Review

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Telomere length determinants in childhood

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Abstract: Telomere length (TL) is a dynamic marker that reflects genetic predispositions together with the environmental conditions of an individual. It is closely related to longevity and a number of pathological conditions. Even though the extent of telomere research in children is limited compared to that of adults, there have been a substantial number of studies providing first insights into child telomere biology and determinants. Recent discoveries revealed evidence that TL is, to a great extent, determined already in childhood and that environmental conditions in adulthood have less impact than first believed. Studies have demonstrated that large inter-individual differences in TL are present among newborns and are determined by diverse factors that influence intrauterine development. The first years of child growth are associated with high cellular turnover, which results in fast shortening of telomeres. The rate of telomere loss becomes stable in early adulthood. In this review article we summarise the existing knowledge on telomere dynamics during the first years of childhood, highlighting the conditions that affect newborn TL. We also warn about the knowledge gaps that should be filled to fully understand the regulation of telomeres, in order to implement them as biomarkers for use in diagnostics or treatment.

Keywords: attrition; children; determinants; dynamics; genetics; telomere length.

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Introduction to telomeres

Telomeres are non-coding repetitive sequences TTAGGG on the ends of the eukaryotic chromosomes [1]. They form a protective cap that conserves the genetic material during cell division and protect it from constitutive exposure to the DNA damage response [2]. In humans, telomeres consist of 4–15 kilobase pairs (kbp) [1]. From the early research it has become clear that they shorten progressively with age [3] as a result of the end replication problem during cell division and/or oxidative stress. Chromosomes with critically short telomeres are recognised by DNA damage response proteins as damaged DNA, which leads the cell to a controlled telomere-initiated senescence [4]. When cells become senescent, they undergo morphological and genetic changes that result in the loss of tissue function.

Cells are capable of maintaining the length of telomeres with the telomerase enzyme, which is extinguished during embryonic differentiation in most somatic cells, but remains active in germline cells, activated lymphocytes and certain types of stem cell populations [5]. During normal human growth and development, telomerase activity is precisely regulated by a number of genes in order to meet the proliferative demand of the specific cellular function [6]. Cancer cells, on the other hand, can acquire the possibility of an infinite number of divisions by enhancing the telomerase activity, thus restraining the shortening of telomeres [7], or with the mechanism of the alternative lengthening of telomeres (ALT) [8].

Telomere length (TL) has been an important topic of research because of its association with longevity, as well as with the occurrence and progression of common chronic diseases. An active role of TL in age-related human diseases arises because short telomeres increase the risk of diseases related to restricted cell proliferation and tissue degradation, such as cardio-vascular diseases (CVD); long telomeres increase the risk of diseases related to increased proliferative growth, such as major cancers [9]. Therefore, TL is considered as a potential biomarker for disease susceptibility or a possible target for a particular treatment. However, its complex biology, mixed with the substantial impact of genetic and environmental factors are making this task difficult [10].

In the early 1990s, the interest in telomeres started to spread, and samples of individuals of different age ranges were used for the studies; among these were also the first samples of children. Knowing the biology behind telomeres in newborns and young children is crucial, as it provides the information about baseline TL [11]. The baseline TL represents a critically important characteristic of an individual's telomere biology, together with a second major determinant – TL attrition over time [9]. A reduction in newborn TL can result in a greater susceptibility for pathophysiological conditions in adulthood [11].

In the following article we summarise the current knowledge of telomeres in childhood. This important period of life is characterised by dynamic biology and strong dependence on genetic and environmental factors, and it plays a crucial role in telomere attrition [12]. We demonstrate why the precise understanding of telomere dynamics in childhood is indispensable for further consideration of telomeres as a biomarker or a treatment target.

Telomeres and diseases

Telomere syndromes

TL and telomerase activity are involved in the pathology of numerous diseases [13], and preterm telomere attrition is associated with reduced longevity [14]. The initial discoveries of the role of telomeres in human diseases primarily came from the studies of rare monogenic disorders of childhood. Monogenic, age-related dysfunctions (i.e. telomere syndromes) are associated with short telomeres, normally caused by genetic mutation in a gene associated with telomere maintenance [15]. Dyskeratosis congenita (DC) is a multisystem inherited syndrome which was first related to defective telomere maintenance [16]. Children inherit short TL from affected parents, together with a mutated copy of a gene encoding products involved in telomere maintenance, such as telomerase core components (*TERT* and *TERC*), factors required for telomerase biogenesis (*DKC1*, *NHP2*, *NOP2*, *NAF1* and *PARN*), telomerase trafficking (*WRAP53*), telomerase recruitment (*ACD*), telomere replication and end structure (*RTEL1*, *CTC1*, *STN1* and *POT1*), and other aspects of telomere biology (*TINF2*) [17]. The three most distinguished subtypes of DC are related to the mentioned genes: X-linked recessive DC with mutations in the *DKC1* gene, encoding a component of H/ACA small nucleolar ribonucleoprotein; autosomal dominant DC with heterozygous mutations in either *TERC* (RNA component of telomerase) or *TERT* (enzymatic component of telomerase); and autosomal recessive DC, for which the genes involved remain largely elusive [18]. The disease affects highly proliferative tissues, which require constant renewal and are normally highly regenerative, such as skin, gut and bone marrow [19, 20]. This leads to mucocutaneous abnormalities, abnormal nails, reticular skin pigmentation, oral leukoplakia and an increased predisposition to cancer. The principle cause of mortality of DC is bone marrow failure or aplastic anaemia; 40% of patients are affected by one of these conditions by the age of 40 [21].

