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ORIGINAL INVESTIGATION

The contribution of parent-to-offspring transmission of telomeres to the heritability of telomere length in humans

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Abstract

Leukocyte telomere length (LTL) is a heritable trait with two potential sources of heritability (h^2): inherited variation in non-telomeric regions (e.g., SNPs that influence telomere maintenance) and variability in the lengths of telomeres in gametes that produce offspring zygotes (i.e., "direct" inheritance). Prior studies of LTL h^2 have not attempted to disentangle these two sources. Here, we use a novel approach for detecting the direct inheritance of telomeres by studying the association between identity-by-descent (IBD) sharing at chromosome ends and phenotypic similarity in LTL. We measured genome-wide SNPs and LTL for a sample of 5069 Bangladeshi adults with substantial relatedness. For each of the 6318 relative pairs identified, we used SNPs near the telomeres to estimate the number of chromosome ends shared IBD, a proxy for the number of telomeres shared IBD (T_{shared}). We then estimated the association between T_{shared} and the squared pairwise difference in LTL ($(\Delta\text{LTL})^2$) within various classes of relatives (siblings, avuncular, cousins, and distant), adjusting for overall genetic relatedness (ϕ). The association between T_{shared} and $(\Delta\text{LTL})^2$ was inverse among all relative pair types. In a meta-analysis including all relative pairs ($\phi > 0.05$), the association between T_{shared} and $(\Delta\text{LTL})^2$ ($P = 0.01$) was stronger than the association between ϕ and $(\Delta\text{LTL})^2$ ($P = 0.43$). Our results provide strong evidence that telomere length (TL) in parental germ cells impacts TL in offspring cells and contributes to LTL h^2 despite telomere "reprogramming" during embryonic development. Applying our method to larger studies will enable robust estimation of LTL h^2 attributable to direct transmission of telomeres.

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Introduction

Telomeres are DNA–protein complexes at the end of mammalian chromosomes that maintain genome stability by preventing recombination, end-to-end fusion, and DNA damage at chromosome ends (Blackburn et al. 2015). In somatic cells, shortening of the DNA component of the telomere occurs with each round of cell division due to the "end-replication problem". In stem cells, extension of telomeres by telomerase counters this shortening, but gradual shortening still occurs with age, with critically short telomeres contributing to cellular senescence. Consequently, leukocyte telomere length (LTL) is considered an indicator of biological aging and a potential biomarker of risk for age-related diseases (Aubert and Lansdorp 2008; Huang et al. 2014). Short LTL is associated with increased risk for several age-related diseases in observational studies, including cardiovascular disease, hypertension, liver disorders, diabetes, atherosclerosis, and overall mortality (Aubert and Lansdorp 2008; Cawthon et al. 2003; Haycock et al. 2014; Willeit et al. 2010a, b). In contrast, Mendelian randomization studies suggest that longer telomeres increase risk for several types of cancer, including lung adenocarcinoma (Zhang et al. 2015), melanoma (Iles et al. 2014), glioma (Walsh et al. 2015), neuroblastoma (Walsh et al. 2016), and chronic lymphocytic leukemia (Ojha et al. 2016).

LTL is a highly heritable trait (Bischoff et al. 2012; Hjelmborg et al. 2015; Honig et al. 2015; Broer et al. 2013b) and is known to be effected by inherited genetic variation in non-telomeric regions (e.g., SNPs or single nucleotide polymorphisms) that influence telomere maintenance (Codd et al. 2013). However, LTL has a unique additional potential source of heritability (h^2): variability in the lengths of telomeres themselves in the parental gametes that produce the offspring zygotes (De Meyer et al. 2014), under the assumption that gamete telomere length (TL) partially determines TL in adult tissues (including leukocytes and germ cells). This second source of h^2 is described as "direct" transmission of telomeres (Fig. 1) and has been hypothesized to be the primary source of TL h^2 (De Meyer et al. 2014). Telomeres undergo "reprogramming" in the embryo, initially by recombination-based mechanisms and then by telomerase at the blastocyst stage and later (Kalmbach et al. 2014), but is unclear to what extent reprogramming alters the impact of parental germ cell TL on TL in offspring cells.

Evidence supporting an impact of parental TL on offspring TL includes the observed associations of advanced paternal age at conception and increased offspring LTL (Broer et al. 2013b; Kalmbach et al. 2013; Liu et al. 2002; Allsopp et al. 1992; Eisenberg et al. 2012; Kimura et al. 2008; Prescott et al. 2012; Achi et al. 2000). This association is often attributed to age-related telomere lengthening in spermatogonia, which express high levels of telomerase (Kalmbach et al. 2013), providing evidence that acquired variation in TL in the male germ line contributes to offspring TL. Also supporting this notion are studies of families with genetic disorders affecting telomere maintenance, in which affected parents who carry a mutation that renders low telomerase activity and short telomeres have non-carrier children whose telomeres appear shorter as compared to the general population, despite not inheriting the mutation (Aubert et al. 2012; Collopy et al. 2015; Chiang et al. 2010). These findings are also supported by studies of telomerase deficient mice (Chiang et al. 2010).

