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Genetic Variants of 11 Telomere-Pathway Gene Loci and the Risk of Incident Type 2 Diabetes Mellitus: The Women's Genome Health Study

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Abstract

Objective—Leukocyte telomere length shortening has recently been associated with type 2 diabetes Mellitus (T2D). Whether this observation was modulated by genetic variation within the telomere-pathway genes remains elusive. To date, no prospective epidemiological data on the relationship of telomere-pathway gene variation with T2D are available.

Methods—The association between 150 tagging-SNPs (tSNPs) of 11 telomere-pathway genes (*TERC*, *UCP1*, *TERT*, *POT1*, *TNKS*, *TERF1*, *TNKS2*, *TEP1*, *ACD*, *TERF2* and *TERF2IP*) and incident T2D was investigated in 22,715 Caucasian female participants of the prospective Women's Genome Health Study. All were free of known cardiovascular disease, cancer and diabetes at baseline. During a 13-year follow-up period, 1,445 participants developed an incident T2D. Multivariable Cox regression analysis was performed to investigate the relationship between genotypes and T2D risk assuming an additive genetic model. Haplotype block analysis was also performed.

Results—A total of eleven tSNPs within *TERF1*, *TNKS*, *TEP1*, *ACD*, and *TERF2* were associated with T2D risk (all p-uncorrected <0.050). Further investigation using the haplotype-block analysis again revealed an association of several prespecified haplotypes of *TERF1*, and *TEP1* with T2D risk (all p-uncorrected <0.040).

Conclusion—If corroborated in other prospective studies, the present findings suggest that genetic variation within the telomere-pathway gene loci examined may be useful predictor for T2D risk assessment.

1. Introduction

Biological factors such as inflammatory responses and oxidative stress accelerate leukocyte telomere-length shortening (1), and heightened oxidative stress is noted in patients with diabetes (2, 3). Recent studies have shown an association of leukocyte telomere shortening in patients with T2D (4, 5). Furthermore, the study by Salpea and coauthors reported an

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Conflict of Interest
None declared

association of the Uncoupling protein 2 (mitochondrial, proton carrier) functional promoter variant (−866G>A; dbSNP rs659366) with leukocyte telomere-length suggesting a link between mitochondrial production of reactive oxygen species and shorter telomere length in T2D (4). Based on their observation, we carried out an investigation examining the potential involvement of 14 UCP cluster gene variants (*UCP2-UCP3*) and incident T2D in a large-scale, prospective setting, and found no evidence for an association (6). Moreover, common single nucleotide polymorphisms (SNPs) in telomere-associated pathway genes could alter chromosomal integrity by influencing gene expression and inducing structural aberrations of telomere-maintenance proteins. This alteration could in turn lead to telomere dysfunction, and ultimately modulates differential susceptibility to T2D.

To data, no prospective epidemiological data are available on examining the importance of telomere-associated pathway genes as potential risk markers for T2D. We, therefore, further examined the potential involvement of 150 tagging-single nucleotide polymorphisms (tSNPs) in 11 telomere-associated pathway genes (Supplementary Data Tables IA and IB) with (i) baseline glycosylated hemoglobin (HbA1c) and C-reactive protein (CRP) levels, and (ii) T2D risk, in a large prospective cohort of 22,715 initially healthy Caucasian middle-aged women.

2. Material and Methods

2.1. Study design

Details of the study design have been previously described (7). In brief, participants in the Women's Genome Health Study (WGHS) – a genetic substudy of the Women's Health Study– included initially healthy North American women aged 45 or older with no previous history of cardiovascular disease, cancer, or other major chronic illness. A baseline blood sample was collected during the enrollment phase of the Women's Health Study between 1992 and 1995. Study participants, who gave an informed consent for blood-based analyses related to risks of incident chronic diseases, were followed up for incident events that were adjudicated by an endpoints committee using standardized criteria and full medical record review. The present investigation included 22,715 Caucasian participants of the WGHS; all were free of known cardiovascular disease, cancer and diabetes at baseline (6). During a 13-year follow-up period, 1445 cases of newly diagnosed T2D were identified. As described elsewhere, DNA extracted from the baseline WGHS blood samples underwent tSNP (r^2 ≈0.80) genotyping using the genome-wide Illumina Infinium II Human HAP300 panel (6). The Brigham and Women's Hospital Institutional Review Board for Human Subjects Research approved the study protocol.

