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Placental telomere length decreases with gestational age and is influenced by parity: A study of third trimester live-born twins

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ABSTRACT

Background: In contrast to the postnatal period, little is known about telomere length (TL) during prenatal life. The decrease in placental TL remains unknown, although intra uterine growth retardation and preeclampsia are associated with shorter placental TL. The aim of this study is to assess the decrease of placental TL during the third trimester of gestation and to explore the role of potential "growth influencing factors".

Methods: The study sample consisted of 329 live-born twins from the East Flanders Prospective Twin Survey. TL was determined using a multiplex quantitative PCR method. Gestational age, sex, birth order, placental characteristics, parity, maternal and paternal age, diabetes, hypertension, smoking, alcohol use, and socio economic status (SES) were considered "growth influencing factors". Bivariable multilevel regression analysis with "growth influencing factors" was performed.

Results: Placental TL ranged from 4.3 kbp to 84.4 kbp with a median of 10.8 kbp. Ln(TL) decreased in a linear fashion with an estimated TL decreasing from 13.98 kbp at 28 weeks to 10.56 kbp at 42 weeks. The regression coefficient of gestational age became smaller if considered together with SES ($b = -0.017$; $p = 0.08$) or diabetes ($b = -0.018$; $p = 0.07$) and bigger if considered together with parity ($b = -0.022$; $p = 0.02$), indicating that part of the association between gestational age and telomere length is explained by these three confounding factors.

Conclusion: Placental TL decreases during the third trimester of gestation of live-born twins with approximately 25% indicating that telomere shortening may play a role in aging of the placenta.

1. Introduction

Telomeres, the nucleoprotein structures at the ends of chromosomes, shorten with each cell division in somatic cells [1], and can be seen as a biological clock. When telomere length reaches a critical value, cells either enter a state of senescence or die. Therefore, telomere length is considered a biomarker of aging, with shorter telomeres indicating an increased biological age [1,2]. A body of evidence has been build up that telomere shortening, as gained during adult life, is a marker of aging and appears to be involved in aging and age-related diseases [3]. However, little information is available on influences of unfavorable circumstances on telomere shortening as early as during the prenatal period in which the placenta plays an important role.

Differentiating normal growth from intra uterine growth retardation (IUGR), in which restricted placental development is a hallmark, is significant, since only IUGR children are at risk of age-related diseases [3]. It has been shown that telomere length is decreased in placental tissue [4–6], but not in cord blood [7] of IUGR children, indicating the importance of the placenta in prenatal growth. In addition, diminished telomerase activity, an enzyme responsible for elongation of telomeres, has been associated with shorter placental telomeres [4,8]. Telomerase activity is shown to be higher in the first and second trimester than in the third trimester of gestation [9,10]. Telomerase activity is also higher in IUGR placentas [4,11]. An increased formation of telomere aggregate was demonstrated to be associated with preeclampsia, a cause of IUGR, as well, independent of telomere length and

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telomerase activity [5,12]. These observations indicate that placental telomere length may decrease during gestation and that preeclampsia enhances this attrition. Besides preeclampsia no other intra uterine growth promoting or restricting factors have been thoroughly investigated in human placentas. However, some studies have focused on telomere length of white blood cells of the neonate instead of placental telomere length.

Between 27 and 32 weeks, the telomere length of white blood cells in cord blood was observed to decline rapidly followed by an insignificant loss thereafter [13]. To the best of our knowledge, only one study has tried to identify associations between telomere length and sex of the neonate, maternal age, and parity. A significant positive association was found with maternal age [14]. Also maternal hypertension or preeclampsia [14] and maternal diabetes, which was investigated by a second study [15], had no effect on telomere length of white blood cells in cord blood of the neonate.