Hoyeraal-Hreidarsson and Revesz syndromes are two very rare disorders with onset in infancy, are linked to very short telomeres, and represent a spectrum of the same disease. They are more severe than DC and are characterised by intrauterine growth retardation, microcephaly, cerebellar hypoplasia, progressive combined immune deficiency and aplastic anaemia. Revesz syndrome can be distinguished from Hoyeraal-Hreidarsson syndrome by the presence of bilateral exudative retinopathy [22].

Telomere disorders most commonly manifest as adult-onset diseases. Mutations in *TERT* and *TERC* can lead to diverse phenotypes in which severity depends on which family generation is affected. Idiopathic pulmonary fibrosis, for example, is normally diagnosed at the age of 50. However, later generations of a susceptible family will more often have children affected with aplastic anaemia along with classic features of DC [23].

Cancer

Telomere biology is very important in paediatric cancers, as most paediatric leukaemias and embryonal solid tumours activate the telomerase enzyme. Therefore, telomeres in paediatric cancer patients are often longer than in healthy subjects, but become shorter after chemotherapeutic treatment, which causes an excessive oxidative stress that harms telomeres. Telomerase was considered as a therapeutic target for paediatric cancers, as the enzyme plays a key role in conferring cellular immortality, is present in most tumours, and is relatively specific for cancer cells [24–26]. Despite all the efforts to develop a telomerase-targeting therapy, there is currently no approved treatment available on the market [27]. This is mainly due to the high number of cell doublings required to induce a tumour suppressive senescence after telomerase inhibition – a long period before a telomerase inhibitor becomes effective against a tumour [28]. In addition, such treatment could prompt a toxic effect on highly proliferative normal stem cells that can express a regulated telomerase activity [29]. A Mendelian randomisation study

on the association between TL and risk of cancer and non-neoplastic diseases showed that increased TL due to germline genetic variation was generally associated with increased risk for site-specific cancers, but reduces the risk for some non-neoplastic diseases, including CVD [30]. Therefore, potential therapeutic applications based on TL should be carefully considered also for the trade-off of the risk for going from one disease to another.

Stress and other diseases

Many childhood clinical disorders can cause increased oxidative stress and chronic inflammation, including perinatal brain damage, asthma, cystic fibrosis, juvenile rheumatoid arthritis, cholestatic liver diseases and diarrhoeal diseases [31]. Chronic inflammation can increase the rate of cell proliferation and increase cellular turnover, therefore facilitating telomere erosion. Also, oxidative stress can increase the size of telomere repeats clipped from the ends of chromosomes, thus promoting telomere DNA double-strand breakage and telomere shortening [32]. Long-term childhood diseases that provoke an increased oxidative stress can therefore cause faster telomere shortening and result in accelerated cellular ageing that may manifest in adulthood as a common age-related pathology [33–36].

It was demonstrated that in childhood, stress originating from childhood maltreatment [37] or violence [38] can cause a premature shortening of telomeres [39]. Similarly, maternal stress during pregnancy can have an impact on TL in children. This subject will be further discussed in the section "Determinants of newborn TL".

Genetic determinants of TL

TL is a highly heritable trait (36%–86% in different family and twin studies) [40–45]. Several large genome-wide association studies (GWAS) were performed to investigate associations between genetic variants and relative TL in an adult population [46–56]. Most of the identified SNPs harbor genes that encode proteins with known functions in telomere biology (i.e. genes directly involved in telomere maintenance). Two GWAS studies were performed in paediatric populations to look for common SNPs related to relative TL (Table 1), and none replicated the results identified in adult cohorts. This suggests that possibly other genetic variants are involved in the regulation of TL in childhood and adolescence than in adulthood [57, 58]. Moreover, none of the identified SNPs was replicated among paediatric populations. One of the possible reasons for this might be the heterogeneity between populations, as studies were performed in healthy African-American children and adolescents [57] and in a healthy European population [58]. Genotyping of the SNPs identified in the European cohort was also performed in newborns in an Asian population, but no significant association for any individual variant was detected [59].

There is no doubt that genes related to telomere biology have a big impact on TL starting at birth; a pure example is the heritable childhood disease DC as discussed already [20]. However, individuals with such diseases are born with the very short telomeres, inherited by their parents, whereas the impact of affected genes can show only later in life. Accelerated loss of telomere base pairs after birth, caused by affected genes, might not be evident in the first decades of life, but would only become significant in adulthood.

The GWAS on paediatric populations are interesting for the identification of genes that reflect the baseline TL before the occurrence of the major attrition. However, paediatric studies to date have used small sample sizes compared to studies in adult cohorts. In order to confirm whether there is an age-specific variation of genetic factors contributing to TL regulation, and to identify novel variants that regulate TL in childhood, larger

Table 1: Genetic variants associated with relative TL in children population.

First author, year, reference	Sample size	SNP	p-Value	Chromosome	Reported gene(s)
Zeiger, 2018 [57]	492 Children (8–20 years)	rs1483898	7.86×10^{-8}	14	*LRFN5*
Stathopoulou, 2015 [58]	322 Children (6–17 years)	rs10496920[a]	5.00×10^{-5}	2	*LRP1B*
		rs528983[a]	2.93×10^{-5}	4	*NDST4*
		rs594119[a]	3.11×10^{-5}	6	*NKAIN2*
		rs12678295[a]	3.53×10^{-5}	8	*MYOM2*
		rs2300383[a]	1.88×10^{-5}	21	*ITSN1*
		rs11703393[a]	5.00×10^{-5}	22	*PARVB*

[a]SNPs did not reach genome-wide significance level, but reached the levels of suggestive association ($p \leq 5\times10^{-5}$).

