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SHORT REPORT

AKT1 fails to replicate as a longevity-associated gene in Danish and German nonagenarians and centenarians

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In addition to *APOE* and *FOXO3*, *AKT1* has recently been suggested as a third consistent longevity gene, with variants in *AKT1* found to be associated with human lifespan in two previous studies. Here, we evaluated *AKT1* as a longevity-associated gene across populations by attempting to replicate the previously identified variant rs3803304 as well as by analyzing six additional *AKT1* single-nucleotide polymorphisms, thus capturing more of the common variation in the gene. The study population was 2996 long-lived individuals (nonagenarians and centenarians) and 1840 younger controls of Danish and German ancestry. None of the seven SNPs tested were significantly associated with longevity in either a case–control or a longitudinal setting, although a supportive nominal indication of a disadvantageous effect of rs3803304 was found in a restricted group of Danish centenarian men. Overall, our results do not support *AKT1* as a universal longevity-associated gene.
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INTRODUCTION

Despite the fact that approximately 25% of variation in human lifespan is attributable to genetic factors,[1] polymorphisms in only a few candidate genes have so far been consistently found to be associated with longevity.

The first to be discovered, and also the most prominent candidate, is the apolipoprotein E (*APOE*) gene. The *APOE* ε4 haplotype has repeatedly been reported as an age-related mortality factor in numerous populations,[2] and recently, the *APOE* ε4 allele was confirmed to negatively affect survival into old age in two genome-wide association studies (GWASs).[3,4]

The forkhead box O3 (*FOXO3*) gene, which encodes a transcription factor involved in the insulin/insulin-like growth factor 1 (IGF1) pathway, has been presented as another well-established candidate. The insulin/IGF1 pathway has repeatedly been implicated in longevity in various model organisms,[5] as well as in humans, where the *FOXO3* association has been demonstrated in a number of populations of diverse ethnic origin.[6–11]

The newest candidate that has been put forward as a third consistent longevity gene is *AKT1*, which is also part of the insulin/IGF1 pathway. Single-nucleotide polymorphisms (SNPs) in this gene have been associated with longevity in two studies: in 2009, Pawlikowska *et al*[9] described the variant rs3803304 as influencing human lifespan in three different cohorts consisting of participants from the Study of Osteoporotic Fractures and the Cardiovascular Health Study, as well as Ashkenazi Jewish Centenarians, whereas the SNP rs2498804 was detected in a recent GWAS of Dutch nonagenarians from the Leiden Longevity Study.[3]

In this study, we evaluated *AKT1* as a longevity-associated gene across populations by analyzing rs3803304 in a large sample of Danish and German long-lived individuals with the aim of replicating the previous finding. Further, we included six additional *AKT1* SNPs in order to cover more of the common variation within the gene.

MATERIALS AND METHODS

Study populations

Danish study. The Danish study population comprised 1383 participants from The Danish 1905 Birth Cohort Study, described in details elsewhere.[12] Briefly, the study was initiated in 1998, when participants were 92–93 years of age and follow-up studies of surviving participants were carried out in 2000, 2003 and 2005, when participants reached 100 years of age.

Genotyping of the candidate SNP rs3803304 was performed in all 1383 participants, of which 190 individuals (women proportion of 80%) lived to be at least 100 years of age, whereas genotype information on the six additional SNPs was available for 1089 individuals as previously described.[13] A total of 143 of the 1089 individuals (women proportion of 79%) lived to be at least 100 years of age.

Vital status was followed until death or until 1 January 2011, resulting in a mean follow-up time for survivors of 12.4 years (range: 12.2–12.6). Information on survival status was retrieved from the Danish Central Population Register, which is continuously updated.[14]

A group of 736 younger control individuals (mean age 50.6 years) were randomly selected from the population-based study of middle-aged Danish twins,[15] which was initiated in 1998 with the random selection of 2640 intact twin pairs born from 1931 to 1952. Here, only one twin from each pair was included.

