the more common (previously unpublished work by Nadia Solovieff, Boston University School of Public Health).

This phenomenon of ethnicity-associated differences is known as population stratification, and it is an important consideration in the analysis of genetic data where experimental and control subjects have different genetic backgrounds and when findings are being compared across ethnic lines.

(c) Prevalence of Disease-Associated and Longevity-Associated Variants Amongst Centenarians. Based upon the demographic selection hypothesis, one would expect that in order to achieve extreme old age, one needs to relatively lack both genetic and environmental variants associated with earlier mortality and perhaps also have variants that are relatively protective. This supposition has recently been challenged by two studies. Both the NECS and the Leiden Longevity Study have found that their subjects have just as many disease-associated genetic variants as their control subjects (Beekman et al. 2010; Sebastiani P et al. 2012). These findings suggest that what makes these subjects different from controls is the existence of longevity-associated variants that could be protective against the effects of disease-associated genetic and environmental variants associated with premature mortality. These findings have many implications for future research into the factors conducive to exceptional longevity, but most immediately they indicate that knowing the presence or absence of a disease-associated allele is likely not enough to accurately predict risk in an individual. A more comprehensive assessment (e.g., as in personal genomics) taking into account other factors that can modify that risk is likely necessary before we can accurately predict risk.

(d) Comprehensive Assessment of Genetic Risk for Exceptional Longevity. The NECS recently reported genome-wide association studies of 801 unrelated centenarians (age range 95 to 119 years; median age = 104 years) and of two replication sets (n=253; age range 89 to 114; median age = 100 years; and n=60, range 100 to 115 years; median age = 107 years) (Sebastiani P et al. 2012). Control data came from a large data set supplied by Illumina (healthy younger subjects) and the NECS controls (children of parents surviving to average life expectancy and spouses of children of centenarians). Principal component analysis was performed to genetically match subjects with controls and to avoid population stratification as a cause of false positive results.

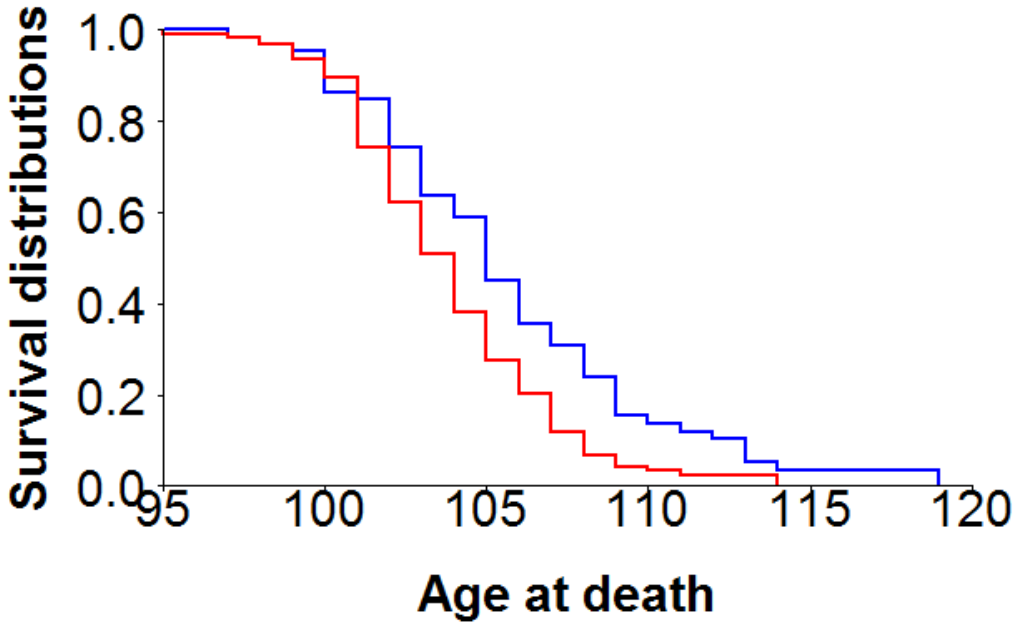
A Bayesian statistical approach was performed in order to take into account the simultaneous effect of genetic factors upon exceptional longevity. Following stringent quality control which likely leads to some loss of true positives, we had access to 243,980 SNPs' worth of data for the analysis. Essentially, a genetic risk model to predict exceptional longevity was built by progressively combining the probabilities for exceptional longevity of nested groups of SNPs until adding another SNP did not enhance both the sensitivity and specificity of the model. Thus, the model began with the SNP that has the strongest association with exceptional longevity, then in a next step, it incorporated the combined probability of the top two most significant SNPs, and this was followed by incorporating the combined probability of the top three most significant SNPs and so on. This process was continued until there was no longer an improvement in both sensitivity (the ability to predict who is a centenarian) and specificity (the ability to predict who is a control), based upon the genetic data alone.

