



Association of *SIRT6* Gene Polymorphisms with Human Longevity

You LI, Jian QIN, Xiao WEI, Guiyun LIANG, Liwei SHI, Meiyu JIANG, Tianlong XIA, Xue LIANG, *Min HE, *Zhiyong ZHANG

Dept. of Occupational and Environmental Health, School of Public Health, Guangxi Medical University, Nanning 530021, Guangxi, China

*Corresponding Authors: Email: rpazz@163.com, m_h_m868@sina.com

(Received 04 Dec 2015; accepted 20 Apr 2016)

Abstract

Background: We aimed to identify the role of *SIRT6* gene polymorphism rs350846 in human longevity.

Methods: *SIRT6* C/G genotypes were determined using Taqman SNP Genotyping Assays in 169 long-lived inhabitants (LG group aged 90-110 yr), 158 healthy internal controls (internal control group; aged 26-82 yr) and 176 healthy external controls (external control group; aged 20-82 yr) without a family history of exceptional longevity. Statistical analysis was conducted using SPSS 16.0.

Results: BMI and TG level were lower in the longevity than in the two control groups, while serum LDL-c and HDL-c and SBP and DBP levels in long-lived individuals were higher than in the two control groups ($P < 0.01$). The waist circumference was obviously different ($P = 0.001$) among the three groups, with the maximum observed in the external group. No statistically significant differences of the gender FBG and TC were seen in long-lived individuals than in the two control groups. Significant genotype differences existed among the different groups except for the longevity and internal control group. The frequency of the minor allele-C was 0.319. The minor allele frequency of rs350846 in *SIRT6* was much higher in the external control than in the other groups. BMI, SBP and HDL-c displayed significant effect on longevity.

Conclusion: The C allele of rs350846 in *SIRT6* gene, CC and CG genotypes as well as BMI, systolic pressure and HDL-c are associated with longevity. Further studies are needed to validate our results.

Keywords: *SIRT6*, Polymorphisms, Longevity

Introduction

Longevity and ageing are a complex and multifactorial process controlled by both environmental and genetic factors (1). Studies reported hundreds of genetic variants that played a role in extension of lifespan. Population studies of longevity in twins suggested that the genes contributed to 15% to 30% of the heritability to the human lifespan (2, 3). However, the mechanisms underlying the role of genetic factors in human longevity and successful ageing are unknown. Over the last decade, sirtuins attracted significant interests in ageing studies. The role of sirtuins has been stu-

died in NAD-dependent lysine deacetylation and a related mono-ADP-ribosylation reaction (4). The histone deacetylase silent information regulator (*Sir2*) is a longevity control gene (5, 6). *Sir2* activity depends on the levels of nicotinamide adenine dinucleotide (NAD) (7). Further, *Sir2* increases longevity by suppressing ribosomal DNA homologous recombination and calorie restriction (6).

Mammalian genomes encode seven *Sir2* homologs (*SIRT1-7*). The human *SIRT6* gene is located on the minus strand of chromosome 19p13.3 and

encodes a 355-amino-acid protein (8). It regulates the expression of several stress-responsive and metabolism related genes at the molecular level (9, 10). In addition, it plays an important biological role in heart disease, diabetes, obesity, inflammation and cancer. Therefore, *SIRT6* activity is crucial in many chronic diseases and healthy longevity (11). *SIRT6* knockout mice manifested kyphosis, cachexia, greying of fur, decreased bone mineral density, reduced weight and subcutaneous fat, hypoglycemia, and chronic inflammation. Their lifespan was reduced to about one month (12). *SIRT6* plays an important role in maintaining normal retinal function and its deficiency causes major chromatin changes in the retina of mice (13). *SIRT6* is a critical regulator of endothelial senescence. Oxidative stress-induced down regulation of *SIRT6* probably mediates the pathogenesis of diabetic retinopathy (14).

