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Successful aging shows linkage to chromosomes 6, 7, and 14 in the Amish

DIGNA R. VELEZ EDWARDS^{1,#}, JOHN R. GILBERT¹, LAN JIANG², PAUL J. GALLINS¹, LAURA CAYWOOD¹, MARILYN CREASON¹, DENISE FUZZELL¹, CLARE KNEBUSCH², CHARLES E. JACKSON³, MARGARET A. PERICAK-VANCE¹, JONATHAN L. HAINES², and WILLIAM K. SCOTT¹

¹Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, FL 33136, USA

²Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA

³Scott & White, Temple, TX

SUMMARY

Successful aging (SA) is a multi-dimensional phenotype involving preservation of cognitive ability, physical function, and social engagement throughout life. Multiple components of SA are heritable, supporting a genetic component. The Old Order Amish are genetically and socially isolated with homogeneous lifestyles, making them a suitable population for studying the genetics of SA. DNA and measures of SA were collected on 214 cognitively intact Amish individuals over age 80. Individuals were grouped into a 13-generation pedigree using the Anabaptist Genealogy Database. A linkage screen of 5,944 single nucleotide polymorphisms (SNPs) was performed using 12 informative sub-pedigrees with an affected-only 2-point and multipoint linkage analysis. Eleven SNPs produced 2-point LOD scores >2, suggestive of linkage. Multipoint linkage analyses, allowing for heterogeneity, detected significant lod scores on chromosomes 6 (HLOD = 4.50), 7 (LOD* = 3.11), and 14 (HLOD = 4.17), suggesting multiple new loci underlying SA.

Keywords

Amish; longevity; genetic epidemiology; family-based study; population isolate

INTRODUCTION

The most rapidly growing segment of the population in the developed world is individuals over age 65¹. According to the United States Census, the proportion of the population over age 65 will increase from 13% in 2010 to 20.2% in 2050 and the number of persons over age 65 is expected to increase from approximately 40.2 million in 2010 to an estimated 88.5 million in 2050¹. The number of persons over age 85 is expected to increase from 5.8 million in 2000 to 19.0 million in 2050. By 2050, the average life span worldwide is predicted to extend another 10 years.

Please address correspondence to: William K. Scott, PhD 1501 NW 10th Avenue BRB 414 (M-860) Miami FL 33136. Telephone: 305-243-2371 fax: 305-243-2523 bscott@med.miami.edu.

#Current Affiliation: Vanderbilt Epidemiology Center, Institute of Medicine and Public Health, Department of Obstetrics and Gynecology, Vanderbilt University, Nashville, TN, USA

There are multiple reasons why this increase in individuals over the age of 80 has been observed. These reasons include but are not limited to: advances in medical care by which individuals with significant chronic diseases are treated with more effective therapies and survive longer with their illnesses, an increase in awareness of good health habits and dietary nutrition, and finally an increase in those surviving past 80 due to more effective screening and treatment of diseases like hypertension and diabetes. However, increased longevity with significant disability will lead to increased health care costs. Thus it is important to identify factors that promote not just longevity, but also full function towards the end of life. A recent report from the National Institute on Aging Biology of Aging Summit 2 reinforced the need to expand phenotypic measures beyond measures of life span by adding measures of health quality and to consider multiple parameters underlying health span in future studies.

Several lines of evidence suggest that SA is influenced by both genetic factors and their interactions with behavioral and environmental influences. One key component of SA, increased longevity, is heritable³⁻⁵. In a study of the Lancaster County Pennsylvania Amish, age at death was estimated to have 25% heritability⁶. Similarly, studies of extreme longevity in samples of centenarians have determined that siblings of centenarians live longer than siblings of individuals who died in their early seventies⁷. Recent studies of the concordance rates in identical (MZ) and fraternal (DZ) twin pairs have suggested that longevity has about 20-25% heritability with little evidence for shared environmental factors^{8,9}.

