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Lower placental telomere length may be attributed to maternal residential traffic exposure; a twin study

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ABSTRACT

Background: High variation in telomere length between individuals is already present before birth and is as wide among newborns as in adults. Environmental exposures likely have an impact on this observation, but remain largely unidentified. We hypothesize that placental telomere length in twins is associated with residential traffic exposure, an important environmental source of free radicals that might accelerate aging. Next, we intend to unravel the nature-nurture contribution to placental telomere length by estimating the heritability of placental telomere length.

Methods: We measured the telomere length in placental tissues of 211 twins in the East Flanders Prospective Twin Survey. Maternal traffic exposure was determined using a geographic information system. Additionally, we estimated the relative importance of genetic and environmental sources of variance.

Results: In this twin study, a variation in telomere length in the placental tissue was mainly determined by the common environment. Maternal residential proximity to a major road was associated with placental telomere length: a doubling in the distance to the nearest major road was associated with a 5.32% (95% CI: 1.90 to 8.86%; $p = 0.003$) longer placental telomere length at birth. In addition, an interquartile increase (22%) in maternal residential surrounding greenness (5 km buffer) was associated with an increase of 3.62% (95% CI: 0.20 to 7.15%; $p = 0.04$) in placental telomere length.

Conclusions: In conclusion, we showed that maternal residential proximity to traffic and lower residential surrounding greenness is associated with shorter placental telomere length at birth. This may explain a significant proportion of air pollution-related adverse health outcomes starting from early life, since shortened telomeres accelerate the progression of many diseases.

1. Introduction

Telomeres consist of TTAGGG tandem repeats and cap chromosomes (Blackburn, 2001). They undergo progressive attrition in somatic cells because DNA polymerase is unable to fully replicate the ends of DNA caused by the unidirectional growth and the requirement for a primer to initiate synthesis (Levy et al., 1992). This is referred to as the end-replication problem. As a result telomeres progressively shorten in somatic cells and a mean leukocyte telomere length has been observed to diminish with age (Benetos et al., 2001; Slagboom et al., 1994). As aging starts before birth, not only establishing the telomere length at birth is a prerequisite, but also investigating environmental and genetic factors influencing telomere length is needed. The placenta plays a pivotal role in fetal development and functions as a barrier between fetal and maternal circulation. In utero telomere attrition is prevented by telomerase activity but as pregnancy progresses its activity in placental tissue declines making telomeres more sensitive to degradation (Chen et al., 2002; Gielen et al.; Kyo et al., 1997). Maternal stress (Class et al., 2011; Lee et al., 2011; Torche, 2011), under nutrition (Schulz, 2010), exposure to cigarette smoke (Ko et al., 2014; Wahabi et al., 2013) and air pollution (Ballester et al., 2010; Brauer et al., 2008; Dadvand et al., 2013; Liu et al., 2003; Pedersen et al., 2013) have been linked to fetal growth retardation, with compromised fetal cerebral development, and might be linked with early onset of insulin resistance (Entringer et al., 2012). It has been suggested that telomere length underlies this fetal programming (Entringer et al., 2012). For instance, exposure to maternal psychosocial stress during intrauterine life has been associated with shorter leukocyte telomere length in young adulthood (Entringer et al., 2011). In adults telomere

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length was observed to be strongly related to the telomere length at birth, which widely varied and showed synchrony with telomere length of different organs (Heidinger et al., 2012; Okuda et al., 2002; Youngren et al., 1998).