However, *SIRT6* overexpression extended the lifespan of male mice by 15%, related to decrease in serum insulin-like growth factor (IGF-1s) and increase in IGF-binding protein 1 (15). Transgenic mice overexpressing *SIRT6* are protected from metabolic disorders associated with triglyceride and accumulation of serum cholesterol and decreased glucose tolerance (16). Therefore, *SIRT6* not only extends lifespan but also improves health quality, which is a major anti-ageing breakthrough (17). Ageing is a normal process in any living mammal. *SIRT6* is related to longevity and ageing in animal studies, with no reported connection between human *SIRT6* and longevity.

The populations of Bama County located in the Hongshuihe River Basin, Guangxi Province, have prolonged lifespan (18, 19). Populations in Hongshuihe River Basin represent the typical groups for studying longevity in Guangxi, China (20, 21). Although studies investigated the relationships between longevity, environmental and genetic factors, the specific mechanisms are still not clear.

In this study, we studied the rs350846 polymorphism of *SIRT6* in the longevity group and the controls in an effort to unravel the genetic basis of longevity.

Methods

Study population

Longevity was defined as survival to age 90 yr or more. Based on the sixth national population census of China in 2010, we selected the Bama County as the sample site, in which there was a higher proportion of the longevous people than other counties. There total of 169 unrelated long-living individuals who satisfied the conditions (43 males and 126 females aged 90-110 yr, the mean ages 94.90 ± 4.73) were randomly selected as longevity group, and 158 unrelated participants (59 males and 99 females aged 26-82 yr, the mean ages 70.59 ± 11.09) from Bama also were enrolled as the internal control group (environmentally matched). The external control group consisted of 176 subjects (56 males and 120 females aged 20-82 yr, the mean ages 49.20 ± 14.50) from Nandan County, which is approximately 160 km away from Bama County (environmentally unmatched). No long-lived individuals existed in both internal and external control groups. Long-lived individuals aware selected based on at least two sibling meetings according to the following inclusion criteria: 1) age 90 or above; 2) subjects with one or more living brother or sister who satisfied the first criterion. Age of the participants was authenticated officially through a certificate of identification or household register and the accounts of their offspring and other socio-demographic events. All of the participants were essentially healthy with no obvious disease or chronic illness. The study was approved by the ethics committee of Guangxi Medical University. Written informed consents were obtained from all participants. The study complied with the tenets of the Declaration of Helsinki.

Anthropometric variables including height and weight were measured in all participants. Body mass index (BMI) was equal to weight (kg)/height (m)². Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were determined using a standard mercury sphygmomanometer. The levels of fasting blood glucose (FBG),

serum total triglyceride (TG), serum total cholesterol (TC), HDL-cholesterol (HDL-c) and LDL-cholesterol (LDL-c) in serum samples were de-

termined by standard enzymatic methods using commercially available kits. The demographic characteristics show in Table 1.

Table 1: The general characteristics between the longevity and two control groups

Index	Longevity (n=169)	Internal control (n=158)	External control (n=176)	F(χ^2)	P
Age (year)	94.90±4.73	70.59±11.09	49.20±14.50	752.425	0.000
Gender (m/f)	43/126	59/99	56/120	5.386	0.068
BMI (kg/m ²)	18.85±2.93	19.70±2.63	22.47±3.33	60.567	0.000
WC (cm)	74.32±8.42	73.47±7.37	78.95±9.58	20.279	0.000
SBP (mmHg)	146.74±23.75	138.56±24.66	119.18±20.02	66.465	0.000
DBP (mmHg)	80.62±13.38	78.92±12.29	75.85±11.85	6.424	0.002
FBG (mmol/L)	5.96±1.95	5.89±1.81	5.85±1.56	0.165	0.848
TC (mmol/L)	4.81±1.04	4.64±0.93	4.80±0.97	1.534	0.217
TG (mmol/L)	1.46±0.93	1.60±1.17	1.93±0.75	11.293	0.000
HDL-C (mmol/L)	1.53±0.75	1.29±0.66	1.08±0.25	24.641	0.000
LDL-C (mmol/L)	2.71±0.71	2.64±0.77	2.48±0.63	5.034	0.007

Note: Values are given as means±SD. BMI=body mass index, WC=waist circumference, SBP=systolic blood pressure, DBP=diastolic blood pressure, FBG=fasting blood glucose, TG=serum total triglyceride, TC=serum total cholesterol, HDL-c=high-density lipoprotein cholesterol, LDL-c =low-density lipoprotein cholesterol.

