Mutation Research 787 (2016) 1–6

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Increased *MTHFR* promoter methylation in mothers of Down syndrome individuals

Fabio Coppedè[a,b,*], Maria Denaro[a], Pierpaola Tannorella[a], Lucia Migliore[a,b]

[a] Department of Translational Research and New Technologies in Medicine and Surgery, Section of Medical Genetics, University of Pisa, Via Roma 55, 56126 Pisa, Italy
[b] Interdepartmental Research Center Nutrafood "Nutraceuticals and Food for Health", University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form 29 January 2016
Accepted 18 February 2016
Available online 22 February 2016

Keywords:
Methylenetetrahydrofolate reductase
MTHFR
Down syndrome
Mothers
Epigenetics
Folate
Methylation
Micronuclei

ABSTRACT

Despite that advanced maternal age at conception represents the major risk factor for the birth of a child with Down syndrome (DS), most of DS babies are born from women aging less than 35 years. Studies performed in peripheral lymphocytes of those women revealed several markers of global genome instability, including an increased frequency of micronuclei, shorter telomeres and impaired global DNA methylation. Furthermore, young mothers of DS individuals (MDS) are at increased risk to develop dementia later in life, suggesting that they might be "biologically older" than mothers of euploid babies of similar age.

Mutations in folate pathway genes, and particularly in the methylenetetrahydrofolate reductase (*MTHFR*) one, have been often associated with maternal risk for a DS birth as well as with risk of dementia in the elderly. Recent studies pointed out that also changes in *MTHFR* methylation levels can contribute to human disease, but nothing is known about *MTHFR* methylation in MDS tissues.

We investigated *MTHFR* promoter methylation in DNA extracted from perypheral lymphocytes of 40 MDS and 44 matched control women that coinceived their children before 35 years of age, observing a significantly increased *MTHFR* promoter methylation in the first group ($33.3 \pm 8.1\%$ vs. $28.3 \pm 5.8\%$; $p = 0.001$). In addition, the frequency of micronucleated lymphocytes was available from the women included in the study, was higher in MDS than control mothers ($16.1 \pm 8.6‰$ vs. $10.5 \pm 4.3‰$; $p = 0.0004$), and correlated with *MTHFR* promoter methylation levels ($r = 0.33$; $p = 0.006$).

Present data suggest that *MTHFR* epimutations are likely to contribute to the increased genomic instability observed in cells from MDS, and could play a role in the risk of birth of a child with DS as well as in the onset of age related diseases in those women.

1. Introduction

Primary trisomy 21 leading to Down syndrome (DS) originates, in the majority of the cases, from the failure of normal chromosome segregation during maternal meiosis (meiotic nondisjunction), and the major risk factor is advanced maternal age at conception [1]. Indeed, after maternal age 35 years, the risk for a DS pregnancy increases for several years proportionally to increasing maternal age [1]. However, most of DS babies are born from women aging less than 35 years at conception, and this has led to an intense investigation of factors that could contribute to DS risk in young women [2].

The micronucleus assay revealed that young mothers of DS individuals (MDS) have an increased susceptibility to chromosome damage and malsegregation events in peripheral lymphocytes than control mothers [3–6]. Additionally, the analysis of telomere lenght in peripheral lymphocytes showed that women who conceived a DS child before 35 years of age have shorter telomeres than matched control mothers, suggesting that they might be "biologically older" than mothers of euploid babies in the same age group [7]. Interestingly, young MDS were also found to have a five-fold increased risk to develop Alzheimer's disease later in life [8,9]. More recently, altered global DNA methylation levels, evaluated as Long Interspersed Nucleotide Element-1 (LINE1) methylation, were observed in the DNA extracted from peripheral blood lymphocytes of young MDS compared to control mothers, suggesting that DNA methylation reactions are impaired in those women and that this might contribute to chromosome 21 malsegregation events [10]. Collectively, those studies revealed that peripheral lymphocytes of

* Corresponding author at: Department of Translational Research and New Technologies in Medicine and Surgery, Medical Genetics Lab., University of Pisa, Medical School, Via Roma 55, Pisa 56126, Italy.
E-mail address: fabio.coppede@med.unipi.it (F. Coppedè).

http://dx.doi.org/10.1016/j.mrfmmm.2016.02.008
0027-5107/

women who conceived a child with DS in young age show several markers of global genome instability.

In 1999 a case-control study conducted in North America suggested that genes encoding enzymes involved in folate metabolism could act as maternal risk factors for the birth of a child with DS [11]. In particular, the authors observed an increased frequency of the methylenetetrahydrofolate reductase (*MTHFR*) 677C>T polymorphism (rs1801133) in MDS than in control mothers [11]. MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is required for the remethylation of homocysteine (Hcy) to methionine in the *trans*-methylation pathway. Methionine can then be converted to *S*-adenosylmethionine (SAM), the intracellular donor of methyl groups for methylation reactions [12]. The *MTHFR* 677T allele results in decreased protein activity leading to increased Hcy levels and impaired DNA methylation [12]. Therefore, James and coworkers [11] hypothesized that impairments of folate metabolism, resulting from the presence of polymorphisms in metabolic genes, could lead to aberrant methylation of *peri*-centromeric regions of chromosome 21, favouring its abnormal segregation during maternal meiosis and leading to the formation of eggs with two copies of chromosome 21 which, if fertilized, would result in a zygote with full trisomy for chromosome 21 [11].