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German study. The German study population included 865 nonagenarians and 748 centenarians (age range 95–110 years, mean age 98.9 years, women proportion of 73.0% in all cases and 80.5% in centenarians). The control individuals were 1104 younger unrelated individuals (age range 60–75 years, mean age 67.2 years). The participants were recruited from different geographic regions of Germany and were all of German ancestry.[16]

Study approvals were received from the Danish National Committee on Biomedical Research Ethics and the Ethics Committee of the University Hospital Schleswig-Holstein, respectively.

Genotyping

The primary SNP for association testing was rs3803304. In addition to this, six supplementary variants, which had previously been selected for coverage of the common genetic variation in *AKT1* using data from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/index.html.en), were included.

Genotyping of rs3803304 in the Danish study population and of rs3803304, rs2494731, rs2494732, rs2498796, rs2494738 and rs1130214 in the German study population was performed using predesigned TaqMan SNP genotyping assays (Applied Biosystems, Pleasonton, CA, USA). The SNP rs2494748 could not be genotyped in the German population because of technical difficulties.

Genotyping of rs2494731, rs2494732, rs2498796, rs2494738, rs2494748 and rs1130214 in the Danish study population was part of a previous study and was performed using the Illumina GoldenGate Technology (Illumina Inc., San Diego, CA, USA) as described by Soerensen *et al.*[13]

Statistical analysis

All analyses were carried out using the statistical software Stata (Stata version 11.2; Stata Corporation, College Station, TX, USA). Logistic regression was used to assess the association between genotype and longevity with dose of minor allele coded as 0, 1 and 2. An additive model was applied to all of the seven *AKT1* SNPs; however, for rs3803304, the potential association with longevity was further explored using a genotypic, dominant and recessive model.

Mortality analysis in the Danish 1905 Birth Cohort was performed on all of the seven *AKT1* SNPs using a left-truncated Cox proportional hazards model to adjust for late entry into the data set according to age.

To account for the multiple testing of seven SNPs, a Bonferroni-corrected significance level of 0.007 (0.05/7) was applied.

Power calculations were performed using the freely available software Quanto (Quanto version 1.2.4, http://hydra.usc.edu/gxe/), assuming a 1 in 20 chance of becoming 92–93 years old, a 1 in 400 chance of becoming a centenarian and a Bonferroni-corrected significance level of 0.007.

RESULTS

Characteristics of the Danish and German study populations are summarized in Table 1.

All genotyped SNPs were in Hardy–Weinberg equilibrium in both control groups, except for rs2494738, which displayed an excess of heterozygotes in the Danish control group ($P = 0.02$).

The set of tested *AKT1* SNPs included the candidate variant rs3803304 and six additional SNPs, of which three are tagging SNPs (rs2494731, rs2494738 and rs1130214). The remaining SNPs (rs2494732, rs2498796 and rs2494748) are distributed evenly along the gene and were added to enable a more comprehensive coverage of the gene.

Case–control analysis of all SNPs was performed for the Danish and German population separately, as well as for the combined study population. The substantial number of individuals in the combined study population allowed for the detection of effects as small as OR = 1.2 (for rs2494738, OR = 1.3 due to a smaller minor allele frequency) with a power of at least 87%. Applying an additive model, none of the seven SNPs demonstrated a significant association with longevity in either of the populations (Table 2).

Table 1 Characteristics of the study populations

	Danish study population		*German study population*	
	Cases	*Controls*	*Cases*	*Controls*
Number, LLI	1383/1089[a]	736	1613	1104
Women, LLI (%)	70.7/71.3[a]	49.6	73.0	74.4
Age at intake, LLI	92–93	46–55	95–110	60–75
Number, centenarians	190/143[a]	—	748	—
Women, centenarians (%)	80.0/79.0[a]	—	80.5	—

Abbreviation: LLI, long-lived individuals.
[a]The number to the left applies to rs3803304.