In addition to chronological age, other twin studies have demonstrated that measures of physical function (another domain of SA) are also heritable, perhaps more strongly than age at death or longevity. These studies have shown that several indicators of upper and lower extremity function are about 50% heritable: hand-grip strength (40-50%)¹⁰⁻¹², lower extremity function (57%)¹³, balance performance (46%)¹⁴. These results suggest that using these measures to define a composite phenotype, “successful aging”, might facilitate identification of SA genes.

The value of conducting genetic studies in isolated populations such as the Amish has long been recognized¹⁵. This value derives from the recent expansion of the population (since the 1820s) from a small number of founders and isolation that restricts the introduction of additional genetic variation¹⁶. These factors often lead to large and stable pedigrees for genetic linkage analysis, while the increased level of linkage disequilibrium (LD) around trait-associated alleles potentially enhances detection of allelic association. In this report we conducted a SNP-based genome-wide linkage screen (GWLS) in a sample of older Amish individuals to examine linkage and association with a composite SA phenotype.

MATERIALS AND METHODS

Study population

This study is a part of the larger ongoing Collaborative Aging and Memory Project (CAMP), a multi-institutional prospective population-based study of aging and dementia in the Amish communities of Adams, Elkhart, and LaGrange counties in Indiana and Holmes county in Ohio, conducted from 2002-present. These communities were formed in several waves of migration in the eighteenth and nineteenth centuries¹⁷. The Amish originally emigrated from Europe to Pennsylvania in the 1700's and a further westward expansion of the Amish population occurred in the early 1800s, when a subset of the Pennsylvania population migrated to Ohio and Indiana. A second wave of migration from Switzerland arrived in the nineteenth century, eventually settling in Adams County, Indiana, in the 1850s. Present-day Amish in Holmes County, Ohio and Elkhart and LaGrange Counties, Indiana, are largely

descended from the first wave of westward immigration from Pennsylvania, while the Adams County, Indiana settlements are largely descended from the second wave of immigration that passed through Pennsylvania¹⁷. Both sets of communities, therefore, have a degree of shared history and ancestry with the Pennsylvania Amish communities. Written and informed consent was obtained for all participants and their legal guardians.

For the SA arm of the CAMP study, individuals over age 80 were identified through public directories published by individual Amish communities and referral from individuals already enrolled in the study. Once individuals were identified a door-to-door interview was performed to obtain a baseline examination. The only exclusion criterion for the SA arm of the study was cognitive impairment (individuals screening cognitively impaired were referred to the dementia arm of the study). The experiments reported here were conducted in the sample obtained in the first three and one half years of the CAMP study (January 2002-July 2006). Of the 249 cognitively intact individuals over age 80 contacted for the study by July 2006, 233 agreed to participate in the baseline examination (94% participation rate). Of these, 214 of these agreed to provide a blood sample (92% participation in the genetic study among those cognitively intact). The recruitment and ascertainment methods for CAMP have been previously described^{18, 19}.

Using an “all common paths” query of the Anabaptist Genealogy Database²⁰, all sampled individuals were placed in a 13-generation, 4998 person pedigree with a single common ancestral couple for 46 out of the 48 SA individuals in the earliest generation of the pedigree. There is no evidence of a more recent founder effect among the 48 SA individuals. The first individuals in this pedigree born in Indiana or Ohio were born in the 1820s, 4-5 generations before the oldest sampled individuals in this study.

