

Nucleic acid therapy for lifespan prolongation: Present and future

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Lifespan prolongation is a common desire of the human race. With advances in biotechnology, the mechanism of aging has been gradually unraveled, laying the theoretical basis of nucleic acid therapy for lifespan prolongation. Regretfully, clinically applicable interventions do not exist without the efforts of converting theory into action, and it is the latter that has been far from adequately addressed at the moment. This was demonstrated by a database search on PubMed and Web of Science, from which only seven studies published between 2000 and 2010 were found to directly touch on the development of nucleic acid therapy for anti-aging and/or longevity enhancing purposes. In light of this, the objective of this article is to overview the current understanding of the intimate association between genes and longevity, and to bring the prospect of nucleic acid therapy for lifespan prolongation to light.

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As said by Bernard Williams (1975, p 417), ‘it would be not only always better to live, but better to live always, that is, never to die.’ Over the years, copious tactics have been proposed to battle for enhancing longevity (Lai and Chan 2011), and have exhibited various degrees of success. For instance, caloric restriction was reported to delay mortality and the onset of diseases such as arcopenia in rhesus monkeys (Balazsi 2010; Colman *et al.* 2009; McKiernan *et al.* 2011), whereas hormonal replacement was found to promote healthy longevity (Kahn 2005; Yonei *et al.* 2007) and be therapeutic to hypergonadotropic hypogonadism in females (Dragojević-Dikić *et al.* 2009). Other important examples of longevity enhancing tactics include ‘Strategies for Engineered Negligible Senescence’ (de Grey 2005) and antioxidant treatments (Baxter 2008; Head 2009). In addition to animal and human studies, research has been done on plant longevity, which was thought to be determined partially by stem cell immortality, vascular autonomy and epicormic branching (Borges 2009). Given this research basis and the ongoing advances in unraveling the etiology of aging, here I would like to highlight the prospect of an unheeded area – nucleic acid (NA) therapy – in prevailing biogerontological research as a new avenue for future endeavours.

As far as aging is concerned, it involves both extrinsic (e.g. oxidative damage) and intrinsic factors (e.g. anomalous

epigenetic signalling) (Gilbert 2009; Holzenberger *et al.* 2003). For the latter, DNA methylation is an important example. Its role in aging was first proposed by Vanyushin *et al.* (1973) based on an observation of age-related changes in the 5-methylcytosine (5-MeCyt) content of DNA from certain organs (e.g. brain, heart and spleen) in mice. This ideology was corroborated by subsequent research, in which a loss of global DNA methylation was found to correlate with aging (Wilson and Jones 1983). Lately, studies on monozygotic twins have suggested that epigenetic changes accumulate with age but independently of the genetic sequence (Fraga *et al.* 2005). Such findings have exposed the complexity of aging by displaying how environmental factors and gene functions may interact. Other than DNA methylation, post-transcriptional histone modifications (via phosphorylation, ubiquitylation, methylation and acetylation) are another key pathway in epigenetic signalling. They have been extensively reviewed in the recent years (Fraga and Esteller 2007; Gilbert 2009).

From the turn of the century, an increasing number of ‘longevity’ or ‘anti-aging’ genes have begun to be identified (Kennedy 2008; Lee 2006; Minois *et al.* 2010), thanks to high-throughput technologies and genetic techniques. Examples of relevant studies have been listed in table 1. With our better understanding of the

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Table 1. Studies on the genetics of aging and longevity

