Absence of mutation in *miR-34a* gene in a Chinese longevity population

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DEAR EDITOR:

Centenarians are a typical longevity model characterized by delayed onset of morbidity in age-related diseases such as cancer, cardiovascular disease, dementia, and stroke (Andersen et al, 2012). Though there may be a number of underlying mechanisms behind this longevity, curiously it seems that the survival advantage persists in their offspring (Terry et al, 2003), suggesting a potentially important role for genetic factors. Previous studies suggested that the heritability of human longevity may be ~25% (Herskind et al, 1996; Mcgue et al, 1993), whicih is consistent with other studies on model organisms that identified several longevity-related genes, such as *age-1, daf-2, daf-16, and sir-2* (Friedman & Johnson, 1988; Kenyon et al, 1993; Lin et al, 1997; Tissenbaum & Guarente, 2001). Likewise, several studies have reported the existence of many mutations related to human longevity (Holstege et al, 2014; Sebastiani et al, 2012).

Alongside gene mutations, microRNAs have been shown to influence gene function in general, and more particularly have been implicated in various age-related diseases (Boehm & Slack, 2005; Bonauer et al, 2010; de Lencastre et al, 2010; Esquela-Kerscher & Slack, 2006; Eacker et al, 2009; Jordan et al, 2011; Provost, 2010; Somel et al, 2010; Schraml & Grillari, 2012). Typically, the expression of most miRNAs are downregulated with human age (Noren Hooten et al, 2010), but are upregulated in centenarians as compared with the octogenarians, being somewhat similar to those of younger people (Serna et al, 2012). Consequently, it is not surprising that miRNAs have been suggested to play crucial roles in longevity (Ibáñez-Ventoso et al, 2006; Pincus et al, 2011). Of these miRNAs, *miR-34a* was reported to determine life span of *C. elegans* and modulate aging in *Drosophlia* (de Lencastre et al, 2010; Liu et al, 2012; Yang et al, 2013). Mutations located in the region of pri-*miR-34a* were shown to affect the function of miR-34a (Gong et al, 2012; Locke et al, 2014). *MiR-34a* itself plays an important role in development and various diseases (Rokavec et al, 2014); previously, it was reported to determine life-span and modulate aging in model organisms (de Lencastre et al, 2010; Liu et al, 2012; Yang et al, 2013). The inhibition of *miR-34a* regulates cardiac aging through silencing or genetic deletion in mice (Boon et al, 2013). Likewise, *miR-34a* was recognized as a tumor suppressor gene in multiple kinds of cancers (Chim et al, 2010; Cole et al, 2008; Li et al, 2009; Welch et al, 2007; Wiggins et al, 2010). [1]

Based on these evidences, we speculated that *miR-34a* gene may be associated with human longevity. Mutations in the *miR-34a* gene were revealed to affect its function (Gong et al, 2012; Locke et al, 2014). Data from 1000G Database showed 5 rare mutations (rs201359809, rs72631823, rs35301225, rs369892834, and rs372904298) located in the *miR-34a* gene (http://www.1000genomes.org/), two of which have been shown to associate with the *miR-34a* function (Gong et al, 2012; Locke et al, 2014). The minor allele A of rs72631823 in pre-*miR-34a* resulted in higher level of mature *miR-34a* expression, which increased apoptosis in pancreatic beta-cell (Locke et al, 2014). The rs35301225 residing in the miR-34a mature sequence shows an effect on its target binding (Gong et al, 2012). Despite these suggestive lines of evidence, to our knowledge, there has not been any data available on the association of *miR-34a* SNPs with longevity.

Here, we aim to locate SNPs potentially associated with longevity by sequencing the *miR-34a* gene. In total, 439 genetically unrelated subjects of Han nationality (203 centenarians and 236 normal control subjects) were recruited

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from Hainan, China. Both centenarians and controls were in good healthy states without severe diseases (He et al, 2014a, b). The age of centenarians ranged from 100 to 109 years with an average of 102.7±2.3 years, and the average age of controls was 48.1±11.3 years. Total genomic DNA was extracted from the whole blood using the standard phenol-chloroform method (Sambrook & Russell, 2006). The purity and concentration of the extracted DNA were determined by Synergy H1 hybrid multimode microplate reader (BioTek, USA).

The *miR-34a* coding gene is located in the region of Chromosome 1p36.23 with a length of 110 bp. The *miR-34a* gene was amplified using primers as follows: the forward primer, 5'- ACTTCTCCCAGCCAAAAGCC-3'; reverse primer, 5'-TTATCAACAGGTGCTGGGGA-3'. The polymerase chain reaction (PCR) conditions were as follows: 3 min at 94 °C for one circle; followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min; final extension was completed at 72 °C for 7 min. The PCR products were sequenced on an ABI3730xl Genetic Analyzer (ABI, USA).

After sequencing the *miR-34a* gene among the 439 Chinese subjects, comprised of 203 centenarians and 236 young controls, we did not find any SNPs in the *miR-34a* gene (Table 1), though this may be attributable to the sample size. This failure to observe variations in *miR-34a* gene suggests that other mechanisms affecting the function of *miR-34a* may exist, such as epigenetic silencing, aberrant miRNA processing or other molecular ways (Garzon et al, 2009; Liang et al, 2009). Further targeted studies to explore this possibility would be invaluable in elucidating the potential association of *miR-34a* functionality and longevity.

Table1 Sequencing results of *miR-34a* gene in Hainan population by using DNA direct sequencing

Sample	Number (*n*)	Mean age (years)	SNPs				
			rs201359809	rs72631823	rs35301225	rs369892834	rs372904298
Controls	236	48.1	0	0	0	0	0
Centenarians	203	102.7	0	0	0	0	0
1000G	-	-	0	0	-	0	0
NCBI	-	-	0.02%	0.01%	-	-	-

SNPs: Single nucleotide polymorphisms; 1000G: 1000 Genomes Project (http://www.1000genomes.org/); NCBI: National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/); Dash indicates that the data was unavailable.

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