In light of the evidence supporting the contribution of "direct" transmission of telomeres to LTL h^2, in this work, we attempt to detect the effect of direct transmission on LTL

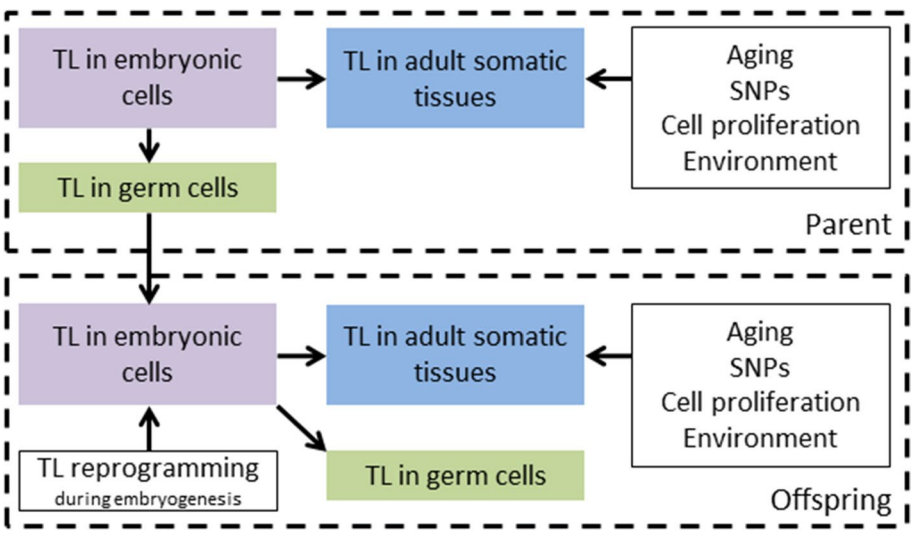

Fig. 1 Causal diagram of transgenerational (i.e., "direct") inheritance of telomere length. TL in adult tissues, including germ cells, is influenced by TL in embryonic cells. TL in embryonic cells of offspring is influenced by the TL in parental germ cells. TL in offspring undergoes "reprogramming" during the pre-implantation stage of embryonic development. Other sources of variation in TL include age, SNPs, cell proliferation rates, and environmental factors

in a population-based setting. Our approach is to estimate the association between identity-by-descent (IBD) sharing at chromosome ends (a proxy for sharing telomeres IBD) and phenotypic similarity in LTL for relative pairs. Prior family-based and SNP-based h^2 studies of LTL have not attempted to disentangle the effects of "direct" inheritance of LTL from other sources of h^2, and it remains unclear whether the h^2 of LTL is primarily driven by inherited genetic variation in non-telomeric regions (e.g., SNPs that impact telomere maintenance) or whether TL is substantially influenced by the length of the telomeres present in the parental germ cells (De Meyer et al. 2014). The latter hypothesis implies that TL is not completely "reset" during embryogenesis and early development.

Experimental methods

Study participants

DNA samples were obtained from participants in the following Bangladeshi studies: The Health Effects of Arsenic Longitudinal Study (HEALS), an expansion of the HEALS cohort (ACE), and The Bangladesh Vitamin E and Selenium Trial (BEST). HEALS is a prospective study of the health outcomes associated with arsenic exposure through drinking local well water in 11,746 adults (18–75 years of age). An expansion of the HEALS cohort (ACE) occurred between 2006 and 2008, and an additional 8287 participants were recruited. Details of HEALS have been described previously (Ahsan et al. 2005). BEST is a chemoprevention trial that assessed the effects of vitamin E and selenium supplementation on non-melanoma skin cancer risk. Details of the BEST study have been described previously (Argos et al. 2013). The HEALS and BEST study participants included in this work are primarily from the Araihazar area, a rural community east of Dhaka. Multiple members of extended families were often recruited, resulting in a substantial number of relative pairs in these cohorts. These studies have been approved by the Ethical Review Committee of International Center for Diarrheal Disease Research, Bangladesh, The Bangladesh medical Research Council, and the Institutional Review Boards of the University of Chicago and Columbia University.

DNA extraction and genotyping

Details of sample collection, DNA extraction and genotyping have been described by Pierce et al. (2012, 2013). In brief, genomic DNA for HEALS samples were extracted from clotted blood using the Flexigene DNA Kit (Cat # 51204) and BEST and ACE samples were extracted from whole blood using the QIAamp 96 DNA Blood Kit (Cat # 51161). DNA samples were processed on HumanCytoSNP-12 v2.1 chips. Prior to quality control, our genotype data consisted of 5499 individuals with 299,140 SNPs measured. We removed individuals with call rates < 97% ($n = 13$), gender mismatches ($n = 79$), and technical replicate samples or duplicates ($n = 53$). We excluded SNPs that had low call rates, i.e. (< 95%) ($n = 20$), were monomorphic ($n = 39{,}798$), did not have rs identifiers ($n = 941$), and had HWE P values $< 10^{-10}$ ($n = 634$). The HWE threshold used is not stringent because our sample includes relatives, leading to inflated HWE test statistics. In an unrelated set of participants, HWE testing confirmed no SNPs used in the analysis had an HWE P value $< 10^{-7}$.

Measurement of LTL using qPCR

For 2203 genotyped HEALS samples, LTL was measured using quantitative(q)-PCR using two different plate designs (design 1 and 2) on 96-well plates. The relative telomere length is represented as a ratio of the telomere repeat copy number to single-gene (*RPLP0*) copy number, i.e. *T*/*S* ratio. Details of this methodology have been described previously (Delgado et al. 2017). Plate design 1 was based on Ehrlenbach et al. (2009) and consisted of triplicates of 14 participant DNA samples per plate and 6 replicates of reference DNA sample, with telomere sequence and *RPLP0* measured on the same plate. Plate design 2 was based on Cawthon et al. (Cawthon 2002), and we used paired plates for amplification of telomere and single-copy gene of each DNA sample, each consisting of triplicates of 31 participant DNA samples and triplicates of reference DNA sample.

The coefficients of variation (CV) were calculated as the standard deviation of the sample replicates divided by the mean of the replicates. To assess the reproducibility of the measured T/S ratios for Plate Design 1 and 2, we re-ran 37 and 31 samples on separate days and calculated the inter-plate CVs to be 11.7% and 9.8%, respectively.

