A Prospective Study of Telomere Length Measured by Monochrome Multiplex Quantitative PCR and Risk of Non-Hodgkin Lymphoma

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Abstract

Purpose: Telomere length plays an important role in the maintenance of chromosomal stability and in tumorigenesis. We hypothesized that telomere length in peripheral WBC DNA obtained from healthy individuals would be a predictor of future risk of developing non-Hodgkin lymphoma.

Experimental Design: Using a new assay to measure relative telomere length, monochrome multiplex quantitative PCR, which strongly correlates with telomere length measured by Southern blot (Spearman r = 0.91, P < 0.0001) and has high precision (coefficient of variation = 7%), we compared telomere length in peripheral WBC DNA in 107 incident male non-Hodgkin lymphoma cases and 107 matched controls within the prospective Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort.

Results: Median (10th, 90th percentile) telomere length was 1.10 (0.79, 1.43) in cases and 1.02 (0.78, 1.26) in controls (P = 0.0017, Wilcoxon sign test). There was a strong dose-response relationship between quartiles of telomere length and risk of non-Hodgkin lymphoma overall [odds ratios (95% confidence intervals) by quartile: 1.0; 1.1 (0.4-2.7); 1.8 (0.7-4.9); and 3.6 (1.4-8.9); P trend = 0.003], and this association was similar across the most common non-Hodgkin lymphoma subtypes present in this study.

Conclusion: These results suggest that longer telomere length may be a potential predictor for future risk of non-Hodgkin lymphoma.

Telomeres are complexes of tandem repeats of the sequence TTAGGG that cap chromosomes. They are essential for protecting chromosomal stability, which is associated with the initiation and progression of human cancers (2). At the same time, many incipient tumors can terminate their own growth by shortening their telomeres sufficiently to trigger replicative senescence or apoptosis (3). However, if sufficient numbers of mutations that promote growth and block cell senescence and apoptotic pathways accumulate in a cell before its telomeres shorten enough to trigger senescence or apoptosis and protect it from cancer, then unlimited proliferation may ensue. It follows that in some cell types, under some circumstances, long telomeres may actually increase the risk of cancer, by allowing more time and more cell divisions during which the cell can accumulate oncogenic mutations.

Most epidemiologic studies have reported that relatively shorter telomere length measured in peripheral WBC and in some instances buccal cells is associated with increased risk of cancer (4–10). In contrast, some recent reports have suggested that longer telomere length may be associated with increased risk of certain tumors, such as breast cancer and melanoma (11, 12). Most studies have used a case-control design (4–8), with several more recent reports using a prospective cohort design (9–11).

Given that peripheral WBC contain lymphocyte subsets that derive from lymphocytic stem cells and immunologically active tissue, we hypothesized that telomere length in peripheral WBC DNA might be particularly informative with regard to risk of developing non-Hodgkin lymphoma. To avoid potential disease bias, we carried out our study within a prospective cohort, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study (13). In addition, we applied a modification of the original PCR-based assay developed by Cawthon (14), referred to as monochrome multiplex quantitative PCR (15), which has high assay precision and is highly correlated (Spearman r = 0.91, P < 0.0001) with the Southern blot method of measuring telomere length.
Translational Relevance

We report that longer telomere length measured in peripheral WBC DNA is associated with future risk of developing non-Hodgkin lymphoma. This work contributes to a growing number of findings that biomarker assays that measure genomic damage and stability in peripheral WBC of healthy people (e.g., telomere length, global methylation, alteration in mitochondrial DNA copy number) can identify individuals who are at higher risk of developing certain types of cancer in the future. The translational implication of our findings, and this overall area of research, is that the general population will eventually be screened not only for inherited variation in genes, but for acquired alterations in DNA as well, which together will be powerfully predictive of future cancer risk. This will identify individuals who could benefit from various preventive strategies (e.g., focused carcinogen avoidance, chemoprevention) as well as targeted early disease detection.

