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Original Article

Telomere length analysis in Down syndrome birth

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ABSTRACT

Human reproductive fitness depends upon telomere chemistry. Maternal age, meiotic nondisjunction error and telomere length of mother of trisomic child are someway associated. Reports exhibiting maternal inheritance of telomere length in Down syndrome child are very scanty. To investigate this, we collected peripheral blood from 170 mothers of Down syndrome child and 186 age matched mothers of euploid child with their newly born babies. Telomere length was measured by restriction digestion – southern blotting technique. Meiotic nondisjunction error was detected by STR genotyping. Subjects are classified by age (old >35 years and young <35 years) and by meiotic error (MI and MII). Linear regression was run to explore the age – telomere length relationship in each maternal groups. The study reveals that with age, telomere erodes in length. Old MII mothers carry the shortest ($p < 0.001$), control mothers have the longest telomere and MI lies in between. Babies from older mother have longer telomere ($p < 0.001$) moreover; telomeres are longer in Down syndrome babies than control babies ($p < 0.001$). To conclude, this study represents not only the relation between maternal aging and telomere length but also explore the maternal heritability of telomere length in families with Down syndrome child.

1. Introduction

The role of maternal aging on Down syndrome (DS) birth is a fascinating unresolved mystery in the field of reproductive biology. Down syndrome is the most prevalent form of live born aneuploidy in human accounting for 1 in 700 live births (Mueller and Young 1995). Overwhelming proportion (~95% cases) of nondisjunction (NDJ) i.e., erroneous separation of chromosome 21 (Ch21) occurs in oogenesis (Antonarakis, 1991; Zhang et al., 2008). Correlation between advance maternal age and higher incidence of trisomy 21 conception was initially reported by Penrose (1933) and later confirmed in other studies (Smith and Record, 1955; Chu et al.2016). Early ovarian aging in women is a caveat for DS birth (Allen et al., 2009). Researches suggest that there are certain risk factors that underlay the ovarian aging and these are genetic, micro environmental and pathological in origin (Younis, 2011). Several genes that are involved in subcellular mechanisms like mitochondrial function, chromosome stability, oogenesis and fertilization have been reported to express differentially in old murine oocyte than younger one (Hamatani et al., 2004). Moreover, increasing maternal age shows correlation with the higher incidence of cytogenetic abnormalities in human oocyte (Pellestor et al., 2005, 2006). Aging accumulates insults in vital cellular processes like sister chromatid adhesion, maintenance of meiotic check points and spindle formation etc and causes NDJ in meiosis I or in meiosis II (Yoon et al., 1996). Age related degradation of cohesin, that holds the sister chromatid together, causes NDJ which in turn leads to erroneous chromosome number in gametes (Angell, 1991). Mutation in mitochondrial DNA accumulates in ovarian cells with aging (Seifer et al., 2002). Antioxidant depletion, activation of apoptotic pathways (Perez and Tilly, 1997; Perez et al., 2005; Tatone et al., 2006) and erroneous spindle formation (Eichenlaub-Ritter et al., 2004) are triggered by damaged mitochondria in aged oocytes. Increased intra-follicular oxidative stress has been recognised as a culprit for oocyte degeneration with age (Tamura et al., 2008; Tatone et al., 2008). Moreover, the defence system against ROS, like estrogen hormone (Lass et al., 1997), glutathione antioxidants (Van Blerkom, 1996) etc gradually decreases with age. Besides that, age related

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accumulation of advanced glycation end products (AGEs) impairs the perifollicular vascularisation and perturb the supply of oxygen and paracrine regulators to ovarian follicles (Li et al., 2012).

The end of the eukaryotic chromosome is capped with telomere, a neucleotide repeat sequence TTAGGG, guarding the ends from fusion with neighbouring chromosomes (Allshire et al., 1989). Telomere erodes with each cell division and replenished by a special reverse transcriptase called telomerase (Shampay and Blackburn, 1988). Telomere is considered as a potent molecular marker for aging as its length degrades progressively with age (Aviv, 2008). Decreased TL is positively correlated with shorter reproductive lifespan due to ovarian aging (Aydos et al., 2005). Several conjectures have been put forward the implication of telomere biology in reproduction.According to telomere theory of reproductive senescence, exposure to reactive oxygen species (ROS) for a long period accelerates telomere erosion in aged women (Keefe et al., 2006). Activity of enzyme telomerase is reversely correlated with ovarian aging (Kinugawa et al., 2000).