Genotyping

Venous blood with ethylenediamine tetraacetic acid (EDTA) anticoagulant was used to extract genomic DNA by the Chelex-100 Method (22). SNPs were selected based on their minor allele frequencies reported in the HapMap or SNP database (<http://www.ims.u-tokyo.ac.jp>). TaqMan (Applied Biosystems) reagents were purchased from ABI. SNPs were selected with a frequency >0.1 in the Chinese population.

PCR was performed in a volume of 10µl containing 1µl of genomic DNA, 0.25µl of Assay-on-Demand SNP Genotyping Assay Mix (40×) (Applied Biosystems Co., Ltd. US), 3.75µl ddH₂O and 5µl TaqMan Universal PCR Master Mix, No AmpErase UNG (2×). The PCR cycle profile was as follow: predenaturation at 95 °C for 10 min, followed by 43 cycles of denaturation at 92 °C for 15 sec, annealing at 60 °C for 1 min, with fluorescence acquisition during each stage of annealing and extension. *SIRT6* genes were genotyped with VIC and FAM as fluorescent tags using the Applied Biosystems 7300 Real - Time PCR System.

Statistical analysis

Statistical analyses were conducted using SPSS 16.0 (Chicago, IL, USA). ANOVA (analysis of variance) was used to compare continuous variables (BMI, SBP, DBP, FBG, TC, TG, HDL-c and LDL-c) and Chi-square test was used for categorical variables. We used the Pearson's chi-square test to evaluate the Hardy–Weinberg equilibrium (HWE). The frequency of the rs350846 *SIRT6* gene polymorphisms in the three groups was assessed. *P*-values were corrected for multiple comparison by Bonferroni analysis ($P=0.05/\text{number of comparisons}$), yielding a new *P*-value ($P<0.017$, number of comparisons =3). To estimate the strength of longevity association, the *P*-value and the odds ratios with 95% confidence interval were estimated using multinomial logistic regression models. Statistical significance was set at $P<0.05$.

Results

Population profile

As shown in Table 1, the BMI and TG levels were decreased in the long-living group than in the two control groups, while serum concentrations of LDL-c and HDL-c and the levels of SBP

and DBP in long-lived individuals were higher than in the two control groups ($P < 0.01$). The waist circumference was different ($P = 0.000$) among the three groups and the maximum waist circumference was observed in the external group. No statistically significant differences in gender, FBG and TC levels were noticed in long-lived individuals.

Hardy Weinberg equilibrium test of different groups

Chi-square test revealed that all genotypes were within the limits of the Hardy-Weinberg law using HWE software ($P > 0.05$) (Table 2).

Genotypic frequency

GG was the dominant genotype in longevity and internal control groups with a frequency of 0.53 and 0.54, respectively. However, CG was the dominant genotype in the external control group with a frequency of 0.5. Statistically significant differences were observed among the different genotypes except for the longevity and internal control groups (Table 3).

Minor allele frequency, MAF

The frequency of the minor allele-C was 0.319. The minor allele frequency of rs350846 in SIRT6 was significantly higher in the external control group than in other groups (Table 4).

Table 2: Chi-square test of Hardy-Weinberg equilibrium of rs350846 in SIRT6 genes

Group	n	Genotype			χ^2	P
		CC	CG	GG		
Longevity	169	17	63	89	1.3418	0.5112
Internal control	158	13	59	86	0.4025	0.8177
External control	176	24	88	64	0.5221	0.7702
Total	503	54	210	239	0.5949	0.7427

Table 3: Genotype frequency of the SIRT6 polymorphism n (%)

Group	n	Genotype n (%)			χ^2	P
		CC	CG	GG		
Longevity	169	17(10.06)	63(37.28)	89(52.66)	0.346 ^a	0.841
Internal control	158	13(8.23)	59(37.34)	86(54.43)	11.281 ^b	0.004
External control	176	24(13.64)	88(50.00)	64(36.36)	9.281 ^c	0.010
Total	503	54(10.74)	210(41.75)	239(47.51)	13.930 ^d	0.008

Note: **a** represents longevity vs. internal control, **b** denotes internal control vs. external control, **c** suggests longevity vs. external control, $P < 0.017$ indicates statistical significance, **d** means total χ^2 value.