As rs3803304 was previously found to be associated with longevity,[9] it was investigated more thoroughly using a genotypic, dominant and recessive model. No significant associations were found with any of the models applied (data not shown).

To scrutinize for potential effects of variation in *AKT1* on extreme survival, the analysis was next restricted to include centenarians only (Table 3), resulting in the ability to detect effects of size OR = 1.25 (for rs2494738, OR = 1.4) in the combined study population with a power of at least 80%. One nominally significant indication ($P = 0.03$) of a disadvantageous effect of rs3803304 on longevity was found in Danish men, and this indication was supported when applying genotypic and dominant models ($P = 0.02$ and 0.01, respectively).

In addition to applying a cross-sectional approach, the effects of the seven SNPs on survival during old age were investigated in the Danish 1905 Birth Cohort. Regardless of the genetic model applied, no associations with survival during the follow-up of >12 years were found. Restriction of analysis to include centenarians only did not change this result (data not shown).

DISCUSSION

AKT1 has recently been suggested as a third universal longevity gene, together with *APOE* and *FOXO3*. To further elucidate this potential role of *AKT1*, case–control studies involving Danish and German long-lived individuals and younger controls were performed. In addition to the previously described candidate variation rs3803304,[9] our study was expanded to include a number of additional SNPs to ensure better coverage of the known allelic variance in *AKT1*.

The main finding of this study was a lack of formal replication of the association of rs3803304 with longevity as previously found in three independent populations.[9] In the initial report by Pawlikowska *et al*,[9] they found that the association with longevity appeared driven by the minor allele homozygous genotype, as subjects homozygous for the minor allele were underrepresented among long-lived cases. In line with this, restricting the analysis to centenarians revealed a trend of supportive evidence in Danish men, with the same direction of effect as identified before.[9] It should be noted, though, that the sample size of the refined group of centenarian men was very limited ($n = 37$), and when applying a Bonferroni correction for multiple statistical testing, the association did not remain significant.

To facilitate a more exhaustive exploration of the possible relevance of *AKT1* variations in longevity, six additional SNPs available from a previous study[13] were included and tested in both the Danish and German study population. Of note, one of these, rs2494731, is in perfect linkage disequilibrium with the *AKT1* SNP rs2498804, which was recently found to be associated with lifespan in a population of Dutch nonagenarians.[3] Nevertheless, we did not replicate an association with longevity, whether applying a cross-sectional

