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Short report

Sirtuin 1 *(SIRT1)* sequence variation is not associated with exceptional human longevity

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Abstract

The *SIR2/Sirt*1 gene has been demonstrated as regulating lifespan in many model organisms, including yeast, *Caenorhabditis elegans* and rodents. These findings render the human homologue, *SIRT*1, a very plausible candidate as a modifier of human life expectancy. We therefore sought to investigate whether common allelic variation in the *SIRT1* gene was associated with human longevity. Five single nucleotide polymorphisms (SNPs), distributed across the entire gene, including the promoter region, were genotyped in our extensive DNA collections of 1573 long-lived individuals (centenarians and nonagenarians) and matched younger controls. Four of the markers were haplotype-tagging SNPs (htSNPs) that defined five common haplotypes. No evidence for an association was detected between any of the tested SNPs and the longevity phenotype at the allele, genotype or haplotype levels. These findings, based on an htSNP approach, suggest that there is no noteworthy influence of *SIRT1* sequence variation on exceptional human longevity in the German population. However, this does not rule out the possibility that allelic variants in direct regulators or downstream substrates of *SIRT1* could play critical roles in extending lifespan in humans.

Keywords: Case–control association design; Centenarians; Long-lived individuals; Ageing; Single nucleotide polymorphism (SNP); Haplotype-tagging; Lifespan extension; Model organisms; Calorie restriction

1. Introduction

The yeast *SIR*2 (silent mating type information regulator 2) gene was the first described member of a large gene family that encodes NAD^+-dependent protein deacetylases. These enzymes are involved in gene silencing, chromatin structure, DNA repair, and control of cellular survival (Guarente, 2000). Expression of extra copies of *SIR*2 and the nematode homologue *sir*-2.1 increases lifespan in yeast (Kaeberlein et al., 1999) and *Caenorhabditis elegans* (Tissenbaum and Guarente, 2001), respectively. The same effects—up-regulation of *SIR*2 with subsequent life extension—can also be achieved by exposing yeast cells to a low calorie diet (Lin et al., 2000). The life-prolonging effects of calorie restriction (CR) have also been seen in more complex species, ranging from *C. elegans* to mammals (Masoro, 2000). Based on these observations, it has been suggested that the evolutionarily conserved *SIR*2 genes could play a similar role as modulators of lifespan in higher organisms (Guarente, 2000; Hekimi and Guarente, 2003).

Seven mammalian homologues, collectively known as 'sirtuins' (Sirts), have been identified, of which *Sirt*1 is the most closely related to yeast *SIR*2 (Frye, 1999; 2000). There is indeed mounting evidence that the mammalian SIRT1 protein may also act as a potential modifier of longevity by slowing ageing during times of adversity. A recent study by Cohen et al. (2004) demonstrated that various tissues from rats on CR had increased amounts of SIRT1 protein compared to animals on a normal diet. The underlying mechanism leading to SIRT1 over-expression is still unknown but it may be mediated, in part, by the insulin-IGF1 signaling pathway (Cohen et al., 2004). SIRT1 protein upregulated through food withdrawal was found to bind and repress the fat regulator PPAR-gamma in murine adipocytes, thus stimulating fat breakdown (Picard et al., 2004). Because murine lifespan can be extended by reducing fat stores, these results suggest that activation of

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SIRT1 mediation by energy restriction could be a possible molecular mechanism of mammalian lifespan regulation (Picard et al., 2004). Interestingly, plant-derived polyphenols, such as the red-grape component resveratrol, induce *SIR2*/*SIRT*1 gene activation and mimic the effects of CR in yeast, *Drosophila* and in human embryonic kidney 293 cell cultures (Howitz et al., 2003; Wood et al., 2004).

In response to CR, radiation or oxidative stress, increased SIRT1 activity has also been shown to reduce p53-, Bax- or forkhead-dependent apoptosis (Luo et al., 2001; Vaziri et al., 2001; Brunet et al., 2004; Cohen et al., 2004; Motta et al., 2004; Nemoto et al., 2004). A strong association has been reported between ageing and enhanced susceptibility to apoptosis (Higami and Shimokawa, 2000; Pollack et al., 2002). The SIRT1-mediated resistance to apoptosis may therefore promote long-term cell survival and mitigate the age-related progressive erosion of cells, tissues and organs. More recent findings in mouse embryonic fibroblasts have revealed an additional SIRT1 function in handling chronic cellular stress: the protein triggers replicative senescence (Chua et al., 2005). This role is in marked contrast to that reported for yeast cells where SIR2p promotes cell division. However, the ability of SIRT1 to induce permanent cell cycle arrest is only triggered by chronic, but not acute, genotoxic stress (Chua et al., 2005). Further work is needed to elucidate how the mammalian SIRT1 helps cells respond to adverse conditions and how this mechanism influences the ageing process.

The human *SIR*2 homologue *SIRT*3, which encodes a mitochondrial protein (Onyango et al., 2002), has previously been subject to a longevity association study in Italians from Calabria (Rose et al., 2003). By analyzing the genotype-specific survival function, an association between a silent mutation in the coding region of *SIRT*3 and survivorship was detected, albeit only in males. This finding suggests that variation in *SIRT*3 itself, or in a gene that is tightly linked to it, may modulate life expectancy in humans (Rose et al., 2003).