Measurement of LTL using quantigene chemistry on a luminex platform

The Affymetric-Panomics QuantiGene Plex is a probe-based non-PCR assay developed in our lab and used to quantify LTL in 1825 BEST and 1047 ACE participants. Like qPCR, the Luminex-method method produces a relative telomere length measure represented by a ratio of telomere probe to reference gene probe, known as the Telomere Quantity Index (TQI). The details of this methodology have been described previously (Kibriya et al. 2014). The Luminex method has been validated in a blinded comparison to Southern Blot (Pierce et al. 2016), the gold standard for TL measurement in DNA samples. In a prior comparison analysis of qPCR and Luminex (Kibriya et al. 2016), the coefficients of variation

(CV) of the telomere quantity index (TQI) were calculated as the standard deviation divided by the mean and were expressed as percentages. The CV for Luminex ranged from 7.2 to 8.4%. The Luminex assay also shows high agreement with singleplex qPCR run in triplicate ($r = 0.7–0.8$) (Kibriya et al. 2014).

LTL standardization

To remove experimental variation in our measures of LTL due to differences in TL measurement and DNA extraction methods, we utilized linear mixed-effects models (lme package in R), where position and plate were treated as fixed and random effects, respectively. We performed three separate mixed effects models; one for the Luminex-based LTL measures and one for each qPCR plate design. To harmonize the LTL measures from the two experimental methods, we standardized the residuals from mixed-effects regressions and adjusted for age, sex, and cohort in a separate regression model. These standardized residuals were used in all downstream analyses. The details of these mixed-effects models and standardization methods have been described in detail previously in Delgado et al. (2017). Additionally, we generated two variables that represent the difference in LTL between relative pairs: the squared difference $(\Delta LTL)^2$ was our primary outcome (due to its utility for h^2 estimation (see below)), and the absolute difference $|\Delta LTL|$ was our secondary outcome (presented as supplementary results).

Estimating telomeres shared

We performed LD pruning on 257,747 genotyped SNPs to produce a subset of uncorrelated markers (i.e. linkage equilibrium). In PLINK, we used the "indep-pairwise" command, where we specified three parameters: a 100-kb window size, a 25-kb count to shift the window at the end of each step, and a pairwise squared correlation (r^2) threshold of 0.1 to eliminate SNP pairs with an $r^2 > 0.1$ in each window, resulting in 52,066 uncorrelated SNPs. Using this set of uncorrelated SNPs, we restricted to the 180 SNPs closest to the end of each chromosome to estimate identity-by-descent (IBD) probabilities for each chromosome end, for all possible pairs of individuals (~ 12.8 million pairs). We chose to use 180 SNPs because this number gave us very clear separation among pairs who shared exactly 2 chromosome ends, exactly 1 end, and < 1 end for all chromosome arms (Figure S1). When using fewer than 180 SNPs (e.g., 150 SNPs), for some chromosomes, we did not see a clear gap in the IBD proportion between the pairs sharing exactly one end and the pairs sharing < 1 end. The genetic and physical distances spanned by these SNPs at each chromosome end are described in Table S1 and the frequency of pairs sharing chromosome ends IBD are described in Table S2.

We used the IBD proportion for each chromosome end, obtained from PLINK, to define a discrete value for the number of chromosome ends shared IBD, which represents likely sharing of telomeres IBD. For each chromosome end, we defined 0 shared telomeres if the IBD proportion was < 0.45, 1 shared telomere if the proportion was between 0.45 and 0.9, and 2 shared telomeres if the proportion was ≥ 0.9. We summed the number of shared telomeres across all autosomal chromosome p- and q-arms to obtain a total number of ends shared IBD for each participant pair, which we take as an estimate of the number of telomeres shared IBD (T_{shared}) (Fig. 2). We excluded the p-arm of chromosomes 13–15, 21, and 22 from our T_{shared} count, because those arms had a > 14 Mb gap between the most telomeric SNP and the chromosome end [as defined by the Genome Reference Consortium Human build 37 (GRCh37/hg19)]. For all other chromosome ends, this gap was < 0.3 Mb, except 1p which had a gap of 0.75 Mb. Recombination events are extremely rare in intervals of this size, implying that sharing a segment IBD at a chromosome end (based on 180 SNPs) is a strong proxy for sharing a telomere IBD. Thus, using only 39 chromosome ends results in a theoretical maximum of $T_{\text{shared}} = 78$ (for twins). We divided T_{shared} by 78 to represent the estimated proportion of telomeres shared IBD.

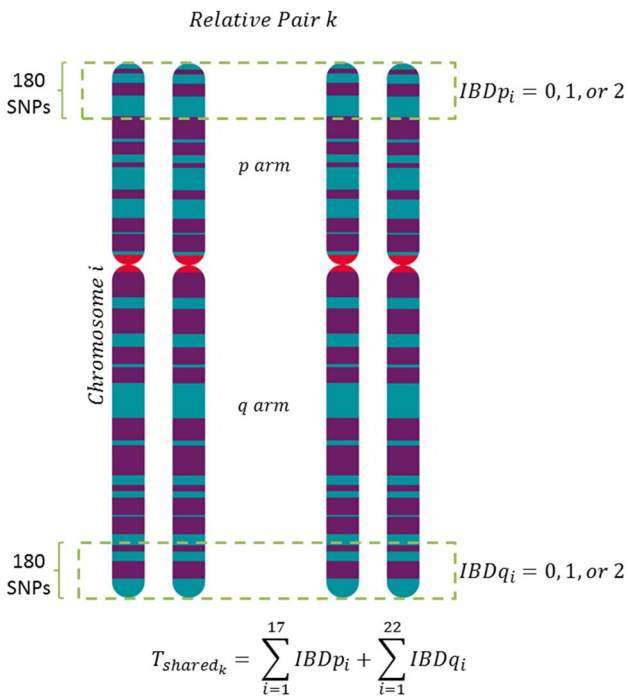

Fig. 2 Calculation of T_{shared} for a relative pair. Chromosome end sharing is used as a proxy for telomere sharing. 180 uncorrelated SNPs from each chromosome end were used to calculate the number of ends shared IBD (0, 1, or 2) at each chromosome end for each relative pair. The sum of the IBD count (0, 1, or 2) over all chromosome ends (17 p-arm ends (5 excluded) and 22 q-arm ends) resulted in a pair's T_{shared} value