GWAS studies should be performed on the paediatric population.

Assessment of TL in healthy and diseased neonates and children

In the following section we will present the studies that focussed on determination of newborn TL and rate of TL attrition throughout childhood (Table 2) and summarise the important conclusions acquired from the researchers.

One of the first investigations that used a paediatric sample to study TL dates from 1993. The study compared lymphocyte TL of 21 individuals with Down syndrome (DS), aged 0–45 years, to 119 healthy controls aged 0–107 years. The rate of telomere loss was calculated as the decrease in mean telomere restriction fragment (TRF) length as a function of donor age. The study demonstrated that DS patients experience significantly higher rates of telomere loss (133 ± 15 bp/year) compared to healthy age-matched controls (41 ± 7 bp/year). Moreover, separation of samples according to gender showed faster rates of male telomere loss compared to female, but the difference was not statistically significant. The difference in the rate of TL shortening from birth until adulthood was not discussed [60].

The first study to focus on the rate of telomere sequence loss in children's leucocytes was published by Frenck et al. in 1998. The research was conducted on 75 individuals that comprised 12 unrelated healthy newborns and their relatives (parents, grandparents). Comparison among three generations showed a larger difference in TL between the newborns and their parents (4.8 kbp) than between parents and grandparents (2.0 kbp), even though the age interval was similar for both generations (25 years). Further research was conducted in order to observe the rate of telomere shortening during the first few years of life. Because of a disagreement of the Institutional Review board, the study could not provide the follow-up of the same children, but it compared 10 unrelated healthy children of age 5–48 months. Researchers discovered that the rate of telomere shortening is not stable, but appears in three characteristic phases: (1) from birth to the age of 4, characterised by a rapid decline in the average TL, (2) stable TL from the age of 4 until early adulthood, (3) gradual decline in mean TL associated with advancing age. The authors assumed that a high rate of proliferation appears in the most immature subset of haematopoietic progenitors in the first months of life. Haematopoiesis throughout life is later initiated from progenitors that have already undergone a substantial amount of telomeric loss [61]. The weak point of the study was a low number of the individuals (10 children) by which the whole theory was estimated. It is important to mention that variation in TL among newborns is as wide as the variation among adults [12]. Therefore, comparisons between non-related children of different ages might not perfectly describe the process of telomere shortening that is happening in the same individual, as inter-individual differences between the subjects of the same age can be significant and could cause biased results when using a small sample size.

This problem was overcome in the study published by Zeichner et al. in 1999, in which nine newborns of HIV-infected mothers were followed up for 3 years. Two adults with the risk of HIV infection with 8- and 10-year follow-up were used for comparison. The study detected a significant difference between TL measured in peripheral blood mononuclear cells (PBMCs) at 1 month and 36 months of age. The average rate for the TL shortening was 270 bp/year from 0 to 36 months, which was significantly higher than telomere loss in adult PBMCs (50 bp/year). Compared to Frenck et al., Zeichner et al. reported only two characteristic phases of telomere loss: (1) a phase of fast telomere sequence loss in the first 3 years, followed by (2) a relatively constant gradual loss of telomere base pairs [62]. The strong point of the study was the longitudinal follow-up of individuals, which enabled a much more refined view of telomere biology than cross-sectional studies. However, the follow-up of the children was conducted only until the age of 3, and the researchers estimated the telomere loss after the age of 3 by calculating the average age loss from the last two samples obtained from each child. The calculated value for the telomere loss was 50 bp/year, consistent with the results in adults; therefore, researchers assumed that from the age of 3 onward the telomere loss is constant [62].

The plateau of TL at the age of 4, first mentioned by Frenck et al., was further investigated in 70 children of Latino origin. Leucocyte telomere length (LTL) was assessed twice in a year, at the age of 4 and 5. The study reported LTL maintenance in most of the children during the period of 1 year, which is consistent with the results obtained by Frenck et al. [61].

In order to have an insight into foetal haematopoiesis and telomere attrition, changes in LTL were also investigated in preterm and full-term newborns. The findings of the cord blood TRF analysis of 15 preterm and 11 full-term newborns showed no significant differences between the groups, though a trend of shorter telomeres with increasing gestational age was observed. A rapid and significant decline between 27 and 32 weeks of gestation was noticed,

Table 2: Studies assessing the TL dynamics in newborns and children.