2.2. Statistical analysis

Genotype frequencies were compared with values predicted by Hardy-Weinberg equilibrium using the chi-square test with one degree of freedom. Multivariable linear regression analysis, adjusting for age, body-mass index (BMI), current smoking status and current (any) hormone use, was performed to assess the relationship of the tSNPs with baseline $\log_e$-glycosylated hemoglobin (lnHbA1c) and $\log_e$-C-reactive protein (lnCRP) levels. Hazard ratios (HRs) of T2D associated with each of the individual tSNPs were calculated separately by Cox regression analysis adjusting for age, current smoking status, and further adjusting for BMI, randomized treatment assignment, history of hypertension, and hyperlipidemia, current hormone use, baseline CRP and HbA1c, assuming an additive model for genetic effects.

Haplotype estimation and inference were determined by expectation-maximization algorithm. Haplotype blocks were defined using the software Haploview v4.1. In addition,

the relationship between haplotypes and T2D was examined by a referent haplotype-based Cox regression analysis, adjusting for the same potential covariables used in the single-tSNP analysis. All analyses were carried out using SAS v9.1 package (SAS Institute Inc). A 2-tailed p-value of 0.05 was considered a statistically significant result. Genotyping call rates were >99% per SNP.

3. Results

The baseline characteristics of the 22,715 initially healthy Caucasian women are shown in Supplementary Data Table II. Fourteen out of the 150 SNPs evaluated were not in Hardy-Weinberg equilibrium with uncorrected p-values <0.0500 (Supplementary Data Table III). In the multivariable linear regression analysis, ten tSNPs (one *TERT*, nine *TNKS*, and one *TEP1*) and nine tSNPs (four *TERT*, one *TNKS*, and four *TEP1*) were differentially associated with baseline lnCRP, and lnHbA1c, respectively (p-uncorrected <0.050; Table 1). However, none of these remained significant after Bonferroni correction for multiple testing. Results from the multivariable Cox regression analysis showed evidence for differential associations of eleven tSNPs (four *TERF1*, one *TNKS2*, four *TEP1*, one *ACD*, and one *TERF2*) with T2D risk (p-uncorrected <0.050; Table 2). One SNP, the *TERF2* rs4783704 (p-uncorrected=0.0001; Table 2), remained significant after Bonferroni correction. Supplementary Data Table IV presents the Cox regression results for all 150 tSNPs. The association of two Haploview-defined haplotypes within *TERF1* and *TEP1*, respectively, with T2D risk was observed (p-uncorrected <0.050; Supplementary Data Table V).

4. Discussion

To the best of our knowledge, the present prospective investigation is the first to examine the possible involvement of telomere-pathway gene loci in the risk of T2D, and we found (suggestive) evidence for an association of the telomere-associated genes evaluated, in particular, *TERF1* and *TEP1*, with T2D risk.

Type 2 diabetes mellitus is a major cause of morbidity and mortality around the world, and genetic variation within the telomere-associated/maintenance genes have been implicated in age-depending common, complex disorders including essential hypertension, non-insulin-dependant diabetes mellitus, atherosclerosis, and cancer (8, 9). The telomere-pathway genes evaluated in the present study encode proteins for the telomere-specific nucleoprotein complex, which regulates and maintains telomere stability and thus the overall genomic integrity. Unfortunately, most if not all of the published reports examined the potential involvement of telomere-associated pathway gene variation with various types of cancer, and, to the best of our knowledge, none on the risk of incident T2D. This lack of information on the relevance of telomere pathway genes with T2D not only makes a cross-reference comparison with the present findings difficult, but also signifies the need of further investigations into the importance of telomere-pathway genes in aging-related disorders such as T2D. Nevertheless, the present investigation suggests that telomere-pathway genes, in particular, *TERF1 and TEP1* gene variation may play a role in the underlying pathogenesis of T2D.