We classified each participant pair according to their relationship type (i.e., parent-offspring, sibling, cousin, avuncular, etc.) using the estimated genetic relationship (ϕ) and the IBD probabilities (P(IBD) = 0 and P(IBD = 1)), all estimated using the PLINK genome command applied to genome-wide SNPs (Fig. 3). Pairs with $\phi = 0.25$ could in theory be avuncular pairs, grandparent-grandchild pairs, or half siblings, but based on what we know regarding the demography of the population groups we are studying and the age difference of these pairs, the vast majority of these pairs should be avuncular pairs, so we classified them as such. The ϕ threshold separating cousin pairs from "distant" pairs ($\phi = 0.125$) was somewhat arbitrary due to lack of clear separation between relative groups (in terms ϕ) at lower ϕ values; thus, there is likely contamination of the "distant" group with cousin pairs. We conducted sensitivity analyses (see below) to ensure our results were not sensitive to arbitrary decisions regarding this threshold. Additionally, an outlier individual with 949 distinct distant relationships was removed from all analyses.

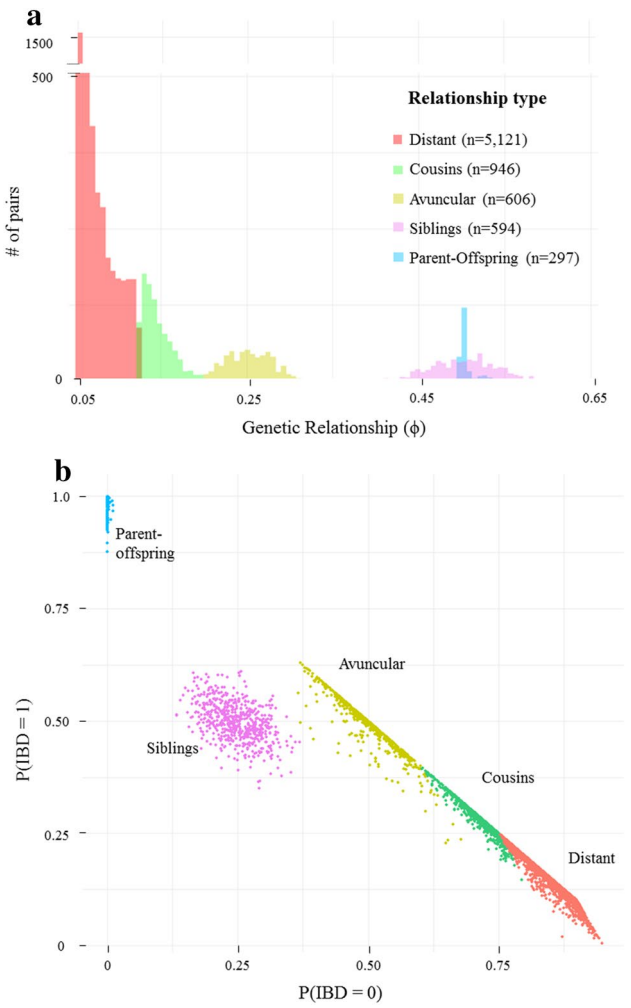

Fig. 3 Genetic relationships present in the Bangladeshi cohort. **a** Distribution of the genetic relationship (ϕ) estimated using genome-wide SNPs, restricting to 6318 pairs with $\phi > 0.05$. **b** Scatterplot of P(IBD = 0) (*x* axis) versus P(IBD = 1) (*y* axis) for 6318 relative pairs

Statistical analyses

For each relative pair type, we used linear regression to estimate the association between the T_{shared} proportion and the squared difference in LTL ($(\Delta LTL)^2$), adjusting for each pair's ϕ value. These regressions were performed for 594 sibling pairs, 606 avuncular pairs, 944 cousin pairs, and 4174 distant relative pairs. Parent-offspring pairs were not analyzed because we expect no variation in the T_{shared} proportion (or ϕ) among these pairs. To estimate the association between T_{shared} proportion and $(\Delta LTL)^2$ using data on all types of relative pairs, we combined the association estimates for each relationship type in a fixed-effects meta-analysis using the 'metafor' package in R. Fixed-effects meta-analysis requires two key assumptions: (1) homogenous effect-size across all groups and (2) no between-group variance. Additionally, we conducted a linear regression to test whether mean $(\Delta LTL)^2$ is different across relationship types (including parent-offspring pairs).

The relative pairs used in our analyses are quasi-independent pairs, meaning that a given individual can be included in multiple pairs, but pairs are treated as independent. This could result in a potentially inflated type I error rate. To address this issue, we obtained *P* values using a permutation-based approach. Specifically, we conducted 1000 permutations of T_{shared} within each relative group, breaking the correlation between T_{shared} and $(\Delta LTL)^2$. For each of the 1000 rounds of permutation, we conducted regression analyses within each relative group assessing the association between $(\Delta LTL)^2$ and T_{shared}, adjusting for overall genetic relatedness (ϕ). From each of these within-group regressions, we extracted the β estimates (e.g. 1000 β values) and standard errors (SE) for T_{shared} regressed on $(\Delta LTL)^2$. These βs and SEs were then used to conduct meta-analysis across all four relative groups for each round of permutation. We calculated the SD of the 1000 β coefficients for each relative group and the meta-analysis, and divided our original/observed β estimates by the SD to obtain "corrected" (permutation-based) t-statistics and corresponding *P* values.