First author, year, reference	Sample size	Age	TL measurement	Findings	Comments	Cells
Vaziri, 1993 [60]	140	0–107 years	Mean TRF length	DS patients shorter TL than controls; shortening of TL with age; men shorter TL than women	No detailed report in children	Lymphocytes
Frenck, 1998 [61]	75 Family members, 10 unrelated children	Three generations 5–48 months	Mean TRF length	Fast decrease in first years of childhood, plateau at age of 4, graduated attrition in the adulthood	Small sample size	Leukocytes
Zeichner, 1999 [62]	Nine newborns, two adults	Longitudinal study: 0 m (for 3 years) 28 and 30 years (for 8 and 10 years)	Mean TRF length	Faster shortening during first 3 years of life, then slower rate of shortening	Small sample size	PBMCs
Friedrich, 2001 [63]	15 Preterm, 11 full-term newborns	<37 weeks of gestation, >37 weeks of gestation	Mean TRF length	No big difference in groups of newborns, significant decline of TL with increasing gestational age between 27 and 32 weeks	Comparison of different children	Leukocytes
Okuda, 2002 [12]	168 Newborns	Newborns	Mean TRF length	No significant difference in male and female newborns in TL, synchrony among tissue	TL variation among newborns is as wide as among adults	Leukocytes, umbilical artery cells, foreskin cells
Akkad, 2006 [64]	34 SGA babies, 38 normal-grown newborns	35–42 weeks of gestation	Mean TRF length	Impaired fetal growth is not associated with TL	Small sample size	Leukocytes
Wojcicki, 2016 [65]	77 Children, 70 mothers	4 years old	qPCR	TL at the age of 4–5 maintains unchanged	Technique less precise than TRF	Leukocytes
Vasu, 2017 [66]	47 Preterm, 31 full-term newborns	<32 weeks of gestation, >37 weeks of gestation	qPCR	Preterm infants at term equivalent age have significantly *longer* telomere lengths than term born infants	Small sample size, less precise than TRF	Leukocytes

DS, Down syndrome; PBMCs, peripheral blood mononuclear cells; SGA, small-for gestational-age; TL, telomere length; TRF, terminal restriction fragment.

pointing to an increased proliferation rate during this period of gestation. Moreover, after 32 weeks of gestation a high inter-individual variability of LTL was observed, possibly due to an important genetic influence on TL, as estimated by the researchers [63].

On the contrary, most other studies observed significant differences in preterm and full-term newborn TL. Vasu et al. confirmed that preterm infants had longer LTL than full-term newborns and that relative LTL is highly variable among newborn infants. In addition, they discovered that at birth, preterm infants had significantly longer LTL than full-term babies [66]. Holmes explained that the difference occurs because of the greater proliferative capacity of foetal haematopoietic stem cells in comparison to post-natal stem cells [67].

Finally, telomeres were also assessed in small-for-gestational-age (SGA) babies; however, there was no difference in comparison to normally grown controls [64].

All but two of the mentioned studies used TRF technique for measurement of TL (Table 2). A real-time PCR (qPCR) technique for TL measurement is a less precise method than TRF, and it might impact the obtained results [68]. The newborn's blood was in all cases collected from the umbilical cord, and TL was measured from blood leukocytes or PBMCs. All studies have substantial limitations due to the small sample size and/or lack of longitudinal observations. However, ethical considerations of such studies limit the research design, as it is unethical to follow-up healthy children for research that will not contribute to health improvement [61]. Hence, the follow-up was possible only in the cases of children with significant health risk (such as preterm babies and children of HIV-infected mothers), where closer medical follow-up was indispensable.

Nevertheless, some final conclusions can be drawn for TL dynamics in children:

- TL is highly variable among children of the same age.
- Preterm babies have longer telomeres than full-term babies.
- The rate of telomere loss is higher in the first 3 years of life (approximately 250 bp/year).
- The TL at the age of 3 until young adulthood might be stable, but it is more likely gradually decreasing (up to 50 bp/year).
- The rate of telomere loss becomes constant in young adulthood (approximately 50 bp/year).
- The differences in shortening rates at different periods of childhood are due to an increased cell replication rate in infants, especially during accelerated development of the immune system (expanding of haematopoietic stem cell [HSC] pool).

TL shortening in diverse tissues

In the following section we will discuss the difference in TL among various human tissues. Different views on the topic will be presented and the most pertinent conclusions will be pointed out.

Several studies have agreed that synchrony in TL exists among tissues of the human foetus, but this is lost during extrauterine life [69, 70]. Corresponding to this idea, TL in samples of three tested tissues in newborns (leucocytes, umbilical artery cells and skin cells) were found to be highly synchronised [12]. Indeed, telomerase is active during every stage of development of the embryo, and this enables the maintenance of equally long telomeres [71].

With ageing, cells in different tissues lose the synchrony in TL due to different proliferative activity, which mainly arises during the expanded growth and development in the first two decades of life. For a long time, there was a common opinion that TL in skeletal cells, which form a minimally proliferative tissue, represents the approximate size of TL at birth [72, 73]. Decary et al. demonstrated that skeletal satellite cells lose both proliferative capacity and TL during the first two decades of life. In adulthood, muscle development is complete and TL becomes stable. On the other hand, they realised that skeletal muscle cell myonuclei, generally presented as post-mitotic tissue, do not undergo any shortening of telomeres and stay consistent from birth to late age [73].

Scientists thus assumed that TL in skeletal muscle and the difference between LTL and skeletal muscle telomere length (MTL) could provide a broad account of LTL dynamics over the life course of the individual, as these two variables would represent birth LTL and its age-dependent attrition, respectively [74]. They concluded that the difference between TL in muscle and leucocytes provides a much better account for LTL dynamics than LTL alone (research was performed in dogs) [74].

The research in adult subjects, however, gave unexpected results. TL shortening in four types of somatic tissue cells – leucocytes, muscle, skin and fat – was examined in 87 individuals with an age range of 19–77 years. Despite different replicative status, all tissues displayed similar rates of age-dependent attrition. The difference in TL across the tissues is thus believed to be established during the first two decades of life. Later, stem cell division rates, including those of muscle, are synchronised across the examined somatic tissues. Muscle cells are therefore not an exact indicator of birth TL, which continuously shortens even in adulthood [75]. The authors explained this discovery, which is not consistent with the previous studies of TL in skeletal muscle, as the result of

Table 3: Determinants of newborn TL.