The life-prolonging effects that the *SIR*2 gene and its homologues exert on various model organisms, together with the association finding in the human *SIRT*3 gene, prompted us to investigate whether common allelic variation in the *SIRT*1 gene is associated with exceptional longevity in humans. To this end, we have analyzed five known single nucleotide polymorphisms (SNPs), which capture the majority of the variation in the *SIRT*1 gene via its common haplotypes, in our extensive collection of 1573 long-lived individuals (centenarians and nonagenarians) and appropriately matched younger controls.

2. Subjects and methods

2.1. Subjects

A total sample of 1026 unrelated German long-lived individuals (LLI) was studied. All individuals were between 95 and 109 years of age at the time of recruitment (mean age: 98.3 years). These included 386 centenarians and supercentenarians (mean age: 101.0 years). The gender ratio in the sample was 74% females vs. 26% males. The 547 German control subjects were between 60 and 75 years of age (mean age: 67.2 years) and matched the long-lived individuals by gender, ethnical ancestry and geographical origin within the country. A detailed description of the samples and the recruitment procedure is given in Nebel et al. (2005). All subjects gave informed, written consent prior to participation. The study was approved by the Ethics Committee of the University Hospital Schleswig-Holstein, Kiel and by the local data protection authorities.

2.2. SNP genotyping

DNA samples from long-lived individuals and control subjects were analyzed for five SNPs located in the *SIRT*1 gene (Table 1) using the Taqman Allelic Discrimination method (Applied Biosystems) on an automated platform as previously described (Hampe et al., 2001a,b). Details about the assay designs are available from http://www.appliedbiosystems.com/. HapMap data (http://www.hapmap.org/) revealed that the entire *SIRT1* gene is within a 151-kb region of strong linkage disequilibrium (an 'LD block'). Such LD blocks are typically characterized by a few (2–4) common haplotypes that capture

Table 1
Summary association statistics for the *SIRT*1 polymorphisms analyzed in German long-lived individuals and controls

SNP	SNP location in gene	MAF[a]	LLI[b]		Centenarians	
			P_{allele}[c]	$P_{genotype}$[d]	P_{allele}[e]	$P_{genotype}$[f]
hCV3003909 (rs3758391)	5′ region	0.323	0.93	0.99	0.53	0.82
hCV11642237 (rs1885472)	Intron	0.325	1.00	0.98	0.57	0.82
hCV16179813 (rs2273773)	Exon 5 (silent mutation)	0.067	0.71	0.82	0.69	0.80
hCV25611602 (rs10997870)	Intron	0.340	0.88	0.95	0.62	0.85
hCV11642299 (rs2234975)	3′UTR	0.082	0.64	0.42	0.53	0.27

[a] Minor allele frequency of SNP in the control sample.
[b] Long-lived individuals aged 95 years and older.
[c] *P* value from a χ^2 test (1df) for allele frequency difference between LLI and controls.
[d] *P* value from a χ^2 test (2df) for genotype frequency differences between LLI and controls.
[e] *P* value from a χ^2 test (1df) for allele frequency difference between centenarians and controls.
[f] *P* value from a χ^2 test (2df) for genotype frequency differences between centenarians and controls.

the majority (typically > 90%) of the genetic variation present within the LD block (Daly et al., 2001). Consequently, by selecting five markers of varying minor allele frequency that are spread across the SIRT1 region we could be fairly confident of capturing the common haplotypes and the majority of the genetic variation. The tested polymorphisms are spaced across the coding region, the 3′UTR and the promoter of the gene (Table 1). By means of the 'First Exon and Promoter Prediction Program for Human DNA' (http://rulai.cshl.org/tools/FirstEF/) the promoter of the *SIRT*1 gene was localized within the genomic sequence NM_012238 to between about 500 and 1000 bp downstream of hCV3003909, i.e. the promoter lies between the two markers hCV3003909 and hCV11642237.

2.3. Statistical analysis

Single marker case–control analyses on allele and genotype frequency data were performed using contingency table χ^2 statistics at the appropriate degrees of freedom using the Web-based Simple Interactive Statistical Analysis (SISA) tool, available from http://home.clara.net/sisa. The software program Haploview, version 3.2 (http://www.broad.mit.edu/mpg/haploview/) was used to assess all polymorphisms for significant deviation from the Hardy-Weinberg equilibrium, to calculate LD (D′) between each marker pair and to visualize haplotype structure (Barrett et al., 2005). Haplotype frequencies were inferred using the expectation maximization (EM) algorithm and the sliding window option as implemented in the program COCAPHASE, version 2.403 which is part of the UNPHASED suite (http://portal.litbio.org/; Dudbridge, 2003). COCAPHASE was also used to evaluate the statistical significance of haplotype frequency differences between cases and controls.

