Project Title: Development of a new Telomere length measurement protocol by digital PCR method and application to the HK community elderly cohort

(US Provisional Application No. 62/856,449-A method to quantify telomere length and genomic motifs)

Abstract

Telomere length (a biological marker of aging) is available from a subset of the original cohort. Repeated measurement (on those who are still alive) would enable the relationship between the ageing process itself (represented by the rate of telomere shortening) and the influence of personal, environmental, and health system factors. This research question represents work at the cutting edge of ageing research, since there is poor correlation between genes and human longevity and aging trajectories [Singer 2015], and the importance of genetic factors likely lie in environmental factors possibly regulating gene expression (epigenetics) rather than in certain patterns of SNPs directly affecting the process. A recent study by Daniel Belsky published in PNAS discussed how telomere length is one of the biological markers in mid-life that indicated the speed of the aging process [Belsky et al 2015]. Blood specimens telomere assay could be included in the follow up interviews.

Telomere length as a marker of biological aging

Aging process may be exacerbated by chronic inflammation resulting in enhanced cell turnover particularly those cell types involved in immune response. Attrition of telomere reflects the replicative history of a cell (Stewart and Weinberg 2006, Collado, Blasco et al. 2007). Telomeres are repeats of hexameric nucleotide (TTAGGG) on end of chromosomes in eukaryotic cells (Goronzy, Fujii et al. 2006). They are critical in maintaining stability of chromosome, prevent fusion and atypical recombination and are essential for cell division (Blasco 2007). Telomeres are shortened by 30 to 200 bp after each division and cell with telomeres shorter than a critical limit will go into senescence and apoptosis. The rate of telomere decay is variable between individuals and both genetic and environmental factors have been shown to affect telomere attrition (Aviv, Valdes et al. 2006, Blasco 2007, Zhang 2007).

Furthermore, studies looked into the association between telomere length and dementia or cognitive ageing (Panossian, Porter et al. 2003, Harris, Deary et al. 2006, Honig, Schupf et al. 2006, Jenkins, Velinov et al. 2006, Martin-Ruiz, Dickinson et al. 2006). These data supported a role of telomere attribution in cognitive aging. AD patients had shorter telomere in peripheral blood lymphocytes which was correlated with higher serum TNF α concentration and higher percentage of heat induced apoptosis in T cells (Panossian, Porter et al. 2003). Similarly, in autoimmune diseases like rheumatoid arthritis, cells from both inflamed joints and peripheral leukocytes had shortened telomere (Steer, Williams et al. 2007). Patients with atherosclerosis and its complications also had shorter telomeres in both vascular endothelial cells and peripheral leukocytes compared to healthy control (Brouilette, Moore et al. 2007, Minamino and Komuro 2007, van der Harst, van der Steege et al. 2007). Therefore, telomere length may be a biomarker of biological aging and cell turnover resulting in cellular senescence.

Methods of measurement of telomere length

Many previous studies had also been limited by the labor-intensive assay of telomere length, which has been overcome by the recent development in high throughput telomere length assays by real-time quantitative PCR. The project team has mastered this technique and applied in a population cohort of 2,000 Chinese elderly men and women. With the advance in real time PCR, we have been using real time PCR to measure telomere length in cross sectional studies. The studies of the project team together with other centers provided new understanding in biology of telomere attrition and various life style factors.

The project team has published extensively on telomere length in the Chinese elderly cohort using the real time quantitative PCR technology.

However, it has been apparent to the project team and other now that the method suffers from various limitations. Firstly, the results have a large analytical variance. Secondly, quality of sample affects the results. Therefore, fresh samples are required to give rise to better results. These limitations preclude precise measurements on archival samples in community based studies.

Recently, the development of highly precise PCR method, called droplet digital PCR (ddPCR), allows counting of DNA molecules (or PCR products) at single molecule level. Therefore, it has been widely applied to study of copy number variations (CNV). In fact, telomere length is a special example of CNV, therefore, it is most suitable to be measured by this new technology. Similar approach was used to quantify telomerase activity at single cell level (Ludlow 2014). The project team will modify their idea to make it useful to measure telomere length in archival samples.

The project team proposes to measure telomere length in both baseline (archival) samples and follow up blood samples of the HK Chinese Community cohort. It will allow a longitudinal study of attrition of telomere length and identification of various life style risk factors.

Objectives

- Development of a new telomere length measurement method using latest digital PCR platform.
- Application of the method to HK Community Elderly Cohort and study the association with various life style, environmental and genetic factors.