First author, year, reference	Sample size	Age	TL measurement	Observed variable	Comments	Cells
Gender						
Okuda, 2002 [12]	168 Newborns	Newborns	Mean TRF length	Gender	No significant difference in male and female newborns in TL, synchrony among tissue	Leukocytes, umbilical artery cells, foreskin cells
Wojcicki, 2016 [102]	54 Newborns	Newborns	qPCR	Gender	Female gender associated with longer TL by ~350 base pairs	Cord blood cells
Ethnicity						
Drury, 2015 [103]	71 Newborns	Newborns	qPCR	Ethnicity	Black infants had significantly longer TL than white ($p = 0.0134$), strongest effect observed in black female	Blood spot cells
Vitamins						
Entringer, 2015 [100]	119 Mother-newborn dyads	Newborns	qPCR	Maternal folate concentration in early pregnancy	10 ng/mL increase in folate increased 5.8% in median TL ($p = 0.03$)	CBMCs
Kim, 2017 [99]	106 Mother-newborn dyads	Newborns	qPCR	Maternal vitamin D concentrations	Newborn TL were associated with maternal vitamin D concentrations ($\beta = 0.33$, $p < 0.01$)	Leukocytes
Maternal pre-pregnancy BMI						
Martens, 2016 [101]	$N_{cord} = 743$ $N_{plac} = 702$	Newborns	qPCR	Maternal pre-pregnancy BMI	Each kg/m^2 increase in pre-pregnancy BMI was associated with a –0.50% shorter cord blood TL and a –0.66% shorter placental TL	Cord blood cells, placental cells
Maternal stress						
Entringerer et al., 2013 [104]	27 Mother-newborn dyads	Newborns	Mean TRF length	Maternal psychosocial stress	Independent, linear effect of pregnancy-specific stress on newborn LTL accounted for 25% of the variance of LTL	Leukocytes
Marchetto et al., 2016 [94]	24 Mother-newborn dyads	Newborns	Mean TRF length	Maternal psychosocial stress	Significantly shorter TL in newborns whose mothers experienced a high level of stress during pregnancy	Cord blood cells
Send et al., 2017 [95]	319 Newborns and 318 mothers	Newborns	qPCR	Maternal psychosocial stress	Stress during pregnancy was associated with shorter telomeres in newborns but not with maternal TL	Cord blood cells
Maternal smoking						
Almanzar, 2013 [97]	$N_{Non\text{-}smoking\ mothers} = 111$ $N_{smoking\ mothers} = 58$	Newborns	qPCR	Tobacco exposure	Newborns exposed to tobacco had significantly longer telomeres than non-exposed newborns	Lymphocytes
Salihu et al., 2015 [105]	n = 86 Mother-newborn dyads	Newborns	qPCR	Tobacco exposure	Newborns exposed to tobacco had significantly shorter telomeres than non-exposed newborns	Leukocytes

Table 3 (continued)

First author, year, reference	Sample size	Age	TL measurement	Observed variable	Comments	Cells
Hormones						
Entringer, 2015 [96]	n = 100 Infants	15 months old	qPCR	Maternal E_3 concentrations in early gestation	One-multiple-of-the-median increase in maternal E3 concentration during early pregnancy was associated with a 14.42% increase in infant TL	Buccal cells
Gestational and pre-gestational diabetes						
Cross et al., 2010 [106]	$N_{type1} = 26$ $N_{type2} = 20$ $N_{gestional} = 71$ $N_{control} = 202$	Newborns	Flow cytometry	GD and PGD	No difference in cord blood TL in pregnancies of women with diabetes compared with controls	CBMCs
Xu et al., 2014 [107]	$N_{gestional} = 82$ $N_{control} = 65$	Newborns	qPCR	GD and PGD	In the GD group, TL was significantly shorter than in non-GD pregnancy ($p = 0.028$)	Leukocytes
Gilfillan et al., 2016 [108]	Diabetes: $N_{gestational} = 20$ $N_{pre\text{-}gestational} = 14$ $N_{control} = 18$	Newborns	qPCR	GD and PGD	No significant telomere shortening in the offspring of mothers with PGD or GD	Cord blood cells
Parental age at conception						
Factor-Litvak, 2016 [109]	n = 490 Father-mother-newborn trios	Newborns	TRF	Parental age	1-year increase in father's age results in 0.016 kb increase in newborn LTL	Leukocytes

BMI, body mass index; CBMC, cord blood mononuclear cells; E_3, estriol; GD, gestational diabetes; PGD, pre-gestational diabetes, TL, telomere length; TRF, terminal restriction fragment.

a more accurate method of TL measurement and a bigger sample size used for the investigation.

The difference between skeletal MTL and LTL is primarily an index of HSC telomere shortening due to the expansion of the HSC pool, which becomes stable in adulthood. Some differences in proliferative activities among two tissues can appear but are likely to be modest [76].

Finally, the difference between skeletal MTL and LTL was examined in the foetal and child samples. Surprisingly, the study demonstrated that MTL is longer than LTL already in foetal samples, assuming that TL is similar across tissues only until early embryonic development. Scientists concluded that variation in TL among individuals is wider than intra-individual variation of LTL and MTL, and that individuals with short/long MTL display short/long LTL, as was previously shown [77].

Different tissue cells display different proliferative activities. However, this difference is mainly established until adulthood, when cells require only a small number of divisions to maintain tissue integrity [75].