Table 4: Distribution of alleles and MAF in rs350846 of SIRT6 gene

Group	C	G	MAF	χ^2	P	OR	95%CI
	n (%)	n (%)					
Longevity	97(28.7)	241(71.3)	0.287	0.067	0.796 ^a	1.046	0.742~1.476
Internal control	85(27.8)	221(72.2)	0.278	8.653	0.003 ^b	0.611	0.439~0.849
External control	136(38.6)	216(61.4)	0.386	7.615	0.006 ^c	0.639	0.465~0.879
Total	318(31.9)	678(68.1)	0.319	11.336	0.003 ^d		

Note: **a** denotes longevity vs. internal control; **b** represents internal control vs. external control; **c** represents longevity vs. external control; $P < 0.017$ suggests statistical significance; Data in italics indicates $P < 0.05$, OR = odds ratio, 95%CI = 95% confidence interval; **d** indicates total χ^2 value.

Multi-factor analysis of exposure and longevity

BMI, CM, DBP, SBP, GLU, TC, TG, HDL-c, LDL-c, sex and rs350846 genotype were included in the logistic model. Among these variables, five factors significantly influenced longevity, including BMI (OR: 1.240; 95%CI: 1.114-1.380), CM (OR: 0.939; 95%CI: 0.905-0.974), SBP (OR: 0.984; 95%CI: 0.971-0.996), HDL-c (OR: 0.612; 95%CI: 0.398-0.941) and sex (OR: 1.719; 95%CI: 1.015-2.913) between the longevity and the inter-

nal control groups. Among these variables, six factors significantly influenced longevity, including BMI (OR: 1.702; 95%CI: 1.464-1.977), SBP (OR: 0.915; 95%CI: 0.895-0.936), DBP (OR: 1.051; 95%CI:1.014-1.089) , TC (OR: 2.319; 95%CI: 1.283-4.191) , HDL-c (OR: 0.113; 95%CI: 0.043-0.294), and LDL-c (OR: 0.374; 95%CI: 0.184-0.761) between the longevity and the external control groups (Table 5).

Table 5: Multinomial logistic regression analysis of longevity and two control groups

Variables	<i>â</i>	<i>SE(â)</i>	<i>Wald(χ²)</i>	<i>P</i>	<i>OR</i>	<i>95%CI</i>
Internal group	2.600	1.493	3.035	0.081		
Intercept						
BMI	0.215	0.055	15.493	0.000	1.240	1.114-1.380
CM	-0.063	0.019	11.154	0.001	0.939	0.905-0.974
DBP	0.004	0.012	0.083	0.773	1.004	0.980-1.028
SBP	-0.016	0.007	6.415	0.011	0.984	0.971-0.996
FBG	-0.014	0.067	0.045	0.832	0.986	0.865-1.124
TC	-0.150	0.248	0.368	0.544	0.860	0.530-1.398
TG	0.268	0.155	2.996	0.083	1.308	0.965-1.772
HDL-c	-0.491	0.220	5.000	0.025	0.612	0.398-0.941
LDL-c	0.199	0.303	0.431	0.511	1.220	0.674-2.207
sex=1	0.542	0.269	4.059	0.044	1.719	1.015-2.913
sex=2	0					
rs350846=1	0.160	0.437	0.134	0.714	1.174	0.498-2.767
rs350846=2	0.061	0.451	0.018	0.893	1.063	0.439-2.572
rs350846=3	0					
External group	19.346	237.208	0.000	0.993		
Intercept						
BMI	0.532	0.077	48.073	0.000	1.702	1.464-1.977
DBP	0.050	0.018	7.393	0.007	1.051	1.014-1.089
SBP	-0.089	0.011	59.401	0.000	0.915	0.895-0.936
FBG	-0.030	0.096	0.100	0.752	0.970	0.805-1.170
TC	0.841	0.302	7.763	0.005	2.319	1.283-4.191
TG	0.121	0.204	0.353	0.552	1.129	0.757-1.685
HDL-c	-2.181	0.489	19.932	0.000	0.113	0.043-0.294
LDL-c	-0.984	0.363	7.361	0.007	0.374	0.184-0.761
sex=1	0.030	0.354	0.007	0.933	1.030	0.515-2.060
sex=2	0					
rs350846=1	-0.847	0.551	2.360	0.124	0.429	0.146-1.263
rs350846=2	-0.408	0.558	0.534	0.465	0.665	0.223-1.986
rs350846=3	0					