Materials and Methods

Case and control enrollment. Details of the ATBC cohort study design have been described previously (16). Briefly, 29,133 male smokers, ages 50 to 69 y, were recruited from southwest Finland and randomized from 1985 to 1988. Subjects were provided α-tocopherol, β-carotene, both, or placebo. The study was approved by the Institutional Review Boards at the National Cancer Institute and the National Public Health Institute of Finland. Participants provided written informed consent. Incident non-Hodgkin lymphoma cases were identified using the Finnish Cancer Registry, which provides nearly 100% of case ascertainment from 1985 to 1998. Cases were comparable to control subjects (Table 1). Telomere length was statistically significantly longer among cases than controls [median (10th, 90th percentile), 1.10 (0.79, 1.43) in cases and 1.02 (0.78, 1.26) in controls, Wilcoxon sign test; P = 0.0017; Table 1]. The risk of non-Hodgkin lymphoma was significantly increased with longer telomere length, compared with the lowest quartile of telomere length (P trend = 0.003), and the association was consistent across the predominant non-Hodgkin lymphoma subtypes in this series of cases (Table 2). Adjustment for demographic factors shown in Table 1 had an negligible impact on the results (data not shown).

Results

Cases were comparable to control subjects (Table 1). Telomere length was very weakly and inversely correlated with age (Spearmann correlation r = -0.07, P = 0.46), and weakly and positively correlated with pack-years of smoking (Spearmann correlation, r = 0.12, P = 0.20) among controls, which would be expected given the relatively narrow age range of this older cohort of all tobacco smokers.

Telomere length was statistically significantly longer among cases than controls [median (10th, 90th percentile), 1.10 (0.79, 1.43) in cases and 1.02 (0.78, 1.26) in controls, Wilcoxon sign test; P = 0.0017; Table 1]. The risk of non-Hodgkin lymphoma was significantly increased with longer telomere length, compared with the lowest quartile of telomere length (P trend = 0.003), and the association was consistent across the predominant non-Hodgkin lymphoma subtypes in this series of cases (Table 2). Adjustment for demographic factors shown in Table 1 had a negligible impact on the results (data not shown).
Fig. 1. The principle of telomere length measurement by quantitative PCR. Three pairs of chromosomes are shown. Circles represent centromeres. The bottom pair of chromosomes represents chromosome 11, with the red arrows along the p arm representing the primer pair for amplifying a segment of the single copy gene β-globin locus. The two upper pairs of chromosomes are representative of the remaining 21 pairs of autosomes, as well as the sex chromosomes X and Y. Regions containing telomere repeats (TTAGGG)n are colored green. As shown, telomere lengths can vary between chromosomes and even between the two ends of a single chromosome. The blue arrows represent the primer pairs used to amplify the telomere sequences. The telomere PCR signal is a measure of telomere length, because the number of telomere primers that can bind the telomeric DNA at the beginning of the PCR is directly proportional to the total summed length of all the telomeres in the cell. The single copy gene PCR signal is a measure of the cell count, because exactly two copies of the single copy gene primer pair can bind to each cell’s DNA at the beginning of the PCR. Therefore, the telomere PCR signal divided by the single copy gene PCR signal is proportional to the average telomere length per cell.

To determine if the association might be driven in part by longer telomere length among cases undiagnosed at the time of blood sample collection, we excluded cases diagnosed within the first year of follow-up (n = 17) after blood sample collection, and found that results were very similar, with odds ratio (95% confidence interval) of 0.8 (0.3-3.1), 1.5 (0.5-4.1), and 3.1 (1.2-8.2) for the second, third, and fourth quartiles of telomere length, compared with the lowest quartile of telomere length (P trend = 0.01).

To evaluate the potential effects of the trial vitamin supplementation on the relationship between telomere length and risk of non-Hodgkin lymphoma, we carried out further analyses stratified by α-tocopherol versus no α-tocopherol supplementation, and β-carotene versus no β-carotene supplementation. Risks were similar in each group, and tests for interactions were not statistically significant (data not shown).