The main conclusions of the above discussed studies are the following:

- During embryonic development, cells of different tissues have similar TL due to the activity of telomerase in early intrauterine growth.
- The difference in TL among tissues (e.g. skeletal muscle and leucocytes) appears during late intrauterine development and is mainly established in the first two decades of life.
- TL of minimally proliferative muscle cells shortens much more slowly than TL of highly proliferative leucocyte cells during the first two decades of life.
- In adulthood, skin, fat, muscle and leucocyte cell TL shortening is synchronized.
- The difference in MTL and LTL represents the leucocyte telomere dynamics during early life and corresponds well to telomere dynamics of an individual.
- High LTL variability among individuals is expressed already *in utero*.
- Variation in TL between foetuses and children is as wide as variation among adults of the same age.
- In the same child or adult, a synchrony exists across somatic tissues (an individual with long LTL will have long MTL).

Determinants of newborn TL

As previously mentioned, since birth, a high variability exists in TL among children. One of the most important factors that determine this inter-individual difference is genetics, which was shown in several heritability studies [40–45]. Nevertheless, a substantial number of studies in adult cohorts showed that besides genetics, TL can be influenced by various other factors. Most commonly reported among those are:

- Gender (women have longer TL than men) [78]
- Race (African Americans have longer TL than Europeans) [79, 80]
- Paternal age (older age of the father is associated with longer TL) [81, 82]
- Smoking (smokers have shorter TL than non-smokers) [83, 84]
- Physical activity (non-active have shorter TL than active) [85]
- Traumatic events (cause shortening of TL) [39, 86–88]
- Obesity (causes shortening of TL) [84]
- Oxidative stress (causes shortening of TL) [89, 90].

Antenatal determinants were shown to be a particularly important factor of TL, as early developmental environment conditions the developmental process, which in turn influences the individual's predisposition to developing a complex common disorder [91]. TL in children can be affected by oxidative, immune, endocrine and metabolic pathways in a way that accelerates cellular dysfunction over the lifespan [11, 92], thus reflecting the effect of specific conditions on the later susceptibility to a disease. Maternal stress [93–95], hormones [96], smoking [97], race [98], vitamin concentrations [99, 100], pre-pregnancy body mass index (BMI) [101] and other factors showed correlation with TL and pointed out the importance of *in utero* exposures in the regulation of newborn TL. In this section we will focus on some of the most pertinent antenatal determinants. The summary of the newborn TL determinants is presented in Table 3.

Gender

The fact that women have a predisposition for longer telomeres than men have is well known and has been systematically confirmed in adult telomere studies [78, 110, 111]. At the beginning of the exploration, studies of telomeres in children did not take into account the gender of the individuals or any other covariate that could impact TL. The first study to focus on newborn gender in association with TL showed that at birth, male (10.95 ± 0.088 kb) and female (11.07 ± 0.077 kb, $p = 0.3$) newborns do not statistically differ in the length of their leucocyte telomeres, suggesting that sex difference in TL arises from different rates

of attrition in extrauterine life [12]. This discovery was not in accordance with later studies [103, 112], and the reason for this discrepancy might be that no other potential TL determinant (race, socioeconomic status, etc.) was taken into account.

On the contrary, longer cord blood and placental telomeres were found in female newborns compared to male newborns in studies of Latino and mixed-population infants [102, 109], supposing that hormonal differences between male and female genders could play a role in telomere dynamics *in utero* [113].

It is possible that the impact of gender on TL is smaller in newborns than in adults of the same age. However, it remains an important determinant that should be systematically considered in all telomere studies. The degree of the gender impact on infant TL remains to be fully investigated.

Race

The explanation for differences in TL among different races lies in natural protection developed during the evolution process. The hypothesis is that a cell division limit was developed as a mechanism for tumour suppression [28]. Therefore, the presence of relatively shorter TL in Europeans is a result of polygenic adaptation of the northbound migration out of equatorial Africa that attenuated the risk of melanoma in individuals with light skin pigmentation [79]. TL was compared among 71 Black and White babies, measured in blood spots of newborns. The results showed that infant TL was significantly longer in Black compared to White newborns ($p = 0.0134$), in a model accounting for sex, birth date, birth weight, gestational age, parental age, maternal race and maternal highest level of education as a proxy for socioeconomic status [103]. This study demonstrated that racial differences in TL are significant from birth and highlighted origin as an important TL determinant.

Vitamins

Two studies give an example of the importance of appropriate maternal vitamin intake during pregnancy. First, maternal folate concentration was measured in the first trimester of pregnancy in serum samples of 119 mothers and was analysed in relation to TL measured from the cord blood mononuclear cells [100]. Adjusted covariates were specified *a priori*, including maternal socioeconomic status (annual family income), race/ethnicity, maternal pre-pregnancy BMI, the presence of obstetric complications, maternal age, infant sex, gestational age at birth and birth weight. The results demonstrated that each 10 ng/mL increase in maternal total folate concentration was associated with a 5.8% increase in median TL ($p = 0.03$).

Second, maternal vitamin D concentration was examined for associations with LTL in newborn cord blood samples in a model adjusted for the maternal age, BMI, LTL, white blood cell count, glycosylated haemoglobin level, health behaviours (smoking, exercise, body weight before pregnancy, medical history) and nutritional intake and for the newborn's sex and birthweight. The study concluded that maternal vitamin D levels were also positively associated with newborn LTL [99].