Ambient air pollution is considered as a global public health threat (Adar et al., 2013; Brunekreef and Holgate, 2002; Katsouyanni et al., 1997; Nawrot et al., 2011). Recent studies of maternal exposure to particulate matter (PM), a constituent of ambient air pollution, support evidence for detrimental effects of PM exposure on the health of fetuses (Ballester et al., 2010; Dadvand et al., 2013) and neonates (Ritz et al., 2006; Scheers et al., 2011), and has been associated with cardiovascular morbidity and mortality later in life (Brook et al., 2010; Grahame and Schlesinger, 2012; Nawrot et al., 2011). Oxidative stress and inflammation are mechanisms linking exposure to particulate air pollution with premature aging (Reliene et al., 2005; von Zglinicki, 2000). Accelerated shortening of telomeres is an important pathway by which oxidative stress may accelerate biological aging and age-related diseases (Haycock et al., 2014; Monickaraj et al., 2012). In adults shorter telomere length has been linked with long-term exposure to traffic exposure. Hoxha et al. (2009) reported lower leukocyte telomere length in traffic officers compared to office workers and an inverse association between telomere length and occupational exposures to benzene and toluene (Hoxha et al., 2009). The Veteran study reported faster telomere attrition in 70-year olds with long-term exposures to airborne particles, especially those related to traffic exposure (McCracken et al., 2010). The effect size for an interquartile range (IQR) increase in black carbon was equivalent to a 3.04 year increase in age on telomere attrition. However, the effect of prenatal traffic exposure on the telomere length in the placenta is unknown. Although there is a synchrony in length among tissues of the human fetus, significant variations in telomere length among fetuses have been observed (Youngren et al., 1998). This variation was also found at birth and the variation among newborns appears to be as wide as the variation in adults (Okuda et al., 2002). This variation may be the result of both genetic and environmental factors. Indeed, based on twin studies (Andrew et al., 2006; Bischoff et al., 2005; Slagboom et al., 1994) and familial studies (Nawrot et al., 2004), telomere length has been shown to have genetic determinants. A recent meta-analysis combining estimates of six independent studies reported a heritability coefficient for leukocyte telomere length of 0.70 in adults (95% CI 0.64–0.76) (Broer et al., 2013). However, until now it is largely unknown to what extent genetic versus environmental factors exert their effect in utero and attribute to the variation in telomere length at birth.

As telomere attrition already starts before birth, we examined the association of placental telomere length in twins and residential traffic exposure, an important environmental source of free radicals that might accelerate aging (Iwai et al., 2000). Next, we intend to unravel the nature-nurture contribution to placental telomere length by estimating the heritability of placental telomere length.

2. Materials and methods

2.1. Subjects

The East Flanders Prospective Twin Survey (EFPTS) was initiated in 1964 and is a population based register of multiple births in the province of East Flanders (Belgium). We selected 231 twins of Caucasian origin (99% naturally conceived), born between 1975 and 1982, who participated in a prenatal programming study (Loos et al., 2001). We excluded twins with bad or doubtful DNA quality (n = 13), or no information on maternal residential address (n = 7).

2.2. Zygosity determination and tissue sampling

A trained midwife examined the placentas within 24 h after delivery following a standardized protocol (Derom et al., 1995). Fetal membranes were dissected, and after removing the membranes and blood clots, the fresh unfixed placentas were weighed, and their length and thickness were measured. A blood sample 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 were taken near the insertions of the umbilical cord and stored at −20 °C at a biobank.

Zygosity was determined by sequential analysis based on sex, chorion type, umbilical cord blood groups, placental alkaline phosphatase, and, since 1982, DNA fingerprints (Vlietinck, 1986). After DNA-fingerprinting, a zygosity probability of 0.999 was reached.

2.3. Data collection

Data recorded by the obstetrician at birth included gestational age, birth weight, sex of the twins and parental ages. Gestational age was based on the last menstruation and was calculated as the number of completed weeks of pregnancy.

When the twins were at adult age, the parents of the twins filled out questionnaires. Maternal smoking during pregnancy and 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; no education or primary school, lower secondary education, and higher secondary education and tertiary education. In addition to individual SES data, we gathered information on neighborhood SES. All mothers were assigned to statistical sectors (average area = 1.55 km^2), the smallest administrative entity for which statistical data are produced by the Belgian National Institute of Statistics, based on their home address. Belgian census data (FOD Economie/DG Statistiek) derived from the NIS were used to define neighborhood SES based on annual household income (1994).

2.4. 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. Telomere length was determined using a monochrome multiplex quantitative PCR (Q-PCR) method (Cawthon, 2009). For multiplex QPCR, the telomere primer pair telg and telc (final concentrations of 900 mM) were combined with the beta-globin primer pair hbgu and hbgd (final concentrations of 500 mM each). Reference genomic DNA (Hela 229 cell line) was used to generate two standard curves for each PCR plate, one for the telomere signal and one for the single copy gene signal. Reference samples with known telomere length, i.e. 5.5 kB (Hela S3 cell line) and 14.5 kB (Hela 229 cell line), were included into each run to enable estimation of telomere length in kB. Samples were assayed in triplicate and the average was used. Based on the reference samples 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 were assessed using the Nanodrop 1000 spectrophotometer (Isogen Life Science, Belgium). 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.

2.5. Traffic related exposure and land use data

Residential addresses of the mothers at birth were geocoded. Distances to the nearest major road with traffic counts available and traffic density were determined using the geographic information system (GIS) functions. All GIS analyses were conducted in ArcGIS 9.3. We collected information on two indicators of traffic at the residence: distance to major road, and traffic density. Traffic density in a buffer was equal to the length of each road in a buffer multiplied with the