To address the possibility of bias in the association between the T_{shared} proportion and $(\Delta LTL)^2$ due to the presence of non-independent relative pairs (i.e., individuals present in multiple pairs) we repeated our analyses restricting first to relative pairs containing no duplicate individuals (i.e., independent pairs) and then to a set of pairs where all pairs contained an individual that is present in more than one pair (i.e., non-independent pairs). We compared the β coefficients using a *t* test.

Using the meta-analysis association estimate, we used the approach described in Visscher et al. (2006) to estimate the narrow sense heritability (h^2) attributed to the proportion of shared telomeres IBD (T_{shared}). Similar to Visscher's approach, we also regress the pairwise difference of a continuous trait on a measure of genetic relatedness; this similarity allows us to apply Visscher's h^2 estimation method to our data. The regression parameter (β) for the association between T_{shared} proportion and $(\Delta LTL)^2$ is equal to twice the additive genetic variance, and h^2 is equal to β divided by the twice the total phenotypic variance, multiplied by -1:

$$h^2 = -\beta/(2\hat{\sigma}_p^2).$$

We used this equation to convert the meta-analysis regression parameter β and its upper and lower bound to the h^2 scale.

In order to determine if LTL reflects TL in germ cells (as expected under the causal diagram in Fig. 1), we used tissue-specific TL data from the Genotype-Tissue Expression (GTEx) project (eGTEx Project 2017) to examine the correlation between TL in whole blood and TL in testicular tissue. Tissue collection from post-mortem tissue donors and DNA extraction has been described previously (Carithers et al. 2015). TL was measured for GTEx DNA samples using the Luminex technology described above.

Results

Characteristics of the 5069 Bangladeshi participants included in our regression analyses are described in Table 1. Age was inversely associated with LTL across each cohort (HEALS: $r=-0.20$, $P<10^{-16}$, BEST: $r=-0.23$, $P<10^{-16}$, and ACE: $r=-0.28$, $P<10^{-16}$), as expected. The types of relative pairs observed in our sample are described in Fig. 3. For each relative pair type, LTL showed significant correlation between relative pairs, with stronger correlation generally observed for closer relative pair types (e.g., siblings $r=0.22$, $P=3\times10^{-8}$) compared to more distant relatives (i.e., cousins $r=0.07$, $P=0.04$) (Table 2). Additionally, we observed potential differences in the strength of correlation between offspring type and maternal versus paternal TL, with mother and father–daughter showing the strongest correlations ($r=0.22$, $P=0.07$ and $r=0.25$, $P=0.14$). Similarly, the mean of our primary outcome of interest, $(\Delta LTL)^2$, increased with decreasing level of relatedness (Figure S2). Univariate regression analyses of $(\Delta LTL)^2$ on relationship type showed a similar trend, with a larger effect of relationship type for increasing degree of relatedness (Table S3).

The total number of chromosome ends shared IBD by relative pairs at each chromosome end (described in Table S2) showed evidence of a negative correlation with the cM distance at each chromosome end encompassed by the 180 independent SNPs used to measure IBD sharing (Figure S3). This is expected, as chromosome ends with SNP intervals that span a large genetic distance will have more recombination events occurring in that interval than ends with SNPs

Table 1 Characteristics of Bangladeshi cohorts by type of telomere length measurement method, qPCR and the Luminex-based method ($n=5075$)

Characteristic	qPCR	Luminex method	
	HEALS (2203)	BEST (1825)	ACE (1047)
Sex			
Male	1060 (48.1%)	996 (54.6%)	396 (37.8%)
Female	1143 (51.9%)	829 (45.4%)	651 (62.2%)
Age (years)			
Mean (SD)	38.3 (10.6)	43.7 (10.6)	36.8 (10.7)
BMI (kg/m^2)			
< 18.5	863 (39.6%)	698 (38.3%)	431 (41.4%)
18.5–24.9	1173 (53.8%)	963 (52.8%)	549 (52.7%)
> 25	145 (6.7%)	162 (8.9%)	62 (5.9%)
Smoking			
Never	1298 (58.9%)	1110 (60.8%)	735 (70.2%)
Former	176 (8%)	198 (10.9%)	58 (5.5%)
Current	729 (33.1%)	517 (28.3%)	254 (24.3%)
Telomere length			
Mean *T*/*S* or TQI[a]	0.765 (0.17)	0.672 (0.12)	0.679 (0.12)
Adjusted TL			
Mean of standardized residuals[b]	0.02 (0.99)	0.00 (0.96)	0.00 (1.06)

[a]These are the raw *T*/*S* ratios (for qPCR) and TQI (for the luminex method) measures, prior to mixed effects modeling

[b]Standardized residuals from mixed-effects models used to generate difference in telomere length (TL_{diff}) variable

Table 2 Telomere length correlations for relative pairs by relationship type

Relationship	*n*	r^a	*P*
Parent-offspring	297	0.15	0.01
Mother-offspring	181	0.13	0.08
Mother–daughter	67	0.22	0.07
Mother–son	114	0.07	0.44
Father-offspring	116	0.18	0.06
Father–daughter	37	0.25	0.14
Father–son	79	0.18	0.09
Sibs	594	0.22	3.0×10^{-8}
Avuncular	606	0.09	0.02
Cousins	946	0.07	0.04
Distant	4172	0.05	0.0007

[a]Correlations are based on age/sex-adjusted standardized residuals from mixed-effects models