The effects of maternal and fetal determinants on placental telomere length are still unclear, but may be unraveled when studying twin pregnancies. Twins share the same maternal environment; therefore, the influence of maternal factors such as nutrition will be shared by both fetuses. However, each fetus has its own fetoplacental environment, which may differ substantially from that of its co-twin. Twins are also an interesting study population since they are more often growth retarded than singletons. Moreover, they offer the opportunity to explore the role of the placenta in telomere shortening in depth. The East Flanders Prospective Twin Survey (EFPTS) is a population based register of multiple births in the province of East Flanders (Belgium) which examines the placentas at birth and establishes zygosity and choriontype. Previously we have shown that placental weight was influenced by gestational age, choriontype, fusion of the placentas, birth order and parity [16]. The association between placental characteristics and telomere length in twins is yet unexplored.

The aim of this study is to quantify the decrease of placental telomere length during the third trimester of gestation and to explore which "growth influencing factors" also determine placental telomere length during this trimester. We were specifically interested in the following "growth influencing factors": sex of the twin, birth order, placental characteristics including zygosity and choriontype, obstetrical and maternal characteristics such as age, parity, maternal diabetes or hypertension, smoking and alcohol use, and socio economic status (SES).

2. Methods

2.1. Subjects

The East Flanders Prospective Twin Survey (EFPTS), which started in 1964, is a population based register of multiple births in the province of East Flanders (Belgium). The twins (and higher order births) are ascertained at birth [17]. The study sample consisted of 366 twins of Caucasian origin (99% naturally conceived), born between 1969 and 1982, who participated in a prenatal programming study Details of the selection process of the study sample have been described previously [18]. Informed consent was obtained and ethical approval was given by the Ethics Committee of the Faculty of Medicine of the Katholieke Universiteit Leuven.

2.2. Perinatal data

2.2.1. Placental examination and zygosity determination

A trained midwife examined the placentas within 24 h after delivery following a standardized protocol [19]. Fetal membranes were dissected, and after removing the membranes and blood clots, the fresh unfixed placentas were weighed, and their maximum diameter and thickness were measured. According to the site of the cord insertion six categories were distinguished (central, eccentric, paramarginal, marginal, velamentous: dividing or surrounding membrane) and dichotomized into two groups: (1) insertion on the placenta, and (2) insertion on the placental membrane. Blood was taken from the umbilical cord if the blood groups of the twins had not yet been determined. An obstetrician examined placentas with obvious or suspected abnormalities. Placental biopsies of ≈ 0.5 cm^2 at the maternal site near the insertions of the umbilical cord were taken close to the surface, rinsed with tap water and stored at -20 °C at a biobank under consistent conditions. Zygosity was determined by sequential analysis based on sex, choriontype, umbilical cord blood groups, placental alkaline phosphatase, and, since 1982, DNA fingerprints [20]. After DNA-fingerprinting, a zygosity probability of 0.999 is reached. Monochorionic (MC) twins and same-sexed dichorionic (DC) twins with the same markers were classified as monozygotic (MZ) [17]. In MC pairs only one placenta is present. In DC pairs (dizygotic [DZ] as well as MZ DC), the two placentas can be separate, or fused. For this study we distinguished two groups: (1) one placental mass, either one placenta in case of MC twins or two fused placentas in case of DC pairs; and (2) two separate placentas, either MZDC or DZ pairs. The total weight of the placental mass was recorded and if two separate placentas were present, the individual placental weights were also noted.

2.2.2. Data prospectively collected at birth

At birth obstetric and neonatal characteristics were prospectively collected from medical records. The umbilical cords were cut and ligated by birth order. Data recorded by the obstetrician at birth included gestational age, birth weight, sex of the twins, parental ages, and parity. Gestational age (Menstrual age) was based on the last menstruation and was calculated as the number of completed weeks of pregnancy. Preterm birth was defined as born before 37^{+0} weeks.

2.2.3. Data retrospectively collected at adult age

When the twins were at adult age, as part of the prenatal programming study, the parents of the twins filled out questionnaires. Maternal smoking and alcohol consumption during pregnancy for each trimester (yes/no), the occurrence of hypertension during pregnancy (yes (including preeclampsia)/no), gestational diabetes (the occurrence of sugar in the urine; yes/no/unknown), paternal age, parental education were collected retrospectively in this way. Educational level as a proxy of socio economic status (SES) was categorized into three groups according to the Belgian education system [21]: (1) no education or primary school, (2) lower secondary education, and (3) higher secondary education and tertiary education. When the educational level of both parents was available, the highest level was used in the analysis.

