● PERSPECTIVE

Can leukocyte telomere shortening be a possible biomarker to track Huntington's disease progression?

Huntington's disease (HD): HD is an autosomal dominant neurodegenerative disease, caused by a CAG trinucleotide repeat expansion in the first exon of the HTT gene encoding the huntingtin protein. The mutant protein contains an expanded polyglutamine sequence that confers a toxic gain-of-function and causes neurodegeneration. Moreover, several studies indicate that loss of the normal protein beneficial functions, contribute to the pathology (Schulte and Littleton 2011). Triplet expansion over 40 repeats are fully penetrant and invariably lead to manifest HD in the fourth or fifth decade of life.

Clinically, HD is a debilitating, incurable disorder in which no symptoms reveal genetic status (presymptomatic phase) until signs and symptoms of motor impairment, such as poor coordination or slight involuntary movements and subtle changes in eye movements, begin to appear (prodromal phase). Often the prodromal phase (up to 15 years before the full-blown disorders) includes psychological and psychiatric alterations as irritable mood, depression, difficulty in learning new information or mental planning; nonetheless, affected individuals are usually able to perform their ordinary activities and to continue work. With progression of the disease, however, involuntary jerking or twitching movements (choreic movement) become exacerbated, making walking, speaking, and swallowing increasingly difficult. Also, cognitive and psychiatric symptoms are worsening until dementia (Paulsen, 2010).

The clinical diagnosis of HD is based chiefly on the presence of motor signs and symptoms according to the diagnostic confidence level from the Unified Huntington's Disease rating scale. This standard assessment tool for grading HD symptom severity is based on two relevant subscores: total functional capacity, which tracks the ability to perform daily activities, and the total motor score, which specifically tracks motor abilities (https://www.ncbi.nlm.nih.gov/books/NBK1305/). The diagnostic confidence level ranges from 0 (asymptomatic) to 4 (motor impairments as unambiguous signs of HD). When a clinical diagnosis is established, the disease is manifest and becomes more and more devastating with time. In late stages, motor disability progress from hyperkinesia/chorea to severe hypokinesia/rigidity, rendering the affected individual often totally dependent, mute, and incontinent. Since, right now, there are no disease-modifying treatments, the disease slowly progresses toward death within 15 to 18 years after its onset.

Though no treatment can alter its course, the prodromal stage is fundamental for evaluating the wide spectrum of signs and symptoms of disease progression. In addition, earlier stages of dysfunction offer a potential window for intervention or modification of pathogenic mechanisms. The duration of these stages varies among individuals. The length of CAG repeat is negatively correlated with age at disease onset. Models based on biological age and CAG repeat length are available to approximately estimate the years until onset in mutation carriers (Langbehn et al., 2010). Such testing, although useful, is not accurate in predicting age at clinical onset because CAG length accounts for only about 50–70% of the variation within a range of 40 to 55 CAG repeats. The residual variance is probably due to genetic, stochastic, and environmental factors. Given this variance, there is growing interest in HD research to search for phenotype modifier genes and biomarkers, identify specific changes or "stages" of disease progression, predict disease onset, and accurately track disease progression in premanifest HD (Gusella et al., 2014).

Telomeres as HD biomarkers: The possibility of presymptomatic genetic testing in HD opens the way for evaluating potential disease-modifying treatments in the premanifest phase. Biomarkers that reflect underlying disease processes or progression are needed to monitor changes in clinically asymptomatic individuals. No studies to date have yielded validated biomarkers that can accurately predict either age at HD onset or disease progression, although the neurofilament light protein appears extremely promising (Silajdzic and Bjorkqvist, 2018). At present there are no effective treatments in clinical practice that can prevent the disease, halt its progression or delay its onset. Nonetheless, as the future holds promise for disease-modifying strategies, there is a need for reliably assessable disease progression biomarkers, as well as markers to detect treatment-related changes.

Ideally, a biomarker will be closely linked to the pathophysiology of the disease, reliable, accurate, sensitive, specific, reproducible, inexpensive, non-invasive, and acceptable for the patient (Silajdzic and Bjorkqvist, 2018). Easily accessible peripheral biomarkers from peripheral blood mononuclear cells and plasma have gained increasing attention. Our recent work seems to indicate that leukocyte telomere length (LTL) could possess several of the characteristics required of an ideal biomarker to track HD progression (Scarabino et al., 2019).