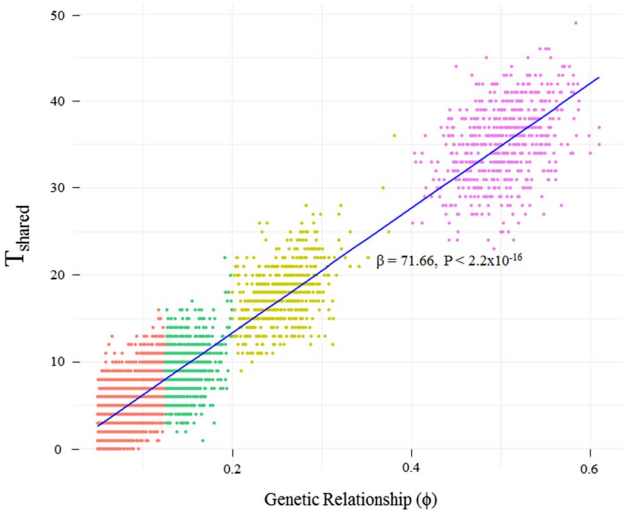

Fig. 4 The association between genetic relatedness (ϕ) and telomere sharing IBD (T_{shared}) for all 6318 relative pairs. Scatterplot shows T_{shared} (y axis) increases with genetic relatedness (ϕ) (x axis). Linear fitted line with the β coefficient and P value are included

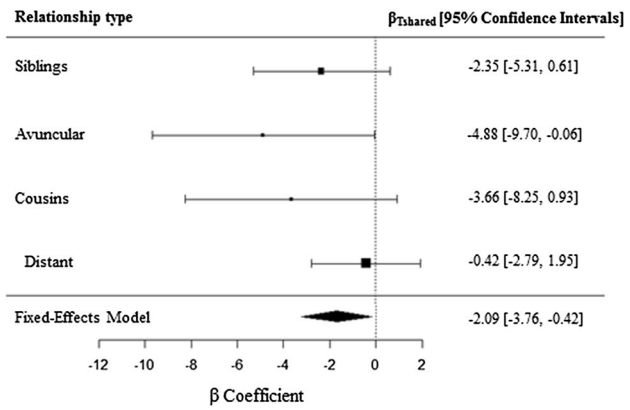

Fig. 5 Forest plot of the association between the proportion of telomere shared (T_{shared} proportion) and the squared pairwise difference in LTL ($(\Delta LTL)^2$), adjusting for genetic relatedness (ϕ). The horizontal bars represent the 95% CI for each association estimate

spanning a shorter genetic distance, resulting in less frequent observation of IBD sharing for the entire segment. The proportion of chromosome ends shared IBD for a relative pair, a proxy for shared telomeres (T_{shared}), showed a strong positive association with the estimated pair-wise genetic relatedness (ϕ, estimated using genome-wide SNPs) for each type of relative pair (Fig. 4). This is also expected, as pairs sharing more of the total genome would be expected to share more telomeres.

Among each relative pair type, the estimate of the proportion of telomeres shared IBD (the T_{shared} proportion) showed a non-significant inverse association with $(\Delta LTL)^2$ (Fig. 5). In contrast, the associations between genome-wide IBD sharing (ϕ) and $(\Delta LTL)^2$ were generally weaker and did not show a consistent direction of association (Table 3). We meta-analyzed the four groups of relative pairs (6318 total pairs) using a fixed-effects model (test of heterogeneity $Q = 2.41$, $P = 0.49$). In the meta-analysis, the T_{shared} proportion showed a significant inverse association with $(\Delta LTL)^2$ ($\beta = -2.09$, $P = 0.01$) (Fig. 5), while ϕ did not ($\beta = -1.22$, $P = 0.43$) (Table 3). The P values obtained from our 1000 permutations appear to be uniformly distributed (Figure S4) and our permutation-based results are comparable to our original (non-permuted) results (Table S4). Using a different threshold to separate cousin pairs from distant pairs ($\phi \leq 0.08$) or combining them into a single group had minimal impact on the results (Figures S5 and S6).

Restricting to independent pairs and non-independent pairs (as described in the "Experimental methods"), we did not observe any clear bias present due to including non-independent relative pairs. The meta-analysis β coefficient for T_{shared} was actually larger in magnitude in the analysis of independent pairs than non-independent pairs, and the t-test comparing the meta-analysis β coefficients was not significant ($t = 0.67$, $P = 0.50$) (Supplementary Table 5).

Regression analyses using $|\Delta LTL|$ as the outcome rather than $(\Delta LTL)^2$ produced similar results ($P = 0.11$) (Table S6, Figure S7). Scatterplots of the T_{shared} proportion against $(\Delta LTL)^2$ for each relative pair type are shown in Figure S8 and Figure S9. Together, these analyses indicate that sharing telomeres IBD contributes to pairwise similarity in LTL.

We conducted similar analyses for five continuous control phenotypes to show that the observed association with T_{shared} was specific to pairwise differences in LTL. These phenotypes were height, BMI, mean arterial pressure, systolic blood pressure, and diastolic blood pressure. Difference measures for each phenotype were taken after each phenotype was adjusted for age, sex, cohort, and batch. We did not observe a statistically significant inverse association between the T_{shared} proportion and the pairwise squared difference or the absolute difference for any of the control phenotypes (Tables S7 and S8).

According to Visscher's method for h^2 estimation using genetic data on full siblings (Visscher et al. 2006), the narrow-sense h^2 for the fixed-effects meta-analysis β coefficient is 1.05 [95% CI (0.11, 1.98)]. This estimate is above the theoretical maximum for h^2, likely due to the very large confidence bounds on this estimate. The lower bound for our h^2 estimate is 0.11, suggesting that at least 11% of the variation in LTL can be explained by sharing telomeres sequence IBD with close relatives. However, variation due to age and other covariates was removed from our TL measures prior to analysis, so the true lower bound is likely lower than 11%.