2.3. Telomere length assay

DNA was isolated from placental tissue using the QIAamp DNeasy blood and tissue kit (Qiagen, Venlo, The Netherlands), following the instructions of the manufacturer for animal tissues. Following extractions, the absorbance at 260 and 280 nm was measured using a NanoDrop spectrophotometer (Thermo Scientific, Breda NL), and the ratios were calculated. Telomere length was determined using a monochrome multiplex quantitative multiplex PCR (Q-PCR) method (Cawthon, 2009) [22] and carried out in triplicate. In brief, in a total volume of 25 μL approximately 20 ng of genomic DNA was mixed with 10 mM Tris–HCl pH 8.3, 50 mM KCl, 3 mM $MgCl_2$, 0.2 mM each dNTP, 1 mM DTT, 1M betaine, 0.75× SYBR Green I, and AmpliTaq Gold DNA polymerase, 0.625 U. Four primers were used (5′–3′): telg (at 900 nM; ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT), telc (at 900 nM;

TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA), hbgu (at 500 nM; CGGCGGCGGGCGGCGCGGGCTGGGCGGcttcatccacgttcaccttg), and hbgd (at 500 nM; GCCCGGCCCGCCGCGCCCG TCCCGCCGgaggagaagtctgccgtt). Four three-fold serial dilutions of a reference genomic DNA sample (HeLa S229 cell line) were used to generate two standard curves for each PCR plate. Reference samples with known telomere length, i.e. 5.5 kbp (Hela S3 cell line) and 14.5 kbp (Hela 229 cell line), were included into each run to enable estimation of TL in kbp. Reference samples were included into each PCR run, and the coefficient of variation was calculated to be 2.5% for within plate measurements and 4.9% for measurements between plates.

Quality and concentration of the isolated placental DNA was assessed using the Nanodrop 1000 spectrophotometer (Isogen Life Science, Belgium). DNA was of sufficient quality of 336 twins (209 pairs). Due to a low DNA yield or to absorption ratios for A260/A280 that were outside the range of 1.8–2.0, 19 twin pairs were excluded from the analyses. In addition, due to PCR values below the detection limit, or due to irreproducible triplicate values for PCR measurements 11 twin pairs were excluded.

2.4. Statistical analyses

Telomere length (kbp) was log-transformed to assure normality and the twins were analyzed as individuals in a multilevel regression analysis to account for relatedness between twin members by adding a random intercept to the model. The variance-covariance structure was allowed to differ between the three zygosity-choriontype groups. First, to assess whether telomere length decreases during gestation, univariable multilevel regression analysis was conducted. Deviations from linearity were tested by polynomial regression. Second, univariable multilevel regression analyses with the following "growth influencing factors": sex of the twin, birth order, zygosity and choriontype, fusion of placentas, site of the insertion of umbilical cord, placental weight, maternal and paternal age, parity, maternal diabetes, maternal hypertension, smoking per trimester, alcohol use per trimester, and SES were performed. To control for the fact that the differences in duration of storage of the placental biopsies might influence TL, we also analyzed the influence of storage time. Finally, in bivariable multilevel regression analyses we tested whether the statistically significant "growth influencing factors" from the univariable regression analysis acted as confounding factors or as effect modifiers in the association between gestational age and telomere length. A "confounding" effect (a change of >10% of regression coefficient of gestational age) was tested by adding a "growth influencing factor" to gestational in the model; an "effect modifier" by putting also the interaction gestational and a "growth influencing factor" in the model. For all analyses listwise deletion was utilized for missing values.

Next, we planned to study the twins as pairs to distinguish between genetic and feto-placental factors by analyzing intra-pair differences of MZ and DZ twins.

The analyses were conducted with the SAS version 9.2 software package (SAS Institute Inc., Cary, NC, USA). All reported p-values are two-sided and were considered statistically significant when $p \leq 0.05$. A trend was considered when $p \leq 0.10$.