Maternal pre-pregnancy BMI

Maternal obesity during pregnancy can reflect an adverse nutritional status that affects an offspring and has a significant deleterious effect on the outcome of pregnancy [114]. This phenomenon was also observed in telomeres. Higher maternal pre-pregnancy BMI was associated with a decline in newborn TL in a study examining 743 samples from cord blood and 702 samples from placental tissue, independent of maternal and paternal age at birth, maternal education, gestational age, newborn's gender, ethnicity, birthweight, maternal smoking status, parity, caesarean section and pregnancy complications. Each kg/m^2 increase in pre-pregnancy BMI was associated with a −0.50% (95% CI, −0.83 to −0.17; $p = 0.003$) shorter cord blood TL and a −0.66% (95% CI, −1.06 to −0.25; $p = 0.002$) shorter placental TL [101]. Higher maternal pre-pregnancy BMI was also associated with increased newborn adiposity and inflammation [115]. Nonetheless, with adequate preventive measures maternal obesity could be reduced. This would result in decreased pregnancy complications and might also impact the overall quality of the child's life.

Maternal stress

Stress plays an important role in foetal development and can result in an altered endocrine and immune response, which serve in the progression of normal gestation. This results in increased oxidative stress that is particularly harmful for telomeres [91]. Psychological stress and psychiatric disorders were previously linked to telomere biology in adults [37, 39, 116, 117]. The significant impact of intrauterine stress exposure on LTL was first

demonstrated in young adults [118]. The fact that exposure to maternal psychosocial stress may exert a "programming" effect on the newborn telomere biology was confirmed in 27 mother-newborn dyads, after accounting for the mother's age at birth, weight, sex and exposure to antepartum obstetric complications. The effect of the pregnancy-specific stress on newborn LTL accounted for 25% of the variance in LTL [104]. Likewise, a significant negative association between maternal stress and newborn TL was observed in a group of 24 mother-newborn dyads. Affected newborns had significantly shorter TL (6.98 ± 0.41 kb) measured in cord blood cells, compared to the newborns of mothers with low stress (8.74 ± 0.24 kb; t = –3.99, p = 0.003) [94]. Finally, it was demonstrated that maternal lifetime history of a psychiatric disorder causes shortening of maternal TL but does not affect newborn TL. Acute stress during pregnancy, on the other hand, is related to shorter TL of a newborn but does not affect the mother's TL [95].

Smoking

A recent systematic review and meta-analysis demonstrated that TL of smokers decreases significantly over time in contrast to non-smokers [119], which implies mechanisms linking tobacco smoke exposure to ageing-related diseases. The impact of intrauterine tobacco exposure on foetal TL was also investigated. First, a study of the effect of cigarette smoking during pregnancy on the lymphocyte subpopulations in newborns showed that newborns of smoking mothers had significantly *longer* telomeres compared to newborns of non-smoking mothers [97]. This unexpected discovery was disproved in a study by Salihu et al. in which evidence of a positive association between shortened LTL and smoking during pregnancy was demonstrated [105], suggesting the possibility of early intrauterine programming for accelerated ageing as a result of tobacco exposure. The reason for the discrepancy in the results of these two studies is not clear; however, there is undeniable proof that link cigarette smoke to telomere shortening caused by increased oxidation stress in cells [120]. We therefore postulate that smoking during pregnancy should be considered as an important environmental factor that causes accelerated chromosomal ageing via increased telomere loss.

Hormones

The hormones have a substantial effect on adult male and female TL, and the impact of hormonal therapy was a subject of numerous studies [121, 122]. Entringer et al. assessed the influence of estriol (E_3) concentration in early gestation on children's TL. TL was measured in buccal cells collected in children aged 14.6 months on average. After accounting for the effects of gestational age at maternal blood draw during pregnancy and the child's age and sex, there was a significant, independent effect of maternal E_3 concentrations on children's TL [96].

Gestational and pre-gestational diabetes

Association studies of a mother's gestational or pre-gestational diabetes with TL showed inconsistent results. First, Cross et al. compared TL of cord blood mononuclear cells of newborns, whose mothers suffered from pre-gestational type 1 diabetes (n = 26), type 2 diabetes (n = 20) or gestational diabetes (n = 71), with newborns of mothers without diabetes (n = 45, n = 76 and n = 81, respectively) [106]. Covariates included maternal smoking status, age of both parents, and offspring's birth weight and sex. The study found no difference in cord blood TL in pregnancies of women with diabetes compared to control subjects, but it identified higher cord blood telomerase activity in type 1 and gestational diabetes. Similarly, a study of 20 cases of mothers with gestational diabetes and 14 cases of pre-gestational diabetes (n = 7 type 1 and n = 7 type 2) compared to 18 control mother-baby pairs also showed no significant difference in the newborn's cord blood TL [108]. On the other hand, another study demonstrated that a group of babies born to mothers with gestational diabetes (n = 82) had significantly shorter LTL than babies born to healthy mothers (p = 0.028). The model was adjusted for maternal age, gestational age at delivery, neonatal birth weight and foetal gender [107]. In conclusion, further studies should be performed to confirm the impact of gestational or pre-gestational diabetes on newborn TL.

Paternal age at conception

Finally, one of the most intriguing observations regarding TL is the fact that children with older fathers have longer telomeres in comparison to their peers with younger fathers. The effect of paternal age at conception (PAC) was also demonstrated in newborns, where a 1-year increase in PAC corresponded to a 0.016 kb increase of newborn LTL [109]. This phenomenon was attributed to elongation of telomeres in the sperm of older men due to a higher telomerase activity [81, 123]. Studies remain inconclusive whether it is maternal or paternal inheritance that has

a greater impact on TL; however, the effects of both are already evident in newborns [92].