Definition of Successful Aging

SA was defined according to tests and measurements taken at the time of baseline enrollment in the study. SA was defined considering functioning in all three domains described by Rowe and Kahn²¹. This trait was created in order to define a composite trait describing successful aging rather than examining individual components that are used to define SA; creating a composite trait also allows for the data to be analyzed with binary-trait genetic linkage and association analyses. The specific criteria we used are outlined in Table 1. The first requirement was survival to age 80. All individuals had to be cognitively intact (education-adjusted modified mini-mental state examination (3MS) > 86). SA individuals did not have significant depressive symptoms (geriatric depression scale (GDS) score < 6). Next, we considered whether individuals met standard cutoffs for “high function” on the self-reported measures of physical function: total scores of 0 or 1 on the activities of daily living (ADL) and instrumental ADLs (IADL) scales, indicating no assistance or partial assistance on only a single item needed; Nagi score of 0 or 1 indicating no difficulty or difficulty on only one item; Rosow-Breslau score of 3 or 4, indicating limitation on zero or just one item. Lower extremity function was considered by limiting SA to individuals scoring in the top 1/3 of the sample on the Established Populations for Epidemiologic Studies of the Elderly (EPESE) short physical performance battery summary score (>8 on a 12 point scale). Finally, individuals considered SA were satisfied or very satisfied with life. Of the 214 cognitively intact individuals over age 80 (40% male, mean age 83), 48 met criteria for SA (69% male, mean age 83) (Table 1). The remaining 166 individuals over age 80 (31.3% male and 83.8 mean age, Table 2) were cognitively intact but impaired on at least one other domain of SA.

Collection of clinical and demographic data

The baseline examination included collection of demographic characteristics and a screening of cognitive and physical function.

Participants underwent cognitive screening using a revision of the 3MS examination²², developed for use in the Cache County Memory Study²³. We established the 3MS cutoff point of <87 (after education adjustment) indicating potential cognitive impairment after examining the Receiver Operating Characteristic (ROC) curves in predicting subsequent dementia and prodromal AD in the Cache County Memory Study. This cutpoint was selected as having maximal utility in detection of dementia²⁴, optimizing the sensitivity of case detection (98.4%). Depressive symptoms were screened using the 15-item GDS²⁵, and life satisfaction was probed using a single question “Overall how satisfying is your life: very satisfying, satisfying, not so satisfying?”

Self-rated physical function was assessed by several well-established instruments. Basic self-care was assessed using a modified Katz ADL scale consisting of 8 items: bathing, dressing, grooming, toileting, continence, transferring, walking, and eating²⁶. Difficulty with musculoskeletal function was assessed by a 5-item scale based on the work of Nagi²⁷ used in the EPESE 28: pushing large objects, stooping or kneeling, carrying weights over 10 pounds, extending arms above shoulder level, and writing. Advanced daily living tasks were evaluated by a 3-item modified Rosow-Breslau scale²⁹ assessing difficulty performing heavy work around the house or farm, walking up and down a flight of stairs, and walking a half-mile without help. A final scale assessing more integrative functions IADL consisted of 6 of the 8 items suggested by Lawton and Brody³⁰: traveling, shopping, preparing meals, doing housework, managing medications, and handling money.

In addition to these self-reported measures of functioning, a short physical performance test developed for use in the EPESE was used to assess lower extremity function²⁸. The tasks include three measures of balance (side by side, semi-tandem, and tandem) for 10 seconds, the time required to sit and stand from a chair 5 times without using the hands and the time required to walk 10 feet.

Genotyping

In conjunction with the CAMP dementia study, we conducted a SNP-based genome-wide linkage screen (GWLS) of 672 Amish individuals using the 6,008 SNP Illumina Human Linkage Panel IVb. After quality control (QC) checks (removing SNPs not polymorphic in the sample, with poorly clustered genotyping, or significant departures ($p < 0.001$) from Hardy-Weinberg equilibrium (HWE) in the 125 most unrelated individuals) genotypes from 5,645 markers were available for analysis. SNP allele frequencies for use in linkage analysis were estimated from all 672 individuals. The present analysis focused on the 214 cognitively intact individuals over age 80 enrolled in the SA arm of the CAMP study. In addition to the 214 individuals over age 80, 11 full siblings younger than 80 were used (without phenotypes) to establish linkage phase as described below.