**Discussion**

To the best of our knowledge, this is the first prospective study addressing the relationship between telomere length and the risk of non-Hodgkin lymphoma. Although the sample size is modest, we detected relatively strong effects, which were consistent across major non-Hodgkin lymphoma subtypes. One previous report of telomere length and non-Hodgkin lymphoma by Widmann et al. of 40 cases and 40 controls using a case-control design found that shorter telomere length was associated with increased risk of aggressive non-Hodgkin lymphoma (7). The reasons for this discrepant result with our study are not immediately apparent. Given that relatively shorter telomere length was present in DNA extracted from B cells, T cells, and granulocytes in cases versus controls in the initial report (7), it would be expected that measuring telomere length in an aggregated “buffy coat” containing all peripheral leukocytes, as we did in our study, would give similar results. Widmann et al. measured telomere length by the Flow-Fish method whereas we used a new PCR-based method, which correlates almost perfectly with the Southern blot assay of telomere length. The correlation between the assay used in our report and the Flow-Fish method is not known. The prospective nature of our study is another potential difference between the two reports.

It is possible that tumor cells were present in the peripheral blood of undiagnosed patients in our study, particularly those with the more indolent non-Hodgkin lymphoma histologies. There is much evidence, however, that telomere length is shorter in lymphoma cells (19-21). As a consequence, if tumor cells were circulating in the blood of undiagnosed patients, the average telomere length of their peripheral WBC DNA would tend to be shorter, not longer. This would have biased our results towards the null, rather than create the relatively strong association we report with longer telomere length. At the same time, given that we observed similar associations between longer telomere length and risk of non-Hodgkin lymphoma for a
relatively indolent (i.e., chronic lymphocytic leukemia/small lymphocytic lymphoma) and a more aggressive histology (i.e., diffuse large B-cell lymphoma; Table 2), it is unlikely that our results have been attenuated by this potential bias. Therefore, our overall interpretation of these results is that in a healthy population there is a normal range of variation in the average WBC telomere length among individuals, and those individuals with relatively long telomeres are at higher risk of later developing lymphoma.

Clearly, telomere shortening is an early and frequently observed finding in malignant transformation (22). The question is to what extent would the tendency to have relatively shorter or longer telomeres in normal tissue, which seems to reflect both genetic and environmental factors (23), be associated with future risk of developing cancer in general, and of developing specific tumor types in particular. From a theoretical perspective, one can argue that the tendency to have shorter or longer telomeres could each contribute to carcinogenesis (3). Optimal telomere length is a balance of cell proliferation, senescence, and control. Shorter telomeres may result in a greater tendency towards chromosomal instability, which could reflect a constitutional or acquired tendency towards carcinogenesis (5, 6). Alternatively, given that telomeres become shorter with cell proliferation, ultimately triggering senescence or apoptosis, it is possible that the tendency to have cells with shorter telomeres may have an advantage for suppressing tumorigenesis when their telomeres reach a critical minimal length. It follows that cells with longer telomeres may favor a delayed senescence (3), and such cells could have more opportunity to acquire genetic abnormalities and be at higher risk of transformation.

In addition, the relationship between telomere length and risk of cancer may vary by cell type. Recently, Han et al. reported that shorter telomere length measured in buffy coats in the prospective Nurses’ Health Study (11) by the original PCR-based assay developed by Cawthon (15), which strongly correlates with the new monochrome quantitative multiplex PCR used in this report (Spearman $r = 0.83$, $P < 0.0001$), was associated with a decreased number of moles and risk of melanoma and an increased risk of basal cell cancer (9).

In summary, in this prospective cohort, we found that longer telomere length was associated with increased risk of non-Hodgkin lymphoma. These findings require replication in larger studies that avoid or carefully address potential disease bias and that incorporate comparable methods to assess telomere length. Overall, our findings suggest that the relationship between telomere length measured in healthy tissue and risk of cancer may be complex and may vary by disease and possibly study design.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Acknowledgments

We thank Jackie King and the other members of the BioReliance BioRepository (Rockville, MD) for blood sample handling, storage, and shipping, and for assisting with laboratory analysis monitoring.

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doi:10.1158/1078-0432.CCR-09-0845

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