To conclude, Entringer et al. understood the process of newborn TL development as a foetal programming of health and disease risk: '… intrauterine life represents a particularly sensitive time period when the effects of maternal states and conditions around conception and across pregnancy may be transmitted to the developing embryo/foetus.' TL is a key cellular target of these effects and carries important implications in long-term health and susceptibility to common age-related disorders [11].

Conclusions

During the last three decades, extensive studies were continually providing the evidences that linked shortened telomeres with common age-related diseases, disease risk factors and longevity. Telomeres were considered as a potential biomarker that could assess susceptibility to a specific pathology, or even as a mitotic clock that would limit cell life span and predict one's longevity. Even though there are reasonable explanations behind these theories, there is still missing knowledge in telomere biology that is restraining the implementation of telomeres as biomarkers in clinical practice.

Only recently, scientists agreed that the best model to describe telomere dynamics is not simply measuring TL *per se*, but determining baseline (newborn) TL and telomere attrition over time and how it is affected by the number of cell divisions, oxidative stress and other exposures that may reduce TL.

Baseline TL of newborns significantly differs among individuals, corresponding to the variation of TL found among adults of the same age. Twin studies, however, showed a synchrony among TL in siblings and therefore provided proof of strong genetic determination of telomere biology. Genetic influence on TL was confirmed with the identification of genetic variants that were significantly related to TL in adult cohorts. Surprisingly, GWAS in the child population did not identify the same significant variants, implying that genetic regulation of TL could be age dependent. Genetic loci detected in adult studies probably modify TL over the life span, while different genetic mechanisms affect the TL of newborns.

Baseline TL significantly differs also among foetuses of the same gestational age. During early intrauterine life, cells divide excessively but maintain their TL with the telomerase enzyme. This results in a synchrony of TL among cells of different tissues within the same individual. The telomerase activity is mainly extinguished after early embryonic development, and telomeres of cells with different proliferating activities start to shorten at different rates. Because leucocytes represent highly proliferative tissue with a high rate of turnover compared to minimally proliferative muscle cells, a shorter LTL of a full-term newborn compared to the MTL of the same individual can be detected.

Cumulative risk exposures, especially during early life, together with genetic make-up account for the likelihood of developing a common age-related pathology. During intrauterine development, the foetus is continuously exposed to numerous environmental factors which can transfer through distinct biological pathways and affect baseline TL in a process of so-called foetal programming. Baseline TL reflects the early environmental conditions and represents an important cellular marker for susceptibility to common age-related pathologies. TL attrition can accelerate under conditions that cause increased oxidative stress and chronic inflammation and can determine newborn TL during pregnancy. Some of the unfavourable conditions can be modified: avoiding smoking during pregnancy, maintaining appropriate BMI before and during pregnancy, and ensuring a proper vitamin intake. Therefore, it is important to be aware of the influence of such conditions on the child's health. Maternal stress and gestational diabetes are less easily avoided but can be managed with proper clinical help. Finally, some of the determinants of newborn TL, such as the child's sex, race or genetics cannot be modified, but it is important to understand and take into account their impact on TL, especially in designing the studies exploring new telomere determinants or association of telomeres with pathologies.

Besides baseline TL, telomere attrition over time represents another important element in telomere dynamics. However, telomeric sequence loss is not constant throughout life; the first 4 years of childhood are characterised by a rapid decline in TL due to a large turnover of highly proliferative cells. In this period, the main difference in TL of tissues with different proliferative capacities is established. Later, telomeres in most cells are gradually and synchronically shortened. The variance among cells in different tissues of an individual can be significant, but it remains smaller than the variance in TL among unrelated individuals.

The dynamics of TL between 4 years of age and early adulthood remain to be elucidated. Some discoveries suggest that telomeres during this life period reach a plateau and maintain their length, whereas other results point to a gradual shortening of approximately 50 bp/year

that starts after the age of 4 and remains stable throughout life. Indeed, the biggest problem of the first studies of TL in children and newborns is the small sample size and the lack of longitudinal follow-up. Despite that, these studies made some consistent conclusions which provided the first insights into telomere dynamics from the first phases of gestation until adulthood. TL at birth reflects TL in adulthood: children with short telomeres will most likely grow into adults with short telomeres. Environmental factors in adulthood may affect TL, but this influence is probably smaller than in the dynamics at birth and in the first years of life.

Knowing the importance of TL in childhood, it is crucial to have a good understanding of the dynamics of telomeres in intrauterine life and in the first years of life. This could help to prevent early attrition and thus decrease the risk for later pathophysiological conditions. It is important to keep in mind that the spectrum of diseases related to relative TL in adulthood is vast, and though these pathologies manifest only in adulthood, early developmental environment plays a significant role in their generation.

In view of this, we can conclude that there are still some missing gaps that need further exploration for a general understanding of telomere biology. First, larger genetics studies in the child population are warranted to detect genetic variants that influence baseline TL. This may result in the discovery of novel genetic loci involved in the pathways of telomere biology and help understanding biological regulation of telomere length in childhood. Second, longitudinal studies on telomere dynamics from birth until adulthood are necessary. Follow-up of individuals through long periods would give answers to the missing information of telomere dynamics after the age of 4 and would confirm the effects of early environmental conditions on adult susceptibility to common diseases. Finally, new studies on newborn telomere determinants are required, to confirm previously discovered relations and to find new potential conditions that significantly influence newborn TL.

With precise knowledge of the biology and dynamics of telomeres, we might expect that one day TL will be used as a common biomarker in everyday clinical practice.

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