Statistical methods

We used the software program PedCut³¹ to cluster all 48 SA cases and 28 full siblings (17 over age 80, 11 younger) into 12 sub-pedigrees informative for linkage (e.g. at least 2 SA individuals in each pedigree). Pedigrees were specified to be no more than 24 “bits,” and were thus small enough to allow Merlin to generate exact 2-point and multipoint LOD scores using the Lander-Green algorithm³². A detailed description of the subpedigrees (including number of individuals, number of genotyped SA cases and number of genotyped non-SA siblings) is provided in Supplemental Table 1. The subpedigrees ranged in size from

17 to 45 individuals. The pedigrees were largely independent; only two pedigrees shared a total of 6 individuals – all untyped, connecting ancestors. Multipoint LOD scores allowing for heterogeneity (HLOD) across all pedigrees were calculated using affected-only dominant (trait allele frequency=0.001) and recessive (trait allele frequency=0.2) models. Multipoint non-parametric allele-sharing (LOD*) models were also generated. Marker allele frequencies were estimated from the data by the Merlin analysis software. Inter-marker distances were determined using the physical and genetic maps provided by Illumina for the Linkage IV panel. The non-SA siblings included in the study were only used to establish linkage phase in the subpedigrees (e.g. to assist in determining sharing of alleles identical-by-descent in SA individuals) but do not contribute to the linkage statistic in the affecteds-only model. Therefore, linkage analysis results only reflect allele sharing between the SA individuals in each subpedigree.

RESULTS

A summary of the clinical and demographic data for our study population is provided in Table 2A for all of the variables examined, stratified by SA status. The SA and non-SA comparison groups have similar mean ages, but more men are SA and a higher proportion of the non-SA comparison group than SA are from Indiana (Table 2A). A summary of the twelve sub-pedigrees created is provided on Table 2B. All pedigrees represent individuals from Elkhart and Holmes counties, as only non-SA individuals were available from Adams County. On average there were four SA individuals per pedigree and two sibs.

Examination of the 2-point LOD scores showed that 11 SNPs produced suggestive evidence (LOD >2) for linkage (Table 3), although none of these SNPs had significant LOD scores >3. Two chromosomes generated multipoint LOD scores allowing for heterogeneity (HLOD) >3 under the dominant HLOD model, and one chromosome generated a non-parametric LOD* >3 (Figure 1): chromosome 6 with a dominant model (maximum HLOD = 4.49 at rs1409014 (54 megabases (Mb) based on human genome build 36/hg18) which had a total of 143 genotyped markers under the linkage peak (Figure 2A), 1-LOD-down support interval 52-65 Mb), chromosome 7 with a nonparametric model (maximum LOD* = 3.11 at rs517258 (64.18 Mb), 1-LOD-down support interval: 49 – 75 Mb)(Figure 3C) which included a total of 22 genotyped markers under the linkage peak, and chromosome 14 with a nonparametric model (maximum HLOD = 3.28 and maximum LOD* = 4.17 at rs764602 (48 Mb), 1-LOD-down support interval: 36 - 56 Mb, based on HLOD; 42 – 53 Mb based on LOD*) which had a total of 53 genotyped markers under the linkage peak (Figure 4).

These results would indicate that additional association-based fine-mapping is necessary to identify high-priority candidate genes for further study.

DISCUSSION

We performed a GWLS looking for chromosome regions linked to SA in the Amish and observed strong evidence for linkage on chromosomes 6, 7, and 14. The regions on chromosomes 6 and 14 are novel, while the region on chromosome 7 is 6 Mb away from a SNP (rs2371208 position at 81,982,510 base pairs) associated with age at death in the Framingham Study³³. Further examination of the literature demonstrated that these three linkage peaks are in the vicinity of several potential longevity and aging candidate genes including bone morphogenic protein 5 (*BMP5*) located on 6p12.1, and bone morphogenic protein 4 (*BMP4*) located on chromosome 14q22-q23. These regions will be further examined through fine mapping to determine if any of the linkage peaks can be explained by an association with these candidate genes.