The analysis produced a model containing 281 SNPs that reached 89 percent sensitivity and specificity in the discovery set, but less specificity (58 percent) and sensitivity (61 percent)

in the first replication set. The lower sensitivity, compared to the discovery set, was likely in great part due to the substantially younger median age of the first replication set. To address this, we assembled a second smaller, though much older, replication set, and the sensitivity was 78 percent. Consistent with the hypothesis that there is an increasingly stronger genetic influence upon survival with older and older ages beyond 100 years, we found that the sensitivity of the model was 71 percent to determine if a subject was older than 102 years, and 85 percent to determine if a subject was older than 105 years. We also replicated the specificity in a set of 2,863 unmatched controls and reached a specificity of 61.2 percent. The lower specificity of the model is likely due to greater heterogeneity of the phenotype because the control sets may include subjects with varying life spans.

As described above, for each of the 281 nested sets of SNPs, there was an associated prediction of exceptional longevity. Those predictions of exceptional longevity can be graphically portrayed as a genetic risk profile. We can then use cluster analysis to determine if any subjects have the same profiles. More than 90 percent of the 801 profiles fit into 26 common genetic signatures. The other 10 percent had what we called rare profiles, each shared by fewer than seven centenarians. Some profiles were notable for having nearly all of each of the 281 nested SNP models associated with exceptional longevity (what we otherwise call longevity-associated variants, LAVs). When we looked at what else the subjects with these particular signatures had in common, we also found that the genetic signature most predictive of exceptional longevity included centenarians with the longest survivals and this result was replicated in an independent set. Figure 2 shows an example of survival distributions for two genetic signatures that are most predictive of exceptional longevity. This would suggest that as age of survival increases, particularly above age 105 years, enrichment for LAVs becomes increasingly more likely and more prominent. We found that other signatures pointed to subjects with other subphenotypes in common beyond exceptional longevity, such as delayed age of onset of dementia and cardiovascular disease. However, other than the signatures associated with the most extreme ages, we did not have sufficient data to attempt replication of the associations with other subphenotypes, and therefore these latter associations particularly need further study in other samples comparable to the discovery set (old enough and large enough to have statistically enough subjects that developed these other subphenotypes).

Figure 2



Survival distributions of centenarians with two different genetic signatures. The blue line shows the survival distribution for 66 centenarians allocated to the signature that is most predictive of exceptional longevity (median age at death 105, 95% CI 103; 106). The red line shows the survival distribution for 94 centenarians that were grouped in the second most predictive signature (median age at death 104, 95% CI 103, 104).

The idea of beginning with a genetic signature that subjects have in common and then determining what other phenotypic characteristics they also have in common is backwards from what geneticists usually do. Normally one begins with a phenotype and then attempts to determine what genetic variants are associated or linked with the phenotype.

3. Implications and Conclusions

(i) Increased power to discover genetic variants associated with exceptional longevity. The NECS genome-wide association study joins work by University of Southern Denmark researchers suggesting that there is increased power for discovering genetic associations with exceptional longevity and its subphenotypes (e.g., intact cognition) as the age of the subjects increases beyond the mid-90s (Tan et al. 2008). While centenarians have been noted to be phenotypically heterogeneous (Evert et al. 2003; Terry et al. 2008), the NECS has noted supercentenarians to be both extremely rare and homogeneous in terms of the marked delay of age-related diseases normally associated with increased mortality risk (Andersen SL et al. 2012; Schoenhofen et al. 2006). Such homogeneity should also be associated with increased power to

discover genetic associations with such survival and support concerted efforts to enroll supercentenarians for studies of genetics of exceptional longevity.

(ii) Longevity-associated variants. At least two studies have now indicated that the genetic advantage related to exceptional longevity appears to rely more upon longevity-associated or protective genetic variants rather than the lack of disease-associated variants. Deciphering which genes these are and understanding how they confer a survival advantage could lead to novel screening and prevention strategies and perhaps, in the distant future, therapeutics. As noted above, it also appears that relying upon the presence of disease variants alone (e.g., current personal genomics screens for age-related diseases such as cardiovascular disease and diabetes) in determining risk may be highly deceptive given the potential presence of other genetic variants that can cancel out or attenuate the effect of such deleterious variants.

(iii) Genetic signatures could prove useful in the promising field of personal genomics, which until now has been successfully utilized in solitary gene effects and therefore very specific genetic-disease or genetic-drug efficacy associations. With even more comprehensive genomic arrays, inclusion of different populations, and with more concerted efforts to include as old as possible subjects, genetic signatures of exceptional longevity should become even more specific and sensitive. The potential utility of these signatures is broad, ranging from tailored prevention and screening, to a new tool for discovering gene-modulated pathways that play roles in exceptional survival.

(iv) As genetic signatures become more accurate, determining the prevalence of signatures highly predictive of exceptional longevity versus those that are not in population-based controls might provide an estimate of the proportion of the general population that is predisposed to exceptional longevity. The ability to make such estimates could underlie public policy regarding the dedication of resources towards early prevention and screening that could lead to longer life expectancies and delayed disability.

(v) The potential misuse of such predictive capabilities must be diligently considered and safeguards must be put in place. The Genetic Information Nondiscrimination Act (GINA) is an important step along these lines.

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