Molecular and Cellular Probes 30 (2016) 79–82

Contents lists available at ScienceDirect

Molecular and Cellular Probes

journal homepage: www.elsevier.com/locate/ymcpr

Original research article

Lack of association between polymorphisms in the *SIRT6* gene and longevity in a Chinese population

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ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form
19 January 2016
Accepted 19 January 2016
Available online 21 January 2016

Keywords:
SIRT6
Longevity
Polymorphism
Association study

ABSTRACT

Sirtuin 6 (SIRT6) has recently been demonstrated to play an important role in the regulation of longevity in mammals. We therefore aimed to determine whether common variations in the *SIRT6* gene are associated with human longevity. Five tag single nucleotide polymorphisms (SNPs) across the *SIRT6* gene and its 5 kb up-/downstream region, including rs350852, rs350844, rs352493, rs4807546 and rs3760905, have been successfully determined in 616 unrelated Chinese long-lived individuals (LLIs) (mean age: 102.4 ± 2.3 years) and 846 younger controls (mean age: 48.9 ± 10.6 years) from Hainan Island, China. The allele and genotype frequencies of the five SNPs showed no statistically significant difference between the LLIs and controls (all $P > 0.05$). The five SNPs were in strong linkage disequilibrium and defined seven common haplotypes. Likewise, no association between these haplotypes and longevity was observed (all $P > 0.05$). The present study reveals that common genetic variations in the *SIRT6* gene are not associated with human longevity.

1. Introduction

Aging is known to be the major risk factor for multiple diseases [1]. A lot of theories have been advanced to account for the regulation of mammalian lifespan and has important therapeutic implications for age-related diseases. Sirtuin 6, or SIRT6, has recently been identified as an important regulator of longevity in mammals. In two independent *Sirt6*-transgenic mouse strains, exogenous mouse SIRT6 overexpression extends the median lifespan of males by 14.5% and 9.9%, respectively [2]. Conversely, SIRT6 knockout mouse cells exhibit DNA damage hypersensitivity and genomic instability [3]. These SIRT6 knockout mice develop a severe degenerative phenotype quite similar to that of premature aging. More recently, SIRT6 was shown to regulate miR-766 transcription via a feedback regulatory loop, which has implications for the modulation of SIRT6 expression in reprogramming of aging cells [4].

SIRT6 is mostly found in the nucleus and tightly bound to chromatin. However, SIRT6 translocates into the cytoplasm under stress and interacts with and promotes dephosphorylation of GTPase activating protein (SH3 domain) binding protein. It functions as a NAD + -dependent deacetylase of both acetyl groups and long-chain fatty acyl groups, and ADP-ribosylase [5]. These cellular functions impact upon cellular homeostasis by regulating DNA repair, telomere maintenance, stress-granule formation, and glucose and lipid homeostasis. Thus, modulation of SIRT6 activity has the potential to influence multiple diseases such diabetes, obesity, heart disease, inflammation, and cancer. Such roles may contribute to the overall organismal health and longevity [6].

To better predict variation in human longevity and verify the

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http://dx.doi.org/10.1016/j.mcp.2016.01.005
0890-8508/

Table 1
Basic information about candidate SNPs genotyped in this study.

	SNPs	Chromosomal location[a]	Position in the *SIRT6* gene	Major > Minor allele	Call rate	HWE (control)	Alleles captured
1	rs350852	chr19:4170001	3′ flanking	A > G	0.9986	0.48	rs350852, rs350854, rs3760907
	rs11878868[b]	chr19:4173640	3′ flanking				rs11878868, rs74172624, rs35523266, rs8104543
2	rs350844	chr19:4177054	Intron 4	G > A	0.9973	1	rs386806096, rs350846, rs350845, rs107251, rs350844
3	rs352493	chr19:4180839	Exon 2: S46N	T > C	0.9959	0.93	rs168423, rs11085060, rs352117, rs352492, rs7260071, rs352493
4	rs4807546	chr19:4182063	Intron 1	T > C	1	0.38	rs350851, rs350848, rs350843, rs4807546
5	rs3760905	chr19:4182942	5′ flanking	T > G	0.9993	0.62	rs350853, rs350850, rs350849, rs3760908, rs2159982, rs2159983, rs3760905

The 5 SNPs successfully genotyped were able to capture 25 common variants of the *SIRT6* gene and its 5 kb up-/downstream region (chromosome 19: 4169110.0.4187599 18.49 kbp) at r^2 greater than 0.80 in the CHB and CHS population based on the HapMap and 1000 Genomes Project databases.
[a] Chromosome locations based on human genome reference assembly GRCh38/hg38.
[b] SNPs which failed in genotyping and was removed from the analysis.

link between *SIRT6* and longevity, we considered *SIRT6* as a candidate gene and performed an association study to test whether *SIRT6* polymorphisms are related to human longevity.