3. Results

3.1. Descriptive analysis

The characteristics of the 329 third trimester live-born twins are depicted in Table 1. Telomere length was highly variable and ranged from 4.3 kbp to 84.4 kbp (Fig. 1) with a median of 10.8 (Inter Quartile Range 9.1–13.9) kbp. Gestational age ranged from 28 to 42 weeks with a mean gestational age of 37.2 weeks (SD 2.37).

Table 1
Characteristics of the third trimester live-born twins ($n = 329$).

	Mean (SD)
TL kbp	12.70 (7.05)/median 10.82 (IQR 9.17–13.97)
Gestational age (weeks)	37.2 (2.4)
Placental weight (grams) (13 missing)	381 (76)
Placental weight (grams) not fused ($n = 87$)	409 (88)
Maternal age (12 missing)	27.2 (4.4)
Paternal age (69 missing)	29.0 (5.2)
	***n* (%)**
Collected at birth	
Preterm (<37 weeks)/Term	117 (36%)/212 (64%)
Male/Female	154 (46%)/175 (54%)
First born/second born	150 (46%)/179 (54%)
Zygosity choriontype: DZ/MZDC/MZMC	120 (36.5%)/99 (30%)/110 (33.5%)
Primiparity/Multiparity (4 missing)	151 (46%)/174 (54%)
Placental characteristics	
One placental mass/two separate placentas (13 missing)	229 (72%)/87 (28%)
Umbilical cord on fetal membranes no/yes (4 missing)	292 (90%)/33 (10%)
Placental weight below 10th centile/normal All (13 missing)	25 (8%)/291 (92%)
Not fused ($n = 87$)	6 (7%)/81 (93%)
Collected at adult age	
Diabetes yes/no (and unknown) (14 missing)	10 (3%)/305 (97%)
Hypertension incl. (pre)eclampsia yes/no (15 missing)	42 (14%)/272 (86%)
Smoking yes/no (14 missing) first trimester	41 (13%)/274 (87%)
Second trimester	37 (12%)/278 (88%)
Third trimester	35 (11%)/280 (89%)
Alcohol yes/no (2 missing) first trimester	31 (9%)/296 (91%)
Second trimester	29 (9%)/298 (91%)
Third trimester	26 (8%)/301 (92%)
Socio Economic Status low – middle – high (21 missing)	22 (7%)/44 (14%)/242 (79%)

3.2. Shape of the telomere length attrition

The model fit of the univariable multilevel regression analysis and the polynomial analysis revealed to be the same (AIC 282.5 vs. 283.8) indicating a linear decrease of Ln(TL) with gestational age (Table 2). TL decreased from an estimated 13.98 kbp at 28 weeks of gestation to 11.67 kbp at 37 weeks and 10.56 kbp at 42 weeks of gestation ($beta_{uni} = -0.020$; $p = 0.03$) (Table 2; Fig. 2), which is a total decrease of 24.5%.

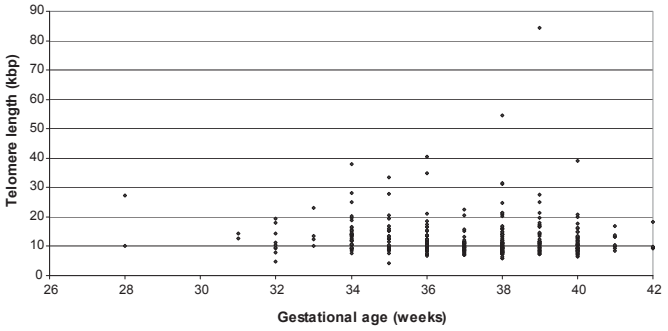

Fig. 1. Placental telomere length of live-born twins during the third trimester: raw data.

Table 2
Univariable and polynomial multilevel regression analyses with gestational age: regression coefficients, model fit and estimated telomere length.