Telomeres are the terminal ends of the chromosomes and play a role in preserving genome stability. Telomere shortening occurs progressively with repeated cell division because of the inability of DNA polymerase to replicate the 3′ end of the DNA strand. A cellular multiprotein complex, called telomerase, counteracts telomere shortening, but its activity, usually present in the early stages of embryonic development, is silenced in several human somatic tissues immediately after birth. As a consequence, the telomeres shorten progressively with increasing age in the replicating cells of adult tissues (Blackburn et al., 2015). This phenomenon may indicate cellular senescence and reflect an organism's biological age. Peripheral blood mononuclear cells provide an easily accessible source of cells in which telomere length can be analyzed.

Shortened LTL has been found to be associated with aging and various age-related diseases, including cardiovascular diseases, Alzheimer's disease (AD), and stroke-associated dementia. Studies have demonstrated rapid leukocyte telomere shortening in diseases associated with increased systemic oxidative stress and chronic inflammation (von Zglinicki, 2002). In addition, telomere loss may be induced by increased immune cell turnover associated with inflammation. When LTL was investigated in neurodegenerative diseases, shorter LTL was frequently found in association with cognitive decline/dementia and AD. A significant progressive leukocyte telomere reduction detected in patients with mild cognitive impairment (MCI) and AD, as compared with controls (Scarabino et al., 2017), suggests that LTL measurement could be a useful means to follow dementia progression as it converts from prodromal (MCI) to manifest AD. AD and HD share several common features, including loss of neurons, accumulation of aggregated and misfolded proteins, and cognitive decline (Clark and Kodadek, 2016). Unlike subjects with MCI in which the evolution of the disease towards AD is uncertain, premanifest individuals with HD will inexorably develop the disease, although the age at disease onset varies widely.

Our recent study (Scarabino et al., 2019) analyzed LTL in a sample of patients with premanifest (pre-HD, *n* = 38), manifest HD (HD, *n* = 62), and healthy control (*n* = 76) to gain a clearer picture of the relationship between telomere length and HD progression. The mean LTL differed significantly ($P < 0.00001$) across the three groups, with the highest mean values observed in the control group, intermediate values in the premanifest HD, and the lowest values in the manifest HD patients (**Figure 1A**), suggesting a relationship between telomere shortening and manifest HD development. The mean LTL values observed in manifest HD were nearly half those observed in the age-matched controls and slightly higher than the minimum length reported to be necessary to ensure human telomere protective stability in white blood cells (Blackburn et al., 2015). Comparison of the regression lines showed that the decreasing trends for telomere length with age were significantly different among the three groups (**Figure 1B**). As expected, there was a downward trend of LTL with increasing age in the controls, which began to increase with advancing ages. In very young premanifest HD patients, LTL was very similar to that reported for the age-matched controls but with and fast decrease after the age of 30. The youngest manifest HD subjects showed already very short telomeres, with only a slight decrease in the most advanced ages (**Figure 1B**).