2. Material and methods

2.1. Study population

This study was conducted with 616 unrelated Chinese long-lived individuals (LLIs) and 846 younger individuals as a control group from Hainan Island. According to the sixth Chinese census database in 2010, in China's 31 provincial-level regions (provinces, autonomous regions and municipalities), the highest number of centenarians per 10,000 inhabitants aged ≥65 years is in Hainan (16.64), followed by Guangxi (7.00) and Guangdong (6.07) [7]. The LLIs were 98 years old or over at the time of recruitment (mean age: 102.4 ± 2.3 years). In the case group, 83.4% were females, 93.8% were Han Chinese and others were Li Chinese. The 846 Chinese control subjects were between ages 30 and 70 years (mean age: 48.9 ± 10.6 years, 69 Li and 777 Han people, 159 males and 687 females). The study was approved by the Ethics Committee of Hainan Medical College and by the local data protection authorities. All subjects provided written informed consent.

2.2. SNP selection and genotyping

Six tag single nucleotide polymorphisms (SNPs) across the *SIRT6* gene and its 5 kb up-/downstream region (chromosome 19: 4169110..4187599 18.49 kbp, human genome reference assembly GRCh38/hg38) were selected on the basis of linkage disequilibrium (LD) patterns observed in the Han Chinese in Beijing (CHB) and Southern Han Chinese (CHS) samples genotyped as part of the International HapMap and 1000 Genomes Projects. Only SNPs with minor allele frequency (MAF) greater than 5% were considered. One coding SNP (rs352493 coding S46N), one 5′flanking SNP rs3760905, as well as two 3′ flanking SNPs rs11878868 and rs350852 were forced included to capture as much functional variation as possible. In addition, two intronic SNPs rs350844 and rs4807546 were selected. These six tag SNPs captured 29 common SNPs with an r^2 of at least 0.80 and were genotyped, blind to case–control status, by a custom-by-design 48-Plex SNPscan™ Kit (Cat#:G0104; Genesky Biotechnologies Inc., Shanghai, China), which was developed according to patented SNP genotyping technology by Genesky Biotechnologies Inc.. As described by Chen et al. [8], it was based on double ligation and multiplex fluorescence polymerase chain reactions.

Finally, SNP rs11878868 failed genotyping and was removed

Table 2
Genotype and allele frequencies of *SIRT6* polymorphisms in the long-lived individuals and controls.

Polymorphisms	Genotype/Allele	Case	Control	OR (95%CI)	*P*
rs350852	A/A	279 (45.3%)	387 (45.9%)	1.00	
	G/A	277 (45%)	376 (44.5%)	1.02 (0.82–1.27)	0.98
	G/G	60 (9.7%)	81 (9.6%)	1.03 (0.71–1.48)	
	A	835 (67.8%)	1150 (68.1%)	1.00	
	G	397 (32.2%)	538 (31.9%)	1.02 (0.87–1.19)	0.84
rs350844	G/G	297 (48.3%)	399 (47.3%)	1.00	
	G/A	264 (42.9%)	362 (42.9%)	0.98 (0.79–1.22)	0.81
	A/A	54 (8.8%)	82 (9.7%)	0.88 (0.61–1.29)	
	G	858 (69.8%)	1160 (68.8%)	1.00	
	A	372 (30.2%)	526 (31.2%)	0.96 (0.81–1.12)	0.58
rs352493	T/T	331 (53.9%)	451 (53.6%)	1.00	
	C/T	237 (38.6%)	330 (39.2%)	0.98 (0.79–1.22)	0.97
	C/C	46 (7.5%)	61 (7.2%)	1.03 (0.68–1.55)	
	T	899 (73.2%)	1232 (73.2%)	1.00	
	C	329 (26.8%)	452 (26.8%)	1.00 (0.85–1.18)	0.98
rs4807546	T/T	231 (37.5%)	328 (38.8%)	1.00	
	C/T	297 (48.2%)	388 (45.9%)	1.09 (0.87–1.36)	0.65
	C/C	88 (14.3%)	130 (15.4%)	0.96 (0.70–1.32)	
	T	759 (61.6%)	1044 (61.7%)	1.00	
	C	473 (38.4%)	648 (38.3%)	1.00 (0.86–1.17)	0.96
rs3760905	T/T	196 (31.8%)	287 (34%)	1.00	0.48
	G/T	315 (51.1%)	405 (47.9%)	1.14 (0.90–1.44)	
	G/G	105 (17.1%)	153 (18.1%)	1.00 (0.74–1.37)	
	T	707 (57.4%)	979 (57.9%)	1.00	
	G	525 (42.6%)	711 (42.1%)	1.02 (0.88–1.19)	0.77