Ln (TL)	Beta	SE	P	AIC	Gestation	Estimated TL (95% C.I.)
Model 1						
Gestational age	−0.0201	0.0092	0.03	282.5	28 weeks	13.98 (11.76–16.63)
					32 weeks	12.90 (11.63–14.32)
					37 weeks	11.67 (11.17–12.19)
					42 weeks	10.56 (9.58–11.64)
Model 2						
Gestational age	−0.1825	0.1890	0.34	283.8	28 weeks	16.12 (11.10–23.41)
Gestational age* gestational age	0.0022	0.0026	0.40		32 weeks	13.28 (11.73–15.02)
					37 weeks	11.52 (10.92–12.15)
					42 weeks	11.17 (9.49–13.14)

TL = Telomere length.

3.3. Growth influencing factors

Of the considered "growth influencing factors" parity, only maternal diabetes, and SES were associated with placental TL ($beta_{uni\ primiparity} = -0.098$ ($p = 0.03$); $beta_{uni\ diabetes} = 0.309$ ($p = 0.02$); $beta_{uni\ SES\ low} = 0.231$ ($p = 0.01$), $beta_{uni\ SES\ middle} = 0.094$ ($p = 0.15$), overall $p_{SES} = 0.02$) (Table 3). Placental telomeres of primiparous mothers were 1.14 kbp shorter than those of multiparous mothers (11.07 vs 12.21 kbp); placental telomere length of diabetic mothers was 4.15 kbp longer than telomere length of non-diabetic mothers (15.63 vs 11.47 kbp); placental telomere length of mothers with lowest SES was 1.82 kbp longer than telomere length of middle SES who in turn were 1.12 kbp longer than those of high SES (14.26 vs. 12.44 vs. 11.33 kbp).

Bivariable multilevel regression analyses revealed that parity, diabetes and SES acted as confounders (Table 4). The regression coefficient of gestational age became smaller if considered together with SES ($b = -0.017$; $p = 0.08$) or diabetes ($b = -0.018$; $p = 0.07$) and bigger if considered together with parity ($b = -0.022$; $p = 0.02$), indicating that part of the association between gestational age and telomere length is explained by these three confounding factors. Placental TL of multiparous mothers decreased with an estimated 4.06 kbp from 28 to 42 weeks of gestation (a decrease of 27%). For primiparous mothers the decrease was 3.35 kbp (25%). The difference in TL between multipara and primipara at 28 weeks was 1.52 kbp and at 42 weeks 1.21 kbp. For the other two confounders, decrease in telomere length ranged from 21% (SES) to 22% (diabetes). Parity, maternal diabetes and SES were no effect modifiers. Unfortunately, pairwise analyses could not be conducted, because the only statistically significant "growth influencing factors" showed, because of their nature, no intra-pair differences.

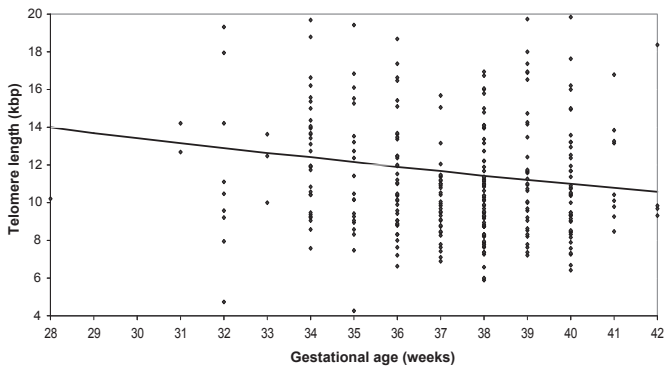

Fig. 2. Placental telomere length of live-born twins during the third trimester: raw data and estimated length based on univariable multilevel regression analysis.

Table 3
Univariable multilevel regression analyses: regression coefficients of potential "growth influencing factors".