Chromosome 6 notably had a minor linkage peak covering the fragile site 6 region on chromosome 6 that includes the parkin gene (*PARK2*). The linkage peak had a LOD score ranging between 1.10-2.09 within the 161.68 to 170.62 Mb region of the chromosome with a recessive model. *PARK2* is a longevity and aging candidate gene and has been associated with several aging-related phenotypes including Parkinson disease, general neuron degeneration, and several forms of cancer. *PARK2* has been described as a tumor suppressor gene, consistent with the theory that preserved DNA repair and tumor suppression activity are essential mechanisms for cancer-free longevity and successful aging^{34, 35}.

Previous genetic studies of SA have focused on three primary categories of genes selected primarily for their relevance to aging associated pathophysiological processes or that are known to be activated/deactivated during the aging process³⁶. These categories include: 1) genes involved in the maintenance of cholesterol, lipid, and/or lipoproteins³⁷⁻⁴¹; 2) genes that influence inflammation and immune response, particularly cytokines many of which also influence cell cycling, growing, motility, and signaling⁴²⁻⁴⁶; 3) genes involved in drug metabolism⁴⁷. To date few of the candidate genes associated with SA have been successfully replicated, suggesting that the true causal variants are yet to be discovered. For a comprehensive description of previously published associations pertaining to SA please refer to Glatt et al³⁶. None of the candidate genes that have been previously established were directly located under one of our three linkage peaks, suggesting that our findings are novel.

In our study we observed a higher rate of SA in males relative to females. These results have not been observed consistently in previous literature, and contrast with the prior study of longevity in the Pennsylvania Amish, which reported no effect of sex on age at death or heritability of lifespan (Mitchell et al., 2001). Consistent with our results are studies by Seeman et al (1994) who reported in the MacArthur Studies of Successful Aging that males were more likely to have better physical performance at baseline in a cohort of 70-79 year olds with high function²⁸. This would suggest that our observations may reflect that at baseline SA males had better physical function. However, the studies by McLaughlin et al 2009 of adults aged 65 and older did not observe an inverse association between SA and gender⁴⁸. One explanation for these differences could be the specific definition of SA used. We attempted to use as comprehensive a definition as possible, integrating factors pertaining to physical and cognitive function, social engagement, and health whereas other studies would more often use only one or two of these components. Another possible explanation for the larger number of SA males relative to females could be the effect of marital status on successful aging⁴⁹. In our sample, a greater proportion of men were successfully aged (52% of men vs. 17% of women) and currently married (78% of men vs. 45% of women). While more women were widowed (48%) than men (22%), all men in the sample were either currently or previously married, while 7% of the women were never married. However, the relationship between gender, marriage, and SA has not been consistent across studies, with the majority finding no association⁵⁰. Further work is necessary to fully understand the significance of these findings.

Although these findings are interesting, the SA linkage regions detected are broad with large numbers of candidate genes within each. The regions identified will be further examined through fine mapping to determine if any of the linkage peaks can be explained by segregation of a functional variant in any biologically relevant genes with SA in a larger sample of Amish that includes these pedigrees.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Genome-wide multipoint linkage results for the successful aging phenotype

LOD scores allowing for heterogeneity (HLOD) for affected-only dominant (Figure 1A) and recessive (Figure 1B) models and the non-parametric allele-sharing LOD* score (Figure 1C) are presented. Three chromosomes (6, 7, and 14) produce LOD scores > 3. The X-axis has a genetic map in units of centimorgans and the Y-axis shows the LOD or HLOD score.

Figure 2. Linkage results for chromosome 6

HLOD for the dominant multipoint model (Figure 2A) recessive HLOD (Figure 2B), and nonparametric multipoint LOD* (Figure 2C) are presented for chromosomes 6. The X-axis has a genetic map in units of centimorgans and the Y-axis shows the LOD or HLOD score.

Figure 3. Linkage results for chromosome 7

HLOD for the dominant multipoint model (Figure 3A) recessive HLOD (Figure 3B), and nonparametric multipoint LOD* (Figure 3C) are presented for chromosomes 7. The X-axis has a genetic map in units of centimorgans and the Y-axis shows the LOD or HLOD score.