In order to provide evidence that TL in germ cells and in blood cells reflect TL in the embryonic cells from which both cell types descend (Fig. 1), we used data on 150 tissue

Table 3 Association of proportion of telomere sharing (T_{shared}) and genetic relatedness (ϕ) with squared pairwise difference in LTL ($(\Delta LTL)^2$) by relationship

Relative type[a]	*n*	Variables	β	SE	*P*	95% CI
Siblings	594	T_{shared}[b]	−2.35	1.51	0.12	(−5.31, 0.61)
		ϕ	−1.36	2.48	0.58	(−6.22, 3.50)
Avuncular	606	T_{shared}[b]	−4.88	2.46	0.05	(−9.70, −0.06)
		ϕ	1.34	4.21	0.75	(−6.91, 9.59)
Cousins	944	T_{shared}[b]	−3.66	2.34	0.12	(−8.25, 0.93)
		ϕ	−4.78	5.33	0.37	(−15.23, 5.67)
Distant	4174	T_{shared}[b]	−0.42	1.21	0.73	(−2.79, 1.95)
		ϕ	−1.19	2.42	0.62	(−5.93, 3.55)
Fixed-effects meta-analysis						
All relative types	6318	T_{shared}[b]	−2.09	0.85	0.014	(−3.76, −0.42)
		ϕ	−1.22	1.53	0.43	(−4.22, 1.79)

[a]Each relationship type was evaluated separately in a multivariate model that adjusted for genetic relatedness based on genome-wide SNPs

[b]Standard errors (SE), *P* values, and 95% CI were obtained based on 1000 permutations of T_{shared} for each relationship type

donors from the GTEx project to examine the within-person correlation between TL in whole blood and TL in testicular tissue, which primarily consists of spermatagonia, spermatocytes, or spermatids (~80% of cells). Whole blood TL was positively correlated with TL measured in testicular tissue ($r=0.19$, $P=0.02$) (Figure S10). This observation supports the hypothesized causal relationships in Fig. 1 and the interpretation of our results as trans-generational, direct inheritance of TL.

Discussion

In this study of LTL measured in a genotyped cohort with substantial relatedness, we show that the extent of sharing chromosome ends IBD, a proxy for sharing telomere sequence IBD, is associated with similarity in LTL (smaller value of $(\Delta LTL)^2$) measured in a DNA sample. Our results provide evidence that direct transmission of telomeres from parent to offspring accounts for a substantial proportion of LTL h^2. This implies that variation in TL in parental germ cells impacts TL in offspring embryonic cells (and adult cells) despite telomere reprogramming occurring during embryonic development. The novel approach we describe and apply in this work represents the first attempt to estimate the h^2 in LTL due to direct transmission of telomeres in a human population.

The association between telomere sharing (T_{shared}) and pairwise LTL difference was estimated among sibling, avuncular, cousin, and distant relative pairs. Each of these analyses showed a consistent inverse association, and meta-analysis of all relative pair types produced a statistically significant inverse association. This association was consistently larger than the association between overall genetic relatedness (genome-wide) and LTL difference, suggesting the contribution of telomere sharing to LTL similarity between close relatives is larger than the contribution of sharing inherited variation in non-telomeric regions (e.g., SNPs that influence telomere maintenance).

Genome-wide association (GWA) studies of LTL have demonstrated that common genetic variants impact LTL (Delgado et al. 2017; Codd et al. 2010; Do et al. 2015; Levy et al. 2010; Mangino et al. 2012, 2015 ; Pooley et al. 2013; Prescott et al. 2011; Shen et al. 2011; Soerensen et al. 2012). However, the SNPs identified to date account for a < 4% of the variation in LTL (Codd et al. 2013). Heritability estimates of LTL typically range between 34–50% in family studies and are as high as 82% in twin studies (Bischoff et al. 2012; Hjelmborg et al. 2015; Honig et al. 2015; Broer et al. 2013b). SNP-based heritability (i.e., variation in TL explained by common SNPs) has been estimated to be ~28% (Faul et al. 2016). These findings suggest additional sources of h^2, such as the direct transmission of telomeres investigated in this work.

Several prior studies provide support for the hypothesis that direct transmission of telomeres impacts TL. First, there are multiple studies that report a link between paternal age at conception and offspring LTL (Eisenberg et al. 2012; Kimura et al. 2008; Unryn et al. 2005; Broer et al. 2013a; De Meyer et al. 2007). In light of the evidence that telomeres in sperm lengthen as men age, these results suggest that acquired variation in paternal germ cell TL impacts offspring TL in somatic tissues. Additionally, Eisenberg and colleagues (Eisenberg et al. 2012) found the paternal age effect is cumulative across at least two generations, with grandfather's paternal age (at father's birth) significantly predicting grandchildren's TL independent of father's age at reproduction (Eisenberg et al. 2012). Stindl (2016) offers a

different explanation for the paternal age effect that does not involve age-related telomere lengthening in sperm. Stindl proposes that the association between paternal age and offspring TL is the result of confounding by birth cohort, claiming that TL in the female germline decreases with age and oocytes used first (at younger ages) have longer TL. Consequently, older men have longer sperm TL because they are part of a generation born to younger mothers (Stindl 2016). Both explanations for the paternal age effect support the hypothesis that parental transmission of telomeres contributes to TL h^2.