After adjusting LTL for age, the effect of CAG repeat number on LTL was analyzed as well. An inverse relationship between mean LTL values and number of CAG repeats was found in the individuals with premanifest HD but not in those with manifest HD, indicating that in premanifest HD the number of CAG repeats contributes to telomere attrition independently of age (Scarabino et al., 2019). These findings indicate that in premanifest HD leukocyte telomeres begin to shorten gradually and markedly after age 30 years, depending on increasing age and CAG number, up to the values that are typically observed in patients with manifest HD. In premanifest HD individuals, LTL was then examined in relation to the estimated time to clinical diagnosis. The estimated age at onset was calculated according to the formula of Lanbehn et al. (2010), at different probability values, given CAG repeat number and age at blood sampling. The years to clinical diagnosis were calculated as the difference between estimated onset and age at blood sampling. A
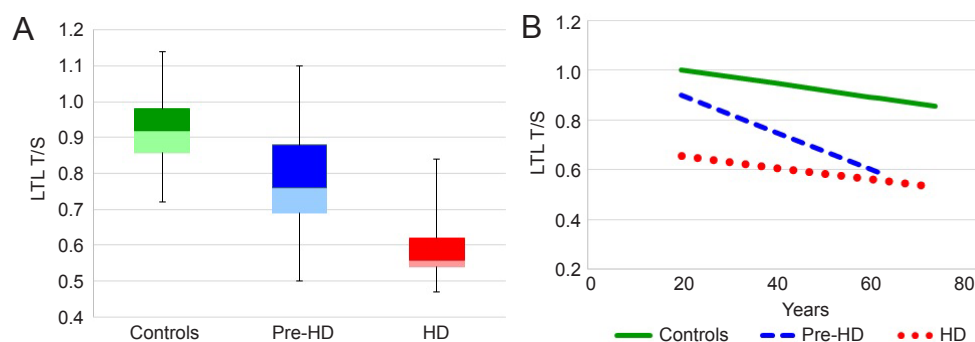
Figure 1 Distribution of LTL in HD patients.
(A) Box Plot showing the distribution of LTL (T/S ratio) in controls, premanifest HD (Pre-HD), and manifest HD patients (HD). LTL was measured by real-time PCR. T/S ratio: Number of copies of telomeric repeats (T) as compared to single copy gene (S). (B) LTL expressed as T/S ratio as a function of age in controls, in premanifest HD (pre-HD) and manifest HD patients (HD) (Scarabino et al., 2019). LTL: Leukocyte telomere length; HD: Huntington's disease.

significant linear positive relationship was observed between LTL and estimated years to diagnosis, indicating that the fewer the estimated years to HD onset, the shorter the LTL and vice-versa (Scarabino et al., 2019). To date, two other studies on LTL in HD have confirmed significantly shorter LTL in HD patients as compared with controls (Kota et al., 2015; Castaldo et al., 2019).

Taken together, the data seem to indicate that LTL could possess several characteristics required of an ideal biomarker of HD progression (Silajdzic and Bjorkqvist, 2018). It can be easily obtained by inexpensive blood sampling, and it is readily quantifiable and highly reproducible. Moreover, LTL is closely linked to the pathophysiology of HD. Indeed, the discovery of brain lymphatic vessels, the new role of microglia and astrocytes in survival, and metabolism of neurons are changing the idea of the pathogenesis of neurodegenerative diseases. There is now strong evidence that the mechanism leading to neuron death is substantially influenced by the expression of the mutant protein by non-neuronal cells (microglia-astrocytes, non-cell autonomous pathogenesis). Since microglial cells are regulated and regulate lymphocytes (Clark and Kodadek, 2016), we reason that peripheral lymphocyte stress might be a faithful mirror of microglia-astrocytes-neuron stress. Moreover, also leukocyte telomere shortening could reflect microglial activation and the resulting neuroinflammatory state. In this context, we may speculate that leukocyte telomere shortening could be an indicator of the above-mentioned phenomena that accompany disease progression from the premanifest to manifest HD.

Conclusions and future perspectives: LTL measurement seems to possess distinctive features required for a suitable biomarker to detect HD progression: it is easy to obtain, readily quantifiable and reproducible, and closely linked to the pathophysiology of HD. In premanifest HD individuals, LTL shows a very significant linear relationship with the estimated years to the clinical onset of HD and could predict the time at clinical diagnosis with good probability levels. Using LTL as a biomarker in HD could have multiple potential applications:

1) characterize the different phases (premanifest and overt disease);
2) monitor the progression of pathogenic events leading to manifest disease;
3) find peripheral patterns that may mirror brain dysfunctions;
4) contribute to constructing a simple and reliable model, perhaps by incorporating novel biomarkers (LTL, circulating micro-RNAs and snoRNAs, neurofilament light protein), to predict disease onset and to define a temporal window in which patients could be effectively treated. Previous models, which focused only on the length of CAG trinucleotide repeat expansion, were univariate predictors of clinical age;
5) monitoring the efficacy of potential novel therapies.

In this context, a non-invasive procedure to obtain samples and relatively simple analytical assays are desirable as biomarkers for trials and care in premanifest HD individuals. Extending the analysis to a larger sample with a longitudinal study design would be useful for investigating the real properties of LTL as a biomarker for HD progression.

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