Note: Reference was defined as sex= 1, and 2 for male and female; rs350846= 1, 2 and 3 denote GG, CG and CC genotypes, respectively

Discussion

Our data suggest that the basic characteristics of the populations including age, BMI, TG, SBP, DBP, HDL-C, and LDL-C were significantly dif-

ferent between the longevity and the two control groups, which is consistent with other geriatric studies (23, 24). The BMI and TG levels were lower in the long-living population. Lower BMI and TG levels may promote longevity. Longevity

was associated with higher levels of SBP, DBP, HDL-c and LDL-c than in the two control groups. All these signs were related to nutrition and lifestyle factors. Although, other investigations (24) showed several changes in diet and lifestyle in Hongshuihe River Basin including increased intake of fats, proteins, and carbohydrates and additional alcoholic beverages than before. Longevity is mainly associated with easily digestible plant-based foods such as whole grains or corn with less fat and animal protein, and intake of multiple small meals. Caloric restriction can prolong life (25, 26).

In the present study, the genotypes in Table 3 were significantly different among the external control and the other two groups. The GG was the predominant genotype in both the longevity group and the internal control group compared with the CG type in the external control group. The C allele may promote longevity according to the MAF results. We found no significant gender differences in the final model, in contrast to other longevity studies (27). BMI, SBP and serum TG levels represented the risk or protective factors for longevity. No *SIRT6* rs350846 genotypes were associated with longevity, although previous studies reported that *SIRT6* was associated with aging (17). The main studies focused on the somatic cells and mice demonstrating that *SIRT6* prolonged the life of male mice (15). Cell senescence may be associated with NF- κ B signaling pathways (28).

No studies are available correlating *SIRT6* and human longevity. Our findings suggest that the *SIRT6* rs350846 gene polymorphism was not associated with longevity, due to relative stability in the population and limited sample size. Therefore, the association of *SIRT6* rs350846 polymorphism with longevity needs to be confirmed with larger sample sizes and other polymorphisms.

Conclusion

The C allele of rs350846 in *SIRT6* gene, CC and CG genotypes are associated with longevity, with BMI, systolic pressure and HDL-c may be impor-

tant factors representing key factors in the long-lived populations of Bama County.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgments

This work was supported by the National Natural Science Foundation of China grant award to Dr Zh. Zhang (NSFC-81260423, NSFC- 61161009). The authors declare that there is no conflict of interest.

References

1. Witten TM, Bonchev D (2007). Predicting aging/longevity-related genes in the nematode *Caenorhabditis elegans*. *Chem Biodivers*, 4(11): 2639-55.
2. Herskind AM, McGue M, Holm NV, et al (1996). The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870-1900. *Hum Genet*, 97(3): 319-23.
3. Iachine IA, Holm NV, Harris JR, et al (1998). How heritable is individual susceptibility to death? The results of an analysis of survival data on Danish, Swedish and Finnish twins. *Twin Res*, 1(4): 196-205.
4. Liszt G, Ford E, Kurtev M, et al (2005). Mouse Sir2 homolog SIRT6 is a nuclear ADP-ribosyltransferase. *J Biol Chem*, 280(22): 21313-20.
5. Tissenbaum HA, Guarente L (2001). Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. *Nature*, 410(6825): 227-30.
6. Lin SJ, Defossez PA, Guarente L (2000). Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science*, 289(5487): 2126-28.