Ln(TL)	Univariable analysis		
	Beta	SE	*p*
Placental characteristics			
Choriontype			0.45
MZMC	ref		
MZDC	−0.0628	0.0531	0.23
DZ	−0.0116	0.0547	0.83
Fusion of placentas: separate placentas	−0.0386	0.0505	0.45
Insertion of umbilical cord on placenta	0.0449	0.0654	0.49
Placental weight (continuous) gram*	0.00004	0.0003	0.91
Male	−0.0004	0.0434	0.99
First born	0.0237	0.0350	0.50
Maternal/paternal characteristics			
Paternal age years	−0.0038	0.0050	0.45
Maternal age years	0.0057	0.0054	0.29
Primiparity	−0.0983	0.0442	**0.03**
Diabetes	0.3090	0.1312	**0.02**
Hypertension incl (pre)eclampsia	0.0115	0.0693	0.87
Smoking in first trimester	0.0111	0.0684	0.87
Second trimester	0.0311	0.0712	0.66
Third trimester	0.0316	0.0725	0.66
Alcohol use in first trimester	0.0024	0.0716	0.97
Second trimester	−0.0243	0.0750	0.75
Third trimester	−0.0748	0.0773	0.34
Socio Economic Status			**0.02**
Low	0.2305	0.0908	0.01
Middle	0.0940	0.0646	0.15
High	Ref		
Storage time	−0.0099	0.0079	0.20

Table 4
Regression coefficients and estimated telomere length for bivariable multilevel regression analyses.

Ln (TL)	Bivariable analysis		*p*	Gestation	Estimated TL	
	Beta	SE				
					Multiparity	*Primiparity*
Gestational age weeks	−0.0224	0.0092	**0.02**	28 weeks	15.07	13.55
Primiparity	−0.1063	0.0439	**0.02**	32 weeks	13.77	12.39
				37 weeks	12.32	11.07
				42 weeks	11.01	9.90
					Diabetes	*No Diabetes*
Gestational age weeks	−0.0175	0.0095	0.07	28 weeks	17.79	13.50
Diabetes	0.2756	0.1315	**0.04**	32 weeks	16.58	12.59
				37 weeks	15.19	11.53
				42 weeks	13.92	10.56
					Low SES	*High SES*
Gestational age weeks	−0.0170	0.0096	0.08	28 weeks	16.54	13.27
Socio Economic Status			**0.04**	32 weeks	15.46	12.40
Low	0.2203	0.0905	0.02	37 weeks	14.20	11.39
Middle	0.0809	0.0646	0.21	42 weeks	13.04	10.47
High	Ref					

4. Discussion

We are the first to show that placental telomere length significantly decreases during the third trimester of gestation in a relatively large sample of live-born twins. Telomere shortening seems to be ongoing in a linear fashion until delivery and multiparity appears to delay placental telomere shortening. Of the other "growth influencing factors" only maternal diabetes and low SES were associated with longer placental telomere length.

Placental telomeres shorten approximately 25% during the third trimester from an estimated TL of 13.98 at 28 weeks to an estimated TL of 10.56 kbp at 42 weeks. Given that synchronization in telomere length exists among multiple organs, including the placenta of the human fetus [23], the observed attrition is very strong compared with the leukocyte shortening of telomeres in the first year of life (0.70 kbp [24]) and even stronger compared to attrition in adults (attrition rate of 0.024 kb/year [23] or 0.031 [24]). Absolute placental telomere length shortens in an almost linear fashion up to 42 weeks without slowing, suggesting a continuous and ongoing cell division. The placental proliferative capacity remains even beyond term, which is between 36 and 37 weeks for twins [13,25].

Placental telomere length of multiparous mothers was longer than that of primiparous mothers. To the best of our knowledge, only one study has been published on changes in relation to parity. It has been shown by Khong et al. that spiral arteries play a vital role in supplying nutrients to the placenta and fetus and are remodeled into dilated vessels during pregnancy. Duplication and fragmentation of the internal elastic lamina and the proportion of non-muscular tissue increases with increasing parity [26]. These permanent anatomical changes may enhance the remodeling of spiral arteries into dilated vessels and the nutrient supply to placenta and fetus [26]. This could explain why placentas of multiparous mothers are heavier [16] and why telomeres of multiparous mother are longer. The fact that attrition is approximately the same for primipara and multipara in the third trimester additionally supports this idea [27].