Strengths of the present study are the overall sample size, the prospective design and the complete long-term follow-up. Nonetheless, some potential limitations of our study require discussion. Our sample population was limited to Caucasian female healthcare professionals from the US. Thus, our results may not be generalizable to other racial/ethnic or socio-economic groups, geographical regions, or to males. Cautious interpretation of our present (uncorrected) findings should be exercised, and confirmation in other studies is needed.

In our study, we had the ability to detect, based on the present sample size, assuming 80% power, at an alpha of 0.05, an effect estimate of greater than 1.10 if the minor allele frequency is 0.50, and of greater than 1.90 if the minor allele frequency is 0.01.

5. Conclusion

The present prospective data from a large cohort of apparently healthy Caucasian US females provide suggestive evidence for an association between the gene variants tested and risk of T2D. If corroborated in other large prospective studies, our data further suggest that the telomere-pathway gene loci assessed may be informative for risk assessment of T2D.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Multivariable linear regression analysis.

Baseline lnCRP							
Gene	**dbSNP rs**	**MA**	**MAF**	**Parameter estimate**	**Standard error**	**t value**	**p-Uncorrected**
TERT	2736100	A	0.4970	−0.0200	0.0095	−2.11	0.0352
TNKS	6601327	G	0.3785	0.0252	0.0098	2.58	0.0100
TNKS	17150237	A	0.2301	0.0243	0.0113	2.15	0.0315
TNKS	9644677	G	0.2763	0.0229	0.0106	2.16	0.0311
TNKS	12549064	C	0.1997	0.0249	0.0124	2.01	0.0448
TNKS	11787063	A	0.2668	−0.0224	0.0108	−2.08	0.0377
TNKS	9644704	A	0.3629	0.0228	0.0099	2.31	0.0210
TNKS	12155819	A	0.3642	0.0203	0.0099	2.06	0.0396
TNKS	6994574	G	0.3651	0.0202	0.0099	2.05	0.0406
TEP1	1760921	G	0.0401	0.0509	0.0241	2.10	0.0354
Baseline lnHbA1c							
Gene	dbSNP rs	MA	MAF	Parameter estimate	Standard error	t value	p-Uncorrected
TERT	7445640	C	0.4079	0.0013	0.0006	2.13	0.0336
TERT	7447815	G	0.3942	0.0015	0.0006	2.57	0.0103
TERT	33954691	A	0.1033	0.0022	0.0009	2.27	0.0235
TERT	2853677	G	0.4272	0.0013	0.0006	2.27	0.0232
TNKS	6990116	G	0.0645	0.0024	0.0012	1.97	0.0485
TEP1	7140768	G	0.4289	−0.0013	0.0006	−2.25	0.0246
TEP1	7145318	G	0.4284	−0.0014	0.0006	−2.43	0.0151
TEP1	8017603	A	0.4950	0.0014	0.0006	2.43	0.0151
TEP1	1878705	G	0.3782	0.0015	0.0006	2.58	0.0099

Adjusted for age, body-mass index, current smoking, and current hormone use,.

MA, minor allele; MAF, minor allele frequency.

Table 2

Cox regression analysis of incident T2D.

Gene	dbSNP rs	MA	MAF	HR	95%CI	p- Uncorrected
TERF1	2010441	A	0.2833	1.131	1.042–1.227	0.0032
	10099824	A	0.4539	0.904	0.838–1.005	0.0088
	3863242	A	0.4356	0.903	0.837–0.974	0.0083
	2291219	A	0.2979	1.096	1.011–1.189	0.0265
TNKS2	1539041	A	0.3087	0.904	0.833–0.981	0.0160
TEP1	3093872	A	0.0538	1.182	1.010–1.383	0.0369
	3093921	G	0.0231	1.264	1.017–1.571	0.0346
	1713423	A	0.4762	0.927	0.859–1.000	0.0496
	1713434	G	0.4385	1.087	1.009–1.172	0.0291
ACD	7202185	A	0.0715	1.217	1.062–1.396	0.0049
TERF2	4783704	A	0.0572	0.710	0.596–0.845	0.0001

Adjusted for age, body-mass index, randomized treatment assignment, history of hypertension, hyperlipidemia, current smoking, current hormone use, plasma C-reactive protein and HbA1c levels.

MA, minor allele; MAF, minor allele frequency.