Precocious aging is also common in Down syndrome patients. However, the regulation of telomere dynamics is unsolved till now. Gruszecka et al. (2015) showed that juvenile DS patients have longer telomeres than age matched control. Whereas, Wenger et al. (2014) found shorter telomere in DS babies. Longitudinal study with adult DS patients revealed that telomere shortening can be a potent biomarker for their dementia status (Jenkins et al., 2006; Jenkins et al., 2012). Again, Nakamura et al. (2014) cannot differentiate the telomere length between trisomy 21 and normal diploid individuals.

With an objective to solve this enigma, our present study includes mothers stratified according to age and stage of meiotic errors (MI or MII) and their newly born DS child as well as age matched controls for telomere length analysis. Our previous study (Ghosh et al., 2010a, 2010b) revealed that older mothers of DS child are "genetically older" than control. However, the present cross-sectional case-control study gives us the idea about the complex relationship in between maternal and offspring TL exploring the role of telomere dynamics in DS birth.

2. Materials and methods

2.1. Subjects

A total of 170 mothers having DS child and 186 age matched control women witheuploid child were recruited randomly from number of medical colleges and hospitals around the vicinity of Kolkata, India. To get rid of the telomere erosion bias in samples we collected peripheral blood from case and control mother-offspring pairs within a week of delivery. Only eight families with DS child were found with paternal inheritance of 21st chromosome and were excluded from this study. The enlisted families are chiefly Bengali speaking and socio-economically and religiously discrete. Sample collection is strictly done after taking detail family history and informed consent. The women were categorised in two broad groups viz. young (<35 years) and old (□35 years).The study design was approved by institutional ethical committee and follows the principles mentioned in the Declaration of Helsinki.

2.2. Cytogenetic analysis

Conventional karyotyping was done for each sample to confirm the chromosomal profile. At least 30 metaphase plates were examined. Mosaic cases were excluded from and only free trisomy 21 samples are included as cases.

2.3. Genotyping

The inheritance of the supernumerary Ch21 and the stage of meiotic NDJ (MI or MII) were determined by short tandem repeat (STR) genotyping using polymerase chain reaction (PCR). Ten highly polymorphic STR markers orderly spanned from centromere to telomere of Ch21 were used to define the inheritance; they are centromere–D21S1432–D21S11–D21S1437–D21S1270–D21S167 –D21S1412–D21S2055–D21S1260–D21S1411–D21S1446–qter. Cases for which maternal NDJ of Ch21 was determined unambiguously at least for two STR markers were considered for further analyses. Additional four pericentric STR markers viz. D21S369, D21S215, D21S258 and D21S120 were used to infer the type of meiotic errors (MI or MII). If maternal heterozygosity of a marker is retained in DS offspring the case was recognised as "nonreduced" and inferred as MI error, whereas, reduction of maternal heterozygosity into homozygosity in DS child is called "reduced" and considered as MII error.

2.4. Telomere length (TL) determination

We used *TeloTAGGG*Telomere length Assay [Cat. No.2 209 136 001] from Roche chemicals to detect TL of the DNA samples isolated from peripheral blood leucocytes of mother-child pairs. Two restriction enzymes HinfI and RsaI (20 U/μl each) were used to digest 10 μg of DNA sample following manufacturer's instruction. The digested DNA was then resolved in 0.8%agarose gel. The gel was denatured and neutralized subsequently. Southern blotting technique was employed for transferring the DNA fragments into nylon membrane. The transferred DNA fragments were then fixed onto membrane by UV irradiation. Hybridization of the membrane was done with the labelled telomere specific probe provided in the kit. The membrane was then exposed to X-Ray film to obtain an autoradiogram. Free online software "image" from NIH, USA was used for recording and calculating the signal intensity or density of the TL DNA smears in the autoradiogram. The signal was optimized and subdivided into 1 kbp intervals. The telomere restriction fragment or TRF length (L) was estimated using the formula $L = \sum(\mathrm{OD}i)/\sum(\mathrm{OD}i)/(Li)$, where OD*i* was the signal intensity and *Li* was the length of the TRF fragment at midpoint of position *i*. More intense signal reflects higher TRF length (TL) accounting for multiple binding of telomere specific probe.

2.5. Statistical analysis

For all analyses women (both cases and controls) were stratified into two broad classes, young (<35 years) and old (>35 years). Further, each case group were sub divided into MI or MII group according to meiotic error.Children from control and case mothers were also classified accordingly. The estimate of TL shortening over the maternal age of conception was done by regression analysis in 'Rattle', a free and open source software package based on "R" programming language.TLwas used as response or dependent variable whereas; age was the explanatory or independent variable. The slope of the regression line was indicative of the telomere erosion rate in terms of kbp/year. We used *t*-test to compare mean TL among different groups. Moreover, to analyse the age-TL relationship among different categories, we measured the correlation between age and TL of mothers, age and TL of babies and finally mother's age and baby's TL.