Table 2 Association analysis of *AKT1* variations in long-lived individuals

		Danish population				*German population*				*Combined population*			
SNP	*Sex*	*MAF Cases/controls*	*OR*	*95% CI*	P	*MAF Cases/controls*	*OR*	*95% CI*	P	*MAF Cases/controls*	*OR*	*95% CI*	P
rs3803304	All	0.25/0.27	0.93	0.80–1.08	0.320	0.27/0.26	1.03	0.91–1.17	0.610	0.26/0.26	0.99	0.90–1.09	0.784
	Men	0.25/0.28	0.87	0.69–1.09	0.229	0.28/0.26	1.09	0.84–1.41	0.515	0.26/0.27	0.96	0.81–1.14	0.673
	Women	0.25/0.26	0.97	0.80–1.18	0.779	0.26/0.26	1.02	0.88–1.18	0.831	0.26/0.26	1.00	0.89–1.12	0.967
rs2494731	All	0.34/0.34	1.00	0.87–1.17	0.953	0.36/0.35	1.05	0.94–1.17	0.393	0.35/0.34	1.03	0.94–1.13	0.503
	Men	0.32/0.35	0.87	0.69–1.11	0.266	0.36/0.33	1.12	0.90–1.39	0.296	0.34/0.34	1.01	0.86–1.18	0.893
	Women	0.34/0.32	1.10	0.91–1.34	0.324	0.36/0.35	1.02	0.90–1.17	0.714	0.35/0.34	1.04	0.93–1.16	0.472
rs2494732	All	0.43/0.43	0.99	0.86–1.14	0.879	0.46/0.44	1.06	0.95–1.18	0.296	0.45/0.44	1.04	0.95–1.13	0.406
	Men	0.39/0.43	0.81	0.64–1.02	0.073	0.46/0.42	1.13	0.92–1.39	0.253	0.43/0.43	0.99	0.85–1.15	0.928
	Women	0.45/0.42	1.12	0.94–1.34	0.214	0.46/0.45	1.03	0.91–1.17	0.594	0.45/0.44	1.06	0.95–1.17	0.287
rs2498796	All	0.30/0.32	0.92	0.79–1.07	0.292	0.32/0.31	1.03	0.92–1.16	0.575	0.31/0.32	0.99	0.90–1.08	0.788
	Men	0.29/0.34	0.80	0.63–1.02	0.075	0.33/0.31	1.10	0.88–1.37	0.411	0.31/0.32	0.96	0.81–1.12	0.573
	Women	0.30/0.30	1.01	0.83–1.23	0.912	0.32/0.32	1.01	0.88–1.16	0.877	0.31/0.31	1.00	0.90–1.12	0.949
rs2494738	All	0.12/0.12	0.99	0.79–1.23	0.912	0.08/0.07	1.06	0.87–1.29	0.581	0.10/0.09	1.03	0.89–1.19	0.718
	Men	0.12/0.13	0.92	0.66–1.29	0.645	0.07/0.07	0.97	0.66–1.44	0.893	0.09/0.11	0.87	0.68–1.12	0.275
	Women	0.12/0.12	1.04	0.77–1.39	0.799	0.08/0.07	1.09	0.86–1.38	0.473	0.10/0.09	1.12	0.93–1.34	0.221
rs2494748	All	0.34/0.35	0.95	0.82–1.09	0.466								
	Men	0.35/0.36	0.96	0.77–1.20	0.728								
	Women	0.33/0.35	0.94	0.78–1.13	0.510								
rs1130214	All	0.31/0.31	1.05	0.91–1.23	0.498	0.30/0.31	0.94	0.83–1.05	0.258	0.30/0.31	0.97	0.89–1.07	0.544
	Men	0.32/0.31	1.06	0.83–1.34	0.653	0.28/0.31	0.86	0.69–1.09	0.214	0.30/0.31	0.94	0.79–1.10	0.429
	Women	0.31/0.30	1.05	0.86–1.28	0.612	0.31/0.31	0.96	0.84–1.10	0.557	0.31/0.31	0.99	0.89–1.10	0.842

Abbreviations: CI, confidence interval; MAF, minor allele frequency; OR, odds ratio (applying an additive model).
Cases are long-lived individuals with an age of >92 years at intake in the Danish population and >95 years in the German population. *P*-values are uncorrected.