We were able to show that maternal diabetes was associated with longer placental telomere length. This result should be interpreted cautiously, since only 10 mothers reported diabetes in a retrospective way. We were unable to confirm that preeclampsia [14,15] enhances placental telomere attrition. One explanation could be that in this study no distinction could be made between mild hypertension and preeclampsia. In diabetes as well as in preeclampsia oxidative stress is considered to play a role [28,29]. Due to the relatively high guanine content in telomere sequences and a deficient repair of single-strand breaks in telomeric DNA, telomeres are sensitive to oxidative stress [30]. Telomere attrition normally can be counteracted by telomerase activity [31] which has been detected in placenta [32]. Therefore telomere length results from the balance between the processes that shorten telomeres and telomerase-dependent elongation of telomeres. In addition, cells with critically short telomeres may lose their ability to divide and become senescent or apoptotic, and the pool of cells with longer telomeres may be able to expand and contribute to an increase in average telomere length, which has also been described [33]. Since oxidative stress and inflammation are both associated with telomere lengthening and shortening, the time-dependent relations between maternal factors such as onset of diabetes or preeclampsia on placental telomere length remain unknown and should be further investigated.

A striking result, which we can not explain, was that low SES was associated with longer telomeres. Also after adjusting for gestational age, placental telomeres of mothers with lowest SES were longer and maternal age did not influence the results. The general concept is that low SES is associated with shorter telomeres. A recently published meta-analysis showed a small association between SES, based on education, and leucocyte TL in adults. A standardized mean difference of 0.06 (95% C.I. 0.002–0.118) between low and high SES was reported in favor of high SES [34]. No difference in TL was found in children [34]. Other studies showed that parental education was associated with child TL, in favor of higher education [35,36].

Surprisingly, none of the placental characteristics (including choriontype) were associated with telomere length. One would expect monochorionic twins to have the shortest telomeres, but we were unable to confirm this hypothesis. Also, fusion of placentas and a subsequent peripheral insertion of the umbilical cord can be seen as asymptomatic growth [33,37,38]. Delivery of nutrients to the placenta is disturbed if the placentas are fused, which (in theory) could influence telomere length [29]. Also, older paternal age was not associated with longer telomere length, although other studies mention elongation [39,40]. Possible explanations could be the fact that the fathers in this study were relatively young (range: 21–44 years of age) or that we measured placental telomere length.

For the present study cross sectional data of third trimester live-born twins were analyzed. It is possible that the lack of associations is caused by the fact that telomere attrition is higher earlier in gestation than in late gestation. The use of cross-sectional data could bias the results since premature birth itself is probably related to an unphysiological state in either mother or fetus [41,42]. We can not exclude that unborn placentas can have longer telomeres than placentas of neonates of the same age. As stated by Holmes et al. it could be argued that, analog to telomere length in white blood cells, in the placentas of preterm born children telomeres can have a more accentuated telomere loss than in unborn placentas of the same age [43]. However, the leading cause of preterm birth is not a pathological condition but twinning itself [44]. [45] [46]. Therefore, extrapolation of the results to singletons should be done with caution. Nevertheless, until now no large studies are available of longitudinal data of placental telomere length during pregnancies. A weakness of this study is that no telomerase activity was measured, as a decreased telomerase activity in fetal growth retarded placentas is reported [4]. Also other mechanisms are possible to elongate telomeres. Some studies found indication of alternative lengthening of telomeres as well (a recombination mechanism that is also used by some tumor cells and that leads to highly variable telomere lengths) [47].

In conclusion, placental telomere length decreases with 25% during the third trimester of gestation of live-born twins indicating that telomere shortening may play a role in aging of the placenta. The differences in utero in multiparous mothers may also positively influence telomere length and take place early in gestation. (Unknown) factors associated with the development of maternal diabetes and low socio economic status may be of importance as well.

Conflict of interest

Herewith I confirm that none of the authors has a conflict of interest in relation to this work.

There are no financial relationships that might bias this work.

There is no financial interest and no direct payment to an author.

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