Source	Gene(s) examined	Model	Results and implications
Fujii <i>et al.</i> 2011	<i>oxy-5</i>	Nematode	Increasing the expression of the OXY-5 protein could promote the sensitivity of the nematodes (<i>Caenorhabditis elegans</i>) to oxygen and decrease the nematodes' longevity.
Berdichevsky <i>et al.</i> 2010	<i>kat-1</i>	Nematode	Loss-of-function mutation of <i>kat-1</i> may shorten the lifespan and result in abnormalities that are characteristic of premature aging.
Chen <i>et al.</i> 2010	<i>ATM</i>	Human	A functional single nucleotide polymorphism in the promoter of <i>ATM</i> associated with longevity.
Tang <i>et al.</i> 2009	<i>dOpa1</i>	Fruit fly	Mutation of <i>dOpa1</i> could shorten the lifespan, increase the susceptibility of fruit flies to oxidative stress, and elevate the production of reactive oxygen species in the insect model.
Madia <i>et al.</i> 2008	<i>SCH9</i>	Yeast	Mutation of <i>SCH9</i> (homologous to <i>AKT</i> and <i>S6K</i>) prevented recombination errors and premature genomic instability, suggesting that modulation of the IGF-I-Akt-56K pathway may also protect against premature aging in mammals.
Zhao <i>et al.</i> 2008	<i>WRN</i>	Human	Mutation of <i>WRN</i> linked with premature aging.
Zheng <i>et al.</i> 2007	<i>Il-2</i> and <i>fas</i>	Mice	Both IL-2 knockout and Fas mutation could prolong the lifespan of Scurfy mice.
Roux <i>et al.</i> 2006	<i>pka1</i> and <i>sck2</i>	Yeast	Pka1 and Sck2 were suggested to regulate chronicle aging in the fission yeast <i>Schizosaccharomyces pombe</i>
Holzenberger <i>et al.</i> 2003	<i>Igf1r</i>	Mice	Heterozygous IGF-1R knockout mice could display greater resistance to oxidative stress, and lived 26% longer than their wild-type littermates.
Fabrizio <i>et al.</i> 2003	<i>SOD2</i> , <i>SOD1</i> and <i>RAS2</i>	Yeast	Sod2 was found to link with survival extension in <i>Saccharomyces cerevisiae</i> . While overexpression of Sod2 (and Sod1) could extend survival by 30%, deletion of the <i>RAS2</i> gene doubled the mean lifespan of the yeast.

longevity-influencing roles of different pathways [e.g. insulin/insulin-like growth factor-1 (IGF-1) signalling] and genes (which encode proteins like Sod2, Ras, protein kinase A, Msn2, Msn4 and adenylate cyclase) (Longo 2004), NA therapy for lifespan prolongation have been made, at least theoretically, possible. In fact, the concept of NA therapy is no longer a hypothetical notion in the clinical arena. Its therapeutic values have been substantiated in different pathological conditions, ranging from β -thalassemia (Bank *et al.* 2005) to X-linked adrenoleukodystrophy (Cartier *et al.* 2009). In clinical trials, NA therapy can mitigate and correct the T cell immunodeficiency in severe combined immunodeficiency (SCID)-X1 patients, obviate the initiation or resumption of enzyme-replacement therapy and give the patients a normal life (Fischer *et al.* 2010). This range of impressive evidence, along with a profoundly increased repertoire of NA delivery technologies (Khosravi-Darani *et al.* 2010; Lai 2011; Lai and Lin 2009; Tros de Ilarduya *et al.* 2010; Yoon and Park 2010), has not only opened up an exciting vista of treating diseases that have been incurable to date (El-Aneed 2004; Fajac *et al.* 1999; Ferrari *et al.* 1999; Olefsky 2000) but has also paved the way for its further extension to longevity enhancement and aging postponement.

In actuality, the plausibility of genetically modulating the aging process and extending life has already been

hinted at in both the *in vitro* and *in vivo* contexts. The former can be illustrated by Wang *et al.*'s earlier observation (2001) that, while suppression of HuR, an ubiquitously expressed Elav-like RNA-binding protein, may elicit cellular senescence (such as lower ^3H -thymidine incorporation, lower basal cyclin-dependent protein kinase activity and higher senescence-associated β -galactosidase activity) in IDH4 human fibroblasts, the phenotype of senescence could be rejuvenated once HuR was overexpressed. In the *in vivo* context, an earlier study conducted by Hsieh and colleagues (2002) reported that mutation of *Pit1* could prolong the lifespan of the Snell dwarf mouse. Likewise, with RNA interference, Li *et al.* (2007) have demonstrated that down-regulation of the expression of dUbln, the *Drosophila* orthologue of human UBQLN1, in the nervous system can shorten the lifespan of fruit flies and elicit age-dependent neurodegeneration. More recently, the intimate association between the lifespan and genes has been further attested by Walker's team (Copeland *et al.* 2009), which has successfully prolonged the lifespan of *Drosophila melanogaster* by suppressing the expression of selected electron transport chain (ETC) genes encoding components of mitochondrial respiratory complexes I, III, IV, and V in flies. All these studies have shed light not only on the genetics of longevity but also on the apparent possibility of lifespan prolongation, which imbues NA

therapy with striking potential to extend life directly rather than merely correcting genomic mutations and rejuvenating the degenerated nervous system as discussed earlier (Lai and Lin 2010).