The work of Aubert et al. (2012), Collopy et al. (2015) and Chiang et al. (2010) also suggests an effect of direct transmission of parental telomeres in humans. These studies report that parents with mutations in *TERT* or *TERC* that render low telomerase activity and shorter telomeres pass their short telomeres to offspring who do not carry the telomerase mutation (Aubert et al. 2012; Collopy et al. 2015). However, these studies were conducted in relatively small samples of parents with telomerase deficiency and their offspring. In addition, these findings were complicated by differences in TL among individuals and the admixing of affected or unaffected paternal and maternal telomeres (Chiang et al. 2010). Thus, these papers provide consistent evidence of the impact of direct transmission on TL, but the implications of these results beyond telomerase-deficient individuals and their families are unclear.

It is worth noting that Chiang et al. (2010) also reached a similar conclusion based on a telomerase deficient ($Tert^{+/-}$) mouse model. These mice had critically short and dysfunctional telomeres, whereas their genotypically normal $Tert^{+/+}$ offspring had functional telomeres that were protected from progressive shortening but had similar lengths to $Tert^{+/-}$ parents.

To our knowledge, this is the first study to attempt to assess the relationship between IBD sharing of telomeres and similarity in LTL. We focused our analysis on specific types of close relative pairs (siblings, avuncular pairs, cousins, and distant relatives) for several reasons. First, the strength of the presumed relationship between telomere sharing and LTL similarity may weaken as relatives become more distant due to "noise" in TL introduced by telomere reprogramming and other factors affecting TL in germ cells. Second, when examining all types of relative pairs in a single analysis, the correlation between genome-wide relatedness and telomere sharing is very strong ($r = 0.97$), and regression models are unable to estimate both parameters; the correlation is weaker for specific relative pair types, enabling estimation of both parameters. Also, very distantly related pairs have an increased number of recombination events occurring since their most recent common ancestor, which makes inferring IBD relationships at chromosome ends more difficult due to more recombination events occurring within the span of SNPs used to estimate IBD. Lastly, traditional SNP-based h^2 analyses that leverage very distant relationships are not ideal for detecting the effects of telomere sharing on TL similarity because we have no evidence showing that the last SNP measured on each chromosome will be in strong LD with variation in the TL on that chromosome (given the gaps can be hundreds of kb).

Our study utilized two different methods for TL measurement, qPCR and a Luminex-based method. Although we have demonstrated that both methods produce measures that are strongly correlated with Southern blot measures (the gold standard for TL measurement using extracted DNA) (Kibriya et al. 2014; Pierce et al. 2016), using multiple methods can introduce heterogeneity into our TL measurement. Therefore, we removed experimental variation from our TL measures through mixed-effects modeling, and we standardized TL distributions across all batches and methods to harmonize these measures. In addition, the measures used in this study are a relative measure of average TL (i.e., telomere abundance) rather than an absolute measure of average TL.

Our study has limited power. Assuming there is attenuation in the effect of T_{shared} on $(\Delta\text{LTL})^2$ with decreasing degree of relatedness, we may be underpowered to detect this difference among relative pair types due to small sample sizes. Our ability to estimate heritability through the method presented by Visscher et al. (2006) is also limited by our sample size. Visscher et al. show that at least 10,000 sibling pairs are required to accurately estimate heritability using the Haseman-Elston-like regression approach. As expected, our h^2 estimate obtained from the meta-analysis β coefficient has a very wide confidence interval, and produced a point estimate greater than 1. Thus, our work essentially provides a lower bound of the narrow-sense h^2. Regarding generalizability, it is important to note that these results were obtained using data from a rural Bangladeshi population and cross-population differences in LTL h^2 could exist due to genetic, environmental, and demographic factors. It is worth noting that some of our study participants have been chronically exposed to arsenic, however this exposure does not appear to impact our results. We have previously investigated the association between TL and arsenic exposure (in water and in urine), and we did not observe a significant association among 1469 Bangladeshi adults (Zhang et al. 2018), providing compelling evidence that arsenic exposure in adulthood does not have a substantial impact of LTL. Additionally, correlations in LTL among relative pairs appear generally weaker than have been observed in prior studies (Bischoff et al. 2012; Hjelmborg et al. 2015; Honig et al. 2015; Broer et al. 2013b), and this could potentially be due to measurement error and/or population differences in the non-genetic determinants of TL.

To better understand the direct transmission of TL from parents to offspring, future studies should analyze larger

numbers of relative pairs, which would enable more accurate estimation of h^2 due to direct transmission. Our method could be applied to UK Biobank data, which includes a substantial number of relative pairs, as well as LTL data in an upcoming release. Larger studies would also allow researchers to determine if the effect of telomere sharing on pairwise TL differences is weaker for more distant relatives. More accurate methods for measuring TL could increase power for these analyses (e.g., Southern blot), and chromosome-specific measures would enable one to study the transmission of specific telomeres and the impact on chromosome-specific TLs, although these approaches are not currently practical for very large cohorts.

In summary, we provide evidence supporting the direct transmission of variation in TL from parent to offspring. This is the first study to attempt to detect and/or estimate the h^2 in LTL that is due to direct transmission. Together with prior evidence (De Meyer et al. 2007, 2014; Eisenberg et al. 2012; Prescott et al. 2012; Achi et al. 2000; Aubert et al. 2012; Unryn et al. 2005; Broer et al. 2013a), our work implies that any effect on TL in germ cells—be it due to genes, environment, or aging—will impact offspring TL. Such effects could potentially shift TL in a population in response to demographic or environmental changes or amplify the impact of natural selection on variation (i.e., SNPs) affecting telomere maintenance (Hansen et al. 2016). These findings are also relevant for complex diseases for which TL has a causal impact on disease risk, as the h^2 for these traits may be impacted by trans-generationally inherited TL, a component of h^2 that cannot be explained by SNPs or other variation in non-telomere sequences. Applying our method to larger studies of relative pairs will enable robust estimation of LTL h^2 attributable to direction transmission.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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