3. Results

The entire collection of 1026 German LLI and the sub-set of 386 centenarians were subjected to a gender-matched case–control analysis of five SNPs in the *SIRT*1 gene. All SNPs were found to be in Hardy-Weinberg equilibrium in both the cases and controls. No evidence for an association was detected between any of the tested SNPs and the longevity phenotype in either sample, neither at the allele nor at the genotype level (Table 1). Similarly, analysis with gender-stratified samples showed no evidence for association.

As expected, the five SNPs comprised a single LD block with high levels of LD between all the markers. The pairwise D′ values ranged from 0.92 to 1.00. The five SNPs defined five haplotypes with a frequency > 1% in the control population and with the three most frequent haplotypes accounting for 92% of the data (Table 2). Four of the markers were haplotype-tagging SNPs (htSNPs) in the sense that they were sufficient to resolve the five haplotypes. In other words, markers hCV3003909 and hCV11642237 were in perfect LD and redundant with respect to each other such that either could be used in conjunction with the other markers to reconstruct the haplotypes and their frequencies. None of the five-SNP haplotypes differed significantly in frequency between the case samples and the controls. Neither did testing of two-, three- and four-SNP haplotypes in a sliding window across the gene reveal any statistically significant frequency differences (data not shown).

4. Discussion

The *SIR2/Sirt*1 gene has been shown to regulate lifespan in many model organisms, including yeast, *C. elegans* and rodents (Hekimi and Guarente, 2003). In mammals, expression of the SIRT1 protein is activated by various stress stimuli, leading to a break-down of fat stores, induction of cell cycle arrest and DNA repair processes, or attenuation of apoptosis (Brunet et al., 2004; Cohen et al., 2004; Nemoto et al., 2004; Picard et al., 2004; Chua et al., 2005). Under adverse circumstances, SIRT1 appears to increase organismal longevity by tipping physiological responses away from cell death and towards cellular stress resistance (Brunet et al., 2004). As effective stress resistance is frequently correlated with extended lifespan in lower eukaryotes, the ability of SIRT1 to modulate reactions to stress in mammalian cells also suggests a potential link between the gene and longevity in mammals (Chua et al., 2005), and therefore possibly humans.

We therefore sought to determine whether common allelic variation in the *SIRT*1 gene was associated with longevity in humans. Five SNPs, four of which are haplotype-tagging, were selected from the public databases and genotyped in our extensive DNA collection of more than 1500 long-lived

Table 2
Frequencies of the common five-SNP haplotypes in the *SIRT*1 gene

Haplotype[a]	Frequency[b] in controls	Frequency[b] in LLI	$P_{haplotype}$	Frequency[b] in centenarians	$P_{haplotype}$
11111	0.580	0.570	0.59	0.558	0.35
22121	0.255	0.261	0.74	0.275	0.34
11112	0.083	0.087	0.70	0.091	0.55
22221	0.064	0.062	0.83	0.062	0.86
11121	0.013	0.018	0.32	0.012	0.80
Global significance			0.58[c]		0.90[c]

[a] The five-SNP haplotype block as defined by Haploview. The SNPs in the haplotypes are listed in the 5′ to 3′ order; 1 denotes the major allele, 2 the minor allele. The first and the second SNP are in perfect LD and provide the same haplotype information. SNPs 2 to 5 are haplotype-tagging.
[b] Frequencies inferred by the EM algorithm using the program COCAPHASE.
[c] Global significance value obtained after 1000 permutations with COCAPHASE.

individuals and controls. Five frequent haplotypes in the *SIRT*1 gene were identified, but no association between these and exceptional longevity was observed. Neither could we detect association at the single-point allele or genotype levels.

The htSNP approach employed here is likely to have captured the common variation present in the *SIRT*1 genomic region for the samples analyzed in this study. By tagging the common variation (haplotypes) within *SIRT*1 we implicitly assume that any undetected rare variants will be carried by one or other of the common background haplotypes and hence be statistically detectable; this is the logic of the htSNP approach. It is of course possible, albeit unlikely, that the selected markers might not tag the common variation comprehensively, and that rare *SIRT*1 polymorphisms, with a weak impact on the longevity phenotype, may exist. Overall however, our findings would suggest that there is no noteworthy influence of *SIRT*1 gene variation on exceptional human longevity in the German population. The possibility that this negative association finding results from population stratification in our samples is unlikely. The validity and efficacy of our extensive study population for genetic longevity research have recently been demonstrated (Nebel et al., 2005).

The conserved function of SIRT1 in invertebrates and mammals predicts that the up-regulation of the *SIRT*1 gene and the subsequent increase in levels of the SIRT1 protein would also exert a life-prolonging effect in humans. In this case, potential mutations affecting the transcription of the gene would be of particular interest. Because the htSNPs used in this study, which are in strong LD, also span the promoter of *SIRT*1, a significant influence of variation in this regulatory region of the gene may be excluded. However, it is possible that allelic variants in direct modulators of *SIRT*1 may be crucial in regulating SIRT1 protein levels. In addition, variation in the broad array of *SIRT*1 downstream targets could also play critical roles in extending lifespan.

In this study, we have demonstrated that common *SIRT*1 variation does not appear to be associated with exceptional human longevity, despite the fact that the gene is considered an important player in pathways that modulate lifespan in a wide range of organisms, and possibly also in humans.

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