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SHORT REPORT

Common variants near *TERC* are associated with leukocyte telomere length in the Chinese Han population

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A recent genome-wide association study has identified an association between leukocyte telomere length (LTL) and a locus at 3q26 that includes *TERC*. In order to evaluate the effects of the SNPs rs12696304 and rs16847897 near *TERC* in the population of mainland China, we conducted an association study of LTL focusing on these two candidate SNPs in a sample of 4016 Chinese Han individuals. Multiple linear regression analyses were performed to evaluate the association of LTL with each SNP adjusted for age, gender and diabetes status. In the study, we confirmed the association of SNP rs12696304 and rs16847897 near *TERC* with LTL in the Chinese Han population ($P \sim 4.5\times10^{-3}$ and 9.5×10^{-5}, respectively). Each copy of the major allele of rs12696304 and rs16847897 was associated with a shorter mean telomere length of 0.024 and 0.031 T/S respectively, which is equivalent to about 3 and 4 years of average age-related telomere attrition. Our short report confirmed the effects of SNPs near *TERC* on LTL in the Chinese Han population for the first time.
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INTRODUCTION

Telomeres are structures at the ends of eukaryotic chromosomes, which are made up of a repetitive sequence and which has a major role in genomic stability. Telomere dysregulation can lead to cell death, cell senescence, or abnormal cell proliferation.[1] In humans, leukocyte telomere length (LTL) is getting shorter progressively with age, and is frequently reported to be relatively shorter in aging-related diseases: such as Alzheimer's disease[2] and vascular dementia.[3] Telomere length varies between individuals of the same age, and is found to be inheritable in quantitative-trait linkage analyses of sib pairs.[4,5] Quantitative trait locus studies have mapped putative loci for telomere length to human chromosomes 3p26.1, 10q26.13, 12q12.22 and 14q23.2.[5–7]

Telomerase is a large, mainly uncharacterized, RNA–protein complex that is responsible for the *de novo* synthesis and maintenance of telomere ends. *TERC* is a main component of telomerase, which serves as a template for addition of multiple 6 bp (TTAGGG) telomere repeats. A recent genome-wide association study (GWAS) identified association of common variants near *TERC* (on 3q26) with telomere length in two large European cohorts of 2917 individuals, followed by replication in three further European cohorts of 9492 individuals.[8] The strongest associations with LTL were observed for SNP rs12696304 and rs16847897 near *TERC* on 3q26, and the association signals across the 3q26 locus between rs12696304 and rs16847897 are located toward opposite ends of an ~87 kb region showing significant association with telomere length. Another GWAS study in an American population also identified an association signal in the *TERC* locus (rs3772190, $P \sim 1.1\times10^{-5}$). This SNP is in strong linkage disequilibrium (LD) with rs12696304 (r^2=0.91).[9] However, it is unclear whether this locus recently identified in Europeans and Americans exerts a similar effect on LTL in the Chinese population. In the present study, we sought to validate these reported associations between SNPs near *TERC* (rs12696304 and rs16847897) and LTL in 4016 unrelated individuals based on the Chinese Han population.

MATERIALS AND METHODS

Participants

Participants in the project, which was designed to be a case-control study for type 2 diabetes, comprised 4016 Han Chinese individuals. All participants were recruited from Shanghai. This study population had been used and described in our previous study.[10] Diabetes status was defined in accordance with WHO criteria and the details of the subgroups are summarized in Table 1 (Materials and Methods).

Genotyping

High-molecular-weight genomic DNA was prepared from venous blood using the QuickGene 610L Automatic DNA/RNA Extraction System (Fijifilm, Tokyo, Japan). All SNP genotyping experiments were done using TaqMan technology on an ABI7900 system (Applied Biosystems, Foster City, CA, USA). The standard 5 μl PCR procedures were carried out using TaqMan Universal PCR Master Mix reagent kits under the guidelines provided. Replicate quality

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control samples (1% samples) were included and genotyped with 100% concordance.

Telomere length measurement

Mean LTL was measured using an established and validated quantitative PCR-based technique.[11] This method expresses telomere length as a T/S ratio with RNase P as a reference (ABI) for each sample (Supplementary Methods). Reactions for telomere and RNase P reference were run in duplicate in 10 μl reactions in the same plate on ABI-Applied Biosystems 7900 HT Thermal Cycler (Applied Biosystems).

Statistical analysis

In this study, mean telomere length was considered as a quantitative trait, and expressed as a T/S ratio. We conducted multivariable linear regression to analyze the association of the T/S ratio with the two SNPs, adjusted for age, gender, and diabetes status. Given the statistical power of our study, we did not exclude the type 2 diabetes subjects, in line with previous genome-wide studies.[12,13] Considering the potential correlation between diabetes and telomere length, we included the diabetes status in the association model as a covariant. Bonferroni correction was used for multiple test correction. According to that there are two SNPs were investigated in this study, a *P*-value <0.025 was considered significant.

RESULTS

LTL was measured successfully in 3817 individuals out of the total of 4016 (success rate $>95\%$). Telomere length was approximately normally distributed in our study population as shown in Supplementary Figure 1. The age relationship of telomere length is shown in Supplementary Figure 2, which shows the expected decline in telomere length according to age. We deduced from our study that the age-telomere declining formula is 'LTL (T/S ratios)$=-0.008\times$YEAR$+1.55$, $R^2=0.055$, $P<10^{-16}$', which indicates that LTL declines on average by 0.008 T/S per year between the ages of 20 and 90.