Figure 4. Linkage results for chromosome 14

HLOD for the dominant multipoint model (Figure 4A) recessive HLOD (Figure 4B), and nonparametric multipoint LOD* (Figure 4C) are presented for chromosomes 14. The X-axis has a genetic map in units of centimorgans and the Y-axis shows the LOD or HLOD score.

Table 1

Criteria to identify “successfully aged” individuals

Criteria	Test/Cut-off
Age	>80
Cognitively intact	3MS >86 (education adjusted)
No significant depressive symptoms	GDS <6
“High function” self-reported measures of physical function	0 or 1 ADL and IADL scales
	0 or 1 Nagi score
	3 or 4 Rosow-Breslau score
Lower extremity function	>8 (12 pt scale) EPESE
Satisfied or very satisfied with life	Questionnaire

Table 2

Demographic and clinical characteristics of study population

A.

Variable	SA (N=48)	Non-SA Comparison Group (N=166)
Gender (% Female)	31.2%	68.7%
Age (Mean(SD1))	82.6 (2.9)	83.8 (3.8)
County (%)	0.0% Adams; 43.8% Elkhart; 56.2% Holmes	18.1% Adams; 53.6% Elkhart; 28.3% Holmes
3MS (Median[Range])	93 [87-103]	91 [58 [*] -101]
GDS (Median[Range])	1 [0-4]	3 [0-14]
ADL (Median[Range])	0 [0-1]	1 [0-9]
IADL (Median[Range])	0 [0-1]	1 [0-10]
Nagi (Median[Range])	0 [0-1]	2 [0-5]
Rosow-Breslau (Median[Range])	3 [3-4]	4 [3-6]
EPESE (Median[Range])	10 [6-12]	7 [0-12]
Grip strength (dominant hand) (Median[Range])	23.5 [8-38]	19 [4-45]
DSSI Subscales		
Social interaction (Median[Range])	6 [2-10]	6 [2-11]
Social network (Median[IQR])	7 [6-9.5]	6 [5-8]
Instrumental support (Median[Range])	12 [8-12]	12 [7-12]
Life satisfaction	100% very satisfied or satisfied	95.8% very satisfied or satisfied

B.

Pedigree	# Individuals ^I	# SA	# Siblings	# Elkhart	# Holmes
1	30	3	6	8	1
2	27	3	5	8	0
3	26	4	4	5	3
4	17	5	2	0	7
5	25	5	2	1	6
6	22	5	1	0	6
7	30	4	2	6	0
8	35	5	1	0	6
9	34	3	3	1	5
10	31	3	2	0	5
11	34	4	0	3	1
12	45	4	0	4	0

^{*} all individuals with 3MS < 86 were determined to be cognitively normal in detailed neuropsychological evaluation, and are thus included in the sample.

^I Total number of individuals (sampled and unsampled) in the pedigree.

Table 3

2 point lod scores > 2 for dominant, recessive, and nonparametric models

Chromosome	Position (cM)	rs#	2pt LOD Score ¹		
			Dom	Rec	Non
3	0.961	rs1516350	1.27	0.41	2.38
3	70.891	rs7061	2.04	0.95	1.23
5	108.89	rs1981846	2.12	0.58	0.10
6	179.41	rs3002430	1.21	0.52	2.09
7	126.46	rs1320393	1.77	1.05	2.55
8	145.56	rs760327	2.08	0.58	0.37
14	42.89	rs735543	1.62	1.04	2.27
15	109.53	rs7176568	1.05	0.40	2.92
16	41.96	rs227761	1.26	0.93	2.18
21	76.23	rs2968	0.65	0.08	2.12
X	151.18	rs17407	2.08	0.83	2.20

¹ LOD scores are presented for dominant (Dom), recessive (Rec), and nonparametric (Non) 2 point linkage analyses.