Table 3 Association analysis of *AKT1* variations in centenarians

		Danish population				*German population*				*Combined population*			
SNP	*Sex*	*MAF Cases/controls*	*OR*	*95% CI*	P	*MAF Cases/controls*	*OR*	*95% CI*	P	*MAF Cases/controls*	*OR*	*95% CI*	P
rs3803304	All	0.23/0.27	0.81	0.61–1.06	0.126	0.26/0.26	1.00	0.86–1.17	0.993	0.26/0.26	0.95	0.83–1.08	0.443
	Men	0.16/0.28	0.49	0.26–0.94	0.033	0.26/0.26	1.00	0.70–1.41	0.980	0.25/0.27	0.84	0.63–1.12	0.230
	Women	0.24/0.26	0.92	0.67–1.25	0.590	0.26/0.26	1.00	0.84–1.19	0.982	0.26/0.26	0.98	0.85–1.14	0.821
rs2494731	All	0.32/0.34	0.97	0.72–1.30	0.821	0.35/0.35	1.01	0.88–1.16	0.871	0.34/0.34	1.01	0.90–1.15	0.837
	Men	0.29/0.35	0.75	0.39–1.42	0.373	0.33/0.33	0.98	0.73–1.31	0.869	0.32/0.34	0.91	0.70–1.18	0.479
	Women	0.33/0.32	1.04	0.75–1.44	0.826	0.36/0.35	1.02	0.87–1.20	0.786	0.35/0.34	1.05	0.91–1.20	0.534
rs2494732	All	0.43/0.43	1.01	0.76–1.33	0.957	0.45/0.44	1.03	0.90–1.17	0.684	0.44/0.44	1.03	0.92–1.16	0.580
	Men	0.35/0.43	0.69	0.38–1.26	0.224	0.44/0.42	1.05	0.79–1.38	0.741	0.40/0.43	0.97	0.77–1.24	0.830
	Women	0.45/0.42	1.12	0.82–1.54	0.468	0.45/0.45	1.02	0.88–1.19	0.776	0.45/0.44	1.05	0.92–1.20	0.454
rs2498796	All	0.28/0.32	0.85	0.63–1.14	0.276	0.32/0.32	0.99	0.86–1.15	0.931	0.31/0.32	0.97	0.85–1.10	0.602
	Men	0.27/0.34	0.69	0.37–1.30	0.253	0.31/0.31	1.01	0.75–1.35	0.957	0.30/0.32	0.90	0.70–1.17	0.446
	Women	0.28/0.30	0.90	0.64–1.26	0.545	0.32/0.32	0.99	0.84–1.16	0.898	0.31/0.31	0.99	0.86–1.14	0.865
rs2494738	All	0.13/0.12	1.14	0.76–1.72	0.525	0.07/0.07	1.00	0.78–1.29	0.990	0.10/0.09	0.91	0.74–1.12	0.368
	Men	0.20/0.13	1.68	0.84–3.38	0.142	0.03/0.07	0.88	0.51–1.54	0.663	0.11/0.11	0.82	0.54–1.24	0.346
	Women	0.11/0.12	0.95	0.58–1.57	0.853	0.08/0.07	1.03	0.78–1.37	0.811	0.10/0.09	0.94	0.74–1.20	0.631
rs2494748	All	0.37/0.35	1.11	0.85–1.45	0.443								
	Men	0.41/0.36	1.23	0.71–2.13	0.457								
	Women	0.37/0.35	1.08	0.79–1.46	0.643								
rs1130214	All	0.33/0.31	1.13	0.84–1.51	0.431	0.29/0.31	0.95	0.83–1.10	0.502	0.31/0.31	0.98	0.87–1.11	0.754
	Men	0.30/0.31	0.97	0.54–1.76	0.926	0.28/0.31	0.88	0.64–1.20	0.416	0.31/0.31	0.89	0.69–1.16	0.389
	Women	0.33/0.30	1.18	0.84–1.66	0.334	0.31/0.31	0.97	0.83–1.14	0.736	0.31/0.31	1.01	0.88–1.16	0.907

Abbreviations: CI, confidence interval; MAF, minor allele frequency; OR, odds ratio (applying an additive model).
Cases are restricted to individuals living to an age of ≥100 years. *P*-values are uncorrected.

approach or taking advantage of the longitudinal data with >12 years of follow-up available for the Danish nonagenarian sample.

In conclusion, despite a substantial power, we did not confirm *AKT1* as a longevity-associated gene in populations of Danish and German nonagenarians and centenarians. This finding does not necessarily rule out a potential relevance of *AKT1* in longevity. First, population genetic differences may explain the lack of replication, although this explanation is not very likely, because all analyzed populations were of European ancestry. Second, because our coverage of allelic variation in *AKT1* is probably not complete, other variations that are not tagged by the included SNPs may be associated with survival differences. However, all in all, our results do suggest that *AKT1* is not a universally acting longevity gene.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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