Notwithstanding the aforementioned prospect, clinically applicable interventions do not exist without the efforts of converting theory into action. Unfortunately, it is the latter that has been far from adequately addressed. This was demonstrated by executing a database search on PubMed and Web of Science (figure 1), from which only seven studies published between 2000 and 2010 were found to directly touch on the development of NA therapy for anti-aging and/or longevity enhancing purposes. Among those studies, over 70% ($n=5$) manipulated only one to two facets of the aging process [such as vascular dysfunction (Brown *et al.* 2006), erectile dysfunction (Melman *et al.* 2008), memory impairment (Mouravlev *et al.* 2006), delayed angiogenesis (Wang *et al.* 2004) and thymic involution (Phillips *et al.* 2004)] rather than combating decrepitude as a whole. Only two out of the seven articles have been devoted to the issue of lifespan extension. However, except Boghossian *et al.*'s research (2007) on adult obese ob/ob mice, whose lifespans have been substantially increased from 55.5 weeks to 106.5 weeks after intracerebroventricular

injection of recombinant adeno-associated virus encoding the leptin gene, the other article attempted simply to extend lifespan in the cellular rather than the organismal context (Chung *et al.* 2007). Such scarcity of research to turn NA therapy for longevity enhancement into practice clearly points to a delay in the development of relevant interventions.

In light of this, the pace of applied research has to be expedited to catch up with the rapid progress of basic biogerontological research. Definitely, challenges are expected. This is partially because lifespan determination is a polygenic trait (Lai and Lin 2010) and being able to manipulate few genes *in vivo* may not necessarily mean that aging postponement and life extension could be successfully achieved in a human body, which is a much more complex biological entity as compared to fruit flies and yeasts. Moreover, the yet-to-be-elucidated physiological price paid by modulation of each individual gene in long term may impose limitations on the extent of experimental work that could be attempted. Nevertheless, the challenges and opportunities of NA therapy for lifespan prolongation have now been brought to light. It is hoped that with more studies and insights, the fantasy of indefinite lifespan extension could one day be turned into reality.

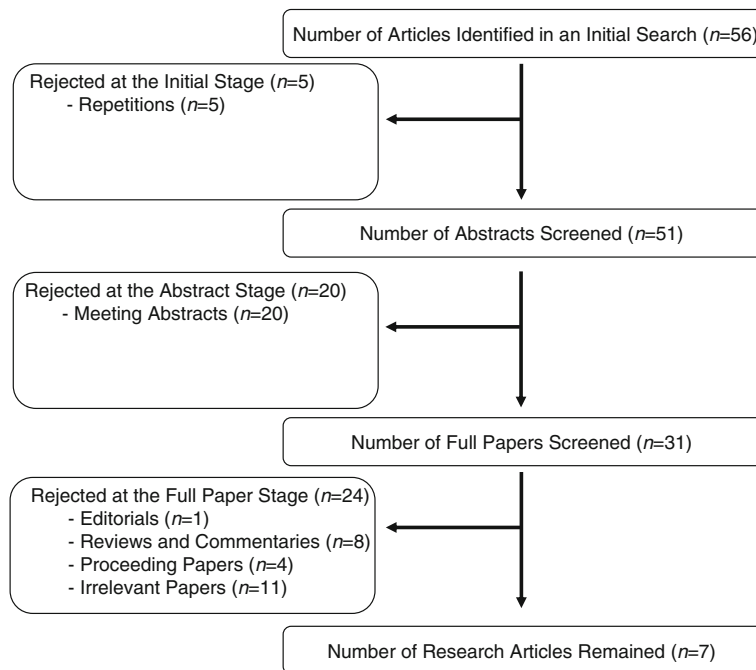


Figure 1. QUOROM flowchart. The systematic review protocol was adopted as previously described (Lai 2011). An initial search was performed by searching the following keywords, as appropriately combined by Boolean operators 'AND' and 'OR', in titles: 'delivery', 'transfer', 'therapy', 'intervention', 'aging', 'aged', 'ageing', 'lifespan', 'longevity', 'gene', 'DNA', 'RNA', 'nucleic', 'plasmid' and 'genetic'. Articles published between 2000 and 2010 are included if and only if they are research reports that touch directly on the development of NA therapy for anti-aging and/or longevity enhancing purposes.

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