That paper was followed by over 50 case-control studies investigating the potential contribution of folate-pathway gene polymorphisms as maternal risk factors for the birth of a child with DS (reviewed in Ref. [13]). Systematic reviews and *meta*-analyses of those papers revealed that the *MTHFR* 677C>T polymorphism is likely to be a maternal risk factor for a DS birth, particularly in women subjected to nutritional and/or environmental factors leading to reduced folate bioavailability [2,13–16].

Accumulating evidence is revealing that the *MTHFR* gene is regulated by promoter methylation, and increased *MTHFR* promoter methylation has been observed in semen DNA of infertile men [17–19], in DNA extracted from cancerous tissues [20,21], in blood DNA of patients with cardiovascular pathology or renal disease [22,23], as well as in blood and placenta DNA of women with pre-eclampsia [24]. Those studies revealed that increased promoter methylation levels of this gene result in reduced MTHFR protein activity, thus increasing the risk of various human illnesses [17–24]. Several metabolic genes show inter-individual variability in promoter methylation levels, resulting in inter-individual changes in protein activity similar to those conferred by genetic polymorphisms [25]. However, to the best of our knowledge, there is no available data on *MTHFR* promoter methylation levels in MDS tissues.

Therefore, in the present study, we investigated *MTHFR* promoter methylation levels searching for difference between DNA extracted from peripheral lymphocytes of MDS and matched control mothers. We also searched for correlation between *MTHFR* promoter methylation and micronucleus frequency, an established biomarker of genome instability.

2. Materials and methods

2.1. Study population

Peripheral blood samples were available from 40 women who had a DS child with karyotipically confirmed trisomy 21 and 44 healthy women matched with the case mothers for age at sampling. Control mothers had at least one healthy child and no experience of miscarriages, abnormal pregnancies, or children affected by genetic disorders in their life. All women were aged less than 35 years when they conceived (mean age at conception 28.4 ± 4.9 years). Blood samples were collected by the medical personnel of either the Pisa University Hospital (Pisa, Italy) or the pediatric Hospital "IRCCS Stella Maris Foundation" (Pisa, Italy), and control mothers were recruited among people working at the above Hospitals, at the University of Pisa, or among healthy volunteers. Blood samples were not collected at the time of birth but often some years later when women brought their children to the recruiting Hospitals for medical checks. Therefore, we paid extreme caution to match case and control mothers for age at sampling (Table 1). Peripheral blood samples from all the women included in the present study were originally collected in the frame of a previous study aimed at evaluating chromosome damage events in peripheral lymphocytes [4]. Therefore, data on the frequency of binucleated micronucleated (BNMN) lymphocytes were collected at the time of blood drawing by means of the cytokinesis-block micronucleus assay according to the procedure previously described by us [3] and were already available from our cohort [4,26]. Table 1 presents the mean frequency of BNMN lymphocytes in MDS and control mothers.

The individuals included in the study have been selected after the administration of a validated questionnaire [3] designed to document their previous conditions in order to apply the adopted exclusion criteria. Particularly, all women included in the study were healthy at the time of blood collection and had no documented medical or occupational history of exposure to physical, biological or chemical agents known or suspected to interfere with DNA methylation or with the micronucleus frequency in the three months preceding blood drawing. For instance, alcohol consumption, viral infection, or the current use of pharmacological products known or suspected to interfere with DNA methylation, such as for example folate or B vitamin supplements or epigenetic drugs, were used as exclusion criteria. Case and control mothers were matched for smoking habits (Table 1).

All mothers (MDS and controls) were white Caucasians and residents of central Italy at interview. Written informed consent for inclusion in the study was obtained from each subject. The Ethics Committee of the Scientific Institute IRCCS Stella Maris Foundation approved the study, and all the samples were processed blind, in accordance with the Declaration of Helsinki.

2.2. Extraction of genomic DNA and bisulfite modification

Peripheral blood samples were collected from each subject in EDTA tubes, and stored at −20 °C until assayed. DNA samples were

Table 1
Demographic characteristics of mothers of DS children (MDS) and control mothers.

Variable	MDS N° = 40	Control mothers N° = 44	*p*-Value
Age at sampling: years (mean ± SD)	46.1 ± 11.5	47.8 ± 7.1	0.41[a]
never-smokers	31 (77.5%)	33 (75.0%)	0.69[b]
ex-smokers	4 (10.0%)	3 (7.0%)	
smokers	5 (12.5%)	8 (18.0%)	
BNMN ‰ (mean ± SD)	16.1 ± 8.6	10.5 ± 4.3	0.0004[c]

[a] Student's T test.
[b] Fisher exact test.
[c] Analysis of variance (ANOVA) with corrections for age at sampling and smoking habits.