The rs12696304 and rs16847897 were genotyped in all 4016 samples with a success rate of 98.6%. Neither of the two SNPs showed statistical deviation from Hardy–Weinberg equilibrium. The G allele frequency of rs12696304 and the C allele frequency of rs16847897 are 0.68 and 0.61 in our sample respectively, whereas in Europeans both of them are 0.26. The frequencies of the two SNPs showed no significant difference between age <61 group and age ≥ 61 group (Supplementary Table 1), which suggest no selection bias in the sample. Table 2 shows the *P* values for the association between both SNPs and LTL adjusted for age, gender and diabetes status under three genetic models. The β-coefficients equate to the mean change in telomere length (T/S) per copy of the major allele for the specific SNP. Markers rs12696304 and rs16847897 near the *TERC* gene were significantly associated with LTL in all genetic models tested except the dominant model of rs12696304. The additive model provided the best fit based on the Akaike information criterion value, which is consistent with previous GWA study.[8] *P*-values for the additive genetic model were 4.5×10^{-3} and 9.5×10^{-5} for rs12696304 and rs16847897, respectively.

The association analysis of rs12696304 and rs16847897 with telomere length in type 2 diabetes and non-type 2 diabetes is shown in Supplementary Table 3.

Table 1 Participants characteristics

	Non-T2D	*T2D*	*All*
Number	2080	1936	4016
Female/male	1452/628	1140/796	2592/1424
Smoker/non-smoker	329/1714	431/1482	760/3196
Drinker/non-drinker	309/1735	309/1603	618/3338
Age (years)[a]	58 (53–66)	64 (58–71)	61 (55–69)
BMI (kg/m^2)[a]	24.5 (22.5–26.7)	25.1 (23–27.3)	24.84 (22.72–27.12)
LTL[a]	1.04 (0.84–1.29)	0.98 (0.81–1.19)	1.01 (0.81–1.26)

Abbreviations: LTL, leukocyte telomere length; T2D, type 2 diabetes.
[a]Data are shown as median (25% quartile ~75% quartile).

DISCUSSION

LTL varies a lot between individuals from birth, and two quantitative-trait linkage analyses have shown it to have a heritability of between 36 and 81.9%.[4,5] Veryan Codd's group recently conducted a GWAS, and identified association of common variants near *TERC* (on 3q26) with telomere length in two large European cohorts. In the present study, we confirmed that the variants rs12696304 and rs16847897 near *TERC* are associated with LTL (*P* values $\sim 4.5\times10^{-3}$ and 9.5×10^{-5}, respectively, in additive genetic model). Each G allele of rs12696304 and C allele of rs16847897 was associated with a shorter mean telomere length of 0.024 and 0.031 T/S, respectively, equivalent to about 3 and 4 years of average age-related telomere attrition. These results are consistent with those of Veryan Codd's study.[8] However, the allele frequencies of rs12696304 and rs16847897 varied considerably between the different populations. The risk alleles for shorter LTL of rs12696304(G) and rs16847897(C), which were minor alleles in Europeans in Veryan Codd's study, were major alleles in our study based on the Chinese population.

The two markers rs12696304 and rs16847897, both showing a significant association with LTL, are located toward opposite ends of a ~87 kb region lying 1.5 kb downstream of *TERC*. Linkage disequilibrium analysis showed a week LD between rs12696304 and rs16847897 ($D'=0.61$, $r^2=0.26$). However, their associations are not independent: the inclusion of rs16847897 as a covariate in the association analysis of rs12696304 negated the latter's association with telomere length ($P\sim0.29$). In turn, the inclusion of rs12696304 as a covariate in the association analysis of rs16847897 weakened the latter's association with LTL ($P\sim0.005$). Haplotype analysis gave no addition information (Supplementary Table 2). The association analysis of rs12696304 and rs16847897 with telomere length in type 2 diabetes and non-type 2 diabetes is shown in Supplementary Table 3.

Table 2 Association of SNPs near *TERC* and leukocyte telomere length

	Major allele	*HWE*	*Genotype distribution*	*Additive model*		*Dominant model*		*Recessive model*	
SNP	*(frequency)*	P-*value*	*(RR/RC/CC)*	β	P-*value**	β	P-*value**	β	P-*value**
rs12696304	G(0.68)	0.83	398/1731/1853	−0.02	4.5E-03	−0.04	3.4E-02	−0.03	1.4E-02
rs16847897	C(0.61)	0.78	600/1884/1452	−0.03	9.6E-05	−0.05	3.3E-03	−0.04	7.4E-04

Abbreviations: CC, homozygous for major allele (common allele); HWE, Hardy–Weinberg equilibrium; RC, heterozygous for minor and major allele; RR, homozygous for minor allele (rare allele)
**P*-values were calculated in multiple regression model adjusted for age, gender and status of type 2 diabetes.

Although the confirmed markers rs12696304 and rs16847897 are not in the coding region of *TERC*, their association with telomere length is likely via an effect on *TERC* expression, given the important function of *TERC*. Alternately, the contribution to telomere length could occur through one of the other genes in the 3q26 locus, which include *MYNN* (encoding myoneurin), *ARPM1* (encoding actin-related protein M1), and three members of the *LRCC* (leucine-rich repeat containing) superfamily (*LRRC34*, *LRRC31* and *LRRIQ4*). *ARPM1* encodes a nuclear protein considered to have a role in the organization of the sperm-specific nucleus in mice.[14] Although very little is directly known about *LRRC34*, *LRRC31* and *LRRIQ4*, the *LRRC* superfamily consists of members with diverse roles, including DNA repair, cell cycle regulation, apoptosis and chromosomal stability.[15]

In conclusion, we confirmed that SNPs near *TERC* on locus 3q26 affect telomere length based on a sample of 4016 subjects of Chinese Han origin. Given the importance of telomeres in cellular function and the central role of telomere length in determining telomere function, our findings could provide a pointer for elucidating the pathogenesis of age-related diseases, and indicate that future studies aimed at accurately mapping this region could be fruitful.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)