7. Imai S, Armstrong CM, Kaeberlein M, et al (2000). Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature*, 403(6771): 795-800.
8. Mahlknecht U, Ho AD, Voelter-Mahlknecht S (2006). Chromosomal organization and fluorescence in situ hybridization of the human Sirtuin 6 gene. *Int J Oncol*, 28(2): 447-56.
9. Zhong L, D'Urso A, Toiber D, et al (2010). The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1alpha. *Cell*, 140(2): 280-93.
10. Kawahara TL, Rapicavoli NA, Wu AR, et al (2011). Dynamic chromatin localization of Sirt6 shapes stress- and aging-related transcriptional networks. *PLoS Genet*, 7(6): e1002153.
11. Kugel S, Mostoslavsky R (2014). Chromatin and beyond: the multitasking roles for SIRT6. *Trends Biochem Sci*, 39(2): 72-81.
12. Lombard DB, Schwer B, Alt FW, et al (2008). SIRT6 in DNA repair, metabolism and aging. *J Intern Med*, 263(2): 128-41.
13. Silberman DM, Ross K, Sande PH, et al (2014). SIRT6 is required for normal retinal function. *PLoS One*, 9(6): e98831.
14. Liu R, Liu H, Ha Y, et al (2014). Oxidative stress induces endothelial cell senescence via down-regulation of Sirt6. *Biomed Res Int*, 2014: 902842.
15. Kanfi Y, Naiman S, Amir G, et al (2012). The sirtuin SIRT6 regulates lifespan in male mice. *Nature*, 483(7388): 218-21.
16. Kanfi Y, Peshti V, Gil R, et al (2010). SIRT6 protects against pathological damage caused by diet-induced obesity. *Aging cell*, 9(2): 162-73.
17. Gertler AA, Cohen HY (2013). SIRT6, a protein with many faces. *Biogerontology*, 14(6): 629-39.
18. Yang J (1992). An analysis of the longevous population in Bama. *Chin J Popul Sci*, 4(4): 351-56.
19. Xiao Z, Xu Q, Yuan Y (1996). Solving the mystery of the status and longevity of centenarians in Bama. *Chin J Popul Sci*, 8(4): 385-94.
20. Yang X, Wang X, Yao H, et al (2012). Mitochondrial DNA polymorphisms are associated with the longevity in the Guangxi Bama population of China. *Mol Biol Rep*, 39(9): 9123-31.
21. Pan SL, Wang F, Lu ZP, et al (2012). Cholesteryl ester transfer protein TaqIB polymorphism and its association with serum lipid levels and longevity in Chinese Bama Zhuang population. *Lipids Health Dis*, 11: 26.
22. Mari D, Mannucci PM, Duca F, et al (1996). Mutant factor V (Arg506Gln) in healthy centenarians. *Lancet*, 347(9007): 1044.
23. Feng J, Zhang J, Liu M, et al (2011). Association of mtDNA haplogroup F with healthy longevity in the female Chuang population, China. *Exp Gerontol*, 46(12): 987-93.
24. Pan SL, Luo XQ, Lu ZP, et al (2012). Microsomal triglyceride transfer protein gene -493G/T polymorphism and its association with serum lipid levels in Bama Zhuang long-living families in China. *Lipids Health Dis*, 11: 177.
25. Arum O, Rasche ZA, Rickman DJ, et al (2013). Prevention of neuromusculoskeletal frailty in slow-aging ames dwarf mice: longitudinal investigation of interaction of longevity genes and caloric restriction. *PLoS one*, 8(10): e72255.
26. Colman RJ, Beasley TM, Kemnitz JW, et al (2014). Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. *Nat Commun*, 5: 3557.
27. Bennati E, Murphy A, Cambien F, et al (2010). BELFAST centenarians: a case of optimised cardiovascular risk? *Curr Pharm Des*, 16(7): 789-95.
28. Kawahara TL, Michishita E, Adler AS, et al (2009). SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene expression and organismal life span. *Cell*, 136(1): 62-74.